CHEMISTRY LETTERS, pp. 51-52, 1994.

Preparation of Anti-Pyrenyl Antibody and Its Chiral Recognition against a Nonnatural Amino Acid Carrying Pyrenyl Group

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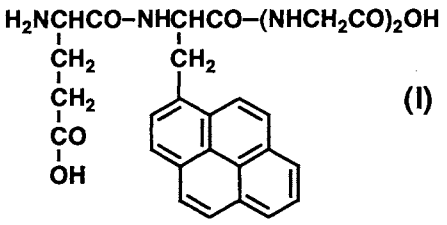
Monoclonal antibody was raised against a tetrapeptide containing L-pyrenylalanine. The antibody bound the tetrapeptide as well as L-pyrenylalanine methyl ester, indicating that it recognizes the pyrenyl group. The antibody, however, did not bind D-pyrenylalanine methyl ester. This finding suggests a potentiality of monoclonal antibodies for chiral separations.

Monoclonal antibodies have been widely used as tools in organic chemistry.[1] We have reported previously that monoclonal antibodies against an amino acid, L-p-phenylazophenylalanine showed on/off switching of the antigen-antibody reaction associated with the photoisomerization of azobenzene group.[2] In this paper we report preparation and chiral recognition of a monoclonal antibody against L-1-pyrenylalanine.



(I)

A monoclonal antibody was raised against a tetrapeptide (I) containing L-1-pyrenylalanine (pyrAla).[3] The tetrapeptide of fully protected form, Boc-Glu(O^{t}Bu)-pyrAla-Gly2-OEt, was synthesized by liquid phase method. After the C-terminal was converted to N-hydroxysuccinimide ester, the protecting groups were removed. The free peptide with active ester was treated with bovine serum albumin (BSA) and the resulting peptide-BSA conjugate carrying 18 peptides on each BSA molecule was used to immunize mice. After conventional procedures,[4] an IgG-type monoclonal antibody (Py-Ab) was obtained.

Fluorescence spectra were measured for mixtures of the antibody (1.0×10^{-6} M) and various concentrations of the free tetrapeptide I in phosphate buffer (17.5 mM). Fluorescence of the antibody that is originated from tryptophyl groups (343 nm) was quenched by the addition of the tetrapeptide, due to an energy transfer from the excited tryptophyl group to the bound pyrenyl group. The fluorescence intensity of the antibody at 343 nm is plotted against the peptide concentration in Fig.1. The fluorescence titration curve showed a turning

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point at the equimolar ratio of the peptide and the binding sites of the antibody (twice the antibody concentration), indicating a specific binding of the peptide to the antibody. Numerical analysis of the plot[5] gave an association constant $K=7\pm2\times10^6\ M^{-1}$.

Similar fluorescence quenching was observed for L-1-pyrenylalanine methyl ester (L-Py-OMe), although no clear turning point was seen at the equimolar point. The titration curve gave $K=4\pm2\times10^6\ M^{-1}$. The association constant of the amino acid ester is on the same order as that of the peptide hapten. Similar titration curve was also obtained when 3-(1-pyrenyl)butyric acid was used as a quencher. These results indicate that the antibody recognizes mainly the pyrenyl group.

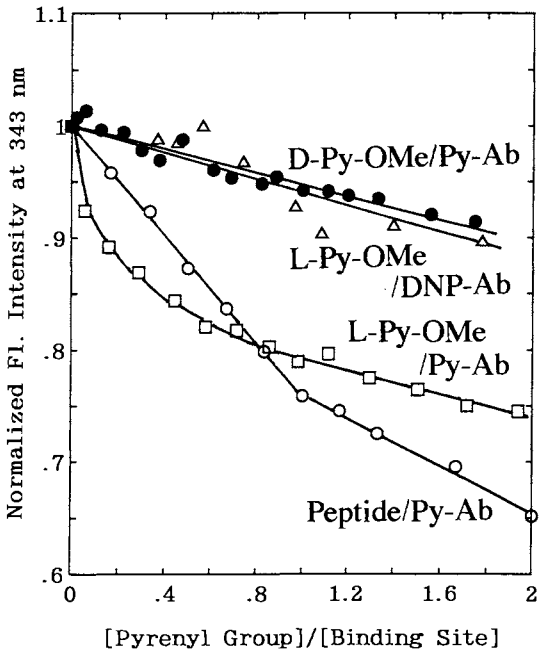

Fig.1. Fluorescence titration curves for various hapten/antibody mixtures.

When D-1-pyrenylalanine methyl ester (D-Py-OMe) was used as an hapten, the fluorescence quenching was less marked thanthe L-isomer. The small quenching by the D-isomer may be due to nonspecific binding of the quencher to the antibody, since similar quenching curve was observed when L-1-pyrenylalanine methyl ester was mixed with an antibody raised against dinitrophenyl group (DNP-Ab). The different affinities against L- and D-pyrenylalanine methyl ester indicate that the antibody recognizes not only the pyrenyl group but also the configuration around the α-carbon atom.

The chiral recognition of antibody provides a basis for a new technique of chiral separation of nonnatural amino acids and other enantiomeric substances. An important advantage of using antibody is that a monoclonal antibody against one of the enantiomers can be obtained from a racemic mixture, since only one hybridoma species is selected in the screening process.

The authors wish to thank Mr. Masataka Harada of Takeda Chemical Industries and Professor Mamoru Nakanishi of Nagoya City University for the cooperation and guidance in the preparation of antibody.

References

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(Received September 29, 1993)