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Optical biosensor analysis in studying herpes simplex virus glycoprotein D binding to target nectin1 receptor

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Abstract

Studies on molecular interactions between cellular receptors of herpes simplex virus (HSV) and the viral glycoproteins showing receptor-binding activity are of great relevance for understanding the molecular basis of virus entry. Information on such interactions further provides the basis for a rational design of antiviral drugs. A variety of biochemical and biophysical methodologies are used for determining the binding parameters of interacting biomolecules. Most of them require relatively high amounts of the analyzed compounds, or the use of labeled target molecules. Here, we report the study of the binding of two recombinant forms of HSV glycoprotein D, gD(Δ290-299t) and gD(305t), and a recombinant form of the human cellular receptor for HSV, nectin1-Fc, by using an optical biosensor (IAsys Plus, Affinity Sensors, UK). This device detects and quantifies the changes in refractive index in the vicinity of the surface of sensor chips to which ligands are immobilized. The changes in the refractive index are proportional to the change in the absorbed mass, thus the analysis allows the monitoring of the interaction process in real-time and the determination of the binding parameters. HSV cellular receptor has been immobilized on the surface of the biosensor cuvette, bearing a carboxymethyl dextran layer. The immobilized receptor cuvette was then used for the binding experiments of the two glycoproteins. A significant difference in their dissociation constants was determined, showing for the gD(Δ290-299t) protein a much higher affinity (K_D, 2.8×10^{-7} M) with respect to gD(305t) (K_D, 2.8×10^{-6} M). The active ligand concentration decreased on time, however the binding properties of the immobilized receptor were maintained over 5 weeks.

Keywords: Biosensor; IAsys; Molecular recognition; Protein binding; Herpes simplex virus; Receptors

1. Introduction

The characterization of the binding process of functional proteins to a target is nowadays of high impact due to the central position of specific molecular recognition events in cell and molecular biology. Various methods are useful for studying

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the interaction process between proteins and target structures [1], however many of these methods require labeling of one of the two biomolecules with a fluorescent or radioactive tag. A recent development in instruments that investigates biomolecular interactions is IAsys, an instrument based on the evanescent wave detection with a biospecific sensor chip, produced by Affinity Sensor (Cambridge, UK) [1]. Several other optical biosensors based on the surface plasmon resonance (SPR) technology have been introduced into the market by a number of companies, including BIAcore (Uppsala, Sweden), Windsor Scientific (Berkshire, UK) and XanTec-bioanalitics (Hong Kong, China) [2–4]. These devices can monitor and quantify biorecognition processes, by detecting changes in refractive index in the vicinity of the surface of sensor chips, to which one of the biomolecules is immobilized, because of the binding of the interacting protein. The changes in refractive index values are proportional to the change in the absorbed mass, thus the analysis allows the monitoring of the interaction process in real-time. Most interestingly, both kinetics and the equilibrium constants can be determined by a single experiment, using small amounts of non-labeled material [5]. Indeed, quantities in the range of nanograms are required for the molecule to anchor to the biosensor surface, while the amount needed for the analysis of the interacting molecule is related to its binding affinity, but usually it is in the nanogram–microgram range. This methodology has been here applied to characterize the binding process between cellular receptors of herpes simplex virus (HSV) and two forms of a viral glycoprotein showing receptor-binding activity. These biorecognition phenomena are of great relevance for understanding the molecular basis of virus entry. Information on these mechanisms further provides the basis for a rational design of antiviral drugs. In particular, here we report the binding study of two recombinant forms of viral glycoprotein D, gD(Δ290-299t) and gD(305t), and a recombinant form of human HSV cellular receptor, nectin1-Fc, by using IAsys optical biosensor. HSV glycoprotein D (gD) is a viral envelope glycoprotein that has been studied extensively by different approaches. It is one of the essential glycoproteins for virus entry, and there are nowadays many evidences that indicate that it functions by interaction with a cellular receptor [6]. A number of gD mutants indicate that the molecule is composed of four functional regions (I–IV). Deletion/substitution in functional region IV generates a truncated form of gD, gD(Δ290-299t), more potent in inhibiting HSV infectivity compared to a soluble gD truncated at residue 305 [gD(305t)]. Here, we report on binding studies between soluble gD and members of the nectin family of cellular receptors. Comparative binding studies showed that despite the high level of conservation, human and murine nectin1 receptors differ with respect to the binding properties to HSV virions and gD. HSV cellular receptors, i.e. human and murine nectin1, have been immobilized on the surface of the biosensor cuvette, bearing a carboxymethyl dextran (CMD) layer. The immobilized receptor cuvettes were then used for the binding experiments with the two recombinant forms of glycoprotein D, gD(305t) and gD(Δ290-299t). The active receptor concentration was checked over time in order to verify the applicability of this method for a large screening of functional proteins for their binding to the target structure. Furthermore, the reliability of measuring the binding parameters was verified by repeated experiments over time and using different receptor anchored cuvettes.

