Halide Permeation in Wild-Type and Mutant Cystic Fibrosis Transmembrane Conductance Regulator Chloride Channels

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ABSTRACT Permeation of cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channels by halide ions was studied in stably transfected Chinese hamster ovary cells by using the patch clamp technique. In cell-attached patches with a high Cl^- pipette solution, the CFTR channel displayed outwardly rectifying currents and had a conductance near the membrane potential of 6.0 pS at 22°C or 8.7 pS at 37°C. The current–voltage relationship became linear when patches were excised into symmetrical, *N*-tris(hydroxymethyl)methyl-2-aminomethane sulfonate (TES)-buffered solutions. Under these conditions, conductance increased from 7.0 pS at 22°C to 10.9 pS at 37°C. The conductance at 22°C was ~1.0 pS higher when TES and HEPES were omitted from the solution, suggesting weak, voltage-independent block by pH buffers. The relationship between conductance and Cl^- activity was hyperbolic and well fitted by a Michaelis-Menten–type function having a K_m of ~38 mM and maximum conductance of 10 pS at 22°C. Dilution potentials measured with NaCl gradients indicated high anion selectivity (P_{Na}/P_{Cl} = 0.003–0.028). Biionic reversal potentials measured immediately after exposure of the cytoplasmic side to various test anions indicated P_I (1.8) > P_{Br} (1.3) > P_{Cl} (1.0) > P_F (0.17), consistent with a "weak field strength" selectivity site. The same sequence was obtained for external halides, although inward F^- flow was not observed. Iodide currents were protocol dependent and became blocked after 1–2 min. This coincided with a large shift in the (extrapolated) reversal potential to values indicating a greatly reduced I^-/Cl^- permeability ratio ($P_I/P_{Cl} < 0.4$). The switch to low I^- permeability was enhanced at potentials that favored Cl^- entry into the pore and was not observed in the R347D mutant, which is thought to lack an anion binding site involved in multi-ion pore behavior. Interactions between Cl^- and I^- ions may influence I^- permeation and be responsible for the wide range of P_I/P_{Cl} ratios that have been reported for the CFTR channel. The low P_I/P_{Cl} ratio usually reported for CFTR only occurred after entry into an altered permeability state and thus may not be comparable with permeability ratios for other anions, which are obtained in the absence of iodide. We propose that CFTR displays a "weak field strength" anion selectivity sequence.

KEY WORDS: iodide permeability • lyotropic sequence

INTRODUCTION

The cystic fibrosis transmembrane conductance regulator (CFTR)[1] belongs to the "ATP binding cassette" superfamily of membrane transport proteins. Although its functions are still debated, several lines of evidence suggest that its primary function is that of a tightly regulated Cl^- channel. Heterologous expression of CFTR generates a low conductance Cl^- channel that is indistinguishable from that found in epithelial cells (Gray et al., 1989; Champigny et al., 1990; Tabcharani et al., 1990; Berger et al., 1991; Kartner et al., 1991; Rommens et al., 1991; Tabcharani et al., 1991). Mutations in CFTR alter the ionic selectivity of the expressed whole cell currents (Anderson et al., 1991), single channel conductance (Sheppard et al., 1993; Tabcharani et al., 1993; McDonough et al., 1994) and sensitivity to channel blockers (Linsdell and Hanrahan, 1996*a*; McDonough et al., 1994). When CFTR is purified to homogeneity and incorporated into bilayers, it forms channels closely resembling those in CFTR-expressing cells (Bear et al., 1992). Mutations in CFTR that cause it to be mislocalized (i.e., ΔF508) or unresponsive (i.e., G551D) are associated with severe forms of cystic fibrosis, whereas mutations that only partially reduce CFTR conductance (R347P,H, Sheppard et al., 1993; Tabcharani et al., 1993), open probability (R117H, Sheppard et al., 1993; Becq et al., 1994; intracellular loop IV mutants, Seibert et al., 1996), or processing (A455E, Sheppard et al., 1995) are associated with milder symptoms. Thus, the primary, though perhaps not exclusive, function of CFTR is to mediate Cl^- permeation.

CFTR gating by nucleotides and regulation by phosphorylation have been characterized in some detail but less is known regarding its permeation properties. Most single channel measurements of CFTR selectivity have not been carried out under biionic conditions, although permeability ratios have been estimated using

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[1]*Abbreviations used in this paper:* CFTR, cystic fibrosis transmembrane conductance regulator; CHO, Chinese hamster ovary; TES, *N*-tris(hydroxymethyl)methyl-2-aminomethane sulfonate.

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Volume 110 October 1997 341–354
http://www.jgp.org

cell-attached and excised membrane patches (Gray et al., 1989; Champigny et al., 1990; Kartner et al., 1991; Bajnath et al., 1993), whole cell patches (Cliff and Frizzell, 1990; Anderson et al., 1991; Bear and Reyes, 1992), cell monolayers (Bell and Quinton, 1992; Kottra, 1996), and fused cells (Schröder and Frömter, 1995). The P_I/P_{Cl} ratios in these studies ranged between 0 and 2.0, with most groups reporting ~0.4. Low I^- permeability has become a diagnostic for identifying CFTR-mediated macroscopic conductance; therefore, it seems important to assess whether the variation in permeability ratios reflects different experimental conditions in the various studies or some intrinsic property of the channel.

In this paper, we describe the halide permeability of single wild-type and mutant CFTR Cl^- channels stably expressed in Chinese hamster ovary cells. CFTR was highly anion selective and the initial halide permeability ratios under biionic conditions were consistent with the lyotropic sequence. Interestingly, the relative I^- permeability was strongly protocol dependent, with high initial P_I/P_{Cl} switching abruptly to a low value similar to the ratios usually reported for macroscopic CFTR currents. This switch to low I^- permeability was accelerated by holding the membrane at potentials that favored Cl^- entry into the channel, and was not observed in R347D CFTR, a mutant shown previously to lack multiion pore behavior in mixtures of Cl^- and thiocyanate. Preliminary reports of this work have appeared (Tabcharani and Hanrahan, 1993).

METHODS

Cells

Chinese hamster ovary (CHO) cells expressing wild-type CFTR or the mutant R347D (arginine 347 in the sixth predicted membrane spanning region mutated to aspartate) have been described previously (Tabcharani et al., 1991; Chang et al., 1993; Tabcharani et al., 1993). They were plated in DMEM containing 8% fetal bovine serum and methotrexate (100 μM) on glass coverslips at a density of ~10^5/cm^2 and maintained at 37°C in 5% CO_2 for 3–5 d before patch clamp experiments.

Solutions

The standard recording solutions in the pipette and bath contained (mM): 150 NaCl, 2 $MgCl_2$, 10 NaTES, pH 7.4. A 100× stock solution of 50 mM dibutyryl cyclic AMP (db-cAMP), 1 mM forskolin, and 1 mM 3-isobutyl-1-methylxanthine (IBMX) was used to raise intracellular cAMP in intact cells during cell-attached recordings. Forskolin, IBMX, db-cAMP, and adenosine triphosphate were from Sigma Chemical Co. (St. Louis, MO). Channel activity was maintained when recording from excised patches using 180 nM protein kinase A catalytic subunit and 1 mM MgATP, as described previously (Tabcharani et al., 1991, 1993). To measure cation permeability, reversal potentials were measured when 110 mM NaCl in the bath or pipette solution was replaced by 220 mM sucrose. Halide permeation was studied under biionic conditions by replacing salts in the pipette or bath solutions with those of the appropriate anion.

Single Channel Recording

Cells were transferred to a chamber containing 0.5 ml bath solution. Pipettes were prepared using a conventional puller (PP-83; Narishige Instruments Lab., Tokyo, Japan) and had 4–6 MΩ resistance when filled with 150 mM NaCl solution. The Ag/AgCl electrodes in the patch pipette and bath solutions were protected from I^- poisoning when necessary by NaCl agar bridges. The bath agar bridge had the same composition as the pipette solution or the agar bridge in the pipette when one was necessary. Liquid junction potentials were determined using a flowing 3-M KCl electrode (Neher, 1992) and reported voltages have been corrected accordingly. Single-channel currents were amplified (Axopatch 1C; Axon Instruments Inc., Foster City, CA), recorded onto video cassette tape by a pulse-coded modulation-type recording adapter (DR384; Neurodata Instrument Co., Delaware Water Gap, PA) and low pass filtered during play back using an 8-pole Bessel-type filter (902 LPF; Frequency Devices Inc., Haverhill, MA). The final recording bandwidth was −3 db at 230 Hz, and records were sampled at 1 kHz and analyzed using a microcomputer as described previously (Tabcharani et al., 1989). V_p refers to the command voltage applied to the pipette interior with reference to the bath during cell-attached patch recording. By convention, the membrane potential (V_m) in excised patches is the potential of the bath solution with reference to that of the pipette.

Current–voltage (i/V) relationships were calculated by a semiautomated procedure in which all-points histograms were computed screen by screen and displayed sideways next to the data so that current amplitudes and peaks could be verified using cursors. Also, the number of data points used in the histogram was adjusted to minimize the effect of any drift so that only those points immediately before and after a transition were included (between 100 ms and 8 s of the record). Histogram peaks were broader when patches contained multiple channels (usually between one and five channels), but this did not affect estimates of unitary amplitudes, which were averages of at least 10 determinations between several levels. Periods of severe baseline drift were not included in the analysis. Open events were measured at each potential and entered into an i/V curve, which was displayed at the end of the run. Reversal potentials were estimated by fitting a polynomial function to the i/V curve. Slope conductance was determined by linear regression over the voltage ranges specified in RESULTS. Permeability ratios were calculated using the equation $P_X/P_{Cl} = \exp(-E_{rev}F/RT)$, where E_{rev} is the reversal potential and the other terms have their usual meanings. The relationship between conductance and symmetrical Cl^- activity was fitted using the Michaelis-Menten equation $\gamma = \gamma_{max}/(1 + [K_m/(Cl^-)])$, where γ is conductance, γ_{max} the saturating conductance of the channel, K_m the apparent affinity of the channel for Cl^- ions, and (Cl^-) the Cl^- activity calculated using the Debye-Hückel theory (Robinson and Stokes, 1970). Single channel experiments were performed at room temperature (~22°C) unless otherwise indicated.

