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Determination of affinities of a panel of IgGs and Fabs for whole enveloped (influenza A) virions using surface plasmon resonance

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Abstract

The affinity of a panel of neutralizing monoclonal IgGs and their Fab fragments has been measured for the first time with an enveloped type A influenza virus, by surface plasmon resonance (SPR) and the BIAlite™ instrument. Equilibrium constants could be calculated for four of the five mAbs tested. These were in the nanomolar range. The ranking order was very similar to that obtained with an affinity ELISA, (an equilibrium system) but as others have found, affinities were 2-10-fold lower as measured by SPR (a flow system). No data were obtained with mAb HC58 although it had one of the highest affinities using an ELISA format, and was 28-fold higher than another mAb (HC10) which gave good data by SPR. This may relate to the orientation of its binding on the virion surface. The $K_{dissoc.}$ of the Fabs was only 3-10-fold higher compared to their IgGs. Fab from the lowest affinity IgG (HC10) could not be measured, possibly because it fell below the threshold for detection.

Keywords: Influenza A virus; IgG; Fab; Affinity; Surface plasmon resonance

1. Introduction

Despite the widespread use of surface plasmon resonance (SPR) in the study of virus-antibody interaction, whole virus particles are not commonly used, and there is only one published example where antibody affinity measurements have been made with whole virus particles (tobacco mosaic virus: TMV) as the antigen (Pellequer and Van Regenmortel, 1993). No antibody affinity data have been published before for whole enveloped virus particles. The applications of SPR to virology were first highlighted (Dubs et al., 1991,

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1992) when it was shown that interactions between antibodies and a number of animal viruses particles (vaccinia virus, poliovirus) and plant viruses (TMV and cowpea mosaic virus) could be studied in real time. However, most virological studies with SPR use subvirion ligands. SPR work falls into four categories. (1) Epitope mapping using human immunodeficiency virus type 1 (HIV-1) gp120 and gp160 (VanCott et al., 1992, 1994; Lucey et al., 1993); transmissible gastroenteritis virus S (spike) protein (Godet et al., 1994); type A influenza virus neuraminidase (N9) (Gruen et al., 1994); infectious pancreatic necrosis virus VP2 (Tarrab et al., 1995); bovine rotavirus VP6 (Tosser et al., 1994); and turnip mosaic virus coat protein (Kantrong et al., 1995). (2) Interactions of viral components with antibody in relation to neutralization including the use of surrogate antigens, such as purified viral protein, viral peptides and anti-idiotype antibodies instead of whole virus. The majority of data relate to HIV-1 and its surface proteins: gp120 (Richalet et al., 1994; VanCott et al., 1994; Roben et al., 1994; Burton et al., 1994) and gp41 (Conley et al., 1994; Mani et al., 1994), and also peptide T which blocks gp120-CD4 binding (Ramsdale et al., 1993). Mouse hepatitis coronavirus has also been studied, but functional affinity data for IgG, F(ab')2 and Fab were obtained using an anti-idiotype mAb as a surrogate viral antigen (Lamarre and Talbot, 1995). (3) Screening of combinatorial Fab fragments prior to neutralization studies using HIV-1 gp120 (Ditzel et al., 1995; Roben et al., 1994; Barbas III et al., 1994; Burton et al., 1994) and respiratory syncytial virus F glycoprotein (Barbas III et al., 1992; Lounsbach et al., 1994). (4) Cell receptor-virus interactions using human rhinovirus serotype 3 with ICAM-1 (CD54) (Casasnovas and Springer, 1995) and HIV-1 gp120 with soluble CD4 (Brigham-Burke et al., 1992).

Viral peptides, recombinant viral proteins and purified viral proteins offer convenient ways of determining antibody affinity values. However, the structure, conformation and interrelationship between such subunits and whole virus particles is likely to differ. Antibody affinities obtained using viral subunits as antigens may give misleading data as there are differences in antigen conformation and density/accessibility of epitopes compared to whole virions. The importance of studying virus-antibody interactions in a manner that is as close to the in vivo situation as is possible has been highlighted recently as differences between the monomeric and oligomeric forms of the envelope protein of HIV-1 have been found (Sattentau and Moore, 1995; Moore et al., 1995).

