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Molecular recognition by monoclonal antibodies of porphyrins

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Monoclonal antibodies for meso-tetrakis(4-carboxyphenyl)porphine (TCPP) and carboxyphenyltriphenylporphine (CATPP) were prepared by immunizing Balb/c mice with TCPP and CATPP keyhold limpet haemocyanin conjugates and fusing the spleen cells with myeloma cells using poly(ethylene glycol). The antibodies bound TCPP and CATPP strongly with dissociation constants of 10^{-6}–10^{-8} M. One of the antibodies caused a shift of the Soret band and Q band of TCPP to a longer wavelength, and caused large induced Cotton effects (ICD) on TCPP and CATPP. Quantitative analyses of the binding of TCPP to the antibody by ICD showed that not only 1:1 binding but higher orders of binding, such as 2:1 (binding site:TCPP), took place with an excess of the antibody over TCPP.

INTRODUCTION

Recently, much attention has been focused on molecular recognition of low molecular weight compounds and ionic species in the field of biomimetic and supramolecular chemistry.[1] Crown ethers, cryptands and cyclophanes have been extensively used as host molecules for such small molecules. However, their guests have been limited to the small and simple ions or molecules. Host molecules that can recognize larger and more complicated molecules are required. Recently, with the advent of hybridoma technology it has become possible to generate chemically homogeneous antibodies, monoclonal antibodies.[2] Antibodies are unique in their ability to recognize a diversity of substrates. More recently, the diversity of the immune system has been well utilized to generate catalysts with specific binding properties, 'catalytic antibodies'.[3] Most of these catalytic antibodies have been produced by immunizing mice with 'transition-state analogue' compounds. Another approach is to use a cofactor as an active site, such as enzymes.[4] One of the most important cofactors is a family of porphyrins, which functions act as oxygen carriers, cause redox reactions, photoinduced electron transfer, and so on. We consider monoclonal antibodies as novel tailor-made hosts for artificial guests. We now report on the preparation, characterization and properties of monoclonal antibodies against meso-tetrakis(4-carboxyphenyl)porphine (TCPP) and carboxyphenyltriphenylporphine (CATPP).

PREPARATION OF MONOCLONAL ANTIBODIES

TCPP and CATPP (Fig 1) were covalently attached to the carrier proteins keyhole limpet hemocyanin (KLH) and bovine serum albumin using water-soluble carbodiimide, 1-(3-dimethylamino)propyl-3-ethylcarbodiimide, or carbonyldiimidazol. The conjugates were purified by column chromatography on Sephadex G-50. The number of porphyrins on a carrier protein was determined by the absorbance at the Soret bands. Balb/c mice were immunized with the KLH conjugate emulsified in complete Freund's adjuvant. A fusion was carried out using Sp2/0 myeloma as the fusion partner and poly(ethylene glycol) as a fusion reagent.[2] Hybridoma was screened, cloned, and propagated in ascites as described.[2] Seven monoclonal antibodies specific for TCPP were obtained. Four antibodies were chosen for further experiments. Monoclonal antibodies

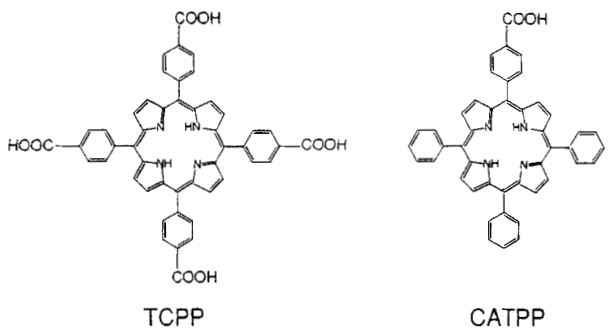

Figure 1 Structures of TCPP and CATPP.

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were purified from ascites fluid by ammonium sulphate precipitation, dialysis against phosphate buffer, and column chromatography on DEAE–Sephacel and protein A column. Antibody purity was determined by 10–15% SDS polyacrylamide gel electrophoresis with Coomasie blue staining.

