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Monitoring Charge Flux to Quantify Unusual Ligand-Induced Ion Channel Activity for Use in Biological Nanopore-Based Sensors

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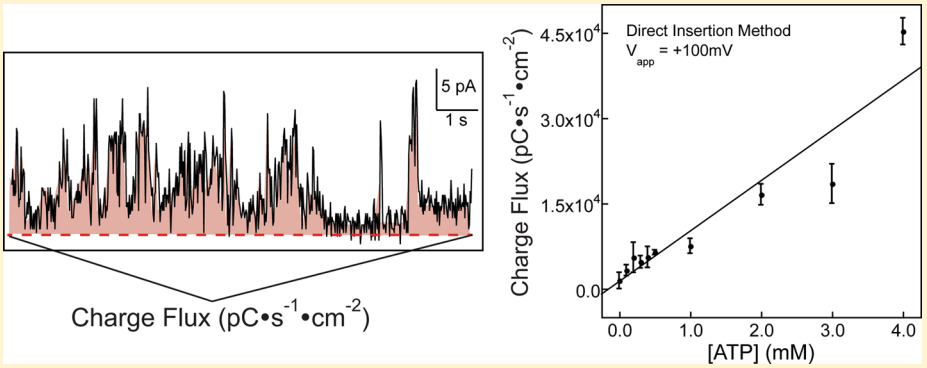
Supporting Information



ABSTRACT: The utility of biological nanopores for the development of sensors has become a growing area of interest in analytical chemistry. Their emerging use in chemical analysis is a result of several ideal characteristics. First, they provide reproducible control over nanoscale pore sizes with an atomic level of precision. Second, they are amenable to resistive-pulse type measurement systems when embedded into an artificial lipid bilayer. A single binding event causes a change in the flow of millions of ions across the membrane per second that is readily measured as a change in current with excellent signal-to-noise ratio. To date, ion channel-based biosensors have been limited to well-behaved proteins. Most demonstrations of using ion channels as sensors have been limited to proteins that remain in the open, conducting state, unless occupied by an analyte of interest. Furthermore, these proteins are nonspecific, requiring chemical, biochemical, or genetic manipulations to impart chemical specificity. Here, we report on the use of the pore-forming abilities of heat shock cognate 70 (Hsc70) to quantify a specific analyte. Hsc70 reconstitutes into phospholipid membranes and opens to form multiple conductance states specifically in the presence of ATP. We introduce the measurement of "charge flux" to characterize the ATP-regulated multiconductance nature of Hsc70, which enables sensitive quantification of ATP (100 μM–4 mM). We believe that monitoring protein-induced charge flux across a bilayer membrane represents a universal method for quantitatively monitoring ion-channel activity. This measurement has the potential to broaden the library of usable proteins in the development of nanopore-based biosensors.

The utilization of the properties of nanopores, both biological and solid state, represents a promising strategy in the development of sensitive biosensors.[1−5] Nanopore-based detection strategies have the ability to provide reproducible and sensitive detection down to the single molecule level.[1,2,4] The success of biological nanopore-based sensors, including ion channels and transmembrane proteins, is afforded by the reproducible nanoscale pore size that biology provides with an atomic level of precision.[5] Furthermore, measuring the conductivity of these pores embedded in an insulating lipid bilayer is readily adaptable to resistive-pulse type measurements for quantitative detection.[2,3] Using resistive pulse methods, the utilization of transmembrane proteins has been reported for the sensitive detection of analytes ranging from divalent metal ions,[6] small molecules,[2−4] and large single stranded polynucleotides.[7] While not as physically robust as their solid-state counterparts,[1,5] several recent reports describe strategies to overcome the limitations of using biological pores, including their susceptibility to pH, salt, and temperature changes,[5,8] and the fragility of the lipid bilayer that supports the protein pore.[2−5] As such, the use of biological nanopores continues to show promise in the development of biosensors.