SPR is an optical phenomenon which permits biomolecular interactions to be monitored in real time and without the need to label the interacting components (Pharmacia BIAtechnology Note 101). SPR arises when light illuminates a thin conducting film under certain conditions. The interaction between electromagnetic vectors in an incident light beam and surface plasmons (free electron constellations in the conducting film) results in a resonance (Welford, 1991). Total internal reflection produces an evanescent wave which is propagated away into the medium of lower refractive index. SPR arises if the interface is coated with a thin conducting layer (in the Pharmacia Biosensor BIAlite™ instrument this is gold). The resonant coupling between the incident light energy and the surface plasmons in the film occurs at a specific angle of incident light. The light energy is absorbed and this causes a drop in the light intensity for this angle. The incident light is focused on the surface as a wedge, and such a drop in intensity at the resonance angle shows up as a shadow in the light wedge that is reflected off the conductor surface. This resonance angle is sensitive to the refractive index of the medium into which the evanescent wave is propagated. The evanescent wave decays exponentially with increasing distance from the interface (Kovacs, 1982), thus for BIAlite™ the maximum distance for detection is 300 nm, i.e. in theory it is suitable for most virus particles. Biomolecular interactions occurring at the sensor surface cause a change in mass which affects the refractive index providing it is within the range of the evanescent wave, thus allowing SPR to be used to monitor such interactions.

Here we present for the first time SPR data derived with whole enveloped (influenza type A) virus particles, from which we have determined the functional affinities of five influenza A virus HA-specific monoclonal IgGs and their Fab fragments. The system should be valuable for the study of ligands binding to other enveloped virions, including HIV-1.

2. Materials and methods

2.1. Virus

Influenza A/fowl plague/Rostock/34 (H7N1) (FPV/R) was grown in the allantoic cavity of 10 day old embryonated chickens' eggs (J.K. Needle and Co., Goffs Oak) and incubated for 20 h at 35°C. Allantoic fluid was clarified by low speed centrifugation and stored at −70°C. The infectivity was determined by plaque assay on MDCK cell monolayers under 0.9% agar (Difco) in 199 medium containing 100 U/ml penicillin (Sigma), 100 μg/ml streptomycin (Sigma) and 5% (v/v) foetal calf serum (Gibco). Monolayers were incubated for 4 days at 37°C before staining with 0.015% crystal violet (BDH) in PBS.

2.2. Purification of influenza virus

Virus was purified from allantoic fluid by differential centrifugation at 4°C (Kelly and Dimmock, 1974). Pelletted virus was loaded onto a 10–45% (w/v) linear sucrose gradient in PBS and centrifuged at 60 000 × *g* for 90 min (Beckman) and the visible band harvested. Virus was stored at −70°C. Infectivity was UV-inactivated for safety reasons before use.

2.3. Monoclonal antibodies

The hybridoma cell lines for the mouse anti-H7 HA monoclonal antibodies (mAbs) HC2 (site A, IgG2a), HC3W (site D, IgG2b), HC10 (site B, IgG2a), HC58 (site D, IgG2a) and HC61 (site D, IgG2a) (Sugrue et al., 1990) were provided by A.R. Douglas and J.J. Skehel (National Institute for Medical Research, London). All were cultured in RPMI 1640 with sodium bicarbonate buffer, supplemented with 0.02 mM glutamine (Flow), 10% (v/v) foetal calf serum, 100 units/ml of penicillin and 100 μg of streptomycin. Only serum containing negligible amounts of protein A-binding bovine proteins was used in order to avoid contaminating the mAbs. Tissue culture supernatants were stored at −20°C. MAbs were purified on protein A-oxirane beads (Sigma), and protein concentrations determined using the Bio-Rad dye-binding assay, and a mouse IgG calibration curve. All mAbs neutralized virus infectivity and inhibited haemagglutination of chicken red blood cells.

