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Cooperative Binding of Porphyrin by Anti-Porphyrin Antibodies

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One of the monoclonal antibodies specific for meso-tetrakis(4-carboxyphenyl)porphine(TCPP) causes a large shift of the Soret band of TCPP to a longer wavelength and large induced Cotton effects(ICD) on TCPP. Quantitative analyses of the binding of TCPP to the antibody by ICD show that not only one-to-one binding but two-to-one(binding site:TCPP) binding occurs in excess of the antibody over TCPP.

Antibodies are unique in their ability to recognize a diversity of substrates.[1] Recently, the diversity of the immune system can be well utilized to generate catalysts with specific binding properties, catalytic antibodies.[2] Most of these catalytic antibodies have been produced by immunizing a mouse with "transition-state analog" compounds.[3] Another approach is to use a cofactor as an active site such as enzymes. One of the most important cofactors is a family of porphyrins, which function as redox reaction catalysts. Anti-metalloporphyrin antibodies have been prepared.[4] Previously we paid attention to an application of the immune system to a preparation of functional polymers, antibodies, and reported the preparation of anti-non-metalated porphyrin antibodies.[5] In this study, five monoclonal antibodies specific for meso-tetrakis(4-carboxyphenyl)porphine(TCPP) were

obtained and one of the monoclonal antibodies(03-1) for TCPP showed a unique binding behavior. In this letter, we report the cooperative binding of TCPP by the antiporphyrin antibody.

The UV-visible absorption spectra of TCPP in the presence of monoclonal antibodies show that four of the five antibodies did not cause any shift of the Soret band of TCPP at 420 nm, although the spectra showed some hypochromicity at the Soret band. In contrast, the absorption spectrum of TCPP in the presence of one of the five antibodies, 03-1, showed a shift of the Soret band of TCPP to a longer wavelength about 10 nm, indicating that the electronic structure of TCPP was modified by binding to the antibody. The circular dichroism spectra of TCPP in the presence of the antibody(03-1) show large induced Cotton effects, although other four monoclonal antibodies show no induced Cotton effects at all. When the concentration of the monoclonal

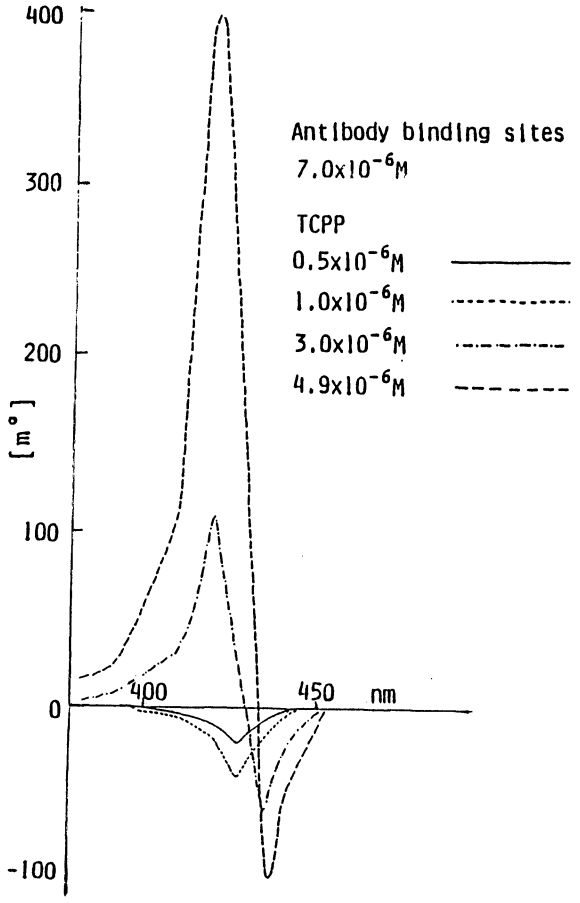

Fig. 1. CD spectra of TCPP in the presence of antibody 03-1.