RESULTS

Fig. 1, *A* and *B* show cell-attached recordings of CFTR channels at 37 and 22°C. The current–voltage (i/V) relationships were essentially linear at positive applied membrane voltages, but outwardly rectified at hyperpolarizing potentials (Fig. 1 *C*). Rectification under these cell-attached conditions presumably reflects (*a*) the low intracellular Cl^- activity anticipated from ion-selective microelectrode studies in other cells, and (*b*) voltage-

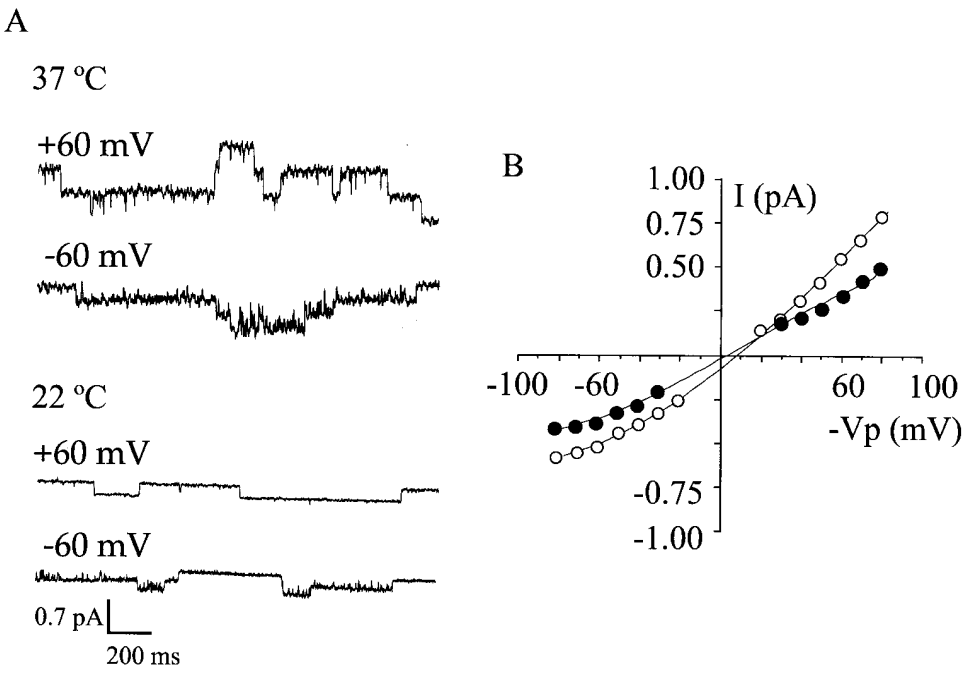


FIGURE 1. CFTR Cl^- channels recorded in excised, inside-out patches bathed with symmetrical 154-mM Cl^- solutions. (*A*) Traces at ±60 mV when the bath was kept at 37 or 22°C. (*B*) Current–voltage relationships for patches shown in *A*.

dependent fast block of CFTR by large intracellular anions (Linsdell and Hanrahan, 1996*b*).

When patches were excised and bathed symmetrically with 150 mM NaCl solutions at different holding potentials (Fig. 2 *A*), single channel conductance was 7.0 ± 0.1 pS. Using different patches to test each Cl^- concentration, the i/V relationship remained linear in the range ±80 mV as Cl^- activity was varied symmetrically (Fig. 2 *B*). Activity coefficients for Cl^- in 15, 30, 45, 75, 150, and 400 mM NaCl solutions were estimated by the modified Debye-Hückel theory to be 0.93, 0.85, 0.87, 0.79, 0.75, and 0.69, respectively (Robinson and

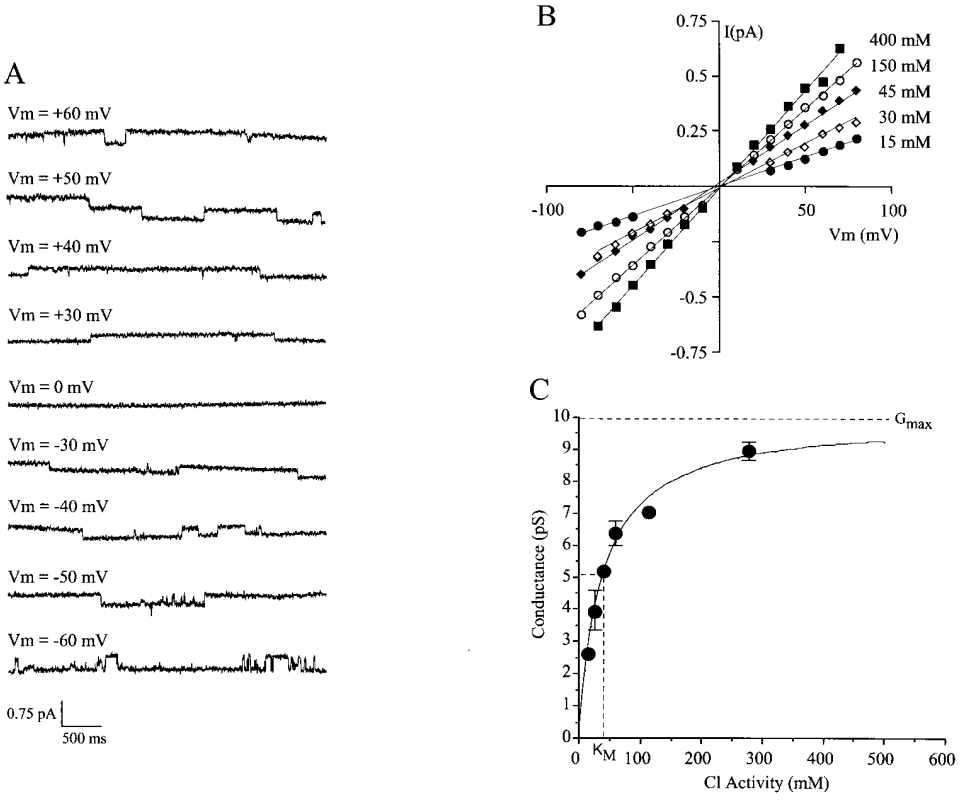


FIGURE 2. Relationship between Cl^- concentration and CFTR conductance. (A) Traces obtained with symmetrical 150-mM NaCl concentrations. (*B*) Current–voltage relationships obtained with different symmetrical NaCl concentrations. All solutions also contained 2 mM $MgCl_2$. (*C*) Relationship between single channel conductance and bilateral Cl^- activity. Solid line is the best fit Michaelis-Menten relationship with K_m = 37.6 and maximum conductance γ_{max} = 10.0 pS.

Stokes, 1970). Plotting mean CFTR conductance at each Cl^- activity yielded a rectangular hyperbola that was well described by the Michaelis-Menten equation when K_m = 37.6 mM Cl^- activity and G_{max} = 10.0 pS (Fig. 2 *C*).

The conductances reported here are consistent with previous reports from this laboratory, although values in the literature vary over a considerable range (4–12 pS), presumably due to different experimental conditions. HEPES and some other N-substituted taurine buffers are known to inhibit the conductance of anion channels (e.g., Hanrahan and Tabcharani, 1990); therefore, we compared i/V curves obtained in solutions buffered symmetrically by the zwitterionic buffers TES and HEPES with those obtained when solutions were weakly buffered using the cationic buffer TRIS (1 mM; Fig. 3). TES and HEPES at concentrations typically used in patch clamp experiments (10–40 mM) reduced single channel conductance by ~14%. This effect was small compared with the inhibition of the outwardly rectifying anion channel that would occur with the same concentration of HEPES (~65%; Hanrahan and Tabcharani, 1990). TES was chosen as the pH buffer in subsequent studies because it is a stronger buffer at pH 7.4 and has low reactivity compared with TRIS; however, it should be noted that CFTR conductances reported in this paper will be reduced at all potentials. The conductance of the CFTR channel bathed symmetrically with 154 Cl^- solutions was increased at 37°C as reported previously for endogenous CFTR channels on T_{84} cells (Tabcharani et al., 1990), and the i/V relationship remained linear (not shown).

CFTR channels were selective for Cl^- over Na^+ according to dilution potential measurements with a NaCl gradient (not shown). Single channel currents reversed at −27.8 ± 0.7 mV (n = 4) with 150 mM NaCl in the pipette and 40 mM NaCl + 220 mM sucrose in the bath solution, which yields P_{Na}/P_{Cl} = 0.028. The zero current potential was +30.0 ± 0.9 mV (n = 2) when the orientation of the salt gradient was reversed, which gave P_{Na}/P_{Cl} = 0.003. These ratios are too low to be measured accurately but are generally consistent with the values reported previously (0.125, Tabcharani et al., 1990; 0.04–0.07, Tabcharani et al., 1991; 0.01–0.16, Anderson et al., 1991; and 0.2, Bear and Reyes, 1992).

Permeation by external Br^- and F^- was studied under biionic conditions at 22°C. Bromide currents were clearly observed at positive potentials with 150 mM NaBr solution in the pipette and 150 mM NaCl solution in the bath (Fig. 4 *A*). The reversal potential was shifted from 0 to −5.2 mV under these conditions, indicating a permeability ratio P_{Br}/P_{Cl} = 1.22. Nevertheless, the mean slope conductance between +60 and +80 mV suggested the ratio G_{Br}/G_{Cl} = 0.48. External Br^- had no apparent effect on Cl^- flow from the cytoplasmic side. With NaF solution in the pipette, large negative currents (−0.2 pA) carried by outward Cl^- flow were observed at 0 mV (Fig. 4 *A*). These Cl^- currents decreased in amplitude as the potential was increased, and became undetectable when it approached +50 mV (<0.05 pA). External F^- was not measurably permeant; therefore, a reversal potential could not be determined. Extrapolation of the i/V curve suggests an upper limit for the permeability ratio ($P_F/P_{Cl} < 0.1$). Ex-