2.4. Preparation of Fab fragments

Fab fragments were produced using immobilised papain (Sigma). Purified IgG (1 mg/ml), was incubated with 6.2 μl fresh 1.0 M cysteine (Sigma) and 20 μl of 20 mM EDTA, 2 U of agarose-immobilised papain, and 200 mM sodium acetate (pH 5.5) for 4 h at 37°C in a shaking water bath. The reaction was stopped by centrifuging out the immobilised papain, and 100 μl of 47 mM iodoacetamide added, and the mix returned to pH 8.0 by the addition of Tris/HCl. A sample was electrophoresed on an 8% SDS-PAGE gel to confirm the presence of Fab, and stained with the highly reactive colloidal Coomassie brilliant blue G-250 (Sigma: Neuhoff et al., 1988). Any undigested IgG and Fc were removed by repeatedly passing the Fabs through a protein A column. The Fabs were then dialysed against PBS overnight at 4°C, and concentrated. The protein concentration is then determined as before. The final Fab preparation was analysed by PAGE gel and colloidal Coomassie brilliant blue G250 staining for contaminating IgG. Polypeptide bands could be detected visually down to 5 ng per band. All Fabs contained $< 0.5\%$ IgG.

2.5. Haemagglutination assay

Virus was titrated by doubling dilutions in 96 well U-bottomed microtitre plates (Greiner), in 100 μl vols, using 0.13% chicken red blood cells (Seralab). This concentration of red cells is lower than normally used and increases sensitivity about

10-fold. The 50% agglutination end point (1 haemagglutination unit: HAU) was estimated by interpolation between complete agglutination and no agglutination. Each HAU of purified virus contains about 10^6 particles and 0.3 ng protein.

2.6. Binding of virus particles to the biosensor chip for SPR measurements

For the real-time kinetic analysis of IgG and Fab with whole virus particles, a double antibody sandwich was used in which antibody 1 was covalently coated to the sensor surface. This was used to capture whole virus particles to which the IgG or Fab to be measured (antibody 2) were bound. After each measurement the sensor chip was regenerated with a pulse of ammonium hydroxide which removed the virus plus antibody 2, but left antibody 1 in place. The cycle could then be repeated.

The gold-coated sensor chip CM-5 is itself coated with a carboxylated dextran polymer matrix and proteins were amine-coupled to it. Carboxyl groups on the dextran layer are activated with 35 μl of a 50:50 (v/v) solution of *N*-hydroxysuccinimide (NHS) and *N*-ethyl-*N'*-(3-diethylaminopropyl)carbodiimide (EDC), at a flow rate of 5 μl/min. The injection loop was washed with 300 μl HBS (BIA-certified HEPES, NaCl, pH 7.4) before addition of 35 μl mAb HC10 (antibody 1) at 30 μg/ml in 10 mM acetate (pH 4.8) with a flow rate of 5 μl/min. The injection loop was washed with HBS and the remaining active binding sites on the chip blocked by the addition of 35 μl of ethanolamine hydrochloride. Purified influenza virus (87 HAU in 35 μl HBS) was injected for capture at a flow rate of 5 μl/min. The injection loop was then washed with 300 μl HBS and the baseline left to stabilise for at least 300 s before any injections of IgG or Fab (antibody 2).

2.7. Measurement of kinetics of binding by SPR

Measurements were made for at least three protein concentrations. Antibody 2 (35 μl) was injected and passed over the virus captured on the chip at a flow rate of 5 μl/min. HBS was then passed over the chip at 5 μl/min, and the dissociation curve was followed for at least 800 s. After the dissociation phase, the captured antibody was regenerated with a 10 μl pulse of 0.1 M ammonium hydroxide (pH 11.5) at a flow rate of 5 μl/min. The baseline was allowed to stabilise for at least 300 s before further use. The SPR detector is the Pharmacia Biosensor BIAlite™ instrument. The refractive index for proteins is proportional to the mass of the protein, and there is little variation between protein species. The change in refractive index is monitored with time, and represented as a sensorgram. The *y*-axis of the sensorgram is the resonance signal (in resonance units: RU). Binding of antibody 2 to the captured virus was analysed using the BIAevaluation software package (v2.1). The rate of antibody 2 dissociation (k_d) was calculated from the dissociation curve and this rate was incorporated into the determination of the rate of antibody 2 association (k_a) along with antibody 2 concentration (M). The equilibrium dissociation constant ($K_{dissoc.}$) is k_d/k_a.

