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Real Time Observation of the Binding of Herpes Simplex Virus Type 1 (HSV-1) to Immobilized Heparan Sulfate and the Neutralization of HSV-1 by Sulfonated Human Immunoglobulin

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A real time biomolecular interaction assay system involving an optical sensor was applied to quantitative analysis of the binding of herpes simplex virus type 1 (HSV-1) to immobilized heparan sulfate, a cellular receptor component of HSV-1, and the neutralization antibody titer against this virus with a commercially available sulfonated human immunoglobulin preparation. The virus titer 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 cell culture.

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

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. That is, the interaction of viral glycoproteins (gC) with cell surface heparan sulfate proteoglycans has been proposed to comprise the mechanism of viral attachment (*3, 4*).

HSV-1 was propagated on Vero cells in Eagle's minimal essential medium with 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 back ground activity. A commercially available sulfonated human immunoglobulin preparation for intravenous injection (hIg, 50 mg/ml, Lot No. SSV 047) was supplied by Teijin (Tokyo), and stored at -80°C before use. For estimation of the neutralization antibody titer, this hIg solution was diluted with PBS(−), and an equal volume was added to the viral solution 30 min before the assay.

As a real time biomolecular interaction assay system, an optical biosensor (IAsys; Fisons Applied Sensor Technology, Cambridge, UK) was used. Heparan sulfate (HS) was immobilized to the wall of a carboxymethylated dextran cuvette *via* a carbon 6 linker. That is, the cuvette was washed with 200 μl of 10 mM PBS(−)/0.05% Tween 20 (PBS/Tween) and then activated three times with 200 μl of an EDC/NHS solution. After washing with PBS/Tween, the C6 linker was fixed with in 200 μl of a 10 mg/ml C6 linker solution three times, and the C6 linker was blocked with 200 μl of 1 M ethanolamine, pH 8.5. After washing with PBS/Tween, activation was carried out with 200 μl of the EDC/NHS solution. After washing with 200 μl of 10 mM acetate buffer, pH 5.3, HS was fixed with in 10 μl of 1 mg/ml HS three times. The HS fixed seemed to be in excess as to the virus applied. The cuvette was kept at 4°C, with 200 μl of PBS/Tween, before use. This cuvette was active for over a month. For viral or antiserum titration, the cuvette was washed with PBS(−), and then 200 μl of a diluted viral solution or a mixture of equal volumes of the viral and diluted hIg solutions was poured into the cuvette and the reaction was monitored. 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 culture till 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 culture for four 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 four 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 to be 1×10^5 units/ml or 2×10^3 units/mg hIg.

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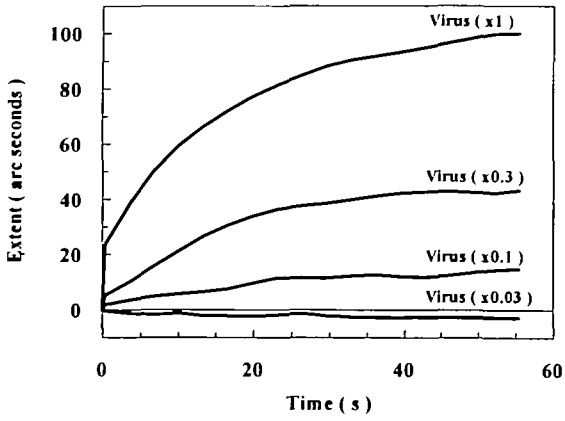

Fig. 1. **The binding of herpes simplex virus type 1 (HSV-1) to immobilized heparan sulfate, a cellular receptor for HSV-1, as monitored with an optical biosensor.** The viral solution employed was prepared from equal volumes of the stock viral solution and fresh MEM. Serially diluted viral solutions (200 μl) were poured into heparan sulfate-conjugated cuvettes and the binding reaction between the virus and the receptor was monitored as the extent of change (arc seconds). The Virus (×1), (×0.3), (×0.1), and (×0.03) curves are for viral solutions diluted with PBS to 1/2, 0.3/2, 0.1/2, and 0.03/2, respectively.

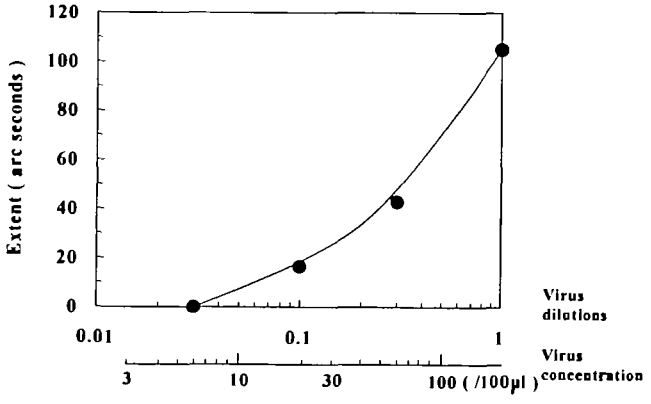

Fig. 2. **Evaluation of response rate constants between the virus and the receptor.** Responses (extent of change) are plotted against the concentrations of HSV-1 (viral dilutions and the viral concentration estimated by the ordinary method). The virus titer was calculated from the curve by the viral concentration at the extent 0.