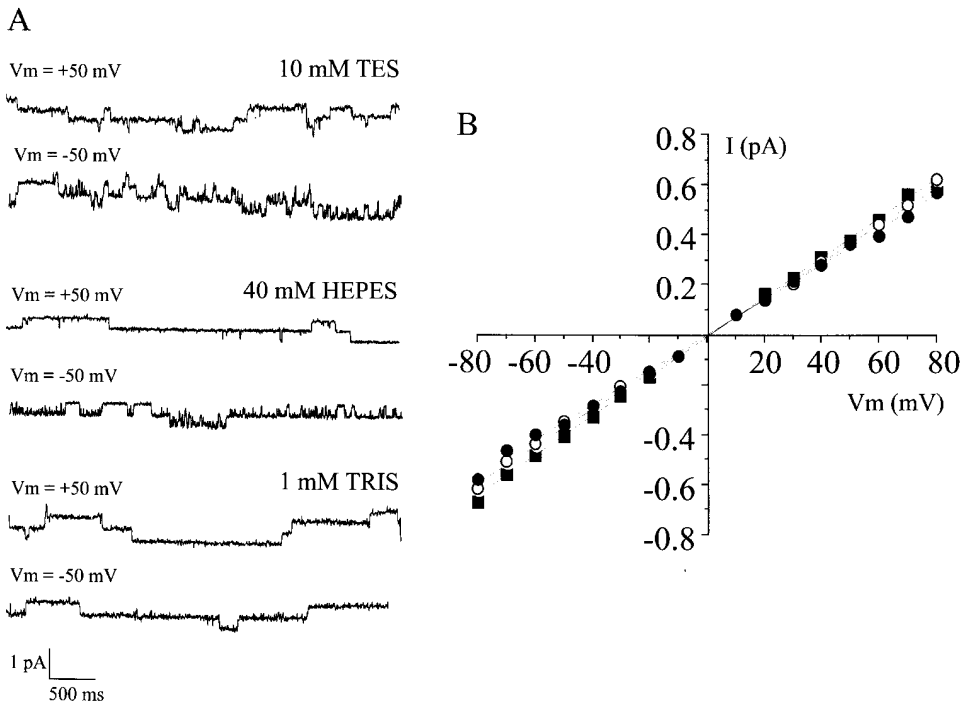
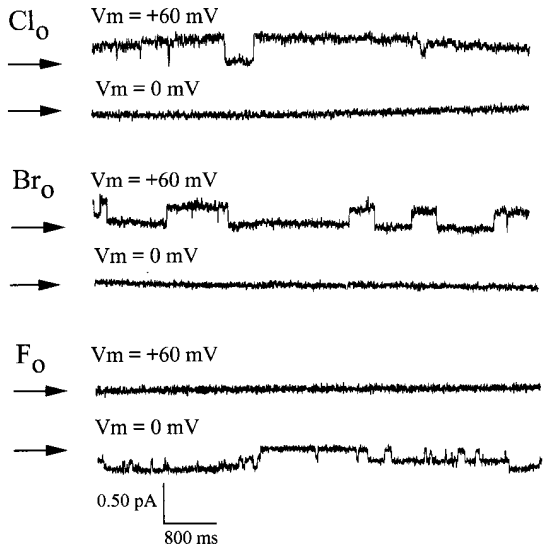


FIGURE 3. Effect of pH buffers on conductance of the CFTR Cl^- channel. (*A*) Traces obtained with 10 mM TES, 40 mM HEPES, and 1 mM TRIS bathing both sides at ±50 mV. Note flickering at negative potentials when bath contained zwitterionic buffers, but not when it contained the cationic buffer TRIS. (*B*) Current–voltage relationships obtained with (○) 10 mM TES, (●) 40 mM HEPES, (■) 1 mM TRIS.



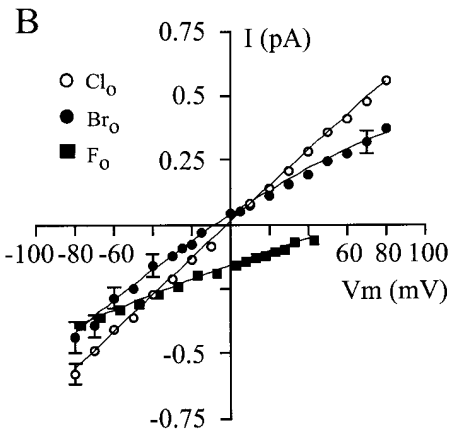
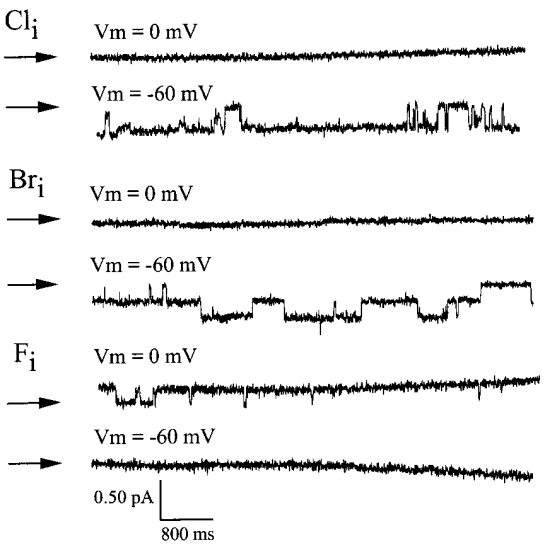


FIGURE 4. Single CFTR channels recorded in excised, inside-out patches with different halides on the extracellular side. (*A*) Traces obtained at +60 and 0 mV with external chloride (Cl_o^-), bromide (Br_o^-), or fluoride (F_o^-). (*B*) Current–voltage relationships obtained under biionic conditions. Reversal potential indicates $P_{Br} > P_{Cl}$; inward rectification indicates $G_{Br} < G_{Cl}$. Note that Cl^- currents at negative potentials are reduced by external Br^-. Currents carried by external fluoride were not detected.



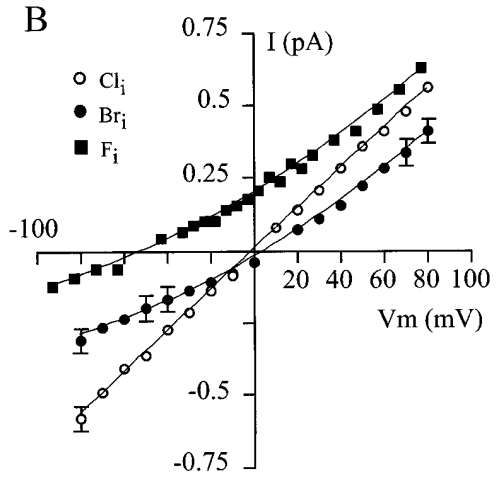


FIGURE 5. Single CFTR channels recorded in excised, inside-out patches with different halides on the intracellular side. (*A*) Traces obtained at −60 and 0 mV with internal chloride (Cl_i^-), bromide (Br_i^-), or fluoride (F_i^-). (*B*) Current–voltage relationships obtained under biionic conditions. Note $P_{Br}/P_{Cl} > 1.0$ and $G_{Br}/G_{Cl} < 1.0$, and the "*trans*" inhibition of Cl^- currents at positive potentials when Br^- solutions bathed the cytoplasmic side. Currents carried by internal fluoride were clearly observed. Note open probability $P_o \cong 1.0$ with 150 mM F_i^-.

ternal F^- also inhibited outward Cl^- flow at negative potentials (Fig. 4 *B*).

Fig. 5 *A* shows single channel currents carried by Br^- under biionic conditions with Br^- on the cytoplasmic side. The i/V relationship was outwardly rectified and reversed at +5.8 ± 3.5 mV (Fig. 5 *B*), indicating $P_{Br}/P_{Cl} = 1.30$. Despite the high permeability ratio, slope conductances at negative potentials suggested $G_{Br}/G_{Cl} = 0.48$, similar to the ratio obtained with external Br^-. The i/V relationship at 37°C with internal Br^- reversed at +8.3 ± 1.3 mV, indicating $P_{Br}/P_{Cl} = 1.36$ (data not shown). Replacing NaCl in the bath with NaF shifted the reversal potential to negative potentials, indicating low F^- permeability (Fig. 5 *B*). However, in contrast to the results obtained with external F^-, currents carried by internal F^- could be observed at appropriate voltages (Fig. 5 *B*). The i/V relationship determined under these biionic conditions reversed at −46.7 mV, indicating $P_F/P_{Cl} = 0.17$. Comparing the slope conductances at negative voltages when patches were bathed with symmetrical Cl^- or with biionic conditions with F^- in the bath and Cl^- in the pipette yielded $G_F/G_{Cl} \cong 0.5$.

The P_I/P_{Cl} ratios that have been reported for CFTR vary over a wide range. Most whole-cell patch clamp and cell monolayer studies have indicated that the P_I/P_{Cl} of CFTR-mediated conductance is between 0.4 and 0.6.

Indeed, $P_I < P_{Cl}$ has been used as a diagnostic property for distinguishing CFTR-mediated conductance from those mediated by other Cl^- channels, such as the outwardly rectifying anion channel. Nevertheless, several studies of CFTR have described substantially higher I^- permeability (e.g., $P_I/P_{Cl} \cong 1.0$, Gray et al., 1990; 1.1, Kartner et al., 1991; 1.9, Tabcharani et al., 1992; 1.3, Dousmanis and Gadsby, 1994).

We examined I^- permeation under biionic conditions and found that the current–voltage relationship was strongly dependent on the voltage protocol. With NaI solution in the pipette, large currents carried by inward I^- flow were initially observed at positive potentials and could be recorded up to ~2 min (Fig. 6 *A*). These iodide currents became smaller as the holding potential was made increasingly negative and reversed polarity near −20 mV, indicating $P_I/P_{Cl} = 2.1$ (Fig. 6 *B*). However, when the membrane potential was subsequently repolarized after briefly recording Cl^- currents at negative potentials, the relationship clearly diverged from the initial i/V curve; i.e., negative currents (carried by outward Cl^- flow) were clearly observed at 0 mV, but positive currents (carried by I^-) could no longer be recorded (Fig. 6 *B*; n = 3–6 patches). The channel remained in this low I^- permeability state as long as I^- was present, but Cl^- currents reappeared when I^- was washed from the bath. The reappearance of Cl^- currents occurred with a time course that was too fast to be resolved with these methods. Extrapolation of the second i/V curve suggested a reversal potential greater than +20 mV; i.e., $P_I < P_{Cl}$, although this reversal could not be measured. We refer to the initial, high I^- permeability state as unblocked (I_{unbl}), and the low I^- permeability state that develops later as blocked (I_{bl}). The switch from I_{unbl} to I_{bl} could not be distinguished during recordings at negative potentials because the amplitudes of negative currents were the same in both states. The switch to low iodide permeability was more rapid at voltages that would drive Cl^- into the pore and was observed in every experiment. Thus, I^- currents were observed at positive potentials for 0.5–2 min before the channel switched to the I_{bl} state. By contrast, I^- currents became blocked within seconds at the same potentials during test pulses from a negative holding potential where Cl^- ions would carry the current. Fig. 7 shows an example of a patch excised into Cl^--containing bath solution exposed to I^- solution in the pipette. Iodide currents disappeared when channels were held at V_p = +20 mV, although Cl^- currents were observed during steps to negative potentials (data not shown).

