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Photoreversible antigen–antibody reactions

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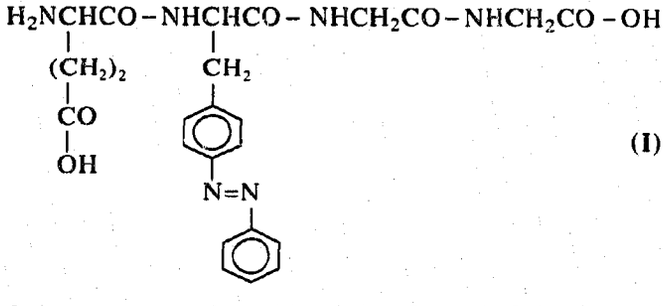
A monoclonal antibody (ZlH0l) for an oligopeptide carrying an azobenzene group, was prepared under conditions where the azobenzene group is in the *trans* form. The antibody bound the hapten peptide effectively when the hapten peptide is in the *trans* form ($K = 5 \times 10^7$ M^{-1}), but the antibody released the hapten under irradiation with UV light where the hapten is in the *cis* form. The antibody bound the hapten again, when the hapten reverted to the *trans* form after irradiation with visible light.

Monoclonal antibody; Photochromic hapten; Azobenzene; Photocontrol

1. INTRODUCTION

One of the major characteristics of antigen–antibody reactions is their extremely precise molecular recognition that can distinguish even a very small change in the antigen structure. In recent years, this precise molecular recognizability is finding applications in biochemical/medical fields as well as in the field of chemistry. However, one of the drawbacks in the chemical application of antigen–antibody reactions is that the binding equilibrium is sometimes strongly biased to the uptake of antigens and no mild conditions are known for the release of the bound antigen. If the uptake and release of antigen can be controlled by some external factors, such as photoirradiation, the antigen–antibody reactions will find much wider application. In this paper, we report a first successful attempt to prepare monoclonal antibodies for a hapten peptide that carries a photochromic group (azobenzene) (Glu-azoAla-Gly_2; I). The azobenzene group is known to undergo a reversible photoisomerization between *trans* and *cis* forms under irradiation with *uv* and visible light. The photoreversible change in molecular structure has been used to control some biochemical reactions [1].



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2. MATERIALS

A fully protected tetrapeptide (Boc-Glu(O^{t}Bu)-azoAla-Gly_2-OEt) was synthesized by a conventional liquid-phase method and checked for purity by thin-layer chromatography, proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, and elemental analysis. L-*p*-Phenylazophenylalanine (azoAla) was synthesized according to Goodman and Kossoy [2]. The *N*-hydroxysuccinimide ester derivative of the deprotected tetrapeptide was reacted with BSA at pH 7. The average number of the peptides linked to each BSA molecule was evaluated as 20. The peptide-BSA conjugate together with Freund's complete adjuvant was used to immunize BALB/c mice. Among the monoclonal antibodies obtained by conventional techniques [3], the IgG_1 (Z1HO1) monoclonal antibody which showed the highest affinity to the hapten peptide will be described in detail.

3. RESULTS AND DISCUSSION

Fluorescence of the antibody (Z1HO1) was effectively quenched by the addition of a small amount of the tetrapeptide in the *trans* form (Fig. 1). The fluorescence quenching was caused by an energy transfer from tryptophan fluorophores in the antibody to the bound azobenzene group. The fluorescence titration curve was numerically analyzed [4] to give an association constant $K(trans) = 5 \times 10^7$ l/mol (25°C). When the same experiment was carried out after irradiating the peptide under UV light (centered at 360 nm), the quenching was significantly suppressed as shown by filled circles in Fig. 1. The suppression of the fluorescence quenching suggests that the antibody cannot bind the peptide in the *cis* form, due to the structural change. ^{1}H NMR analysis indicated that, under the present condition of photoirradiation, about 82% of the hapten peptide is in the *cis* form and the rest of the peptide is in the *trans* form. Therefore, the quenching by the '*cis*' peptide must include the contribution of the quenching by the remaining trans peptide. The fluorescence titration curve extrapolated to 100% *cis* peptide is shown by open triangles in Fig. 1. It seems that the peptide of

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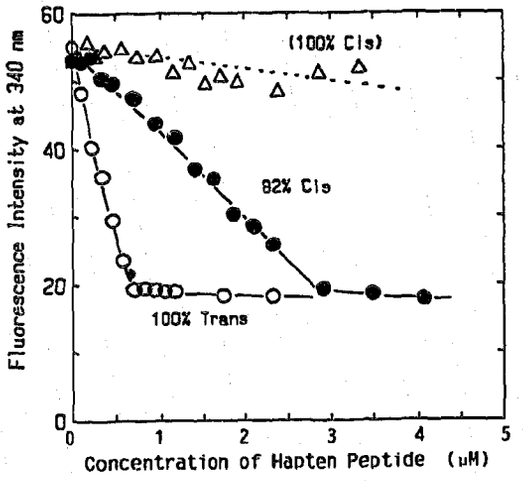

Fig. 1. Fluorescence titration curves of the monoclonal antibody Z1H01 with the hapten peptide 1. Concentration of the antibody = 2.6×10^{-7} M in phosphate buffer (pH = 7.0). The hapten solution was stored in the dark (○, 100% *trans*) or under UV irradiation (●, 82% *cis*). The curve for 100% cis hapten (△) was calculated from the two experimental curves by extrapolation. Excitation wavelength = 280 nm, at 25°C.

