J. Biochem. 121, 633-636 (1997)

Real Time Observation of Binding of Herpes Simplex Virus Type 1 (HSV-1) to Vero Cells and Neutralization of HSV-1 by Sulfonated Human Immunoglobulin

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Received for publication, January 8, 1997

A real time biomolecular interaction assay system involving an optical sensor was applied to the quantitative analysis of the binding of herpes simplex virus type 1 (HSV-1) to Vero cells, and the neutralization antibody titer against this virus with a commercially available sulfonated human immunoglobulin preparation. The virus titers in a viral solution and the neutralizing antibody titer in the human immunoglobulin preparation could be successfully estimated in a short time with this system without any difficult cytopathic effect analysis of cultured cells.

Key words: herpes simplex virus type 1, human immunoglobulin, optical biosensor, virus-cell interaction.

The initial step in viral infection is undoubtedly virion attachment to the cell surface, but we have long been unable to observe this attachment process directly. Recent development of biosensor technology has made it possible to monitor and quantitate interactions of biomolecules in real time (*1, 2*). We tried to apply this method to viral-cell or receptor molecule interactions. Herpes simplex virus type 1 (HSV-1) was chosen for this study, because the viral attachment molecule and cellular receptor are already known. And it was proved that the binding of the virus to the fixed cellular receptor could be successfully observed in real time with this optical biosensor system, and that the virus titer in a viral solution and the neutralizing antibody titer in a human immunoglobulin preparation could be estimated in a short time with this system (*3*). But this system was successfully applied only to the observation of the interaction between the virus and the purified cellular receptor, *i.e.* not to that between the virus and cells. To expand the application of this system to all virus-cell interactions, we tried to develop the new system further.

HSV-1 was propagated on Vero cells in Eagle's minimal essential medium containing 10% fetal bovine serum (MEM; Nissui Pharmaceutical, Tokyo), and cell debris was removed by centrifugation. The viral solution was filtered through a DISMIC-25cs disposable syringe filter unit, 0.2 μm (Toyo Roshi Kaisha, Tokyo), and kept at −80°C before use. The viral solution was diluted with Dulbecco's phosphate-buffered saline solution without a metal salt [PBS(−); Nissui Pharmaceutical]. MEM solutions diluted with PBS(−) to the same concentrations as the viral solutions were used to estimate the background activity. A commercially available sulfonated human immunoglobulin preparation for intravenous injection (hIg, 50 mg/ml, Lot No. SSV 047) was from Teijin (Tokyo), and stored at −80°C before use. For estimation of the neutralization antibody titer, this hIg solution was diluted with PBS(−).

As a real time biomolecular interaction assay system, an optical biosensor (IAsys; Fisons Applied Sensor Technology, Cambridge, UK) was used. Goat anti-Herpes simplex virus type 1 polyclonal antibody (purified IgG, pAb) was purchased from Chemicon International, Temecula, California, USA, and was immobilized on the wall of an aminosilane cuvette *via* the homobifunctional reagent, bis(sulfosuccinimidyl) suberate (BS3) cross linker. That is, the aminosilane cuvette was washed with 200 μl of sterile distilled water (DW) and then activated with 200 μl of a BS3 solution. After washing with DW, the anti-HSV-1 antibody was immobilized with 100 μl of a (100 : 100) pAb solution, and pAb was blocked with 200 μl of 1 M ethanolamine, pH 8.5. After washing with DW, the cuvette was kept at 4°C, with 200 μl of PBS(−), before use. This cuvette remained active for a few days. The immobilized pAb was stable after washing with 200 μl of 20 mM HCl. These immobilization steps are shown as real time reactions in seconds in Fig. 1.

HSV-1 was bound to pAb and then Vero cell binding to the HSV-1 was monitored. That is, the cuvette was washed with PBS(−), and then 200 μl of the HSV-1 solution was added. After washing with PBS(−), another 200 μl of HSV-1 solution was poured into the cuvette, and again the cuvette was washed with 200 μl of PBS(−). About 1×10^5 cells/200 μl of Vero cells were poured into the cuvette, and then the interaction between the virus and cells was monitored. The interaction was stopped by washing with PBS(−). Vero cells and HSV-1 were removed by washing with 200 μl of 20 mM HCl. The real time binding of HSV-1 to pAb, and the interaction between HSV-1 and Vero cell are shown in Fig. 2.

The viral titer was calculated from the extent of binding of the diluted virus to pAb after subtraction of the extent of binding of the diluted MEM medium. The dilution which

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gave no binding was regarded as the breaking point (0/1), and the viral titer estimated with this method was 1×10^2/100 μl (1×10^3/ml) (data not shown).