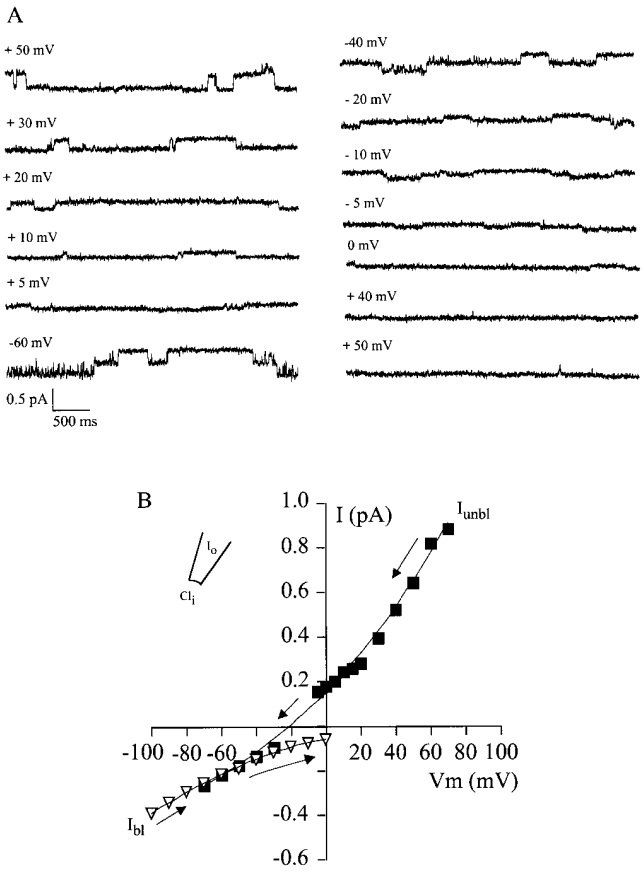


FIGURE 6. Hysteresis of the current–voltage relationship with extracellular iodide solution. (*A*) Representative traces obtained at different holding potentials in the sequence indicated. (*B*) The current–voltage relationship determined from traces such as those shown in *A*. Arrows indicate the protocol. Squares are the currents measured initially when the channel had high I^- permeability (I_{unbl} state). The mean reversal potential under these conditions indicates $P_I/P_{Cl} = 2.1$. Inverted triangles show the currents measured after the switch to low I^- permeability (I_{bl} state). Positive currents, which would be carried by external I^- ions, disappeared after the switch. The extrapolated reversal potential under these conditions indicated $P_I << P_{Cl}$.

Similar hysteresis and block were observed with I^- on the cytoplasmic side (Fig. 8). Iodide currents could be recorded for several minutes with I^- in the bath and Cl^- in the pipette if patches were excised while holding the membrane at negative potentials. When the potential was increased, the resulting i/V relationship was outwardly rectified and reversed near +16 mV, indicating $P_I/P_{Cl} = 1.8$, but transitions abruptly switched from the unblocked (I_{unbl}) state to the "blocked" (I_{bl}) state have ~40% lower conductance. Entering I_{bl} coincided with a negative shift in the (extrapolated) reversal potential to at least −20 mV, consistent with a large decrease in relative iodide permeability to $P_I/P_{Cl} \pm 0.4$ (n = 6–10), similar to that reported in many previous studies of macroscopic I^- conductance (e.g., Anderson et al., 1991; Bell and Quinton, 1992). Each point in Fig. 8 is the mean of 3–10 observations; however, points were collected from at least 20 patches because the unblocked state was transient and not all i/V points could be obtained from each patch. It was not possible to

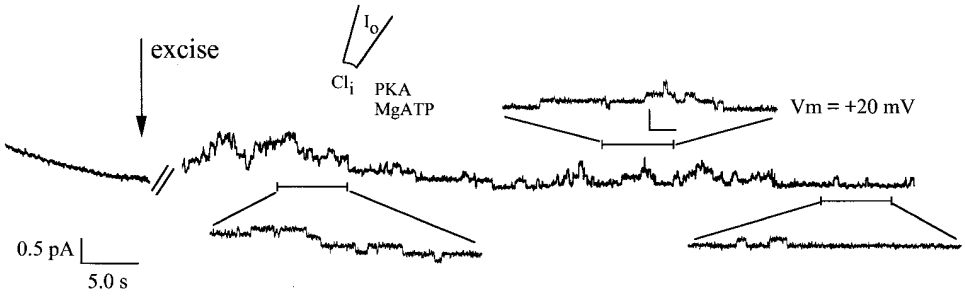


FIGURE 7. Transition from initial high I^- permeability state to the low I^- permeability state while holding at +20 mV. Note that channel currents carried by Cl^- ions were still observed at negative potentials.

record iodide currents after the switch between I_{bl} and I_{unbl} had occurred; however, the number of CFTR channels observed before and after were always similar. When channels had been allowed to enter the I_{bl} state and the bath solution containing NaI, MgATP, and PKA was completely replaced by NaCl, transitions initially disappeared, consistent with the known dependence of CFTR gating on ATP. Restoring MgATP and PKA to the NaCl solution reactivated I_{unbl} transitions, allowing Cl^- currents to be recorded at both positive and negative holding potentials.

The switch from high to low I^- permeability was observed whenever I^- was present on one side of the membrane, and was enhanced by holding at potentials where the current would be carried by Cl^-. Thus, it may depend on the entry of Cl^- ions into the pore. The switch was not instantaneous since I^- currents were sometimes observed for a few seconds after pulses had been applied to drive Cl^- through the channel, as if I^- or Cl^- were binding at a site in the pore where permeating anions are not required to move in single file, preventing permeation by I^- but allowing Cl^- to pass through. This behavior was not symmetrical since Cl^- flow from the "trans" side was reduced by internal (Fig. 6) but not external (Fig. 8) iodide.

The switch from I_{bl} to I_{unbl} was dependent on ionic strength (Fig. 9; see also Tabcharani et al., 1992). When biionic (NaCl/NaI) experiments were carried out using salts at 400 rather than 150 mM, only the high I^- permeability state was observed. With external I^- and internal Cl^-, the mean reversal potential was -19.6 ± 0.2 mV at 400 mM, compared with -18.1 ± 0.01 mV at 150 mM in the I_{unbl} state. With internal I^-, the corresponding reversal potentials were $+17.5 \pm 0.9$ mV and $+15.5 \pm 1.4$ mV, respectively. Both internal and external I^- induced outward rectification under biionic conditions (Fig. 9). Thus, $G_I > G_{Cl}$ when I^- was present externally, whereas $G_I < G_{Cl}$ when I^- was present intracellularly. This discrepancy only occurred when I^- and Cl^- were present on opposite sides of the membrane; the i/V curve with symmetrical I^- solutions was linear and yielded a conductance (5.4 ± 0.15 pS, $n = 4$) that was slightly lower than with symmetrical Cl^- solutions (Fig. 10). The reversal potentials and biionic permeability ratios for wild-type CFTR are summarized in Table I.

Role of Arg^{347} in I^- Permeation

Previous studies have suggested that the sixth membrane spanning region of CFTR lines the pore (Anderson et al., 1991; Sheppard et al., 1993; Tabcharani et al., 1993; McDonough et al., 1994; Cheung and Akabas, 1996; Linsdell and Hanrahan, 1996*a*) and that the arginine at position 347 may form part of an anion binding site. Mutations that remove the positive charge at this site eliminate voltage-dependent block by internal thiocyanate and abolish the anomalous mole fraction effects that are normally observed in mixtures of Cl^- and thiocyanate (Tabcharani et al., 1993). To assess the possible role of this residue in I^- permeation and block, we studied the I^- selectivity of a mutant in which this residue was converted to aspartate (R347D). Fig. 11 compares the i/V relationships obtained when the mutant channel was bathed with symmetrical Cl^- or with external Cl^- and internal I^-. In symmetrical Cl^- solutions, the i/V relationship was linear and conductance was reduced by ~60%, as reported previously (Tabcharani et al., 1993). With I^- on the cytoplasmic side, the reversal potential was +1.0 mV, consistent with an equilibrium permeability ratio $P_I/P_{Cl} = 0.96$. Despite this loss of selectivity between I^- and Cl^-, the mutant resembled the wild-type channel in having low I^- conductance and I^- inhibition of Cl^- flow from the "*trans*" side. Most significantly, the permeability ratio P_I/P_{Cl} was not protocol dependent in the R347D mutant. Iodide currents through R347D could be measured indefinitely after Cl^- currents had been allowed to flow for several minutes. The inability of R347D to distinguish between I^- and Cl^- ions suggests arg^{347} may contribute to the selectivity filter.

DISCUSSION

Comparing the selectivity of whole cell currents generated by wild-type and mutant CFTR helped establish that CFTR forms a Cl^- conductive pathway (Anderson et al., 1991). In studies of macroscopic anion conductances, low P_I/P_{Cl} ratios in the range 0.4–0.6 have be-

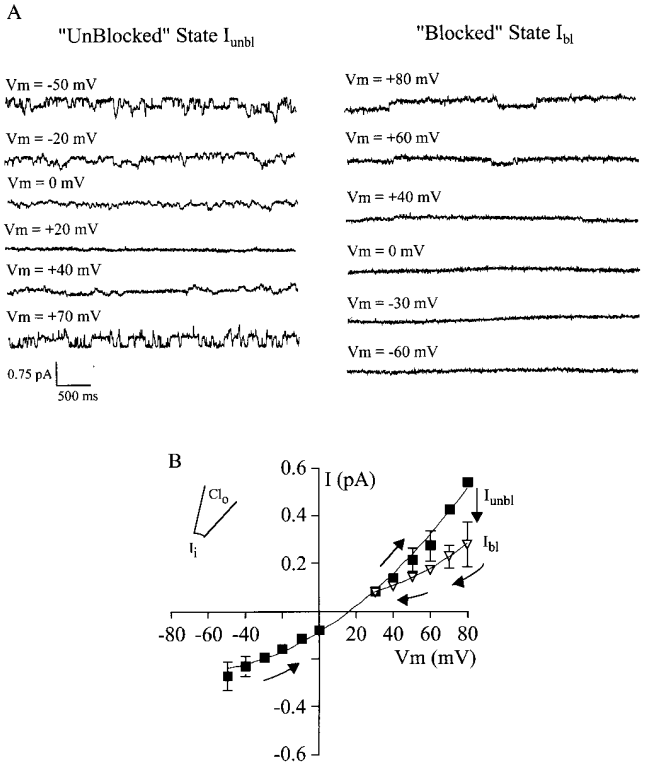


FIGURE 8. Hysteresis of the current–voltage relationship with intracellular iodide solution. (*A*, *left*) Traces recorded at different holding potentials in the sequence are indicated; i.e., starting at negative potentials where currents would initially be carried by iodide. (*right*) Traces obtained from the same patch after the switch to low I^- permeability. Note that current amplitudes decreased by ~40% at +80 mV, and iodide currents could no longer be measured at negative potentials. (*B*) Mean current–voltage relationship obtained under biionic conditions with internal iodide (mean ± SEM, $n = 6$). Arrows indicate the protocol. Squares represent initial currents in the I_{unbl} state, when the mean reversal potential indicated $P_I/P_{Cl} = 2.1$. Inverted triangles show the currents measured after channels switched to low I^- permeability (I_{bl} state). Positive currents, which would be carried by external I^- ions, could no longer be measured under these conditions. The extrapolated reversal potential under these conditions indicated $P_I << P_{Cl}$.