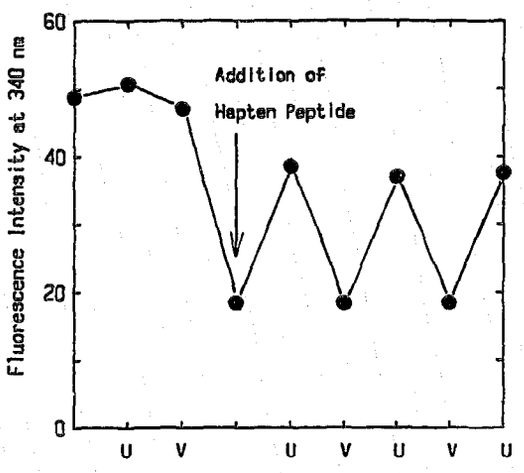

Fig. 2. Fluorescence intensity of the mixture of antibody (Z1H01, 2.6×10^{-7} M) and hapten peptide (7.0×10^{-7} M) in phosphate buffer at room temperature. The solution was alternately irradiated by UV light (U, 360 nm) and visible light (V, 430 nm) and the fluorescence intensity at 340 nm was monitored.

pure *cis* form does not quench the antibody fluorescence at all. The quenching experiment was also carried out using azoAla methyl ester instead of the tetrapeptide as a quencher. The quenching occurred almost equally by the peptide and by the amino acid ester in the *trans* form ($K(trans)=4\times10^7$) and the association constant with the *cis* amino acid ester was very small. This indicates that the monoclonal antibody (Z1H01) specifically recognizes the *trans* azobenzene moiety in the tetrapeptide.

In order to examine the reversibility of the photocontrolled binding, the mixture of hapten and antibody was irradiated alternately with UV light (U: 360 nm, *trans* to *cis*) and with visible light (V: >430 nm, *cis* to *trans*) (Fig. 2). The irradiation did not affect the fluorescence intensity in the absence of the hapten. In the presence of the hapten peptide, the irradiation induced alternate increase and decrease of the fluorescence intensity, suggesting the reversible release and uptake of the hapten.

The photocontrolled uptake and release of the hapten peptide was more directly followed with HPLC analysis. Chromatograms of a mixture of the antibody and the hapten before and after photoirradiation with UV light are shown in Fig. 3a and b. After the photoisomerization to the *cis* form, the amount of free hapten increased. Since the absorption coefficient of the *cis* form at the monitoring wavelength (300 nm) is about 40% of the *trans* form, the concentration of free hapten is therefore about 6 times higher than that before UV irradiation. When the mixture resulting from UV irradiation was irradiated with visible light, the amount of free hapten decreased again (Fig. 3c), in-

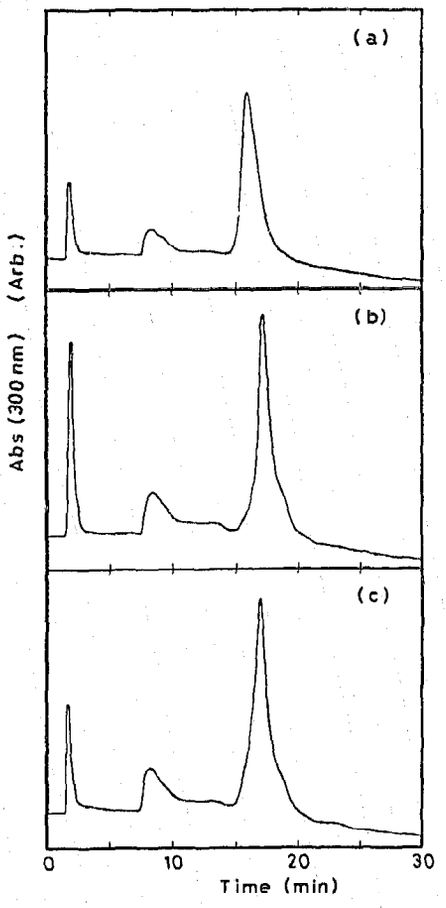

Fig. 3. HPLC charts of a mixture of Z1H01 and the hapten peptide in phosphate solution of increasing concentration from 0.01 M to 0.3 M in 30 min. Column: Shimadzu Shimpack HAC, 1 ml/min. The component eluted after 2 min is a free hapten peptide and that which appeared after 17 min is the antibody. (a) Before irradiation, (b) after irradiation at 330 nm, (c) after irradiation of the latter solution at >430 nm.

dicating photoreversible uptake and release of the hapten.

To conclude, the monoclonal antibody (Z1H01) can bind and release the hapten peptide as well as the amino acid ester reversibly under alternating photoirradiation with UV and visible light. The utilization of this biological photoswitching system in chemical and biological fields will be the subject of future studies.

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