The majority of recent reports using biological nanopores for sensor development have been limited to the use of a handful of well-characterized, well-behaved, and relatively nonspecific protein channels. These channels include α-hemolysin (αHL),[1,9−11] *Mycobacterium smegmatis porin A* (MspA),[1,5] alamethicin,[12] and a more recently studied DNA-packaging nanomotor protein phi29.[1,13] The nonspecific protein channel αHL has dominated the literature in the past decade and presently is the most-studied nanopore channel for small

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molecule detection[1,4,14] and DNA sequencing.[1,5,15] Work with MspA and phi29 has been mostly limited to DNA sequencing.[1,5,13] αHL has become an excellent candidate for ion channel-based biosensors due to its efficient pore-forming capability,[9,11] robust structure,[2,3] and amenability to chemical and biochemical engineering.[2,6,8] The latter has led to sensors for the detection of various analytes, such as divalent metal ions,[2,4,6] organic molecules,[2,4,16] explosive agents,[17,18] enzyme complexes,[19] cancer biomarker,[20] nucleic acids,[4,7,8,15] and proteins.[4,21] Alamethicin has been modified for the detection of protein−ligand interactions[12] and poly(ethylene glycol) polymers.[22] Unlike the other listed examples, alamethicin forms pores with multiple, discrete conductance states based on the number of monomer peptides coming together to form a pore in the lipid membrane.[12,22−25] While mutagenesis and other bioengineering approaches improve the specificity of biological nanopores like αHL, it still remains difficult to achieve high chemical specificity. Biology, on the other hand, provides a wealth of specific, ligand-gated transmembrane proteins. As such, using specific protein channels could provide a means to develop extremely specific sensors. However, examples of using ligand-gated proteins in the development of biosensors are few. These include whole cell-based sensors, serotonin-gated channels (5H3T), and the peptide sequence LKLHL for sugar sensing.[26] The relatively few examples is likely due to the commercial availability, the level of characterization, and the difficult data analysis of the activity of the proteins outside the commonly used channels.

In this study, we present the use of heat shock cognate protein that displays adenosine triphosphate (ATP)-regulated ion channel activity for the specific and quantitative detection of ATP. Specifically, we utilize the channel-like activity of the heat shock cognate protein Hsc70. The ion channel activity of Hsc70 was first reported by Arispe and De Maio who demonstrated that recombinant Hsc70 spontaneously inserts into lipid bilayers and requires ATP for consistent channel activity.[27] While Hsc70 does form stable ion conductance pathways, the protein fluctuates between six open conductance states and the closed state. Furthermore, little quantitative and qualitative information is known about the proteins' ATP-dependent interactions with lipids. We, thus, label this protein as "misbehaved" or "unusual" in relation to the well-behaved and well-characterized channels like αHL.[1] To account for this unusual activity, we develop a new quantitative method that allows reproducible and quantitative analysis of ATP. In particular, we introduce the quantity "charge flux" to suitably characterize the ATP-regulated ion channel-like activity of Hsc70 and specifically quantify ATP as the target analyte. We demonstrate the quantitative relationship of the ion-channel activity of Hsc70 on ATP using a lipid bilayer cup setup. We believe that this new methodology is a universal strategy for monitoring ligand-gated channel activity and could potentially open pathways to using virtually any protein channel in the development of sensitive biological nanopore-based biosensors.

MATERIALS AND METHODS

Chemicals. 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), $MgCl_2$, $CaCl_2$, KCl, and ATP disodium salt were all purchased from Sigma and used as received. HEPES buffer (5.0 mM HEPES, 0.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, pH = 7) was prepared by dissolving the appropriate amount of chemicals in ultrapure water (Milli-Q ultrapure water purification system). The phospholipids, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE), and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (POPS) were obtained from Avanti Polar Lipids (Alabaster, AL) and were stored in a −20 °C freezer until use. Recombinant Hsc70 (Stressgen) was used as received without further purification and was kept at −80 °C.

Preparation of Hsc70 Proteoliposomes. Hsc70 proteoliposomes were prepared following a method described by Arispe et al. with several modifications.[28] Liposomes were prepared by sonicating a 1:1 ratio mixture of POPE/POPS dissolved in 10 mM HEPES−1 M KCl buffer for 5 min. Recombinant Hsc70 (2.5 μM) was then added to the liposome suspension (30 μL) followed by sonication for an additional period of 3−5 min. A 5 μL aliquot of the Hsc70 proteoliposome suspension was added to the cis side of the bilayer chamber and stirred for ion-channel activity measurements in instances when the "liposome method" was used for protein incorporation (described in more detail below).