come a hallmark of cAMP-stimulated Cl^- currents, and have been used to distinguish CFTR-mediated currents from those activated by calcium and cell swelling (Cliff and Frizzell, 1990). Nevertheless, there is confusion at the single channel level and values for P_I/P_{Cl} of zero (Champigny et al., 1990; Tabcharani et al., 1992) and 1.0–1.8 have been reported (Gray et al., 1990; Kartner et al., 1991). The present study shows that P_I/P_{Cl} is strongly protocol dependent, as if I^- were binding in the pore when Cl^- is also present, thereby reducing Cl^- current in one direction and blocking I^- flux in the other. This "low iodide permeability" state is not observed when measuring ratios for other anions; therefore, the P_I/P_{Cl} ratio for this state may not be comparable with P_{Br}/P_{Cl} and P_F/P_{Cl} determined in the absence of I^-. We propose that the high initial iodide permeability in the I_{unbl} state is comparable with ratios for the other anions, and the selectivity sequence for CFTR is $P_I > P_{Br} > P_{Cl} > P_F$; i.e., the lyotropic series observed in many other anion channels. This would be consistent with the high permeability of CFTR to lyotropic polyatomic anions such as SCN^- reported previously (Gray et al., 1989; Tabcharani et al., 1993).

CFTR Conductance

The conductance of CFTR channels (6 pS at 22°C) recorded on CHO cells at the membrane potential, which was not measured during these experiments, is similar to that reported previously in single channel studies of human pancreatic (Gray et al., 1989), intestinal (Tabcharani et al., 1990; Bear and Reyes, 1992; Bajnath et al., 1993), and airway epithelial cells (Haws et al., 1992) expressing endogenous CFTR, and on a variety of heterologous expression systems (e.g., Bear et al., 1991; Berger et al., 1991; Kartner et al., 1991; Tabcharani et al., 1991; Cliff et al., 1992; Sheppard et al., 1993; Venglarik et al., 1994).

The conductance of recombinant CFTR channels at 37°C in this study is similar to previous measurements on T_{84} (8.6 pS cell-attached configuration; Tabcharani et al., 1990) and CHO cells (9.6 pS excised; Tabcharani et al., 1991) and on NIH-3T3 fibroblasts (8.5 pS cell-attached, Fischer and Machen, 1994; 10.1 pS excised, Carson et al., 1994). Outward rectification in cell-attached patches was originally attributed to the Cl^- concentration gradient between the pipette solution and cytoplasm because i/V relations became linear when patches were excised into symmetrical Cl^- solutions (Gray et al., 1989; Tabcharani et al., 1991). Moreover, when CFTR was studied in Sf9 cells infected with a recombinant Baculovirus containing the CFTR gene, both the membrane and reversal potentials for CFTR currents approached 0 mV, and the i/V became linear, suggesting that elevation of cell Cl^- under these conditions was sufficient to eliminate rectification (Kartner et al., 1991). More recent studies indicate CFTR can be blocked by large anions from the cytoplasmic side in a voltage-dependent manner (Linsdell and Hanrahan, 1996*b*), thus flickery block by large intracellular anions may contribute to the rectification seen in the cell-attached patches. Commonly used pH buffers such as HEPES and TES caused a voltage-independent decrease in single channel conductance; however, differences in temperature are considered the most likely explanation for the range of conductances reported for CFTR. There may also be some variation in the conductance of CFTR homologues in other species (e.g., Gray et al., 1988; Riordan et al., 1994).

The dependence of conductance on Cl^- activity was well fitted by the Michaelis-Menten relationship without including a term for surface charge effects (see also

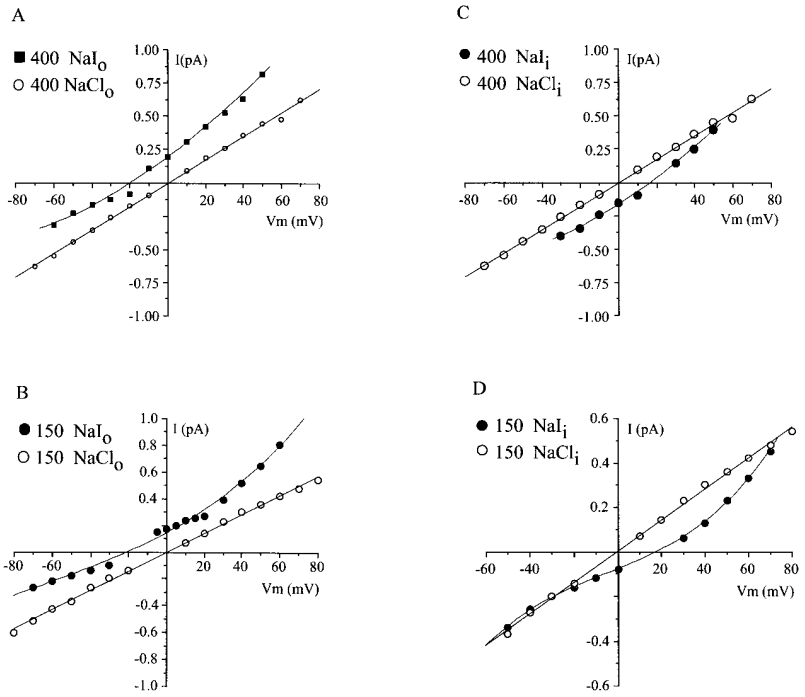


FIGURE 9. Comparison of current–voltage relationships obtained under biionic conditions with external or internal iodide solution. *A* and *B* show results obtained with external NaI and internal NaCl solutions, when pipette and bath solutions were 400 and 150 mM, respectively. *C* and *D* show biionic current–voltage relationships obtained with intracellular I^- when solutions were 400 or 150 mM, respectively.

Linsdell et al., 1997*b*). Interestingly, the K_m estimated from the fits is similar to the intracellular Cl^- activity measured previously in secretory epithelia (e.g., Welsh et al., 1982). Thus, like many enzymes, the affinity of CFTR is close to its substrate concentration. A similar series of experiments carried out to assess the effects of mutations in the sixth membrane spanning region (TM6) yielded similar values for K_m and G_{max} (Linsdell et al., 1997*b*), and were used when fitting barrier models (Linsdell et al., 1997*a*).

Anion–Cation Selectivity

CFTR was highly selective for Cl^- over sodium ($P_{Na}/P_{Cl} < 0.028$). This ratio is somewhat lower than reported previously (Tabcharani et al., 1990; Anderson et al., 1991; Bear, 1992), but is consistent with the observation that CFTR conductance and permeability ratios are not affected when sodium is replaced by *N*-methyl-D-glucamine or tetramethylammonium (Gray et al., 1988; Kartner et al., 1991; Bear, 1992). This insensitivity of Cl^- conductance to the nature of the cation suggests the permeation mechanism in CFTR differs from that described for a neuronal Cl^- channel, where cations permeate relatively well and are proposed to form ion pairs with permeating Cl^- ions in the pore (Franciolini and Non-

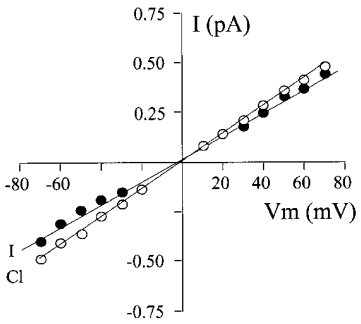


FIGURE 10. Comparison of the mean current–voltage relationships obtained from patches bathed symmetrically with (○) NaCl or (●) NaI solutions.

TABLE I

*Reversal Potentials and Permeability Ratios Determined for Halides under Biionic Conditions**

Ion	Reversal potential	Permeability ratio (P_x/P_{Cl})	*n*
	mV		
Cl	0	1.00	8
Br_o	−5.23 ± 1.04	1.22 ± 0.05	4
Br_i	+5.84 ± 3.53	1.26 ± 0.17	4
F_o	—	<0.04	3
F_i	−46.69 ± 11.69	0.17 ± 0.07	5
$I_{o(unbl)}$‡	−18.10 ± 0.01	2.00 ± 0.11	2
$I_{o(bl)}$§	—	<0.04	6
$I_{o(400\ mM)}$‖	−19.60 ± 0.20	2.08 ± 0.04	2
$I_{i(unbl)}$	+15.50 ± 1.42	1.82 ± 0.10	6
$I_{i(bl)}$	—	<0.04	10
$I_{i(400\ mM)}$	+17.50 ± 0.90	1.9 ± 0.06	6

*Solution containing Cl^- at the same concentration as the test anion bathed the opposite side; ‡unbl refers to the unblocked, high iodide permeability state; §bl is the I^--blocked state; ‖400 refers to biionic conditions with 400-mM salt concentrations bathing both sides.