For anti-serum titration, a cuvette with HSV-1 was washed with PBS(−), and then 200 μl of a diluted hIg solution was poured into the cuvette and the interaction was monitored. After washing with 200 μl of PBS(−), the Vero cell suspension (1×10^5/200 μl) was poured in, and the interaction between HSV-1 and the Vero cells was monitored. Vero cells, HSV-1, and bound hIg were removed by washing with 200 μl of 20 mM HCl. Figure 3 shows the

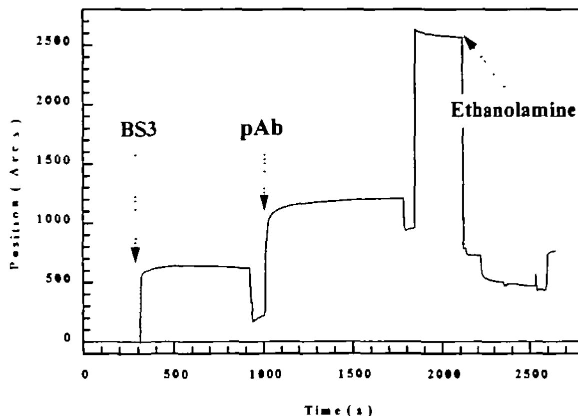

Fig. 1. **Immobilization of the anti-HSV-1 antibody.** Immobilization of the anti-HSV-1 antibody (pAb, IgG) in an aminosilane cuvette was carried out through the following steps in a Fisons IAsys at 25°C. The times indicated are in seconds. Time 0: 200 μl of distilled water (DW) poured into the cuvette for the base line. Time 313: DW removed and 200 μl of 1 mM BS3 solution poured in for activation. Time 928: BS3 removed and 200 μl of DW poured in for wash. Time 1013: DW removed and 100 μl of polyclonal anti-HSV-1 antibody solution (Chemicon International, California, USA, pAb) poured in for immobilization. Time 1790: pAb removed and 200 μl of DW poured in for wash. Time 1850: DW removed and 200 μl of 1 M ethanolamine (EA) poured in for blocking. Time 2113: EA removed and 200 μl of DW poured in for wash. Time 2223: 200 μl of 20 mM HCl poured in for washing. Time 2360: removed HCl and 200 μl of DW poured in for washing. Time 2420: DW removed and 200 μl of 20 mM HCl poured in for washing. Time 2536: HCl removed and 200 μl of DW poured in. Time 2601: DW removed and 200 μl of PBS(−) poured in for storage.

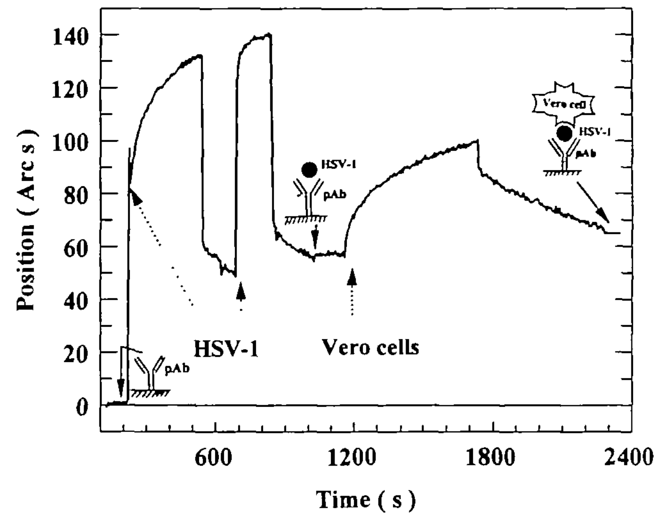

Fig. 2. **Interactions between pAb and HSV-1, and HSV-1 and Vero cells.** An aminosilane cuvette with the immobilized anti-HSV-1 antibody (pAb) was used in a Fisons IAsys at 25°C. The reactions were carried out through the following steps. The times indicated are in seconds. Time 0: old PBS(−) removed and 200 μl of PBS(−) poured in. Time 91: PBS(−) removed and 200 μl of PBS(−) poured in for the base line. Time 222: PBS(−) removed and 200 μl of HSV-1 poured in for association. Time 545: HSV-1 removed and 200 μl of PBS(−) poured in for washing. Time 620: PBS(−) removed and 200 μl of PBS(−) poured in for washing. Time 691: PBS(−) removed and 200 μl of HSV-1 poured in for association. Time 847: HSV-1 removed and 200 μl of PBS(−) poured in for washing. Time 1024: PBS(−) removed and 200 μl of diluted Vero cell suspension (20 : 200) poured in. Time 1160: diluted Vero cell suspension removed and 200 μl of the full Vero cell suspension (200 : 200) poured in for interaction. Time 1740: Vero cell suspension removed and 200 μl of PBS(−) poured in for washing. Time 2591: PBS(−) removed and 200 μl of 20 mM HCl poured in for regeneration.