2. Materials and methods

2.1. Biosensor technology

The resonant mirror biosensor technology (IAsys, Affinity Sensors, UK) [7] permits to monitor ligand–analyte biomolecular interactions in the same time they occur (real-time analysis). This technique offers many advantages with respect to most of the other available methodologies to study biomolecular interactions; above all is the fact that the amount of both ligand and analyte needed to obtain informative results is low (in the nanogram–microgram range) and the time required to perform an assay is very short and moreover the technology is label-free. Usually 20–

25 min is required for ligand immobilization and 4–5 min for a complete characterization of ligand–analyte interactions. The sensor chip can be reused many times and this indeed lowers the costs with the only limitation of verifying the stability of the immobilized ligand.

IAsys is capable of detecting molecular association and dissociation events via changes in mass at the surface of the biosensor. The protein (ligand) is usually covalently bound onto the surface of the biosensor surface cuvette; the most widely used immobilization chemistry for proteins is the coupling between carboxylate groups of CMD to protein ε-amino groups of lysine residues via EDC/NHS chemistry. These primary amino groups are charged, hydrophilic and usually projecting from the surface of the protein as well as lysine residues often represent a high proportion of the total amino acids; that is why covalent linkage is likely to occur without protein denaturation, preserving the native conformation for subsequent interaction with ligate. After immobilization, addition of ligate follows in order to monitor the association process; during this phase, ligand/ligate complex is formed. After the sample solution is replaced by buffer, the dissociation may be monitored. The regeneration phase accelerates this phase and removes completely the bound ligate. Subsequently, it is possible to proceed with another binding cycle (Fig. 1).

When molecules bind to the chip, the refractive index changes, generating an increase of response, measured in arc seconds. This optical technique not only detects, but also quantifies the changes in refractive index in the vicinity of the surface of the sensor chips to which ligand is immobilized. Since the refractive changes are proportional to the changes in the absorbed mass, this technique allows the quantitation of the biomolecules interacting with the ligand immobilized on the sensor chip. IAsys is based on an optical biosensor called the resonant mirror; a laser light is directed at the prism over a range of angles (670 nm is the laser wavelength); light reflected from the inside of the resonant mirror is measured as a function of incident angle. At ''resonance'' angle, when the angle is such as to allow waveguiding to occur, the light couples across the low index layer and it propagates along the surface of the device. To properly resolve the guiding angle, the instrumentation used within IAsys includes polarization components; when the optical components are suitably arranged, light at the guiding angle will undergo a 90° change of polarization, and a simple polarizer in front of the detector is sufficient to clearly distinguish the guiding angle. IAsys ensures rapid sample mixing and complete solution homogeneity throughout the cuvette by means of a patented Vibro-stirrer; this is of extreme importance in order to minimize mass-transport effects. The stirrer vibrates vertically with a frequency of 126 Hz at variable amplitude ensuring a rapid mixing in and an efficient sample delivery to the biospecific surface of the cuvette.

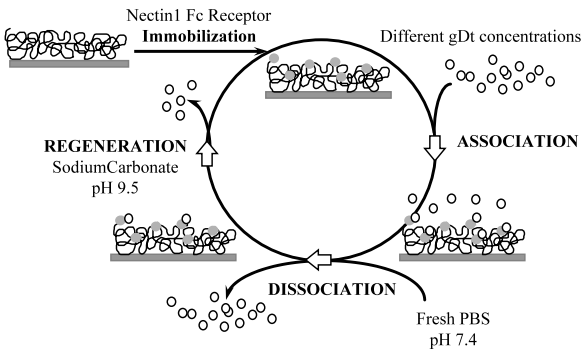

Fig. 1. Cycle scheme of the binding experiments.

2.2. Chemicals

All analyses were performed using IAsys optical biosensor equipped with CMD dual-well hydrogel cuvettes produced by Labsystems Affinity Sensors (Cambridge, England).

All solutions were made using ultrahigh-purity water.