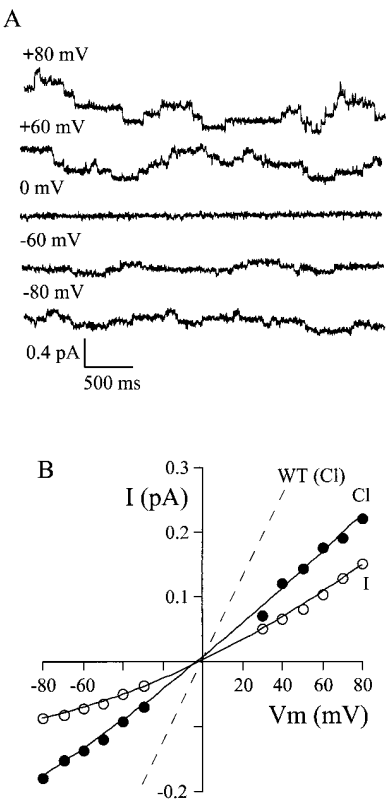


FIGURE 11. Selectivity of the CFTR channel mutant R347D. (*A*) Mutant channels recorded under biionic conditions with NaCl solution in the pipette and NaI in the bath. (*B*) Mean current–voltage relationships obtained when patches containing the R347D mutant are bathed externally with NaCl solution and internally with (●) NaCl or (○) NaI solution. For comparison, the i/V relationship for wild-type CFTR channels bathed with symmetrical NaCl (from Fig. 2) is shown as a dashed line. Cl^- conductance of the R347D mutant was reduced by almost half in symmetrical Cl solution, as reported previously (Tabcharani et al., 1993). When iodide solutions were placed on the cytoplasmic side, conductance was further reduced at all potentials, the reversal potential did not shift significantly, and the switch from I_{bl} to I_{unbl} was abolished.

ner, 1994). The present data do not exclude ionic strength effects on anion:cation permeability ratios.

It has recently been proposed that anion selectivity in CFTR arises at the cytoplasmic end of the pore, based on comparisons of the voltage-dependent rates at which external methanesulfonate reagents react with cysteines engineered at the intracellular end of TM6 (Cheung and Akabas, 1997). In this regard, the mutant R347D had normal anion:cation permeability ratios (Linsdell and Hanrahan, unpublished observations) as did R347E (Anderson et al., 1991), but arg^{352}, which has also been proposed to form part of the anion selectivity filter, remains a candidate.

Bromide and Fluoride Permeation

The Br^-/Cl^- permeability ratio for CFTR was high under biionic conditions (1.3) and was identical whether Br^- was present on the extracellular or cytoplasmic side. It is similar to the ratio estimated previously in cell-attached patches when pipette Cl^- was partially replaced by Br^- ($P_{Br}/P_{Cl} \cong 1$; Gray et al., 1990). The rectifying i/V relations indicate low Br^- conductance, although the ratio calculated at these potentials ($G_{Br}/G_{Cl} = 0.48$) can be only a first approximation if Br^- permeation is affected by Cl^- on the opposite side (see below). That such "*trans*" effects occur in CFTR is evident from the inhibition of outward Cl^- flow by external F^- (Fig. 4), and from the discrepancy between values of G_I/G_{Cl} determined using asymmetrical solutions vs. biionic conditions. Low G_{Br}/G_{Cl} ratios have been reported for single channels under nonbiionic conditions (Gray et al., 1990), but were not observed in whole cell experiments under biionic conditions (Anderson et al., 1991). Although the present results do not explain the high G_{Br}/G_{Cl} ratios in the latter study, they suggest that the discrepancy was not caused by the use of biionic conditions. The high permeability ratio and low conductance ratio suggest that Br^- ions are slowed during permeation by binding within the pore.

Fluoride currents and a biionic reversal potential were observed with internal, but not external F^-. Anderson et al. (1991) reported P_F/P_{Cl} ratios of 0.11 for the apical membrane of T_{84} cells, and 0.3 for whole cell currents in transfected NIH 3T3 cells. Dousmanis and Gadsby (1994) reported ratios of 0.2–0.3 for PKA-stimulated whole-cell Cl^- current in cardiac myocytes. In attempting to reconcile these data, we note that whole cell measurements are expected to be more susceptible to contamination by parallel pathways for F^- movement. On the other hand, single channel recordings are more likely to underestimate permeability when the ion inhibits gating or has very low permeability. Our inability to measure currents carried by external F^- may indeed reflect block by external F^-, since Cl^- currents are also reduced by external F^-.

Evidence for Two States Having Different Iodide Permeabilities

Early studies of CFTR channels on pancreatic duct, which involved partial replacement of pipette Cl^- with I^-, suggested that P_I/P_{Cl} is near 1.0 (Gray et al., 1990). Ratios ≥ 1 were obtained in early studies of recombinant CFTR (1.2, Kartner et al., 1991; 1.7, Tabcharani et al., 1992) and in several cell types that express CFTR endogenously (1.51, Chan et al., 1992; 1.31, Walsh and Long, 1992; 1.29, Dousmanis and Gadsby, 1994). Nevertheless, most permeability ratios obtained from macroscopic currents have been <1.0 (e.g., 0.4, Cliff and Frizzell, 1990; 0.3–0.6, Anderson et al., 1991; 0.4–0.6, Anderson and Welsh, 1991; 0.5, Haws et al., 1992; 0.6, Bear and Reyes, 1992; 0.9, Bell and Quinton, 1992; 0.9, Overholt et al., 1993). The present results indicate that

the I^- permeability ratio depends on the protocol used and that both high and low values can be observed in the same CFTR channel. Thus, I^- currents were recorded for several minutes when patches were excised and held at potentials that would drive I^- through the channel. Under these conditions, the i/V relationship indicated unequivocally that $P_I > P_{Cl}$. After a variable time interval at negative potentials, the channel switched to a different conductance state in which $P_I < P_{Cl}$ and I^- currents disappeared at positive potentials. This state, which we shall call I_{bl}, had an extrapolated reversal potential indicative of $P_I/P_{Cl} << 1$, although the ratio could not be determined because I^- currents were not observed. Impermeability to iodide would agree with some previous single channel studies (Champigny et al., 1990; Cliff et al., 1992). Iodide alone did not induce the I_{bl} state, which did not occur when the channel was bathed in symmetrical I^- solutions. The conductance in symmetrical I^- solutions was 77% of that measured with symmetrical Cl^- (Fig. 10). Low iodide permeability was not caused by oxidized forms of I^- such as I_3^- or I_5^- (Läuger et al., 1967; Finkelstein and Cass, 1968), since it was not affected by addition of the reducing agent thiosulfate (20 mM) to the I^- solution (data not shown). The switch from $P_I > P_{Cl}$ to $P_I < P_{Cl}$ was enhanced at potentials that would favor Cl^- entry into the channel, and once the switch occurred, it was not overcome by reversing the voltage to again drive I^- through the channel.

Mutating Arg^{347} Abolishes the Switch to Low Iodide Permeability

A gross alteration in pore structure (e.g., induced by the simultaneous presence of both Cl^- and I^-) seems unlikely to explain the low I^- permeability of I_{bl} state, considering that Cl^- permeation from the outside was only partially reduced and Cl^- permeation from the inside was unaffected. When studied only from the inside after the switch to I_{bl}, iodide behaved as if it were an impermeant anion. We do not know the precise mechanism of the switch in permeability, but suggest that Cl^- or I^- binds in a non–single file region of the pore, preventing I^- permeation but allowing Cl^- ions to pass through. The notion that the delayed block of I^- currents involves Cl^- entry and ion–ion interactions within the pore is strengthened by the finding that a mutation that eliminates anomalous mole fraction behavior in mixtures of Cl^- and thiocyanate also abolished the switch from I_{unbl} to I_{bl}.

Cl^- currents were restored as quickly as measurements could be made after replacing the I^- solution bathing one side of the patch with Cl^- solution (i.e., within a few seconds; data not shown). Since P_I/P_{Cl} for I_{bl} and I_{unbl} encompass the range of values reported previously, the intermediate ratios that have been obtained in studies of macroscopic currents may have been due to different proportions of channels in each permeability state. The switch to the I_{bl} state and the apparent decrease in P_I/P_{Cl} were abolished when salt concentrations were increased from 150 to 400 mM NaCl/NaI (Fig. 9). Whether this reflects concentration-dependent multi-ion interactions in the pore (e.g., Friel and Tsien, 1989) remains to be determined.

CFTR Has a "Weak Field Strength" Selectivity Sequence

Information on anion selectivity from a large number of biological and nonbiological systems has been used to construct empirical selectivity isotherms (Wright and Diamond, 1977). Based on 81 quantitative anion sequences from various systems, only 5 of 24 possible sequences were observed. Selectivity was modeled by calculating the difference between the free energy of hydration of the anions and electrostatic interactions between each anion and model sites (Eisenman, 1962). Once the equilibrium permeability ratio P_I/P_{Cl} was known for a particular system, the ratios for the other two halides could be predicted using the isotherms (Wright and Diamond, 1977). As shown in Fig. 12 *A*, this holds true for CFTR, but only when P_I/P_{Cl} of the I_{unbl} state is considered. When CFTR switched to low I^- permeability, the "Type I" sequence ($P_I > P_{Br} > P_{Cl} > P_F$) was changed to one that does not resemble any of the known sequences, although it is most consistent with a "moderate field strength" Type III sequence ($P_{Br} > P_{Cl} > P_I > P_F$). Whereas low P_I/P_{Cl} was only observed in the I_{bl} state induced by I^-, P_F/P_{Cl}, and P_{Br}/P_{Cl} were determined in the complete absence of I^- when the channel would presumably be in the I_{unbl} state. Thus, the validity of combining permeability ratios obtained in these different states is questionable and we propose that only the high initial P_I/P_{Cl} ratio obtained immediately after exposure to iodide can be compared with biionic P_{Br}/P_{Cl} and P_F/P_{Cl} ratios.

