Amplification of Detection Signals for Methyl Viologen by Using Supramolecular Formation of Antibody with Viologen Dimer in Surface Plasmon Resonance Sensor

Hiroyasu Yamaguchi and Akira Harada*

Department of Macromolecular Science, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043

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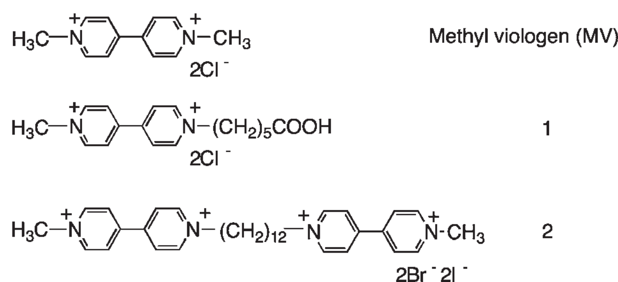
A new method to amplify detection signals for methyl viologen in surface plasmon resonance (SPR) sensor has been devised by using supramolecular formation of anti-viologen antibodies with divalent viologens.

Viologens are well-known functional molecules as a herbicide and an electron acceptor,[1] although they are harmful.[2,3] Anti-viologen antibodies[4–7] may be expected to be useful not only as a medicine for the detoxicant but also as a highly sensitive reagent of viologens or a functional material to control the electron transfer[8] from electron donors to viologens. In a common SPR biosensor technique using corresponding antibodies,[9] the detection of viologens at low concentrations is difficult owing to the low sensitivity (small response). To improve the sensitivity, it is important to detect methyl viologen as a large response signal[10] caused by the antibody bindings. A solution of this problem is thought to be the inhibition of the supramolecular formation[11,12] between the anti-viologen antibody and viologen dimer by methyl viologen. In this method, the amount of methyl viologen (Mw = 257) is expressed as the amount of the antibody (Mw = 150000) that can not form the supramolecules between the viologen dimer and the antibody. We report here the amplification of sensitivity in the viologen sensing system by supramolecular formation of antibodies for 4,4′-bipyridinium, 1-(carboxypentyl)-1′-methyl-dichloride **1** with viologen dimer **2**.



One of the antibodies elicited against hapten **1**, 10D5 (IgG_1) binds hapten **1** with the dissociation constant $K_d = 2.0 \times 10^{-7}$ M. Although antibody 10D5 does not bind dodecamethylenediamine dihydrochloride, it binds methyl viologen and viologen dimer **2**. The antibody 10D5 recognizes the bipyridinium moiety with high specificity. The dissociation constant between antibody 10D5 and viologen dimer **2** was determined by the addition of viologen dimer solutions to the antibody immobilized on the surface of the SPR sensor chip and was found to be $(1.5 \pm 0.5) \times 10^{-7}$ M. The signal intensity on the SPR biosensor increased on the addition of an aqueous solution of antibody 10D5 to the sensor chip on which the viologen dimer-antibody complex was pre-coated. The additional antibody is able to bind the viologen dimer-antibody complex. The intensity enhanced by the binding of antibody to the viologen dimer-antibody complex equals to the amount of the immobilized antibody before the addition of viologen dimer. The viologen dimer molecule is considered to act as a connector between antibodies.

We found that the additional binding of the antibody to the viologen dimer-antibody complex gives a remarkable increase of signal intensities. On the other hand, the addition of methyl viologen (viologen monomer) instead of viologen dimer is expected to block the antigen binding sites and to inhibit the additional antibody binding. A small amount of methyl viologen can be detected as a decrease of signal enhancement due to the inhibition of the complex formation between viologen dimer and antibody by methyl viologen, compared with the signal intensity of complete supramolecular formation between the antibody and viologen dimer. The amount of bound antibody decreased with increase of the concentration of methyl viologen. In the presence of large excess of viologen (11 μM) compared with the concentration of antibody (0.9 μM as a whole antibody, the concentration of the antigen binding site is 1.8 μM), the increase of signal intensity was depressed. However, the increase of signal intensities due to the binding of the additional antibody in the presence of 0.2 and 1.1 μM methyl viologen was in small difference. Methyl viologen up to the equimolar amount of antibody added to the flow cell does not inhibit the complex formation between the antibodies and viologen dimer (Scheme 1 (i)).

To enhance the difference in the signal intensities in the presence of a small amount of methyl viologen, a solution of the antibody-viologen dimer complex was added to the previous state shown in Scheme 1 (i). Figure 1 shows the differences in the response signal intensities ($I_0 - I$) between the complete supramolecular system in the absence of methyl viologen (I_0) and that in the presence of various concentrations of methyl viologen (I) at each step. The differences in signal intensities due to the binding of the additional antibody (0.9 μM) in the presence of methyl viologen ranging the concentration from 0.2 to 1.1 μM was slight. However, further addition of the complex of viologen dimer with the antibody caused a clear difference in the response signal intensities in the same concentration range of methyl viologen. In the absence of methyl viologen, the increase of signal intensity (I_0) was 1250 resonance units (RU). On the other hand, the signal intensity (I) was 550 RU in the presence of 1.1 μM methyl viologen. The sensitivity in this system (700 RU) is 140-fold larger than that in the simple addition of methyl viologen to the antibody immobilized to the surface of the sensor chip (5 RU). Amplification of methyl viologen sensing processes is realized by (i) the inhibition of complex formation between the antibody and viologen dimer-antibody complex and (ii) signal enhancement due to the supramolecular formation of the antibody and viologen