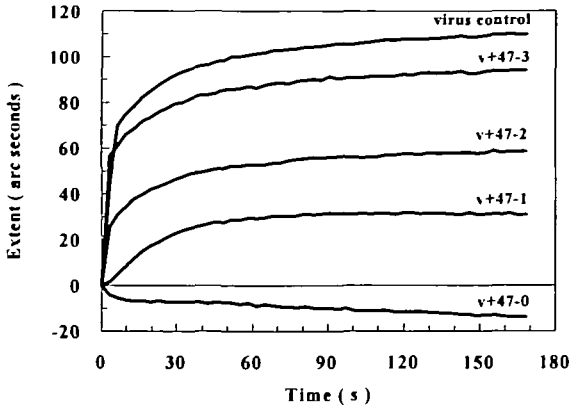

Fig. 3. **The binding of unneutralized HSV-1 with the serially diluted sulfonated human immunoglobulin preparation for intravenous injection (hIg) to immobilized heparan sulfate.** An equal volume of the stock viral solution was added to a serially diluted hIg solution for 30 min, and then 200 μl of the mixture were poured into a heparan sulfate-conjugated cuvettes. The binding reaction between the residual virus and the receptor was monitored with the optical sensor as the extent of change (arc seconds). The virus control curve is for 100 μl of viral solution with 100 μl of PBS, and the v+47-3, -2, -1, and -0 curves are for viral solutions mixed with 10^{-3}, 10^{-2}, 10^{-1}, and 10^{0} dilutions of the hIg solution (Lot. No. SSV 047), respectively.

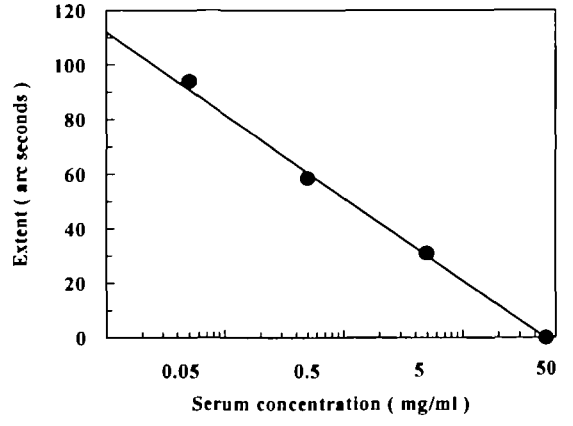

Fig. 4. **Evaluation of response blocking rate constants of hIg against the virus.** Response rates were plotted against the concentrations of hIg. The titer of the neutralizing antibody against HSV-1 was calculated from the curve by the concentrations of hIg at the extent of viral control.

The binding of HSV-1 to the immobilized HS calculated from the extent of viral binding subtracted the extent of background binding of an equally diluted MEM solution is shown in Fig. 1, and the rates of the viral binding reaction calculated are shown in Fig. 2. Binding of the virus to the receptor was found to depend on the viral concentration, and the viral concentration could be obtained from the binding curve as less than 1 unit/100 μl at the point of no binding (Fig. 2). The virus titer of the tested viral solution was estimated to be about 3×10^2 units/ml. This value was a little lower than that estimated by the ordinary biological method. The binding of the unneutralized virus by various concentrations of hIg calculated from the extent of binding of the unneutralized virus subtracted from the extent of binding of an equally diluted hIg solution is shown in Fig. 3, and the rates of neutralization rates of the virus by hIg are shown in Fig. 4. Binding of the virus to the receptor 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/100 μl at the extent of viral control (Fig. 4). The neutralizing antibody titer of the hIg solution was estimated to be 1×10^5 units/ml or 2×10^3 units/mg hIg. These values were almost the same as those estimated by the ordinary biological method. The negative binding observed at low viral concentrations should not be true. It might be caused by components in MEM as well as hIg bound to HS non-specifically. So this could be the reason for the underestimation of viral titers. These components might exhibit higher binding when viral components are not present.

The virus titer as well as the neutralizing antibody titer estimated by this method was almost the same as that estimated from the cytopathic effect on cultured Vero cells by the usual biological method. Since virus binding could be estimated reproducibly with this optical sensor system in a few minutes without any difficult cell culture, this interaction assay system could be a very quick and accurate method for determining virus and antibody titers.

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