Arg^{347} May Contribute to a Weak Field Strength Site for Iodide

High macroscopic P_I/P_{Cl} ratios have been reported previously for CFTR channels in which positively charged residues in the membrane spanning regions were mutated to negatively charged residues (K95E, 1.43; K335E, 1.37; R347E, 0.9; R1030E, 0.81; Anderson et al., 1991). The P_I/P_{Cl} ratio obtained for R347D under biionic conditions is intermediate between I_{unbl} and I_{bl} in the wild-type channel, and is consistent with the value of 0.9 reported previously for R347E (Anderson et al., 1991). This suggests that arg^{347} may play some role in selecting among anions. Arginine has several properties that favor the binding of lyotropic anions. Studies with the model compound *n*-propylguanidine indicate that arginine is by far the most hydrophilic amino acid, with a partition coefficient between water and vapor phases

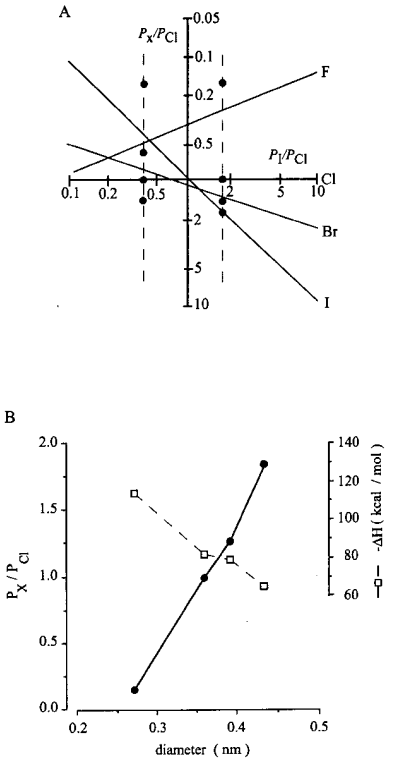


FIGURE 12. Evidence that the CFTR Cl^- channel in the I_{unbl} state has a low field strength selectivity sequence. (*A*) Selectivity isotherms replotted from Wright and Diamond (1977) with permeability ratios obtained for CFTR denoted by ●. Dashed vertical line at right shows that when ratios for other halides are plotted against the high (initial) P_I/P_{Cl} obtained in this study, they follow the isotherms reasonably well and indicate a Type I (low field strength) site. On the other hand, when ratios for other anions are plotted using a low value of P_I/P_{Cl}, they do not fit any of the anion sequences that have been reported for other biological and nonbiological systems. (*B*) Relationship between unhydrated diameters of the halides ($F^- < Cl^- < Br^- < I^-$) and their respective permeability ratios (●). Also shown is the relationship between unhydrated diameter and hydration energy (□; $-\Delta H$).

$\sim 10^6$ higher than those of the charged residues aspartate and lysine (Wolfenden et al., 1981). Despite this, arginine has been suggested to have some hydrophobic character because it appears on the hydrophobic side of amphipathic α helices much more frequently than predicted (Cornette et al., 1987). Indeed, the δ-guanido group of arginine has been suggested to behave as a lyotropic solute itself, increasing the solvation of nearby surfaces within proteins and stabilizing native conformation (Collins and Washabaugh, 1985). The loss of I^- selectivity after mutation of arg^{347} suggests it might contribute to the low field strength site in CFTR. Arginine has low field strength because its positive charge is distributed by resonance amongst the carbon and all three nitrogen atoms of the δ-guanido group. The very large solvent-accessible surface area of the arginine side chain (196 $Å^2$) would be expected to further weaken the field strength of this diffuse charge. The resonance of electrons in the δ-guanido group of arginine would favor induction of a cationic dipole. The relative polarizability of halide crystals (Tessman et al., 1953) follows the sequence I^- (2.17) > Br^- (1.41) > Cl^- (1.0) > F^- (0.22), almost identical to the selectivity sequence for CFTR reported in this paper. Because the side chain and anion are both easily induced to form dipoles, dispersion forces may be important in stabilizing the interaction between the arginine residue and lyotropic anions.

Halide selectivity in CFTR cannot be attributed exclusively to the region around arg^{347}; however, because high P_I/P_{Cl} ratios have been reported previously for other pore mutants (K95D and K335E; Anderson et al., 1991), and because R347D retains some preference for Br^- over Cl^- and selects strongly against F^- (our unpublished observations). P_I/P_{Cl} was altered by a mutation that abolished the anomalous mole fraction effect (AMFE, R347D); however, these properties are not strictly correlated because K335E also had high P_I/P_{Cl} in a previous study (Anderson et al., 1991) and yet displays an AMFE in SCN^-–Cl^- mixtures (Tabcharani et al., 1993).

Lyotropic Selectivity in CFTR

The sequence $I^- > Br^- > Cl^- > F^-$ is part of the lyotropic series first reported by Hofmeister (1888). It is observed when the effects of anions on protein solubility, anion adsorption to lipid bilayers, and surface potentials at air–water interfaces are characterized (for review of early literature, see Dani et al., 1983). Studies with model compounds indicate that such sequences require both an anion-attracting positive charge or dipole and a neighboring hydrophobic group. The fact that many anion channels have nearly identical halide sequences suggests that selectivity may be determined by a common structural element, such as the cationic dipole of the peptide bond, rather than a specific amino acid side chain or arrangement of amino acids. Binding of lyotropic anions to the amide dipole of the peptide backbone in proteins has been proposed previously (Robinson and Jencks, 1965) and amide dipoles in solution are anion attracting and confer lyotropic anion selectivity (Hamabata and Von Hippel, 1973). Lyotropic selectivity also requires a neighboring hydrophobic region where extensive hydrogen bonding between water molecules causes them to be more structured. Preferential association of lyotropic anions such as I^- at these sites will be solvent driven when it is energetically more favorable for water molecules (which would interact only weakly with I^-) to hydrogen bond with this iceberg network on the hydrophobic surface than to associate with the I^- ion. Lyotropic anions bind to lipid bilayers and generate negative surface charge (McLaughlin et al., 1975). If similar binding occurs at

hydrophobic sites in the mouth of the pore, charge screening might account for the loss of unusual effects of iodide at 400-mM salt concentrations.

The finding that a large, weakly hydrated halide such as Br^- permeates through CFTR more readily than a smaller anion such as F^-, which has high hydration energy (ΔG_H; Fig. 12 *B*), suggests that the pore must be sufficiently narrow to require at least partial dehydration of the anion. The following paper estimates the functional diameter of single CFTR channels using polyatomic anions of known dimensions.

We thank Drs. Xiu-Bao Chang, Jack Riordan, Johanna Rommens, and Lap Chee Tsui for providing the CHO cell lines and Jenny Eng and Jie Liao for technical assistance.

This work was supported by the Canadian Cystic Fibrosis Foundation (CCFF), the Medical Research Council (MRC, Canada), and the National Institute of Diabetes and Digestive and Kidney Diseases. P. Linsdell is a fellow of the CCFF. J.W. Hanrahan is an MRC Scientist.

Original version received 10 October 1996 and accepted version received 11 July 1997.

REFERENCES

Anderson, M.P., R.J. Gregory, S. Thompson, D.W. Souza, S. Paul, R.C. Mulligan, A.E. Smith, and M.J. Welsh. 1991. Demonstration that CFTR is a chloride channel by alteration of its anion selectivity. *Science (Wash. DC).* 253:202–205.

Anderson, M.P., and M.J. Welsh. 1991. Calcium and cAMP activate different chloride channels in the apical membrane of normal and cystic fibrosis epithelia. *Proc. Natl. Acad. Sci. USA.* 88:6003–6007.

Bajnath, R.B., J.A. Groot, H.R. De Jonge, M. Kansen, and J. Bijman. 1993. Synergistic activation of non-rectifying small-conductance chloride channels by forskolin and phorbol esters in cell-attached patches of the human colon carcinoma cell line HT-29cl.19A. *Pflügers Arch.* 425:100–108.

Bear, C.E., F. Duguay, A.L. Naismith, N. Kartner, J.W. Hanrahan, and J.R. Riordan. 1991. Cl^- channel activity in *Xenopus* oocytes expressing the cystic fibrosis gene. *J. Biol. Chem.* 266:19142–19145.

Bear, C.E., C. Li, N. Kartner, R.J. Bridges, T.J. Jensen, M. Ramjeesingh, and J.R. Riordan. 1992. Purification and functional reconstitution of the cystic fibrosis transmembrane conductance regulator (CFTR). *Cell.* 68:809–818.

Bear, C.E., and E.F. Reyes. 1992. cAMP-activated chloride conductance in the colonic cell line, Caco-2. *Am. J. Physiol.* 262:C251–C256.

Becq, F., T.J. Jensen, X.-B. Chang, A. Savoia, J.M. Rommens, L.-C. Tsui, M. Buchwald, J.R. Riordan, and J.W. Hanrahan. 1994. Phosphatase inhibitors activate normal and defective CFTR chloride channels. *Proc. Natl. Acad. Sci. USA.* 91:9160–9164.

Bell, C.L., and P.M. Quinton. 1992. T84 cells: anion selectivity demonstrates expression of Cl^- conductance affected in cystic fibrosis. *Am. J. Physiol.* 262:C555–C562.

Berger, H.A., M.P. Anderson, R.J. Gregory, S. Thompson, P.W. Howard, R.A. Maurer, R. Mulligan, A.E. Smith, and M.J. Welsh. 1991. Identification and regulation of the cystic fibrosis transmembrane conductance regulator-generated chloride channel. *J. Clin. Invest.* 88:1422–1431.

Carson, M.R., S.M. Travis, M.C. Winter, D.N. Sheppard, and M.J. Welsh. 1994. Phosphate stimulates CFTR Cl^- channels. *Biophys. J.* 67:1867–1875.

Champigny, G., B. Verrier, C. Gérard, J. Mauchamp, and M. Lazdunski. 1990. Small conductance chloride channels in the apical membrane of thyroid cells. *FEBS Lett.* 259:263–268.

Chan, H.-C., J. Goldstein, and D.J. Nelson. 1992. Alternate pathways for chloride conductance activation in normal and cystic fibrosis airway epithelial cells. *Am. J. Physiol.* 262:C1273–C1283.

Chang, X.-B., J.A. Tabcharani, Y.-X. Hou, T.J. Jensen, N. Kartner, N. Alon, J.W. Hanrahan, and J.R. Riordan. 1993. Protein kinase A (PKA) still activates CFTR chloride channel after mutagenesis of all ten PKA consensus phosphorylation sites. *J. Biol. Chem.* 268: 11304–11311.

Cheung, M., and M.H. Akabas. 1997. Locating the anion selectivity filter of the CFTR chloride channel. *J. Gen. Physiol.* 109:289–299.

Cheung, M., and M.H. Akabas. 1996. Identification of cystic fibrosis transmembrane conductance regulator channel-lining residues in and flanking the M6 membrane-spanning segment. *Biophys. J.* 70:2688–2695.

Cliff, W.H., R.A. Schoumacher, and R.A. Frizzell. 1992. cAMP-activated Cl channels in CFTR-transfected CF pancreatic epithelial cells. *Am. J. Physiol.* 262:C1154–1160.

Cliff, W.H., and R.A. Frizzell. 1990. Separate Cl^- conductances activated by cAMP and Ca^{2+} in Cl^- secreting epithelial cells. *Proc. Natl. Acad. Sci. USA.* 87:4956–4960.