Formation of Black Lipid Membranes. Black lipid membranes (BLMs) were formed in a bilayer chamber/cup setup (Warner Instruments, CT) using a painting method described previously.[29,30] Briefly, the bilayer cup was primed with a lipid suspension containing POPE/POPS (1:1) dissolved in *n*-decane (MD Biochemicals, LLC). After evaporation of excess decane, each compartment of the bilayer chamber was filled with a buffer solution comprising 200 mM KCl, 5 mM HEPES, 1 mM $MgCl_2$, and 0.5 mM $CaCl_2$, pH = 7. A clean plastic gel-loading tip was then lightly dragged across the bilayer cup orifice to paint a lipid bilayer over the micropore. Successful bilayer formation was evaluated through continuous monitoring of the ionic current during the painting procedure, which drops to 0 A upon the formation of a highly resistive bilayer. In addition, the application of ±1 V to induce electrical breakdown of the bilayer was used to test bilayer formation. After electrical breakdown, the bilayer was repainted across the orifice and utilized in ion-channel recordings.

Electrical Measurements and Data Analysis. Ion-channel current recordings were collected using a Dagan Chem-Clamp low-noise potentiostat (Minneapolis, MN) interfaced to a PC through an in-house written data collection program using LabVIEW (National Instruments). Ag/AgCl reference electrodes, prepared by oxidation of clean Ag wires (0.5 mm) in a saturated solution of sodium hypochlorite, were immersed into the ionic solutions on each side of the bilayer chamber. Current−time (i−t) measurements were recorded at a constant applied potential. In this paper, the compartment connected to the working electrode is referred to as the cis side, whereas the chamber housing the reference electrode is regarded as the trans side. Hence, the voltages reported herein are always referenced to the Ag/AgCl electrode positioned at the trans side, thus we are applying a potential to the cis side with respect to the trans side (Supporting Information Scheme 1).

Data analysis was performed following a previously reported technique with minor modifications.[31] In brief, the current recordings were analyzed through integration of the current−time traces per unit time and area of the 150 μm-diameter pore in the bilayer cup. We designate this quantity as the charge flux, J_Q (pC s^{-1} cm^{-2}), or the average amount of charge passing through the lipid bilayer per unit area per unit time. By default, an intact lipid bilayer allows no charge flux across the membrane. In the charge flux analysis, at least three (n = 3)

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10−60 s of current recordings were used to calculate charge flux with and all current measurements were done in triplicate.

RESULTS AND DISCUSSION

In this report, we utilize the reported ion-channel activity of the heat shock cognate protein, Hsc70.[27] While the biological functions of this protein have been the subject of rigorous studies, work performed by Kurucz et al., Welch et al., and Domanico et al. suggest that this protein can associate with, or localize near, the cellular membrane.[27,32−34] For this reason, it has been postulated that heat shock proteins are capable of directly interacting with lipids leading to their ability to assist in the translocation of proteins across lipid membranes. In 1990, Alder et al. initially validated this assumption by demonstrating that Hsp70 induced pores in lipid membranes at low or neutral pH, which promoted leakage of calcein from liposomes.[35] In addition, Alder et al. observed the induction of conductance pathways from interactions of human Hsp70 and groEL with artificial lipid bilayers.[35] More recently, Arispe and De Maio demonstrated that Hsp70, particularly recombinant Hsc70, spontaneously inserts into lipid bilayers and forms stable ion-conductance pathways in the presence of ATP.[27,36] The channels display multiple stable conductance states and fluctuate between open and closed states.[27] It has been proposed that ATP binding and hydrolysis causes a conformational change in the protein to enable interactions with peptides to perform chaperone functions and is hypothesized to cause a channel opening.[27] By measuring the mean amplitude of unitary current events, Arispe and De Maio demonstrate an average conductance for Hsc70 and demonstrate that the channel exhibits slight cationic selectivity. Furthermore, their report demonstrates a qualitative relationship between channel-like activity and ATP through the use of current−time traces. Here, we present a quantitative measure of the ATP-dependent activity of Hsc70.