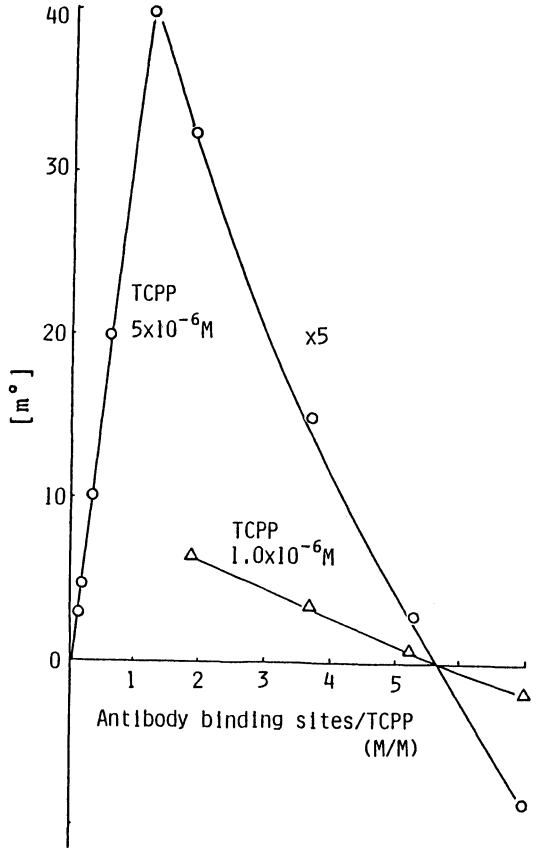

Fig. 2. Effects of molar ratio(antibody binding site:TCPP) on CD spectra.

antibody is fixed (7.0×10^{-6} M) and the concentration of TCPP was raised from 0.5×10^{-6} M to 4.9×10^{-6} M, negative Cotton effects have appeared first and then they changed to positive Cotton effects as the concentrations of TCPP increased.(Fig. 1) These results indicate that there are at least two binding species, one at low concentrations of TCPP which gives minus induced Cotton effects, and the other at high concentrations which gives plus induced Cotton effects. We consider three models to explain this phenomenon. (1) The first binding of TCPP to one of the binding sites of the antibody affects the second binding of TCPP to the same antibody by means of conformational changes or other effects. (2) There are two binding sites, a strong binding site which gives minus Cotton effects, and a weak binding site which causes positive Cotton effects. (3) One-to-one binding, giving plus Cotton effects and two-to-one(binding site:TCPP) binding, which gives minus Cotton effects. When the Fab fragment was used instead of the whole antibody(IgG), similar spectroscopic changes to those by IgG were observed both in the absorption and CD spectra. Therefore the model (1) cannot explain the spectroscopic behavior. When the CD spectrum of TCPP in the presence of equimolar amount of the antibody, large plus Cotton effects were observed. If the model (2) is correct, minus Cotton effects should be observed. So the model (2) does not explain the behavior. When the concentration of TCPP was fixed at 10^{-6} M or 5×10^{-6} M and the concentration of antibody increased, plus Cotton effects increased as the concentrations of antibody increased and the plots show the maximum at about one-to-one(Fig. 2). When the mole ratio exceeds one-to-one, the plus Cotton effect decreases rapidly, and finally minus Cotton effects

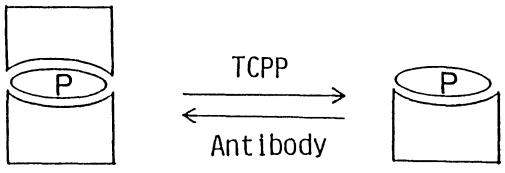

Fig. 3. Two-to-one and one-to-one complex.

appeared. Therefore we propose one-to-one complex at low concentrations and two-to-one complex at high concentrations. Figure 3 shows a proposed structure of the one-to-one complex and the two-to-one complex. TCPP molecules do not aggregate in this concentration range.[6] Zinc tetrakis(4-sulphonatephenyl)porphyrin and carboxyphenyltriphenylporphine were also found to bind to the antibody to give similar induced Cotton effects. These results indicate that antibodies recognize the plane of TCPP which has a plane symmetry instead of carboxyphenyl group. We are now studying a detailed structure of the complex and catalytic behavior of the complexes. Such cooperative binding should lead to the higher order regulation of catalytic reactions.

References

1) G. Kohler and C. Milstein, Nature, 256, 495(1975).

2) P. G. Schultz, R. A. Lerner, and S. J. Benkovic, Chem. Eng. News, 68, 26(1990).

3) P. G. Schultz, Acc. Chem. Res., 22, 287(1989).

4) A. W. Schwabacher, M. I. Weinhouse, M-T. M. Auditor, and R. A. Lerner, J. Am. Chem. Soc., 111, 2344(1989).

5) A. Harada, K. Okamoto, M. Kamachi, T. Honda, and T. Miwatani, Chem. Lett., 1990, 919.

6) R. F. Pasternack, P. R. Huber, P. Boyd, G. Engasser, L. Francesconi, E. Gibbs, P. Fasella, G. C. Venturo, and L. de C. Hinds, J. Am. Chem. Soc., 94, 4511(1972).

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