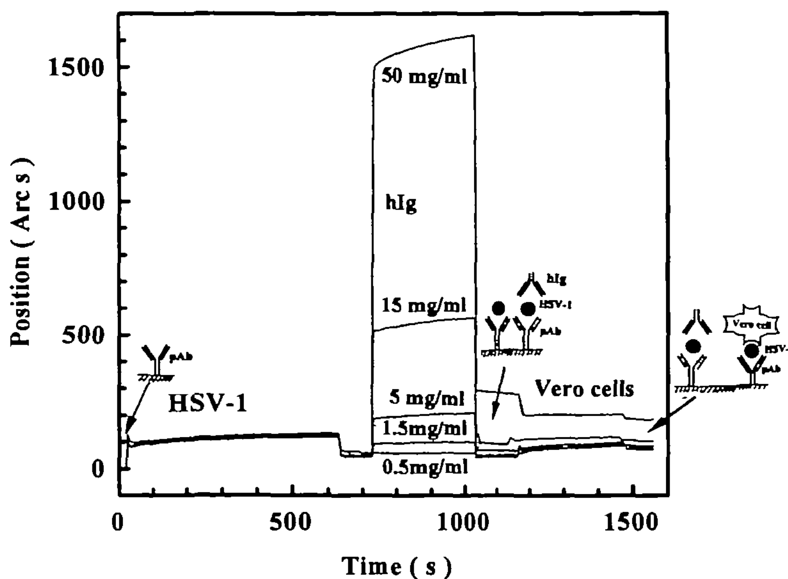

Fig. 3. **The binding of human immunoglobulin (hIg) to the immobilized HSV-1 and the inhibition by hIg of the reaction between HSV-1 and Vero cells.** The binding of hIg to the immobilized HSV-1 and the inhibition of the hIg to virus-cell interaction were monitored with the regenerated anti-HSV-1 antibody immobilized in a cuvette in a Fisons IAsys at 25°C through the following steps. The times indicated are in seconds. Time 0: old PBS(−) removed and 200 μl of PBS(−) poured in for washing. Time 30: PBS(−) removed and 200 μl of HSV-1 poured in for association. Time 630: HSV-1 removed and 200 μl of PBS(−) poured in for washing. Time 730: PBS(−) removed PBS and 200 μl of the various concentrations of hIg solutions (50, 15, 5, 1.5, and 0.5 mg/ml) poured in for inhibition. Time 1030: hIg solution removed and 200 μl of PBS(−) poured in for washing. Time 1150: PBS(−) removed and 200 μl of Vero cell suspension (1×10^5/200 μl) poured for interaction. Time 1450: Vero cell suspension removed and 200 μl of PBS(−) poured in for washing. Time 1700: PBS(−) removed and 200 μl of 20 mM HCl poured in for regeneration.

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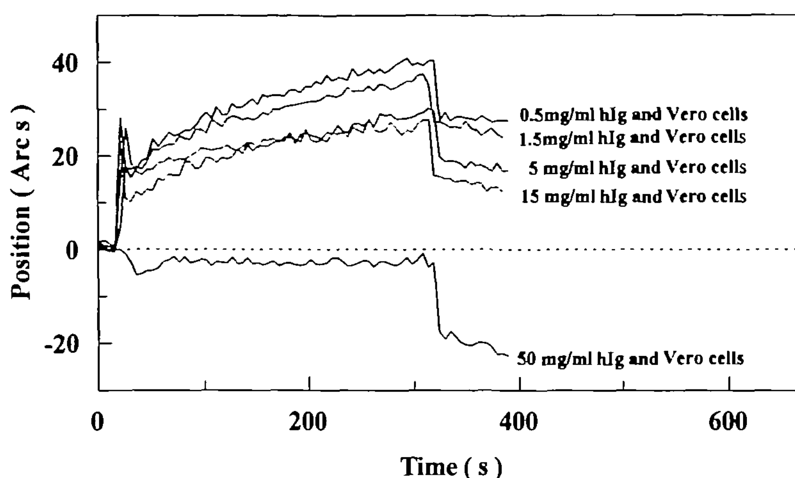

Fig. 4. **Inhibition by hIg of the interaction between HSV-1 and Vero cells.** The extents of reaction between HSV-1 and Vero cells from Time 1100 to 1600 in Fig. 3 are shown as reactions from Time 0 to 500 in this figure.