Phosphate-buffered saline (PBS/T), final pH 7.4, was prepared using Sigma dry powder in foil pouches. Each pouch dissolved in 1 l of deionized water yields 0.01 M PBS: NaCl, 0.138 M; KCl, 0.0027 M; Tween 20, 0.05%; pH 7.4.

PBS was prepared by using KH_2PO_4 and Na_2HPO_4, with NaCl 0.138 M and KCl 0.002 M.

Acetate buffer (Sigma), 10 mM, was adjusted to pH 5 with glacial acetic acid (Sigma). Ethanola-

mine, *N*-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-*N*,*N*-dimethylaminopropyl)carbodiimide hydrochloride (EDC) were purchased as NHS coupling kit from Affinity Sensors. The solutions were prepared and stored as specified into IAsys protocol.

Human nectin1-Fc, murine nectin1-Fc PBS solutions, gD(305t) and gD(Δ299-290t) were kept at −20 °C and thawed immediately prior to use.

2.2.1. Production and purification of soluble recombinant forms of gD

Proteins gD(Δ290-299t) and gD(305t) used were stocked in PBS; the batches had a 5.6–28 μM concentration and a 84 μM, respectively. Production of the two proteins was performed as follows. The gD(305t) soluble recombinant form of HSV glycoprotein D is truncated at amino acid 305. gD(Δ290-299t) has the sequence described by Nicola et al. [8]. The soluble recombinant proteins were produced as described by Menotti et al. [9] for gD(Δ290-299t). Briefly, the open reading frame of gD was PCR-amplified with primers sgDbacB5/2 (CCGCAGCAAGGATCCCTTGGTGGATGCCTC) and sgDbacBg3/3 (GGGTGGCCGGGAGATCTTAAGGCGTCGCGG) for gD(305t), and sgDbacB5/2 (CCGCAGCAAGGATCCCTTGGTGGATGCCTC) and gD290Bg (GGGAGATCTTAAGGCGTCGCGGCGTCCTGAGGGAATATCTTTCCTTGCGGCGCCACCGTCCCC) for gD(Δ290-299t), using purified HSV-1(F) DNA as template. The amplified fragments were cloned in pAcGP67A transfer vector (Pharmingen) for recombination with linearized BaculoGold (Pharmingen) under the control of polyhedrin promoter and gp67 signal sequence for secretion in the medium. Following recombination and baculovirus stock production, Sf9 insect cells were infected at a multiplicity of infection of 4 PFU/cell in serum-free SF-900 medium (Life Technologies). After 4 days, the medium was harvested and the secreted recombinant proteins were purified by affinity chromatography on a column of MAb 30 [10] conjugated to CNBrSe-parose. Fractions were eluted with 3 M KSCN, 10 mM Tris–HCl, pH 7.5, 0.5 M NaCl, dialyzed against PBS and concentrated using YM3 ultrafiltration membranes with an Amicon ultrafiltration device (Millipore).

2.2.2. Production and purification of soluble receptors

Human and murine nectin1-Fc were produced and purified according to the reported procedures [11,12].

2.3. Immobilization of soluble recombinant forms of murine and human nectin1 receptors, fused with human IgG Fc (murine nectin1-Fc and human nectin1-Fc)

To examine the kinetics of binding of gD to human and murine nectin1 by resonant mirror technology, both receptors were immobilized through their surface amine groups via amide bonds with CMD in two different experiments. Stirring was set to 100%, sampling interval to 0.3 s; the running buffer was PBS/T. CMD hydrogel in the cells was activated with a 1:1 EDC/NHS mixture (400 mM EDC and 100 mM NHS) for 7 min.

Two stock solutions of human nectin1-Fc (1.5 μg/μl) and murine nectin1-Fc (1 μg/μl) in PBS were available. Both solutions were diluted into the cuvette to a 0.094 μg/μl concentration in sodium acetate 10 mM, pH 5 and let reacted for 7 min. Addition of samples by dilution into the cuvette avoids disturbance of the baseline. Unreacted NHS-esters were blocked by washing with 1 M ethanolamine, pH 8.5 for 3 min. An alkaline solution of pH 9.5 was added into the chips to wash-off non-covalently bound proteins. The resulting immobilized receptor was 1730 and 3800 arcsec for the human nectin1-Fc, and 3775 arcsec for murine nectin1-Fc; the significant differences for the immobilized value are probably due to the slight differences in the immobilization procedure and/or to the different batch of the receptor samples. The receptors were immobilized only in one channel of the cuvette in order to monitor the possible non-specific bindings by means of the second channel (reference channel). The second channel CMD surface was only activated with 1:1 EDC/NHS mixture, and then treated with ethanolamine to block NHS-ester groups. Human

