

Monoclonal Antibodies as Tailor-Made Hosts for Porphyrins

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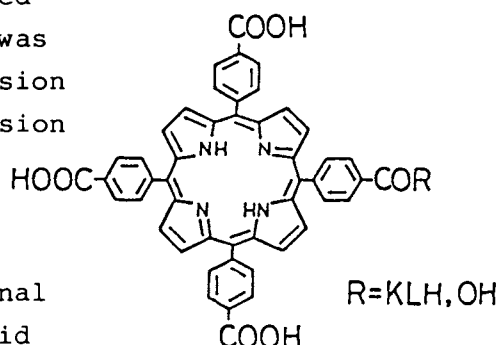
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Monoclonal antibodies against tetracarboxyphenyl-porphyrin(TCPP) were prepared by immunizing Balb/c mice with TCPP bound keyhole limpet hemocyanin covalently and fusing the spleen cells with myeloma cells by using poly(ethylene glycol). The antibodies bind TCPP strongly with the dissociation constants of 10^{-6} - 2.5×10^{-7} M.

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 which 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⁾ More recently, enzyme-like abilities of antibodies have received much attention as "catalytic antibodies".³⁾ We consider monoclonal antibodies as novel tailor-made hosts for artificial guests. We now report the preparation, characterization, and properties of monoclonal antibodies against tetracarboxyphenylporphyrins(TCPP). During our studies, Schwabacher et al. reported the production of monoclonal antibodies against metalloporphyrins.⁴⁾

TCPP was covalently attached to the carrier proteins keyhole limpet hemocyanin(KLH) and bovine serum albumin(BSA) by using water-soluble carbodiimide, 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide, followed by column chromatography on Sephadex G-50. Epitope densities were determined based on the absorbance at 420 nm. 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 and cloned, and propagated in ascites as described. Four monoclonal antibodies specific for TCPP were obtained. Monoclonal antibodies were purified from ascites fluid by ammonium sulfate precipitation, dialysis against phosphate buffer, and column chromatography on DEAE-Sephacel. Antibody purity was determined by 10-15% SDS polyacrylamide gel electrophoresis with Coomassie blue staining.



The binding of TCPP by the monoclonal antibodies was studied by enzyme-linked immunosorbent assay (ELISA).⁵⁾ All four antibodies bind TCPP strongly. The dissociation constants were found to be about 1.0×10^{-6} - 2.5×10^{-7} M for TCPP. (Table 1) The antibodies also combine TCPP metal complex, such as Zn, strongly with the similar dissociation constants to those for unmetallized porphyrin. The UV absorption spectra of TCPP in the presence of the antibody show hypochromicity at the Soret band at 422 nm, suggesting an interaction between TCPP and the monoclonal antibody. The emission spectrum of the monoclonal antibody can be quenched by the addition of TCPP, indicating the interaction between TCPP and aromatic residues of aminoacids, especially tryptophan and/or conformational changes by forming the inclusion complexes.

Table 1. Binding of porphyrins to monoclonal antibodies

	13-1	10-2	12-1	03-1
TCPP	1.4×10^{-6} M	0.25×10^{-6} M	0.7×10^{-6} M	1.0×10^{-6} M
TCPP-Zn	1.2×10^{-6} M	0.2×10^{-6} M	0.7×10^{-6} M	1.1×10^{-6} M

References

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