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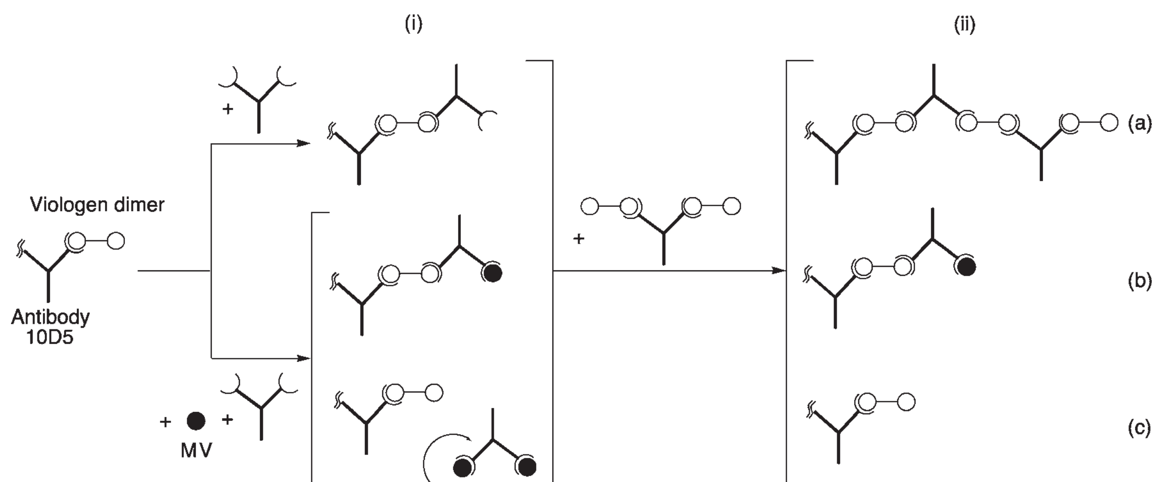

Scheme 1. Amplification of methyl viologen (MV) sensing processes. The inhibition of complex formation between the antibody and viologen dimer-antibody complex by MV (i) and the supramolecular formation of the antibody and viologen dimer (ii). The supramolecular formation between antibody 10D5 and viologen dimer 2 without MV (a), and that in the presence of MV (b) and (c). [MV] $<$ [antibody combining site] (b) and [MV] $\gg$ [antibody combining site] (c).

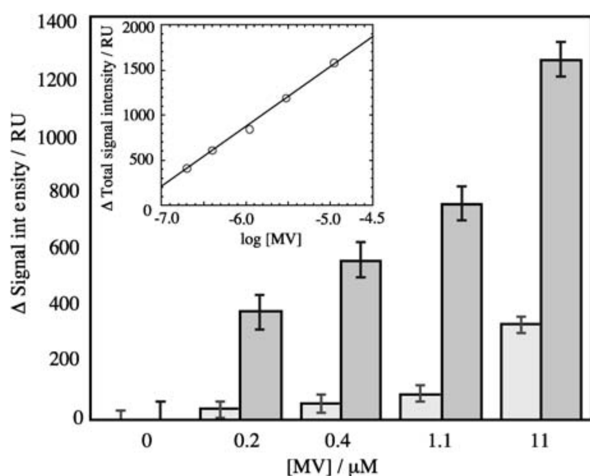

Figure 1. The differences in the response signal intensities between the complete supramolecular system in the absence of methyl viologen and that in the presence of various concentrations of methyl viologen at each step. Changes in the signal intensities by the addition of an aqueous solution of antibody 10D5 and methyl viologen (left side, Scheme 1 (i)) and those by the addition of the solution including the antibody-viologen dimer complex (right side, Scheme 1 (ii)). [Antibody] $= 0.9\,\mu$M and [viologen dimer 2] $= 1.8\,\mu$M. Phosphate borate buffer (0.1 M, pH 9.0) was used. The inserted graph shows the relation between the concentration of methyl viologen and the Δ total signal intensity.

dimer (Scheme 1). The sensor chips can be regenerated with 0.5% SDS buffer and recycled more than ten times with a good reproducibility.

In conclusion, the complex formation between one of the monoclonal antibodies for the viologen derivative **1**, 10D5, and viologen dimer was studied by a biosensor technique. The enhancement of SPR signal intensity was observed by the addition of the antibody to the viologen dimer-antibody complex indicating the formation of linear supramolecules between the antibody and viologen dimer. An amplification method of the detection signals for methyl viologen has been devised by using the signal enhancement in the supramolecular assembly of the antibody and divalent antigen. The sensitivity in this system was found to be larger two orders of magnitude over that in the simple addition of methyl viologen to the antibody immobilized to the surface of the sensor chip. This method can be applied for many compounds (e.g. porphyrins) to be detected with high sensitivity and specificity by using corresponding antibodies.

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