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Binding of HIV gp41 with its Receptors Immobilized at Liquid/Solid Interface Studied by Surface Plasmon Resonance

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A home-made SPR biosensor was used to monitor the interactions of gp41 with its binding proteins in real time. The result indicates that the polystyrene biosensor film is useful for SPR detection. The binding constants indicate both P45 and P62 have a high affinity to the protein gp41, and suggest that P45 and P62 may be the cellular receptors on human T-, B-lymphocytes and monocytes.

INTRODUCTION

Biofunctionalized interfaces are of fundamental importance in the fields of biosensors and bioelectronics[1]. To characterize the structure and function of organized molecules assembled at interface has stimulated the development of novel techniques for detecting biochemical reactions at interface. The surface plasmon resonance (SPR) technique has become particularly useful for its high surface sensitivity and specificity and no need for a special label recently[2]. gp41 is one of glycoproteins on the membrane of human immunodeficiency virus type 1(HIV-1) which is involved in the infection of HIV-1 to the target cell. Both

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gp41-binding proteins (P45 and P62) can inhibit either the binding of gp41 to the target cell or its immunological regulation to the cell[3]. But which protein is the receptor of gp41 needs further investigation.

EXPERIMENTAL

A home-made SPR biosensor[4] based on the Kretschmann configuration was used to detect the interaction of gp41 and its binding protein (P45 and P62). A beam of light from a HL6711G laser (λ=670nm) passes through a prism (n_d=1.8) and is incident on the back surface of a gold film deposited onto a microscope slide. A photodiode is used to detect the reflected light. The sample cell (v=200μl) is fixed on a table which is rotated by a computer-controlled stepper motors. gp41 (rsgp41) was purchased from Biotest (Dreieich, Germany) and P45 and P62 were isolated Raji cell lysates.

RESULTS AND DISCUSSION

KINETIC ANALYSIS

A simple mechanism can be used to describe the reversible interaction between an immobilised ligand(B) and a running ligand(A) in our experiment:

$$A+B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB \quad (1)$$

So the following equations (2) and (3) can be deduced[5] according to this model to calculate the associate rate constant k_a and the dissociate rate constant k_d:

$$dR/dt = k_aCR_{max} - (k_aC + k_d)R \quad (2)$$

R is resonance unit used to indicate the shift in the surface plasmon resonance angle (where 1000 resonance units corresponds to 1° shift in the resonance

angle). C is the concentration of protein A in the bulk solution. As C = 0, the rate of dissociation of the formed complex AB can be described by

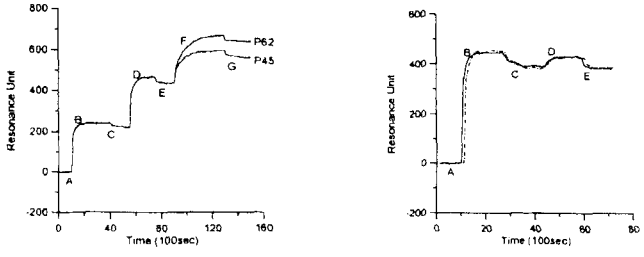
$$dR/dt = -k_d R \tag{3}$$

The association equilibrium constant

$$K_A = [AB]/[A][B] = k_a/k_d \tag{4}$$

POLYSTYRENE BIOSENSOR FILM IS A USEFUL TOOL FOR SPR DETECTION

In the present experiment, the polystyrene biosensor film is first used in SPR detection. The binding constant of the interaction between gp41 and polystyrene film was obtained according to Fig. 1(a)($3.72(\pm 0.02) \times 10^{10} M^{-1}$), and suggested that the interaction of gp41 with the polystyrene surface was very strong. Besides, the immobilizing time of the protein which is about 15 minutes at room temperature which is much shorter than that in ELISA(2-3 hours at 37°C and about 12 hours at 4°C). In the following sections, it can be found that the immobilized gp41 on the polystyrene film still maintains its activity as it can interact with p45 and p62, so the polystyrene biosensor film is suitable for SPR detection.



(a) (b)

FIGURE 1 (a) The interaction between gp41 and P45 or P62. Phase A-B, absorption of gp41; B-C, washing with PBS; C-D, adsorption of gelatin; D-E, washing with PBS; E-F, adsorption of P45 or P62; F-G, washing with PBS. (b)The interaction between gelatin and P45 or P62. Phase A-B, absorption of gelatin; B-C, washing with PBS; C-D, adsorption of P45 or P62; D-E, washing with PBS. (solid line stands for P45 and dot dash line stands for P62).

P45 AND P62 ARE POTENTIAL CELLULAR RECEPTOR OF GP41

Before adding the binding proteins, a high concentration gelatin solution was added in order to cover the exposed part of the polystyrene film not covered by gp41. As a control (Fig. 1(b)), it can be demonstrated that the binding proteins can't bind to the exposed gelatin. So the increase of resonance units after adding the binding proteins in Fig. 1a must be caused by the interaction of gp41 with P45(or, P62). Comparing the kinetic parameters of the two binding proteins (Table 1), it can be found that the association rate constants for both cases are nearly same, and the dissociation rate constant between gp41 and P45 is higher than that between gp41 and P62. Dr. Chen has demonstrated that P45 binds to gp41 only in one of its two active binding sites while P62 can bind to gp41 in the both two active sites. This difference may lead that the dissociation rate of P62-gp41 complex is slower than that of P45-gp41 complex. However, both proteins (P62 and P45) have a high affinity to gp41(Table 1). This result provides another evidence that both P45 and P62 are the potential cellular receptors of HIV-1 gp41.

TABLE 1 The kinetic parameters of the binding of gp41 to P45 and P62

binding protein	$K_A = k_a/k_d\ (M^{-1})$	$k_a\ (M^{-1}S^{-1})$	$k_d\ (S^{-1})$
P45	$2.28(\pm0.007)\times10^{8}$	$1.44(\pm0.004)\times10^{4}$	$6.31(\pm0.003)\times10^{-5}$
P62	$1.08(\pm0.001)\times10^{9}$	$1.52(\pm0.003)\times10^{4}$	$1.41(\pm0.001)\times10^{-5}$

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