3. Results

3.1. Capture of whole influenza A virions on a sensor chip

MAb HC10 was chosen for capturing virus. It bound virus effectively and could be regenerated at high pH, thus allowing the chip to be reused. The latter was critical as the binding of some mAbs to virus was not reversed under these conditions (Schofield and Dimmock, 1996; unpublished data). The sensorgram for the immobilisation of HC10 is shown in Fig. 1. The capture of virus, the binding of antibody 2 (HC2) and its dissociation, and the regeneration of the HC10 ligand with a pulse of ammonium hydroxide which dissociated the virus and any HC2 associated with it is shown in Fig. 2, while Fig. 3 shows the capture of virus following five successive regeneration cycles. The mean of virus bound was 456 ± 21 RU, giving a coefficient of variation of 4.7%.

3.2. Kinetics of binding of HA-specific IgG to influenza A virus particles

The experimentally determined rate constants (k_a and k_d), calculated equilibrium dissociation constant ($K_{dissoc.}$), isotype and antigenic site are shown in Table 1. The association rate constants (k_a) had a 5.2-fold range from 1.7 to 8.8×10^5 M^{-1} s^{-1} which gave a rank order of HC2 > HC3W > HC61 > HC10. The dissociation rate constants (k_d) had a 3.3-fold range from 1.5–5.0×10^{-4} s^{-1} giving a rank order of HC61 > HC2 > HC3W > HC10. There was thus no precise correlation between k_a and k_d, but the differences between the IgGs with respect to either parameter were small. The calculated equilibrium dissociation constant ($K_{dissoc.}$) ranged from 0.98 to 14×10^{-9} M, with a rank order of HC2 > HC61 > HC3W > HC10. However the first three varied by only 2.8-fold, while there was a 14-fold difference between HC2 and HC10. $K_{assoc.}$ is the reciprocal of $K_{dissoc.}$

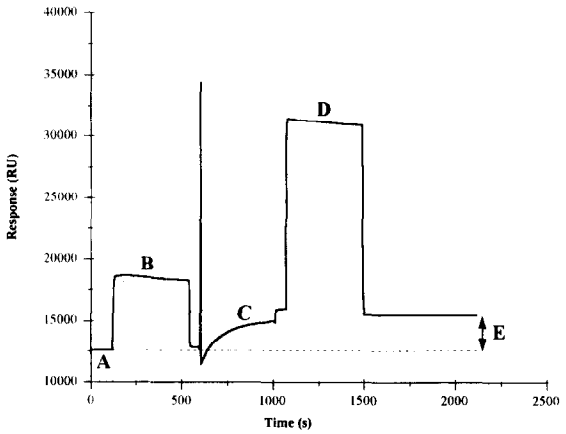

Fig. 1. Sensorgram showing the immobilisation of antibody 1 (HC10) on a sensor chip. A continuous flow of HBS of 5 μl/min was passed over the sensor surface (A). The pulse of NHS/EDC (B) gave an increase of the SPR signal due to the change in bulk refractive index. (C) Corresponds to the injection of 35 μl containing 1 μg of mAb HC10 in 10 mM acetate (pH 4.8). The sensor surface was deactivated with an injection of ethanolamine hydrochloride (D). (E) The baseline was raised for the immobilised mAb HC10.

3.3. Kinetics of binding of HA-specific Fab fragments to influenza A virus particles

Rate constants were determined for HC2, HC3W and HC61 Fab fragments (Table 1). No data were obtained for HC10 Fab. The k_a values ranged by 2.9-fold from 1.3 to 3.7×10^5 M^{-1} s^{-1}, with a rank order of HC2 > HC3W > HC61. HC2 was also the most active IgG. The k_d values ranged by 1.9-fold from 3.2 to 6.0×10^{-4} s^{-1} with a rank order of HC61 > HC2 > HC3W, exactly the same as the IgGs. $K_{dissoc.}$ values ranged by 9-fold from 3.1 to 28×10^{-9} M with a rank order of HC2 > HC61 > HC3W, exactly the same as the IgGs.