To quantitatively evaluate the ion-channel activity of Hsc70, we utilized a setup employing a Delrin cup with an orifice of 150-μm in diameter (Supporting Information Scheme 1). We form an artificial lipid bilayer using the painting method described above with a mixture of POPE and POPS (1:1). This mixture was used by Arispe and De Maio as this lipid composition provides stable lipid bilayers with the ideal fluidity and acidity for incorporation of Hsc70.[27,35,36] We demonstrate two different methods for protein insertion into the lipid bilayer. First, a "direct addition" method is achieved by directly adding a specific amount of recombinant Hsc70 to the ionic solution in the cis side of the bilayer chamber. Alternatively, we employed a "liposome method" in which Hsc70 proteoliposomes were first prepared through sonication of the phospholipids with Hsc70 and were then added to the cis side of the bilayer chamber. With both insertion methods, we observe stable current baselines over the minutes time scale, or lifetime of the bilayer membrane, and reproducible charge flux values at fixed concentrations of protein. As such, we assume the protein distribution is in equilibrium between the membrane and the solution.

ATP-Dependent Ion-Channel Activity of Hsc70. Hsc70 exhibits ATP-regulated ion-channel activity with multiple conductance states in a lipid bilayer. Upon the addition of Hsc70 proteoliposomes to the cis side of the bilayer chamber with high ATP concentration (4 mM), we immediately observed increases in the number of current fluctuations, indicating the flux of ions across the lipid membrane (Figure 1, top). Upon further examination, the current−time trace demonstrates multiple conductance states as previously reported,[27] ranging from 30−600 pS. The multiconductance nature of Hsc70 is postulated to be a result of the protein's ability to self-associate and form different order of oligomers in the absence of peptide targets.[27] Alternatively, conformational fluctuations occurring within the protein channel is a possible cause of the multiconductance nature of Hsc70. It should be noted that in the current−time traces presented here, we could not confirm that there is only a single protein in the lipid membrane at any given time. As we demonstrate below, the observed current fluctuations are dependent on the concentration of the Hsc70 present in solution. The larger conductance values observed could thus be a result of multiple channels that are open simultaneously. Nonetheless, as described below, holding the protein concentration constant allows for quantitative measurements of ATP.

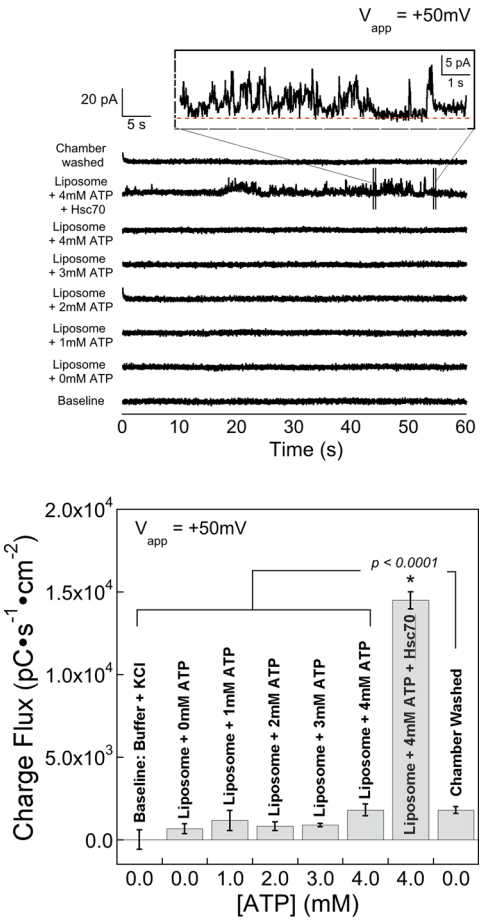

Figure 1. Hsc70 displays ATP-regulated ion-channel activity with multiple conductance states in a black lipid membrane. (Top) Ion-channel activity is only observed when Hsc70 is in solution. Using a bilayer chamber filled with symmetric concentrations of KCl solutions (200 mM) and liposomes without Hsc70 under an applied potential of +50 mV exhibit baseline current recordings. Addition of Hsc70 proteoliposomes with high ATP concentration (4 mM) results in a significant increase in the current fluctuations or ion conductance pathways. The expanded current−time trace reveals multilevel current events, indicating that the Hsc70 channel switches from closed to several open states and/or vice versa. (Bottom) Integrating the current with respect to time and area allows for quantitative determination of the ion-channel activity of Hsc70.