nectin1 immobilization experiment is summarized in the sensorgram shown in Fig. 2. In the initial 14 min (phase 1), a buffer baseline was established. In phase 2, an EDC/NHS mixture was added and the reaction was left to occur for about 7 min. Then, washes with buffer allowed removing from the surface all the unreacted molecules (phase 3). Human nectin1 was prepared in acetate buffer, and so it was necessary (phase 4) to re-equilibrate the cell with acetate buffer before adding the sample. The receptor/surface coupling was let to react for about 6 min (phase 5), washed with PBS to dissociate (phase 6) and then non-coupled activated CMD sites were blocked with ethanolamine (phase 7). Finally, the baseline was stabilized by adding the running buffer (phase 8).

2.4. Binding experiments

Determination of kinetic and equilibrium constants was achieved by adding a wide range of ligate concentrations (approximately $0.1–10K_D$, in the ng–μg/ml range) and allowing association and dissociation to occur (Fig. 3). Equilibrium does not need to be reached. Ligate was replaced with buffer and dissociation occurred. Addition of samples by dilution into the cuvette (usually 1–10) avoids disturbance of the baseline. All experiments were performed with stirring setting to 100,

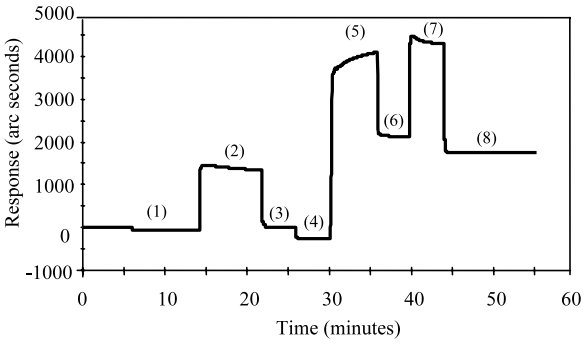

Fig. 2. Human nectin1-Fc receptor immobilization. (1) Buffer baseline stabilization. (2) EDC/NHS mixture add (7 min of reaction). (3) Buffer washes to remove unreacted molecules. (4) Acetate buffer re-equilibration. (5) Human nectin1 in acetate buffer add (6 min of reaction). (6) Buffer washes to allow dissociation. (7) Block of non-coupled activated CMD sites with ethanolamine. (8) Baseline stabilization.

which is the maximum value equivalent to a displacement of 0.5 mm and data were collected at the fastest data collection rate (0.3 s^{-1}); this enables to clearly see where "bulk" effect ends and the binding begins, and that mass transport effects are minimized. Binding of each gDt sample was allowed to occur for 5 min, with the wash delay set for an additional 3–4 min to allow for a smooth dissociation curve. The chip surface was regenerated by adding 0.2 M sodium carbonate, pH 9.5, until the response signal returned to baseline. Regeneration conditions are extremely important since a biosensor surface with immobilized ligand carries out multiple rounds of interaction analysis involving various concentrations of ligate. Dissociation of all ligate rarely occurs. Repeated cycles of analysis need to start with the biosensor surface free of residual material from the previous cycle. This is because it is essential to ensure that the amount of available ligand and binding conditions at the start of each cycle are constant to give consistency of association and dissociation data between analyses. Regeneration is achieved by breaking the non-covalent interactions binding biomolecules to the ligand or matrix. An additional consideration is that the regenerate regime chosen should not result in irreversible denaturation of a significant proportion of the ligand. This will eventually lead to a loss of ligate-binding capability after regeneration. All experiments were repeated several times in different immobilized cuvette surfaces. The regeneration protocol was previously optimized by enzyme-linked immunosorbent assay (ELISA) test. Microwell plates were coated with human nectin1-Fc protein (500 ng/well), or BSA as control, in bicarbonate buffer pH 9.5 at 4 °C overnight. The wells were then washed three times with PBS, and incubated with an acidic solution (0.1 N HCl–glycine, pH 2.2), or with a basic solution (0.2 M bicarbonate buffer, pH 9.5), or PBS as control, for 10 min with stirring. The wells were washed again and reacted with MAb R1.302 [13] which recognizes a discontinuous epitope in the N-terminal V domain of human nectin1. Binding was revealed with anti-mouse peroxidase (Sigma) developing with 0.5 mg/ml *o*-phenylenediamine (Sigma) in 2.5 mM citric acid/5 mM Na_2HPO_4/0.009% H_2O_2, blocking with 1:6