4. Discussion

(Dubs et al., 1992) first used the double antibody sandwich for measuring virus-antibody interactions. This was a valuable innovation for the BIAlite™ system where traditionally small antigens are coated to the sensor chip, and the association/dissociation of larger molecules (e.g. antibodies) is detected. Here we have used IgG to capture a 120 nm diameter influenza A virus particle (Taylor et al., 1987) and monitored the association/dissociation of a 9–15 nm long IgG (Nisonoff et al., 1975), and a 7 nm long Fab fragment (Porta et al., 1994). Despite the relatively large size of these virions, the distance from the sensor surface is still within the 300 nm maximum detection distance defined for the BIAlite™ system. Thus this is the first time that whole enveloped virus particles have been used to study ligand binding in conjunction with SPR, although (Lamarre and Talbot, 1995) admit having tried and failed with murine hepatitis virus. In all only a few non-enveloped virions have been used in antibody binding studies and epitope mapping (see Section 1), although it would seem that whole virus particles in the flow cell of the BIAlite™ system closely mimics the in vivo situation where antibodies in solution pass over virus particles attached to cell receptors.

We found considerable variability in the kinetic data, mainly in the association phase. The coeffi-

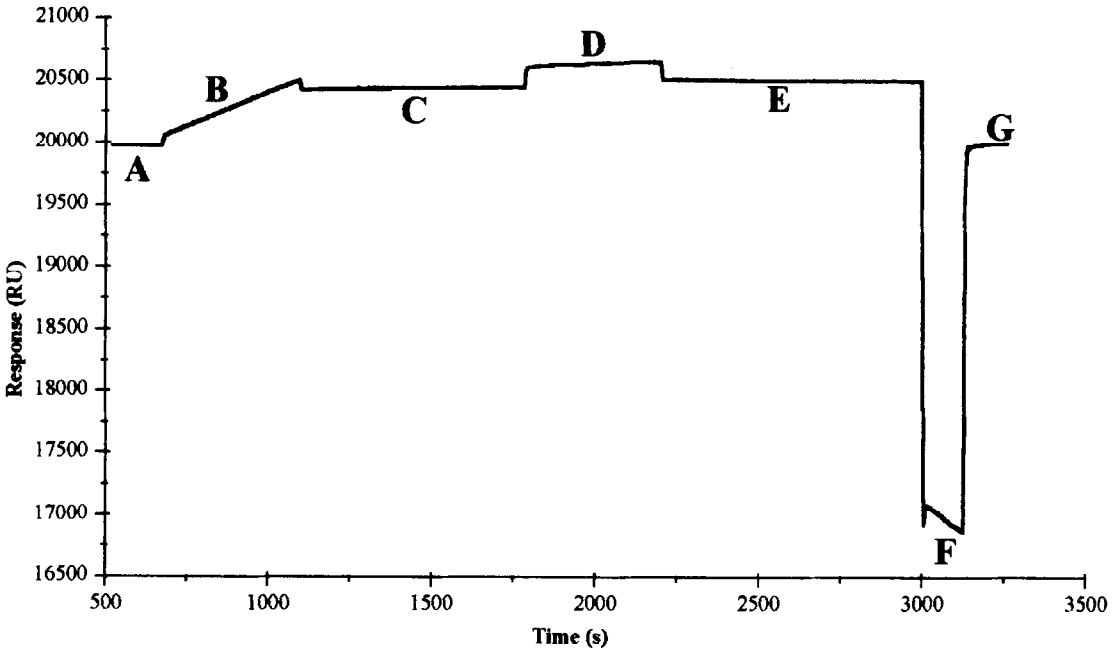

Fig. 2. Sensorgram showing the binding of influenza A virus particles to immobilised mAb HC10, and the subsequent binding of mAb HC2 to the captured virus particles. (A) The baseline before injection of virus. (B) The capture of virus particles (87 HAU in 35 μl HBS at a flow rate of 5 μl/min). (C) The baseline after virus capture following replacement of the diluted virus solution with running buffer. (D) The injection and association phase for antibody 2 (HC2 in 35 μl at a flow rate of 5 μl/min) binding to captured virus particles. (E) The dissociation phase following the replacement of mAb HC2 with running buffer. At (F) 10 μl of 100 mM ammonium hydroxide (pH 11.5) was injected to remove mAb HC2 and the captured virus from mAb HC10 immobilised on the sensor surface. (G) The baseline for the mAb HC10-coated sensor surface.