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Again, while it is clear that Hsc70 creates ion conductance pathways through the artificial lipid bilayer as previously reported,[27] we cannot unequivocally state that the current fluctuations we observe are a result of a single Hsc70 channel. To account for this, we define a new quantitative parameter to describe the activity of the Hsc70 channels. This parameter, termed charge flux (J_Q, pC s^{-1} cm^{-2}), provides a quantitative measurement of the net charge crossing the lipid bilayer per unit area and time. Area was normalized using the area of the orifice in the Delrin cup (150 μm in diameter). The actual area of the bilayer may vary from bilayer to bilayer as the solvent annulus size may vary.[29] These differences may introduce small errors in calculating the absolute value of charge flux. We believe, however, that using the aperture size is a reasonable approximation as we achieve reproducible, quantitative charge flux values. In the charge flux analysis, we typically integrate over 10−60 s of current recordings and perform all current measurements and charge flux calculations in triplicate.

The quantitative evaluation of the charge flux (J_Q) across the lipid bilayer membrane demonstrates that flux is distinctly larger when Hsc70 is incorporated into the lipid bilayer (Figure 1, bottom) and, as is described below, there is a distinct dependence on ATP concentration. This observation, combined with the current fluctuations, confirms that Hsc70 reconstitutes as a stable, multiconductive ion channel across an artificial lipid bilayer. As a control, we demonstrate that adding liposomes alone to the cis chamber while varying the amounts of ATP results in no appreciable current fluctuations, or charge flux, above baseline activity (Figure 1).

Ion-Channel Activity is Dependent on Protein Concentration. The ion-channel activity of Hsc70, and thus charge flux, increases with increasing protein concentration. To control the concentration of protein in the cis chamber, Hsc70 was directly added to the ionic solution with 2 mM ATP. As the concentration of Hsc70 increased, we observed a notable increase in the frequency of current fluctuations indicating an increase in the number of protein conductance pathways. Consequently, with more protein in solution, and thus presumably more reconstituted into the membrane, we observed higher charge flux (Figure 2). Of note, at higher protein concentrations (>30 nM) the bilayer stability diminished dramatically. Typically, bilayers were stable for ∼3−5 min at higher protein concentrations compared to ∼20−30 min at lower protein concentrations. Because of this quantitative relationship between protein concentration and charge flux, we kept the protein concentration constant at 30 nM in the experiments described throughout the remainder of the text.

Charge flux through the membrane also increases with increased applied potential as expected (Figure 2). A larger potential gradient induced by the higher applied potential provides more driving force and thus greater flux of ions across the Hsc70 pathways. The change in charge flux with increasingly higher positive applied potentials, however, is not linear, suggesting that the Hsc70 ion channel displays ion selective behavior (discussed in more detail below).

Ion Selectivity of Hsc70 Channels. The Hsc70 ion channel exhibits slight cation selectivity. We investigated the ion channel activity of Hsc70 by measuring the charge flux at various potentials ranging from −100 mV to +100 mV and various ATP concentrations (Figure 3, top). These experiments were performed using the direct addition method at constant Hsc70 concentration (30 nM) in the cis chamber. Ion-channel activity significantly increased with positive applied potentials as demonstrated by the current−time traces. Characterization of the charge flux as a function of applied potential (J_Q−V plot)