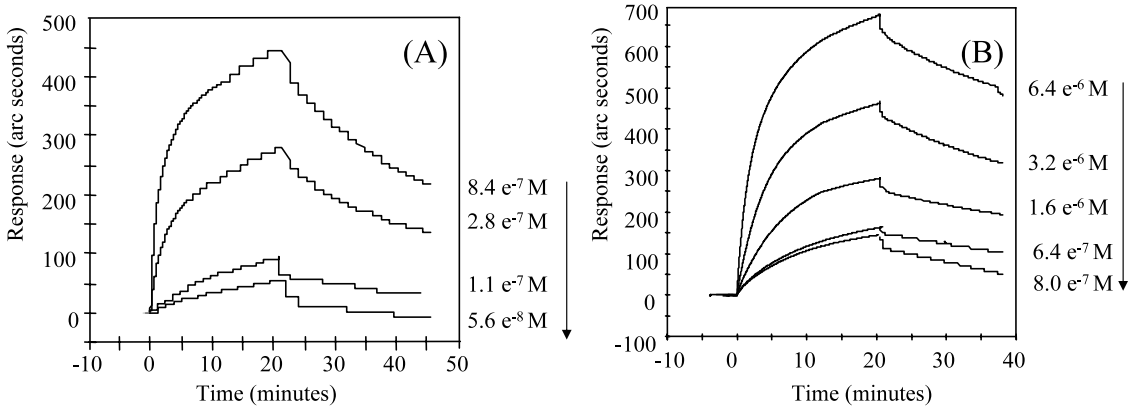

Fig. 3. Corrected sensorgrams overlays for the gDt/hNectin1-Fc interaction. (A) gD(299-290t) binding and dissociation processes at the indicated concentrations; (B) gD(305t) binding and dissociation processes at the indicated concentrations.

H_2SO_4, and monitoring at 490 nm. The results in Fig. 4 show that MAb R1.302 reactivity to the receptor was preserved following both treatments, indicating that the native conformation of the receptor was maintained.

3. Results

Biosensor data were analyzed using a fitting routine with Fast Plot v. 3.1.0.17 (Affinity Sensors by Robin J. Leatherbarrow) and Graph Fit v. 5.0.0.36 software (Erithacus Software limited; author: Robin J. Leatherbarrow). GraphPad Prism 3.0 Software from GraphPad Prism was also used to fit within a linear regression approach Scatchard Plots.

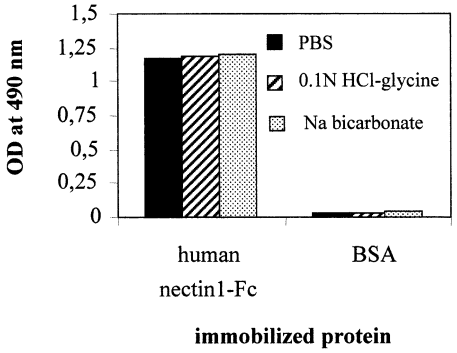

Fig. 4. Effect of the treatment with basic or acid solutions on the stability of human nectin1-Fc receptor. Human nectin1-Fc, or BSA as control, coated onto microwell plates were treated with the indicated solutions, then reacted with R1.302 MAb directed to human nectin1 V amino-terminal domain. Binding was revealed with anti-mouse peroxidase (Sigma) followed by OPD, and monitoring optical density at 490 nm.

3.1. Dissociation equilibrium constant determination

Sensorgrams were all corrected for non-specific binding by subtracting the control channel signal. The classical Langmuirian model of interaction in free solution is used as a foundation for development of analytical methodologies for interaction analysis; in this way, the program determines the value of R_{eq} at a variety of ligate concentrations. Equilibrium (R_{eq}) data may be analyzed by two related approaches as Scatchard analysis as well as binding curve analysis; a comparison of equilibrium constants derived by these two different approaches provides a useful check on the validity of the method. In the Scatchard analysis, a plot of $R_{eq}/[L]$ vs. R_{eq} yields a slope of $-K_A$ with an x-axis intercept of R_{max}. The y-intercept is equivalent to $K_A R_{max}$. For the binding curve analysis approach, a binding curve is made; if the binding curve is Langmuirian, K_D can be obtained directly from the binding curve, as being equal to the ligate concentration at $R_{max}/2$. To generate this plot, experimental data were fitted to the equation

$$R_{eq} = \frac{R_{max}[L]}{K_D + [L]}$$

by means of GraFit v. 5.0.0.36 software. The equation fits equilibrium response (R_{eq}) data at differing ligate concentrations to give the dissociation equilibrium constant (K_D) where R_{max} is the maximum capacity of the ligand for the ligate.