Collins, K.D., and M.W. Washabaugh. 1985. The Hofmeister effect and the behaviour of water at interfaces. *Q. Rev. Biophys.* 18:323–422.

Cornette, J.L., K.B. Cease, H. Margalit, J.L. Spouge, J.A. Berzofsky, and C. DeLisi. 1987. Hydrophobicity scales and computational techniques for detecting amphipathic structures in proteins. *J. Mol. Biol.* 195:659–685.

Dani, J.A., J.A. Sanchez, and B. Hille. 1983. Lyotropic anions. Na channel gating and Ca electrode response. *J. Gen. Physiol.* 81: 255–281.

Dousmanis, A.G., and D.C. Gadsby. 1994. Anion permeability sequence of the open cardiac CFTR Cl channel. *Biophys. J.* 66:*A*421. (Abstr.)

Eisenman, G. 1962. Cation selective glass electrodes and their mode of operation. *Biophys. J.* 2(part 2 Suppl.):259–323.

Finkelstein, A., and A. Cass. 1968. Permeability and electrical properties of thin lipid membranes. *J. Gen. Physiol.* 52:145–173.

Fischer, H., and T.E. Machen. 1994. CFTR displays voltage dependence and two gating modes during stimulation. *J. Gen. Physiol.* 104:541–566.

Franciolini, F., and W. Nonner. 1994. Anion–cation interactions in the pore of neuronal background chloride channels. *J. Gen. Physiol.* 104:711–723.

Friel, D.D., and R.W. Tsien. 1989. Voltage-gated calcium channels: direct observation of the anomalous mole fraction effect at the single-channel level. *Proc. Natl. Acad. Sci. USA.* 86:5207–5211.

Gray, M.A., J.R. Greenwell, and B.E. Argent. 1988. Secretin-regulated chloride channel on the apical plasma membrane of pancreatic duct cells. *J. Membr. Biol.* 105:131–142.

Gray, M.A., A. Harris, L. Coleman, J.R. Greenwell, and B.E. Argent. 1989. Two types of chloride channel on duct cells cultured from human fetal pancreas. *Am. J. Physiol.* 257:C240–C251.

Gray, M.A., C.E. Pollard, A. Harris, L. Coleman, J.R. Greenwell, and B.E. Argent. 1990. Anion selectivity and block of the small conductance chloride channel on pancreatic duct cells. *Am. J. Physiol.* 259:C752–C761.

Hamabata, A., and P.H. Von Hippel. 1973. Model studies on the effects of neutral salts on the conformational stability of biological macromolecules. II. *Biochemistry.* 12:1264–1282.

Hanrahan, J.W., and J.A. Tabcharani. 1990. Inhibition of an outwardly rectifying anion channel by HEPES and related buffers. *J. Membr. Biol.* 116:65–77.

Haws, C., M.E. Krouse, Y. Xia, D.C. Gruenert, and J.J. Wine. 1992. CFTR channels in immortalized human airway cells. *Am. J. Physiol.* 263:L692–L707.

Hofmeister, F. 1888. Zur Lehre von der Wirkung der Salze. Zweite Mittheilung. Ueber Regelmässig keiten in der eiwass fällenden Wirkung der Salze und ihre Besiehung zum physiologischen Verhalten derselben. *Arch. Exp. Pathol. Pharmakol.* 24:247–260.

Kartner, N., J.W. Hanrahan, T.J. Jensen, A.L. Naismith, S. Sun, C.A. Ackerley, E.F. Reyes, L. Tsui, J.M. Rommens, C.E. Bear, and J.R. Riordan. 1991. Expression of the cystic fibrosis gene in non-epithelial invertebrate cells produces a regulated anion conductance. *Cell.* 64:681–691.

Kottra, G. 1996. Calcium is not involved in the cAMP-mediated stimulation of Cl^- conductance in the apical membrane of *Necturus* gallbladder epithelium. *Pflügers Arch.* 429:647–658.

Läuger, P., W. Lesslauer, E. Marti, and J. Richter. 1967. Electrical properties of bimolecular phospholipid membranes. *Biochim. Biophys. Acta.* 135:20–32.

Linsdell, P., J.A. Tabcharani, and J.W. Hanrahan. 1997*a*. A multion mechanism for ion permeation and block in the CFTR chloride channel. *J. Gen. Physiol.* 110:365–377.

Linsdell, P., J.A. Tabcharani, J.M. Rommens, Y.-X. Hou, X.-B. Chang, L.-C. Tsui, J.R. Riordan, and J.W. Hanrahan. 1997*b*. Permeability of wild-type and mutant CFTR chloride channels to polyatomic anions. *J. Gen. Physiol.* 110:355–364.

Linsdell, P., and J.W. Hanrahan. 1996*a*. Disulphonic stilbene block of cystic fibrosis transmembrane conductance regulator Cl^- channels expressed in a mammalian cell line and its regulation by a critical pore residue. *J. Physiol. (Cambr.)* 496:687–693.

Linsdell, P., and J.W. Hanrahan. 1996*b*. Flickery block of single CFTR chloride channels by intracellular anions and osmolytes. *Am. J. Physiol.* 271:C628–C634.

McDonough, S., N. Davidson, H.A. Lester, and N.A. McCarty. 1994. Novel pore-lining residues in CFTR that govern permeation and open-channel block. *Neuron.* 13:623–634.

McLaughlin, S., A. Bruder, S. Chen, and C. Moser. 1975. Chaotropic anions and the surface potential of bilayer membranes. *Biochim. Biophys. Acta.* 394:304–313.

Neher, E. 1992. Correction for liquid junction potentials in patch clamp experiments. *Methods Enzymol.* 27:123–131.

Overholt, J.L., M.E. Hobert, and R.D. Harvey. 1993. On the mechanism of rectification of the isoproterenol-activated chloride current in Guinea-pig ventricular myocytes. *J. Gen. Physiol.* 102:871–895.

Riordan, J.R., B. Forbush III, and J.W. Hanrahan. 1994. The molecular basis of chloride transport in shark rectal gland. *J. Exp. Biol.* 196:405–418.

Robinson, D.R., and W.P. Jencks. 1965. The effect of compounds of the urea-guanidinium class on the activity coeficient of acetyl-tetraglycine ethyl ester and related compounds. *J. Am. Chem. Soc.* 87: 2462–2470.

Robinson, R.A., and R.H. Stokes. 1970. Electrolyte solutions. 2nd revised ed. Butterworth-Heinemann Ltd., London.

Rommens, J.M., S. Dho, C.E. Bear, N. Kartner, D. Kennedy, J.R. Riordan, L. Tsui, and K. Foskett. 1991. cAMP-inducible chloride conductance in mouse fibroblast lines stably expressing human cystic fibrosis transmembrane conductance regulator. *Proc. Natl. Acad. Sci. USA.* 88:7500–7504.

Schröder, U.H., and E. Frömter. 1995. Characterization of two distinct Cl^- conductances in fused human respiratory epithelial cells II. Relation to cystic fibrosis gene product. *Pflügers Arch.* 430: 257–264.

Seibert, F.S., P. Linsdell, T.W. Loo, J.W. Hanrahan, D.M. Clarke, and J.R. Riordan. 1996. Disease-associated mutations in the fourth cytoplasmic loop of cystic fibrosis transmembrane conductance regulator compromise biosynthetic processing and chloride channel activity. *J. Biol. Chem.* 271:15139–15145.

Sheppard, D.N., D.P. Rich, L.S. Ostedgaard, R.J. Gregory, A.E. Smith, and M.J. Welsh. 1993. Mutations in CFTR associated with mild-disease-form Cl^- channels with altered pore properties. *Nature (Lond.).* 362:160–164.

Sheppard, D.N., L.S. Ostedgaard, M.C. Winter, and M.J. Welsh. 1995. Mechanism of dysfunction of two nucleotide binding domain mutations in cystic fibrosis transmembrane conductance regulator that are associated with pancreatic sufficiency. *EMBO (Eur. Mol. Biol. Organ.) J.* 14:876–883.

Tabcharani, J.A., T.J. Jensen, J.R. Riordan, and J.W. Hanrahan. 1989. Bicarbonate permeability of the outwardly rectifying anion channel. *J. Membr. Biol.* 112:109–122.

Tabcharani, J.A., W. Low, D. Elie, and J.W. Hanrahan. 1990. Low-conductance chloride channel activated by cAMP in the epithelial cell line T_{84}. *FEBS Lett.* 270:157–164.

Tabcharani, J.A., X. Chang, J.R. Riordan, and J.W. Hanrahan. 1991. Phosphorylation-regulated Cl^- channel in CHO cells stably expressing the cystic fibrosis gene. *Nature (Lond.).* 352:628–631.

Tabcharani, J.A., X.-B. Chang, J.R. Riordan, and J.W. Hanrahan. 1992. The cystic fibrosis transmembrane conductance regulator chloride channel: iodide block and permeation. *Biophys. J.* 62:1–4.

Tabcharani, J.A., J.M. Rommens, Y.-X. Hou, X.-B. Chang, L.-C. Tsui, J.R. Riordan, and J.W. Hanrahan. 1993. Multi-ion pore behaviour in the CFTR chloride channel. *Nature (Lond.).* 366:79–82.

Tabcharani, J.A., and J.W. Hanrahan. 1993. Permeation in the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel. *Biophys. J.* 64:*A*17. (Abstr.)

Tessman, J.R., A.H. Kahn, and W. Shockley. 1953. Electronic polarizabilities of ions in crystals. *Phys. Rev.* 92:890–895.

Venglarik, C.J., B.D. Schultz, R.A. Frizzell, and R.J. Bridges. 1994. ATP alters current fluctuations of cystic fibrosis transmembrane conductance regulator: evidence for a three state activation mechanism. *J. Gen. Physiol.* 104:123–146.

Walsh, K.B., and K.J. Long. 1992. Inhibition of heart calcium and chloride currents by sodium iodide. *J. Gen. Physiol.* 100:847–865.

Welsh, M.J., P.L. Smith, and R.A. Frizzell. 1982. Chloride secretion by canine tracheal epithelium. II. The cellular electrical potential profile. *J. Membr. Biol.* 70:227–238.

Wolfenden, R., L. Andersson, P.M. Cullis, and C.C. Southgate. 1981. Affinities of amino acid side chains for solvent water. *Biochemistry.* 20:849–855.

Wright, E.M., and J.M. Diamond. 1977. Anion selectivity in biological systems. *Physiol. Rev.* 57:109–157.