PROPERTIES OF MONOCLONAL ANTIBODIES

The binding of TCPP by the monoclonal antibodies was studied by enzyme-linked immunosorbent assay (ELISA).[5] All four antibodies bound TCPP strongly. The dissociation constants were found to be approximately 1.0×10^{-6} to 2.5×10^{-7} M for TCPP (Table 1). The antibodies also combined TCPP metal complexes, such as that of Zn, strongly with similar dissociation constants to those for unmetallysed porphyrin. The UV absorption spectra of TCPP in the presence of the antibody showed hypochromicity at the Soret band at 422 nm, suggesting an interaction between TCPP and the monoclonal antibody. The emission spectrum of the monoclonal antibody could be quenched by the addition of TCPP and TCPPZn, indicating an interaction between TCPP and the aromatic residues of amino acids, especially tryptophan, and/or conformational changes by forming inclusion complexes. (Fig 2).[6]

The UV-visible absorption spectra of TCPP in the presence of monoclonal antibodies showed that three of the four 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 four antibodies, 03-1, showed a shift of the Soret bands of TCPP and TCPPZn to a longer wavelength, by about 10 nm, indicating that the electronic structures of TCPP and TCPPZn were modified by binding to the antibody (Fig 3). The circular dichroism (CD) spectra of TCPP in the presence of antibody 03-1 showed large induced Cotton effects (ICDs), although the other three monoclonal antibodies showed no ICD at all. When the concentration of the monoclonal antibody was fixed (7.0×10^{-6} M) and the concentration of TCPP was raised from 0.5×10^{-6} to 4.9×10^{-6} M, negative ICDs appeared first and then they changed to positive ICDs as the concentration of TCPP increased (Fig 4). These results indicate that there are at least two binding species, one at low concentrations of TCPP which gives negative ICDs, and the other at

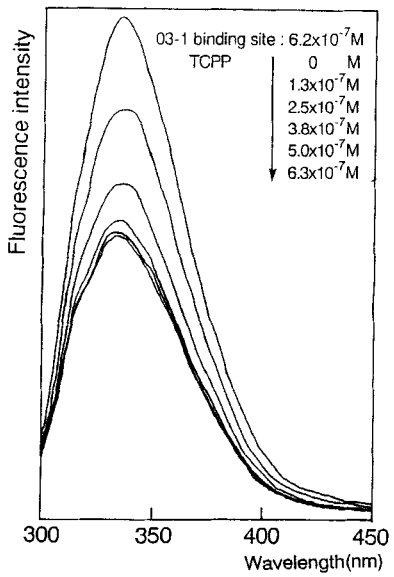

Figure 2 Fluorescence spectra of antibody 03-1 in the presence of TCPP.

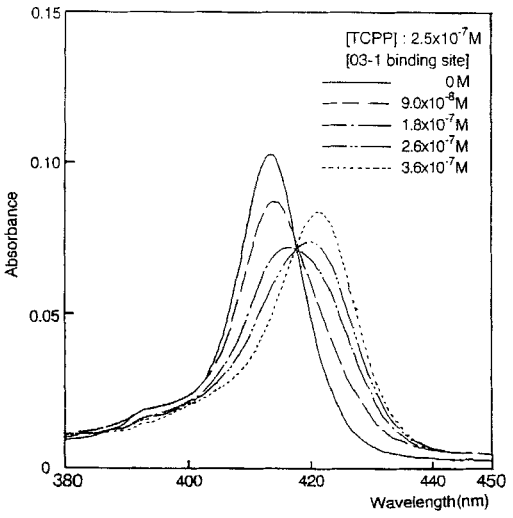

Figure 3 UV-visible spectra of TCPP in the presence of antibody 03-1.