The binding kinetics of gD(305t) was compared with that of gD(Δ299-290t). Data were overlaid with the result of the fitting analysis and are shown in Fig. 3. Based on this methodology, the affinity of gD(Δ290-299t) for human nectin1-Fc was found to be 10-fold higher than the affinity of gD(305t) for human nectin1-Fc. The values for K_D calculated from the resonant mirror biosensor were $2.8 \times 10^{-6} \pm 4.1 \times 10^{-7}$ for gD(305t) and $2.8 \times 10^{-7} \pm 3.3 \times 10^{-8}$ for gD(Δ290-299t). The same data were also analyzed by using Scatchard regression method; this analysis yielded values for K_D of 3.7×10^{-6} and 2.8×10^{-7} for gD(305t) and gD(Δ290-299t), respectively (Fig. 5). Regression values (r^2) for the linear fit of the data were 0.844 and 0.944, respectively. K_D values are essentially the same as those obtained from the binding isotherm analysis. Moreover, graph of k_{on} (pseudo-first-order rate for interaction) vs. ligate concentration (kinetic plot) was also built for both of the proteins (Fig. 6); this graph allows k_{ass} (association rate constant, slope) and k_{diss} (dissociation rate constant, intercept) to be determined. It has to be considered that k_{diss} value is often close to zero with a substantial error and sometimes is inaccurately defined. Being the association and the dissociation rate constants related to K_D ($K_D = k_{diss}/k_{ass}$), we could determine K_D value from kinetic plot as well; multiple k_{on} values were derived from a full interaction experiment allowing to construct the plots. K_D values appeared to be consistent with those determined by Scatchard regression method and binding curve analysis. Affinity determinations of gDt/human nectin1-Fc interaction confirmed and extended previous data obtained by different experimental approaches [9,12,14].

3.2. Receptor binding capacity decays over the time

To determine the reproducibility of the assay, the entire experiment was replicated several times over different receptor surfaces. In order to test the stability of the receptor surfaces, the gD(Δ290-299t) experiments described above were repeated on the same cuvette over 35 days systematically every week. The change in the percentage activity from this surface over time, i.e. the decreasing of the R_{eq} at fixed concentration of ligate is shown in Fig. 7. Within 1 week, the surface lost approximately 10–15% activity. It is worth mentioning that the activity was still relatively high after 5 weeks (Fig. 7). However, the K_D value determined for the gD(Δ290-299t)/human nectin1-Fc interaction was essentially the same over time, being slightly increased only after 35 days. This result

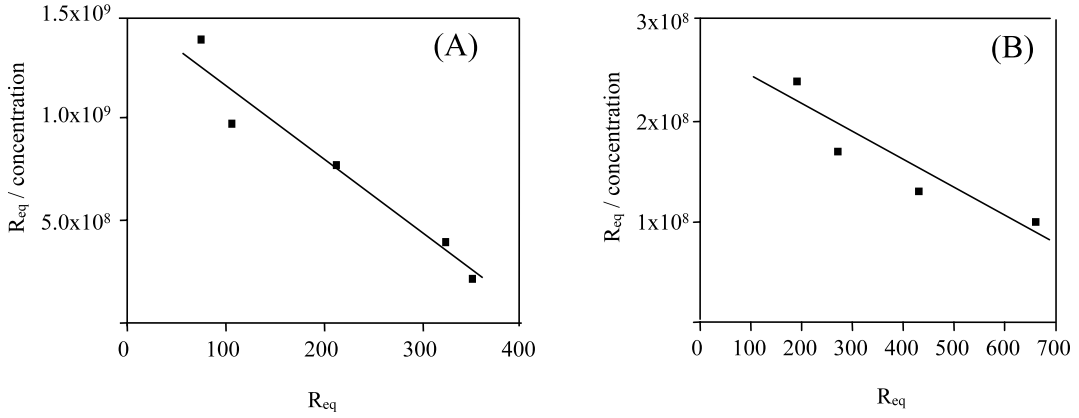

Fig. 5. Scatchard plot, $R_{eq}/[L]$ vs. R_{eq}, the gradient of which is equal to $-K_A$. (A) gD(299-290t) binding (B) gD (305t) binding.