cient of variation (CV) from a mean of three determinations ranged from 16.1 to 67.0%. However, other published data, where given, show a similar CV, e.g. 8.7 to 43.1% for antibody fragments binding to anti-idiotype antibody (Lamarre and Talbot, 1995), 2.0 to 97.0% for recombinant human antibodies to self antigens (Griffiths et al., 1993), 6.0 to 13.0% for ICAM-1 binding to human rhinovirus serotype 3 (Casasnovas and Springer, 1995), and 0.5 to 11.4% for recombinant Fabs to HIV-1 gp120 (Ditzel et al., 1995). It may be that the high CV found here was due to the complex nature of the system under examination, i.e. virus particles possessing a potential 3000 binding sites for antibody on 1000 densely packed HA spikes (Taylor et al., 1987). In contrast protein or peptide capture antigens will be uniformly distributed on the sensor surface, and usually have only one epitope per molecule for the applied ligand.

The differences in $K_{dissoc.}$ values between the IgG and its Fab determined here with whole virions ranged from only 3 to 10-fold, and are similar to those recently reported for mAbs to the gp120 protein of HIV-1 (Cavacini et al., 1994; Burton et al., 1994; Barbas III et al., 1994) and the S protein of murine hepatitis virus (Lamarre and Talbot, 1995). These recent studies show relatively small differences in functional affinity between IgG and Fabs, although only Cavacini et al. (1994) used native protein. This is in contrast to anti-dinitrophenol (DNP) IgG, purified from serum which neutralized bacteriophage conjugated with DNP, as its Fabs had up to 1000-fold less functional affinity than the IgG for the DNP-phage complex (Hornick and Karush, 1972; Blank et al., 1972). Differences in rate constants and equilibrium constants between the IgGs and their Fabs have been ascribed to the bivalent interac-