Table 1 Dissociation constants of porphyrins and monoclonal antibodies[a]

	Dissociation constants (m)			
Monoclonal antibody	*13-1*	*10-2*	*12-1*	*03-1*
TCPP	1.4×10^{-6}	2.5×10^{-7}	7.0×10^{-7}	1.0×10^{-6}
TCPPZn	1.2×10^{-6}	2.0×10^{-7}	7.0×10^{-7}	1.1×10^{-6}

[a] Estimated by ELISA

high concentrations which gives positive ICDs. We considered three models to explain this phenomenon. (1) The first binding of TCPP to one of the binding sites of the antibody effects 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 negative ICDs, and a weak binding site which causes positive ICDs. (3) 1:1 binding (binding site:TCPP), giving positive ICDs and 2:1 binding, which gives negative ICDs. When the Fab fragment was used instead of the whole antibody (IgG), similar spectroscopic changes to those for IgG were observed both in the absorption and CD spectra. Therefore model (1) does not explain the spectroscopic behaviour. When the CD spectrum of TCPP was run in the presence of an equimolar amount of the antibody, large positive ICDs were observed. If model (2) is correct, negative ICDs should be observed. So model (2) does not explain the behaviour either. When the concentration of TCPP was fixed at 10^{-6} or 5×10^{-6} M and the concentration of antibody was increased, positive ICDs increased as the concentration of antibody increased, and the plots show the maximum at approximately 1:1 (Fig 5). When the mole ratio exceeded 1:1, the positive ICD decreased rapidly, and finally negative ICDs appeared. Therefore we propose a 1:1 complex at low concentrations and a 2:1 complex at high concentrations. Figure 6 shows a proposed structure of the 1:1 and the 2:1 complexes. TCPP molecules do not aggregate at this concentration range. Zinc tetrakis(4-sulphonate phenyl)porphyrin and CATPP were also found to bind to the antibody to give similar ICDs. These results indicate that

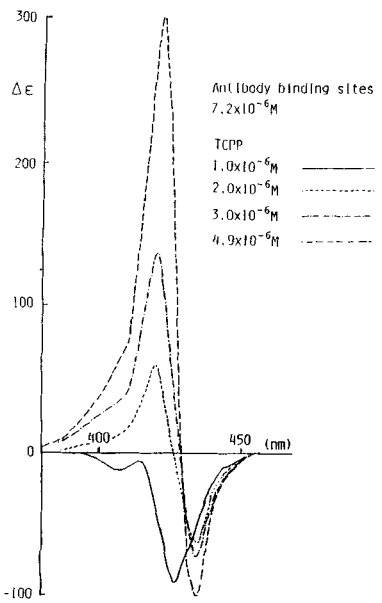

Figure 4 CD spectra of TCPP in the presence of antibody 03-1.

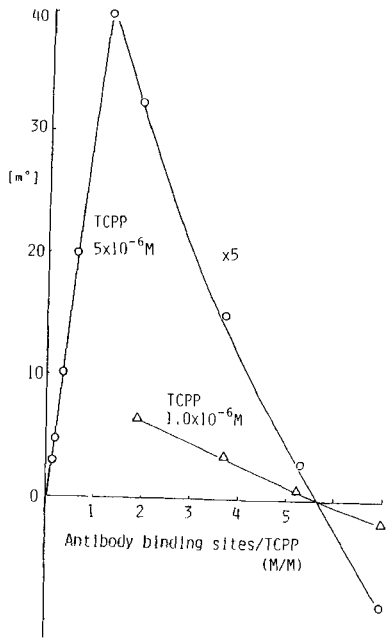

Figure 5 Effects of molar ratio of antibody binding site concentration/TCPP concentration on CD spectra.

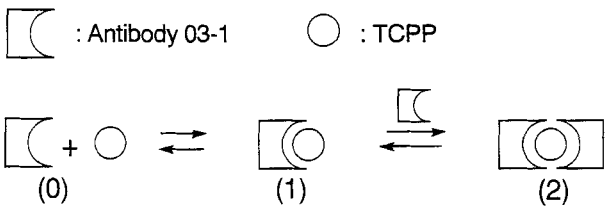

Figure 6 Proposed structures of the complexes of TCPP and antibody 03-1.

antibodies recognize the plane of TCPP (which has a plane symmetry) instead of the carboxyphenyl group. We are now studying a detailed structure of the complex and various properties of the complexes. Such binding should lead to the higher order regulation of catalytic reactions and photoinduced properties.[7]

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