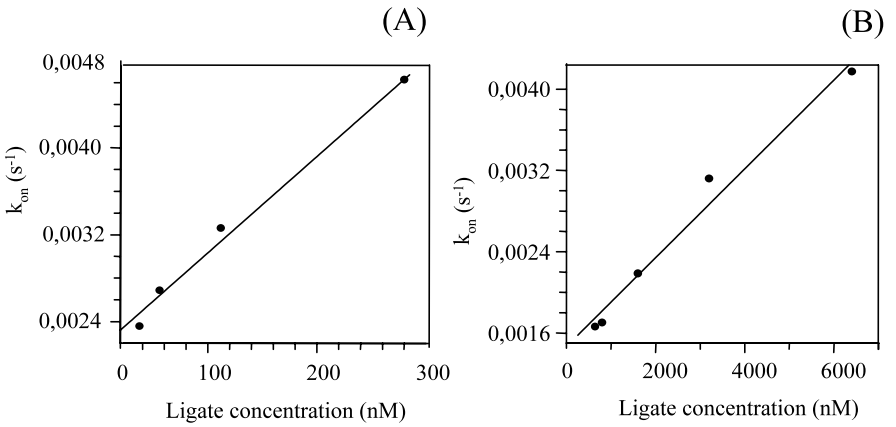

Fig. 6. Kinetic plot, $k_{on}/[L]$, the slope of which is equal to k_{ass} and intercept to k_{diss}. (A) gD(299-290t) kinetic plot; (B) gD(305t) kinetic plot.

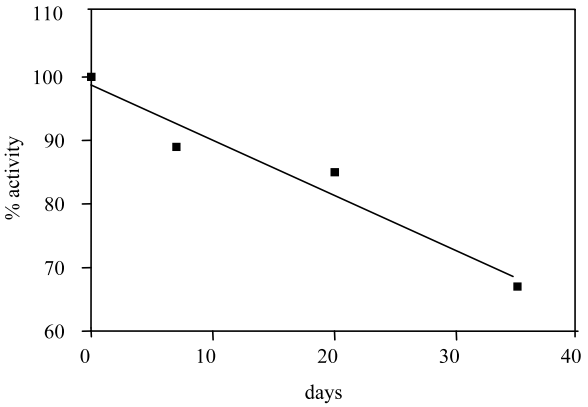

Fig. 7. Change in binding activity of anchored nectin1 human receptor towards gD(290-299t) over time.

could occur because of the significant change of the [ligate]/[ligand] molar ratio due to the decrease of the ligand concentration upon time. All subsequent experiments were performed with freshly prepared receptor surfaces and data were collected immediately. The activity of the immobilized receptor after 5 weeks was proved by the binding of an anti-human Fc antibody (Dako).

3.3. Murine and human receptors comparison

The murine nectin1α receptor (mNectin1α) was also taken into examination. Similar to the human receptor, the murine homolog mediates the entry of HSV into cells, but has been reported that while the binding of hNectin1 to HSV glycoprotein D (gD) is readily detectable, the binding of mNectin1 to gD was barely or not detectable. Biosensor technique was used to verify differences in gD-binding activity to murine- and human-nectin1 receptors. The binding experiments performed onto the human receptor surface were also repeated onto the murine receptor surface. Murine nectin1-Fc immobilization was conducted in exactly the same manner of human nectin1-Fc receptor; the resulting signal was about 3775 arcsec for the murine nectin1-Fc receptor. However, murine nectin1-Fc did not show any significant binding to gD(Δ290-299t) under the same conditions used for human, and even at higher ligate concentrations. This behaviour prevented the determination of K_D. The quality of the immobilized murine nectin1-Fc was checked by means of anti-human Fc antibody (Dako Laboratories), which displayed a very strong binding.

The response obtained with 7.5 μM gD(Δ290-299t) solution on murine nectin1-Fc is reported in Fig. 8, together with the sensorgram obtained by binding the control anti-human Fc antibody (8.6 μM). These results agree with barely detectable gD-binding activity reported previously [11,14].

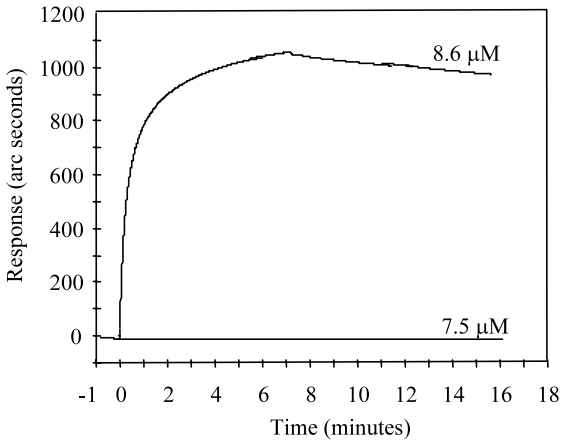

Fig. 8. Corrected sensorgram overlay showing the gD(Δ290-299t)/murine nectin1 receptor interaction and the anti-human Fc antibody/murine nectin1-Fc as receptor activity control experiment.