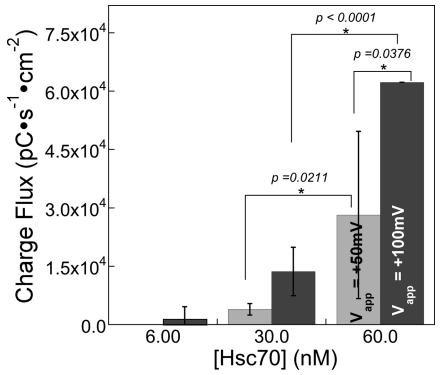

Figure 2. Ion-channel activity of Hsc70 increases with increasing protein concentration. Varying amounts of Hsc70 are added directly to the cis side of a bilayer chamber containing symmetric amounts of 200 mM KCl and 2 mM ATP. The ion channel response, expressed as charge flux, increases monotonically with increasing concentration of Hsc70 at all applied potentials. Of note, the difference in charge flux observed at +50 mV and +100 mV are not directly proportional to each other, suggesting that the ion channel has a preference for some ions (discussed in Figure 3).

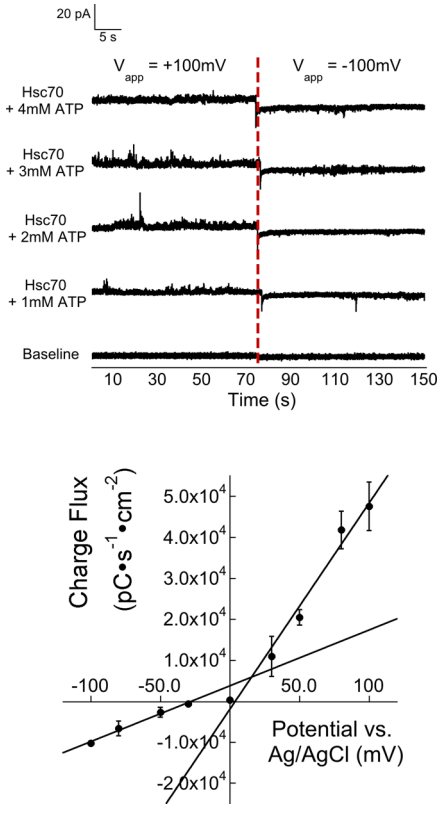

Figure 3. Hsc70 ion channels exhibit cation selectivity. (Top) Current−time traces demonstrate that ion-channel activity increases when the applied potential is positive cis to trans. (Bottom) Varying the applied potentials from +100 mV to −100 mV at a constant ATP concentration (2 mM) generated larger current amplitudes (hence, larger charge flux) at positive applied potentials. The intersection on the negative cis voltage axis demonstrates that Hsc70 preferentially permitted the passage of cations. Lines are drawn to guide the reader's eye.

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shows that charge flux is higher at positive applied potentials (Figure 3, bottom). While the lines in Figure 3 are to guide the reader's eye, this observation, similar to the channel conductance measurements at various potentials observed by Arispe and De Maio,[27] implies that the Hsc70 ion channel is cation selective.

Our observation of cation selectivity is in agreement with the observation that the Hsc70 ion-channel activity is cation-selective. A review of the literature demonstrates that Hsc70, in general, preferentially interacts with hydrophobic peptides, with high specificity for basic or positively charged residues, particularly lysine and arginine.[37−41] Furthermore, it has been shown that positively charged potassium ions significantly enhance the ATPase activity of Hsc70,[27,42] whereas this monovalent cation is also required for the dissociation of denatured proteins from DnaK,[27,43] a member of the Hsc70 chaperone family in bacteria.[37,39,44,45] These previous observations suggest that Hsc70 may have a preference for cations.

Hsc70 Ion-Channel Activity is Quantitatively Dependent on ATP. The ion-channel activity of Hsc70 displays linear dependence on ATP concentrations. We initially employed the liposome method to study the quantitative relationship of ion-channel response on ATP (Supporting Information Figure S1). Using this insertion method, however, resulted in inconsistencies in the current fluctuations, which we hypothesize is a result of liposome aggregation presented by the proteoliposomes (Supporting Information Figure S2). This hypothesis is based on an earlier report that demonstrates that Ca^{2+} ions play a vital role in affecting the ability of Hsc70 to induce liposome aggregation and, thus, potentially with the black lipid membrane.[46] To eliminate significant effects of liposome aggregation, we used the direct addition method to obtain quantitative ATP-dependent information on the ion-channel activity of Hsc70 (Supporting Information Figure S3). For these experiments, Hsc70 was directly added to the cis chamber of the bilayer cup apparatus. The applied potential was held constant at +100 mV. Ion channel activity was monitored while varying the ATP concentration from 0 to 4 mM in the cis chamber (Figure 4, top). As a result, charge flux increased as the concentration of ATP increased (Figure 4, bottom). The current signals steadily increased from 0 to 4 mM, indicating a quantitative relationship between the Hsc70 ion-channel activity and the concentration of ATP. Again, small variations may occur from differences in actual bilayer area leading to deviations in the charge flux measurements. The protein channel was sensitive to micromolar concentrations, where the lowest ATP concentration detected was 100 μM, as seen in the lower region of the titration curve (Figure 4, bottom).