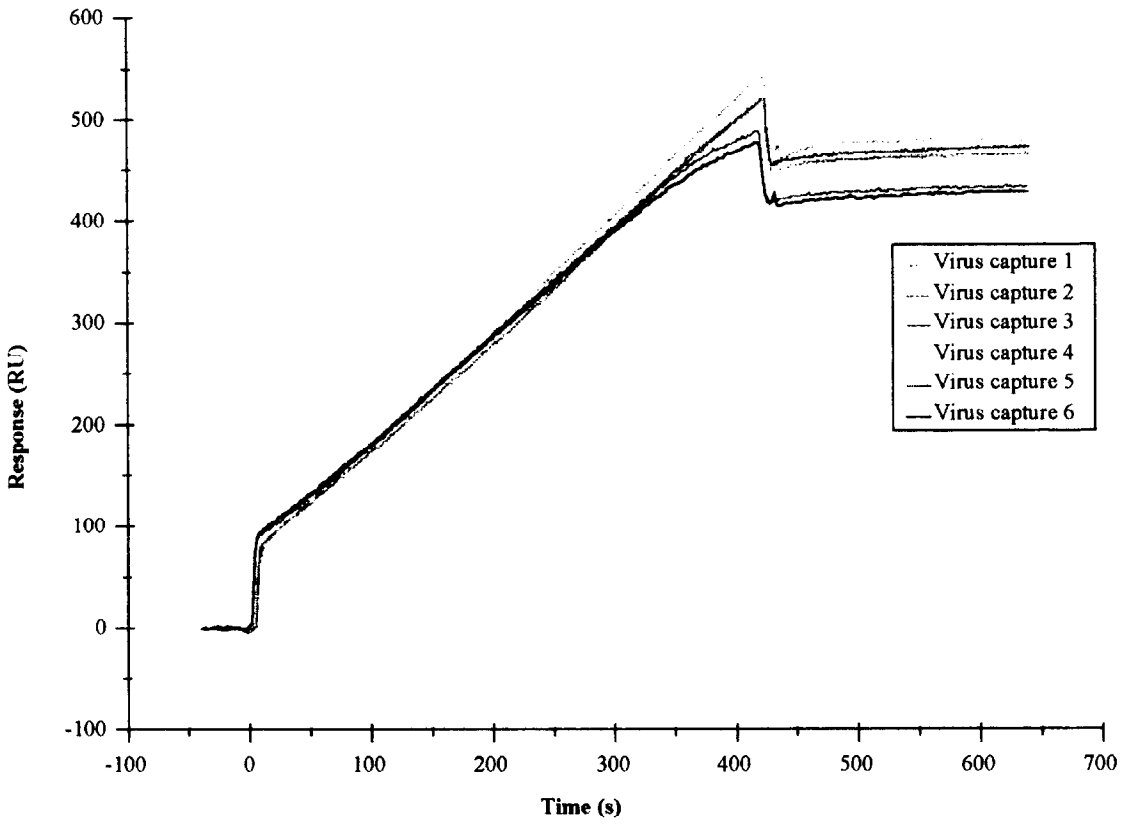

Fig. 3. Variation in the capture of influenza A virus particles by immobilised mAb HC10 (antibody 1) following five successive regeneration cycles with 100 mM ammonium hydroxide (pH 11.5).

tion of IgG versus the monovalent binding of Fab (Cavacini et al., 1994; Lamarre and Talbot, 1995). However for mAbs of very high affinity, bivalent binding may have an insignificant effect on functional affinity, and Parham (1983) suggested that a loss of bivalency became significant only at a $K_{assoc.}$ of $< 10^8$ M^{-1}. This may account for the large differences in affinity between IgG and Fab seen by Hornick and Karush (1972) and Blank et al. (1972) as the association constants for the IgGs examined were around 10^7 M^{-1}. On the other hand the BIAlite™ system may favour monovalent binding by preventing its conversion to bivalent binding. Dissociation of antibody would then probably result in its removal from the flow cell, if it was unable to bind to another immobilised virus on the sensor surface. Alternatively a small proportion of the IgG molecules may be binding bivalently while most are binding monovalently. The slight differences observed between IgG and Fab would then be due to the slower dissociation of bivalently bound IgG in a background of faster dissociating monovalently bound IgG. Using the BIAlite™ system Malmborg et al. (1992) saw a slightly biphasic dissociation curve for IgG, but not for Fab, which they attributed to bivalent interaction with the antigen (tetanus toxoid) on the sensor chip. However, dissociation of the antigen was monophasic in both cases when Fab or IgG was coated to the sensor chip. It has been suggested (Malmborg et al., 1992) that a mixture of bi- and monovalently bound IgG would give a biphasic dissociation curve. In the system described here the rate of dissociation of all the mAbs was slow, and it was not possible to assess if the dissociation curves were mono- or biphasic.

Table 1
Comparison of the rates of association (k_a) and dissociation (k_d), and the equilibrium dissociation constants ($K_{dissoc.}$) for HA-specific monoclonal IgGs and their Fabs bound to captured influenza A virus particles using surface plasmon resonance

MAb	Agic site	Isotype	k_a (10^5 M^{-1} s^{-1})	k_d (10^{-4} s^{-1})	$K_{dissoc.}$ (10^{-9} M)[a]	IgG:Fab $K_{dissoc.}$
HC2	A	IgG2a	1.7 ± 0.8 (1)[b]	1.6 ± 0.4 (2)	0.98	
		Fab	1.3 ± 0.6 (1)	3.9 ± 0.6 (2)	3.1	+3
HC3W	D	IgG2b	0.9 ± 0.5 (2)	2.4 ± 1.3 (3)	2.7	
		Fab	0.2 ± 0.1 (3)	6.0 ± 1.4 (3)	28.0	+10
HC10	B	IgG2a	0.4 ± 0.1 (4)	5.0 ± 1.2 (4)	14.0	
		Fab	na[c]	na	na	na
HC61	D	IgG2a	0.6 ± 0.3 (3)	1.5 ± 0.4 (1)	2.5	
		Fab	0.4 ± 0.1 (2)	3.2 ± 0.7 (1)	8.7	+4

[a]Equilibrium dissociation constant ($K_{dissoc.}$) = k_d/k_a
[b]Numbers in the parenthesis give the ranking order for the IgGs or the Fabs with respect to the parameter at the column head.
[c]Not available as no data could be obtained.