4. Conclusions

The glycoprotein gD(Δ290-299t), a recombinant soluble gD variant, lacking a portion in region IV, showed 10-fold higher human nectin1-Fc affinity with respect to the soluble form truncated at amino acid 305 [gD(305t)], while no significant binding was observed for the same proteins with murine nectin1-Fc. The evanescent wave detection-based IAsys optical biosensor appeared to be a high sensitive system for detecting and quantifying the binding of the functional proteins to the receptor, without labeling the interacting material. Advantages of this optical technique involve speed and reproducibility of the measurements. Furthermore, the system uses a very low amount of immobilized receptor and interacting material, and the functionalized surface cuvettes can be used for a quite long time, if the immobilized surface is proved to be stable. Then, a large number of experiments can be carried out when, as in the present investigation, the receptor maintained its specific binding properties over 1 month, even if the activity decreased on time.

Finally, the optical biosensor monitors the binding process in real-time, then allowing not only determining the binding parameters but also the kinetics of the process. This aspect, i.e. the possibility of monitoring the association and dissociation phases, is fundamental for obtaining a better insight on the molecular interactions between the receptor and the glycoproteins involved in the mechanism of virus infection.

All these advantages make the optical biosensor very well suited for the screening of the glycoproteins showing receptor-binding activity, as well as of phage displayed peptides with the aim to give the basis for a rational design of antiviral drugs.

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References

[1] W.D. Wilson, Science 295 (2002) 2103–2105.
[2] R. Gambari, Curr. Med. Chem. 1 (2001) 277–291.
[3] K.N. Baker, M.H. Rendall, A. Patel, P. Boyd, M. Hoare, R.B. Freedman, D.C. James, Trends Biotechnol. 20 (2002) 149–156.
[4] R.L. Rich, D.G. Myszka, J. Mol. Recognit. 13 (2000) 388–407.
[5] G. Canziani, W. Zhang, D. Cines, A. Rux, S. Willis, G. Cohen, R. Eisenberg, I. Chaiken, Methods 19 (1999) 253–269.
[6] G. Campadelli-Fiume, F. Cocchi, L. Menotti, M. Lopez, Rev. Med. Virol. 10 (2000) 305–319.
[7] P.A. Lowe, T.J.H. Alwyn Clark, R.J. Davies, P.R. Edwards, T. Kinning, D. Yeung, J. Mol. Recognit. 11 (1998) 194–199.
[8] A.V. Nicola, C. Peng, H. Lou, G.H. Cohen, R.J. Eisenberg, J. Virol. 71 (1997) 2940–2946.
[9] L. Menotti, R. Casadio, C. Bertucci, M. Lopez, G. Campadelli-Fiume, J. Virol. 71 (2002) 5463–5471.
[10] R. Brandimarti, T. Huang, B. Roizman, G. Campadelli Fiume, Proc. Natl. Acad. Sci. USA 91 (1994) 5406–5410.
[11] L. Menotti, M. Lopez, E. Avitabile, A. Stefan, F. Cocchi, J. Adelaide, E. Lecocq, P. Dubreuil, G. Campadelli Fiume, Proc. Natl. Acad. Sci. USA 97 (2000) 4867–4872.
[12] F. Cocchi, M. Lopez, L. Menotti, M. Aoubala, P. Dubreuil, G. Campadelli-Fiume, Proc. Natl. Acad. Sci. USA 95 (1998) 15700–15705.

[13] M. Lopez, F. Jordier, F. Bardin, L. Coulombel, C. Chabannon, P. Dubreuil, in: T. Kishimoto, H. Kikutani, A.E.G. von dem Borne, S.M. Goyert, D.Y. Mason, M. Miyasaka, A. Moretta, K. Okumura, S. Shaw, T.A. Springer, K. Sugamura, H. Zola (Eds.), Leukocyte Typing VI, White Cells Differentiation Antigens, Garland Publishing Inc., New York, 1997.

[14] L. Menotti, E. Avitabile, P. Dubreuil, M. Lopez, G. Campadelli-Fiume, Virology 282 (2001) 256–266.