As mentioned above, the quantitative relationship of the ion-channel activity on ATP can be postulated to be a result of conformational changes occurring within the protein channel induced by interaction with ATP,[27] possibly leading to the quantitative dependence of the ion-channel response of Hsc70 on ATP. Our findings show compelling evidence that through charge flux analysis of the multiconductive ion-channel activity of Hsc70, we can quantitatively detect milli- and micromolar concentrations of ATP with appreciable sensitivity, selectivity, and high specificity.

Ion-Channel Activity is Specifically Regulated by ATP. ATP specifically regulates the ion-channel activity of Hsc70. We employed a closely related purine nucleoside triphosphate molecule, GTP, as a competing substrate for the Hsc70 channel. To test the specificity of Hsc70 ion-channel activity,

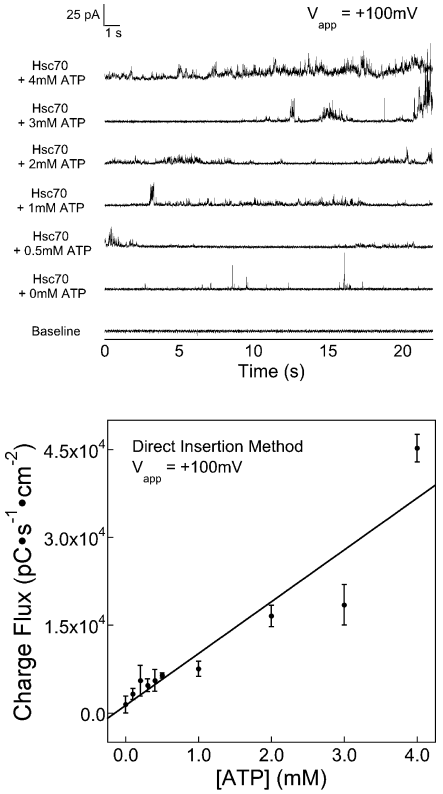

Figure 4. Ion-channel activity of Hsc70 displays quantitative dependence on ATP. (Top) Titration with ATP is performed in a bilayer chamber where the bilayer separates two symmetric buffer solutions of 200 mM KCl. Hsc70 is directly added to the side that did not contain ATP (cis). A membrane potential of +100 mV is applied, and measurements are taken as ATP concentration is increased from 0 to 4 mM. (Bottom) The ATP titration curve, showing the charge flux generated across the membrane, shows a quantitative relationship between the ion-channel activity of Hsc70 and the concentration of ATP, where larger charge fluxes are observed in the presence of increasing amounts of ATP (line of best fit equation: $f(x) = 8857.2 + 1280.9$, $R = 0.939$). Examination of the lower region of the titration curve demonstrates ATP detection at the micromolar range (100 μM).

the protein was directly added to the cis chamber of the bilayer cup apparatus, followed by incremental amounts of GTP and ATP in an alternating fashion. The current−time traces show clear distinctions in the current fluctuations produced by GTP and ATP (Figure 5, top). The charge flux is higher when ATP is in solution and only shows appreciable changes whenever the ATP concentration is altered and not when the GTP concentration is changed (Figure 5, bottom). These results imply that ATP specifically regulated the ion-channel activity of Hsc70 and that GTP, despite its structural resemblance with ATP, does not influence the ability of Hsc70 to form conductance pathways. Through these experimental findings, we have therefore established that the Hsc70 ion channel has specificity for ATP, which can be a valuable characteristic when this protein pore is employed in ion-channel based biosensors for the selective and specific quantification of ATP.