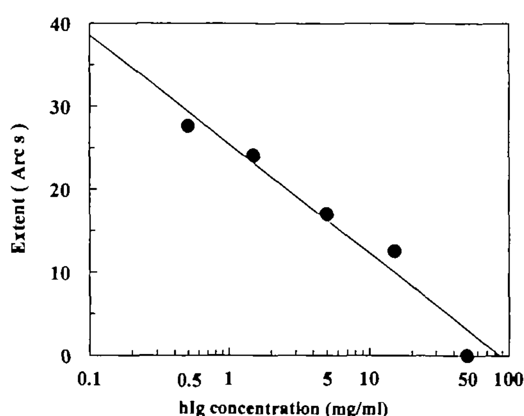

Fig. 5. **Evaluation of the response inhibiting rate constants of hIg against the virus.** The response rates are plotted against the concentrations of hIg. The titer of the neutralizing antibody against HSV-1 was calculated from the curve by means of the concentrations of hIg at the extent of no hIg control (45 Arc s).

extents of binding of hIg in real time when the protein concentrations of the hIg solutions added were 50, 15, 5, 1.5, and 0.5 mg/ml from top to down, respectively, and Fig. 4 shows the extents of binding of Vero cells to HSV-1 treated with the indicated concentrations of hIg in real time.

The same viral and neutralized viral solutions were added to cultured Vero cell monolayers, and the virus titer of a viral solution and the antibody titer of the hIg solution were also estimated from the usual cytopathic effect (CPE) on cultured Vero cells. That is, for viral titration, 100 μl of 10^5 cells/ml of Vero cells in MEM was dispensed into a 96-well cell culture plate, followed by culturing till the cells reached about 50% confluent growth in a CO_2 incubator. After removing the culture supernatant, 100 μl of a serially diluted viral solution was dispensed into each of 10 wells, followed by culturing for 4 days to see CPE. For antibody titration, 1 ml of a serially diluted hIg solution in MEM was added to 1 ml of a viral solution, and then 200 μl of the mixture was dispensed into each of 10 wells containing cultured Vero cells. CPE was observed 4 days later. The viral titer of the original solution was estimated to be 2×10^3/ml, and the neutralization antibody titer of the hIg solution was 1×10^5 units/ml or 2×10^3 units/mg hIg.

The binding of HSV-1 to the immobilized pAb calculated from the extent of viral binding with the extent of background binding of an equally diluted MEM solution subtracted was estimated and the rates of the viral binding reaction were calculated. Binding of the virus to the specific antibody was found to depend on the viral concentration, and the viral concentration could be obtained from the binding curve at the point of no binding as less than 1 unit/100 μl. The virus titer of the tested viral solution was estimated to be about 1×10^3 units/ml. This value was a little lower than that estimated with the ordinary biological method (2×10^3 units/ml).

The extents of binding of the various concentrations of hIg to the bound HSV-1 were calculated from the real time binding shown in Fig. 3. The concentration of hIg which gave no binding was 0.5 mg/ml (0.1 mg/200 μl). The specific antibody titer against HSV-1 calculated was 100 units/mg (5×10^3 units/ml) (data not shown).

The extents of inhibition of the various concentrations of hIg of the interaction between HSV-1 and Vero cells were calculated from the real time binding of the unneutralized virus to Vero cells shown in Fig. 4, and evaluation of the response inhibiting rate constants of hIg is shown in Fig. 5. Binding of the virus to the cells was found to be blocked by hIg in an exponential manner, and the neutralizing antibody titer of the hIg solution could be calculated from the blocking rate curve as 1 unit/200 μl at the point of no hIg control (45 Arc s). The neutralizing antibody titer of the hIg solution was estimated to be 5×10^3 units/ml or 1×10^2 units/mg hIg. The specific antibody titers estimated as both the binding of hIg to HSV-1 and the inhibition by hIg of the interaction between the virus and cells were found to be almost same. These values were lower than those estimated with the ordinary biological method. This might be due to the following reasons. This optical sensor detects all hit numbers on the detection surface at the bottom of the cuvette as reactions. Different from small molecules such as antigens, Vero cells were too heavy to maintain a stable suspension state, but sank to the bottom in a non-specific manner. This non-specific hit was subtracted as the background. When the specific reaction was slight, the background hit in a hIg free cuvettes subtracted was far higher than the hit in the protein-rich hIg cuvettes, because the

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presence of the high amount of non-specific globulin in the sample cuvettes gave a far less chances of collision for Vero cells. The negative reaction observed on the reaction of 50 mg/ml hIg and Vero cells in Fig. 4 was the result of the background subtraction. The reaction at this point might be positive, but this point should be regarded as no reaction (Fig. 5).

Since virus binding could be estimated reproducibly with this optical sensor system in a few minutes without any difficult CPE analysis, this interaction assay system could be a very quick and accurate method for determining virus and antibody titers, and be applicable to all viruses.

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