Under conditions which were adequate for the other IgGs and Fabs, no data could be obtained for HC58 IgG and its Fab, or HC10 Fab. This was unexpected as the $K_{dissoc.}$ of HC58 by affinity ELISA was comparable with the best of the others, and 28-fold higher than HC10 which could be assayed by SPR (Schofield and Dimmock, 1996). However, our model for the binding of HC58 IgG/Fab proposes that antibody molecules are orientated at around 90° to the HA, so that the bulk of the IgG lies between HA globular heads and parallel with the surface of the virion, whereas other IgGs bind at about 180° to the extremity of the HA (Schofield and Dimmock, unpublished data). The necessity to interdigitate at right angles with the HA might make it difficult for the IgG to access its epitope in a flow cell, and might account for the failure of HC58 IgG or its Fab to bind to virions. HC10 had the highest $K_{dissoc.}$ (lowest affinity) of the mAbs studied here and it seems likely that its Fab would have an even lower affinity as did all the other mAbs. This may have taken the affinity below the threshold for detection in the SPR system. Coincidentally, the $K_{assoc.}$ of mAb HC10 (7.1×10^8 M^{-1}) is close to the value which Parham (1983) suggested would be associated with a major reduction in affinity on conversion of the bivalent IgG to the monovalent Fab.

The rankings for the $K_{dissoc.}$ values determined for our IgGs by the BIAlite™ and ELISA meth-

Table 2
Comparison of the apparent equilibrium dissociation constants ($K_{dissoc.}$) for IgG bound to whole influenza A virus particles as determined by surface plasmon resonance and an affinity ELISA

MAb	Antigenic site	SPR $K_{dissoc.}$ (10^{-9} M)	ELISA[a] $K_{dissoc.}$ (10^{-9} M)	SPR:ELISA ($K_{dissoc.}$)[b]
HC2	A	0.98 (1)[c]	0.49 (2)	+2
HC3W	D	2.70 (3)	0.53 (4)	+5
HC10	B	14.00 (4)	1.40 (5)	+10
HC58	D	na[d]	0.50 (3)	na
HC61	D	2.50 (2)	0.41 (1)	+6

[a]Data from Schofield and Dimmock (1996).
[b]Indicates that the SPR $K_{dissoc.}$ values were higher than the ELISA $K_{dissoc.}$ values, and hence that the affinity values measured in the SPR system were lower.
[c]Numbers in the parenthesis give the ranking order for the IgGs or the Fabs with respect to the parameter at the column head.
[d]Not available as no data could be obtained.

ods (Schofield and Dimmock, 1996) were similar, except that the positions of HC2 and HC61 were interchanged (Table 2). $K_{dissoc.}$ values for IgG determined in the BIAlite™ system were higher (i.e. more dissociation) than those determined by the ELISA method, by 2-fold for HC2, to 10-fold for HC10. A similar situation was reported previously (Malmborg et al., 1992) for the binding of tetanus toxoid to IgG and Fab using the BIAlite™ and affinity ELISA systems. In our BIAlite™ system IgG and Fab k_a values were of the same order of magnitude, while the Fabs had k_d values approximately 2-fold higher than their IgGs. $K_{dissoc.}$ values of Fabs were from 3- to 10-fold higher than their IgGs (Table 1). IgG $K_{dissoc.}$ values obtained by ELISA were consistently from 2- to 10-fold lower (less dissociation) than the BIAlite™ values (Table 2).

The data presented here demonstrate that antibody affinity measurements can be made with a large enveloped virus by SPR. Further development and refinement of this assay system is likely to lead to the more frequent use of whole virus particles in a variety of ligand-binding studies.

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