■ CONCLUSION

In this report, we presented the use of charge flux analysis as a method to quantitatively analyze ligand-gated ion channel activity. As a proof of concept, we utilized the ATP-dependent pore-forming capability of Hsc70 across a lipid bilayer. Previous

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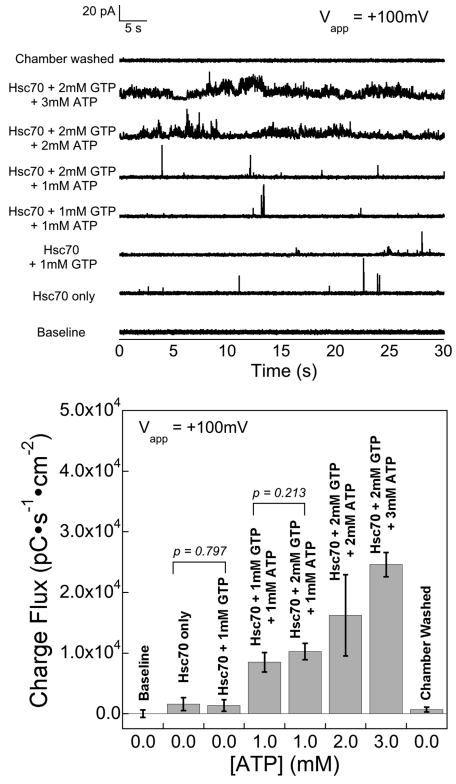

Figure 5. Hsc70 ion-channel activity is specifically regulated by ATP. (Top) A black lipid membrane is prepared using symmetric amounts of 200 mM KCl and subjected to a maintained electric potential of +100 mV. Hsc70 is directly added to the cis side of the chamber, followed by incremental amounts of GTP and ATP in an alternating fashion. (Bottom) The bar graph shows distinct current responses of the Hsc70 channel in the presence of GTP, ATP, or both. The charge flux values only change with changes in ATP concentrations.

reports indicated that Hsc70 reconstitutes into lipid bilayers and opens to create multiple conductance states that displays slight cation selectivity in the presence of ATP.[27] We demonstrated, for the first time, the quantitative relationship of Hsc70 ion-channel activity on ATP, through quantitative analysis of ATP-induced charge flux produced by Hsc70 across a lipid bilayer. This new method of quantifying the Hsc70 ion-channel activity through charge flux analysis allowed sensitive detection of ATP in the micromolar range (100 μM − 4 mM). Finally, when channel response was assessed in the presence of both ATP and GTP, Hsc70 exhibits specific regulation by ATP without showing significant response to GTP. These data demonstrate that the channel-like activity of Hsc70 can be used to provide quantitative information about ATP.

Overall, we compellingly demonstrate that this charge flux quantification method, using Hsc70 ion channel as the biosensing element, permits sensitive, specific, and quantitative detection of ATP, and thus, holds considerable potential for use in biological nanopore-based sensing. A similar approach was reported by Mayer et al. for the characterization of a modified multiconductive alamethicin channel as it binds to proteins.[12] This channel exhibits well-characterized, relatively long-lived discrete conductance states as a function of the number of monomer units associated with forming the pore in contrast to the unknown origin of the fluctuations observed with the Hsc70 protein. The authors measure charge flux for a given time period to quantify protein−protein interactions. The charge flux at each concentration of analyte protein is normalized to the maximum charge flux observed at high analyte concentration. This method, however, requires measuring a maximum flux whereas our method does not require knowing a maximum flux value. Nonetheless, the measurement of charge flux appears to open the possibility of using essentially any protein channel in the development of sensitive ion-channel based biosensors. The utility of sensitive and selective protein channels provided by nature can ultimately offer a new route for the development of extremely sensitive sensors capable of functioning in complex biological matrixes.

■ ASSOCIATED CONTENT

Supporting Information

Schematic of the bilayer setup (Scheme 1) and additional figures (Figures S1−S3) demonstrating liposome-based protein insertion as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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