Association of an Antibody-Conjugated Enzyme with Synthetic Polymers Dissolved in Organic Solvents

M. Kumakura

Department of Bioscience, The Nishi-Tokyo University, Uenohara, Kitatsuru, Yamanashi 409-01, Japan

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An antibody of a tumor marker (α-fetoprotein) conjugated with peroxidase was dispersed in organic solvents including synthetic polymers, and the interaction of the antibody with the polymers was investigated. It was found that the antibody-conjugated enzyme is associated with the polymers dissolved in hydrophobic organic solvents. The association correlated with the polymerization degree, concentration, and nature of the polymers.

Introduction

The adsorption of plasma proteins such as an antibody onto solid surfaces is of great importance in the development of artificial internal organs. Also, knowledge of interfacial interaction of macromolecules with plasma proteins is an important criteria to establish polymer biocompatibility. Therefore, many workers have investigated the adsorption of plasma proteins onto various solid surfaces (Andrade *et al.,* 1979). These investigations have been carried out relating to interaction between plasma proteins and solid surfaces in water. However, the behavior of plasma proteins in organic solvents has not been investigated.

Organic synthesis reactions with enzymes such as lipase, protease in organic solvents have been studied by some workers (Freeman and Lilly, 1987; Isowa and Ichikawa, 1979; Stokes and Oehlschlager, 1987). Lipase is a typical enzyme that has an affinity to organic solvents (Stokes and Oehlschlager, 1987). Peptide synthesis with proteases in organic solvents are known, in which the concentration of water in the system affects the conversion yield (Margolin *et al.,* 1987). Peroxidases which are sensitive to organic solvents have been studied in organic synthesis by immobilizing the enzymes (Randolph *et al.,* 1988). Due to its high specific activity, peroxidase has been often used as a marker enzyme in enzyme immunoassays.

In this study, an antibody of a tumor marker (α-fetoprotein) conjugated with peroxidase was suspended in organic solvents together with synthetic polymers, and the interaction of the antibody with the polymers was investigated. The author describes that the antibodies are associated with the polymers – the first reported observation of the association of antibodies in organic solvents.

Materials and Methods

Materials

Antibody (anti-α-fetoprotein) used in this work was labelled by horse-radish peroxidase conjugated with anti-human α-fetoprotein. The association of the antibody in organic solvents in the presence of various macromolecules (polymers) were studied by using chloroform.

As polymers, polystyrene (PST) and polymethyl methacrylate (PMMA) were used. Polyethyleneglycol dimethacrylate-
$[CH_2C(CH_3)CO(OCH_2CH_2)_nOCOC(CH_3)CH_2]$
such as monoethyleneglycol dimethacrylate (n = 1), diethyleneglycol dimethacrylate (n = 2), triethyleneglycol dimethacrylate (n = 3) and tetraethyleneglycol dimethacrylate (n = 4) were used as additives; n is the number of oxyethylene units.

Polymerization degree of polymethyl methacrylate (PMMA)

The polymerization degree of PMMA was controlled by a radiation degradation method. The polymerization degree is generally decreased by increasing irradiation dose. PMMA was irradiated by γ-rays from a ^{60}Co source with an irradiation dose rate of 1×10^6 R/h at 20 °C. After irradiation with various irradiation doses, the polymerization degree of the irradiated polymers were measured by a viscosity method.

Preparation of solution containing polymer and antibody

A certain amount of the polymer is dissolved in the solvent (chloroform) with a stirrer, the total

Reprint requests to Dr. M. Kumakura.
Telefax: 0554 (63) 4431.

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weight of the solution was 10 g. The antibody (immunoglobulin concentration, 5 mg/ml; molar ratio IgG/peroxidase, 1.0) was diluted with 1/15 M phosphate buffer solution, pH 7.2, to obtain a concentration of 500 μg/ml of the antibody solution. The antibody solution (0.5 ml) was mixed with a stirrer at 20 °C. After stirring, the solution containing chloroform, polymer and antibody, was in a transparent homogeneous state, indicating that each component is perfectly dissolved in chloroform. After additional stirring for 10 min, the solution was poured into a vessel (300 ml) containing methanol (200 ml) and immediately mixed strongly to precipitate the polymers. The polymer obtained by precipitation was washed once with 1/15 M phosphate buffer solution, pH 7.2, then the content of the antibody associated with the polymer was determined by measurement of the antibody by the peroxidase reaction. The washed polymer was put into the buffer solution (1 ml) including hydrogen peroxidase (0.3 mg/ml) and *o*-phenylenediamine (3 mg/ml). The enzyme reaction was carried out at room temperature for 30 min and terminated by adding 1 ml of 1 M hydrochloric acid, and measured at 492 nm. The amount of the antibody was determined by a reference curve between the amount of antibody and enzyme activity.

Results and Discussion

The amount of the antibody increased with increasing concentration of the polymers as shown in Fig. 1. The start of the curves in Fig. 1 was zero indicating that the antibody molecule was not associated with the solvent. The result means that the antibody is associated with the polymer. Since the polymers were perfectly dissolved in the solvent, the molecules of the antibody should be associated with the polymer, the molecular weight of the antibody being comparable with that of the polymers (about 10^5). In Fig. 1, the amount of the antibody in PMMA was greater than that in PST though the molecular weight of both polymers were similar. This suggests that the chain structure of PMMA is linear without a branch (side) group, while that of PST has a benzene ring as branch group by which the chain of PMMA could be easily associated with the antibody. The antibody having amino and carboxyl groups in the Fab and Fc

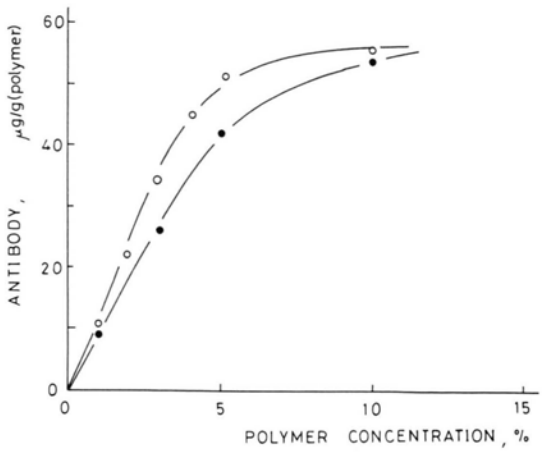

Fig. 1. Relationship between amount of antibody and concentration of polymer. Polymer: (●) polystyrene; (○) polymethyl methacrylate.

chains is a hydrophilic molecule. Therefore the antibody is not associated with a hydrophobic organic solvent such as chloroform. This may produce a favorable condition for the association of the antibody, although chloroform is a polar molecule.

The relationship between the amount of antibody and irradiation dose was investigated. The polymerization degree of PMMA was controlled by radiation degradation, because PMMA is a typical polymer that can be degraded easily by radiation at room temperature (Alexander *et al.*, 1954). The values of the molecular weight of non-irradiated PMMA, 20 kGy irradiated PMMA and 80 kGy irradiated PMMA were found by the irradiation experiments to be *ca.* 10^7, 7×10^6 and 8×10^5, respectively. The amount of the associated antibody was decreased by increasing irradiation dose, indicating that the association of the antibody is reduced by lowering the molecular weight of PMMA. Antibody and polymer are twisted together. Such an association should depend on the molecular weight of polymer corresponding to the size of molecule. This work reveals the meaningful information that the association of the polymer molecule depends on molecular weight, because it is comparable with that of the antibody. The association between antibody and polymer in the present system is due to van der Waals forces. This force is affected by an electric parameter of the

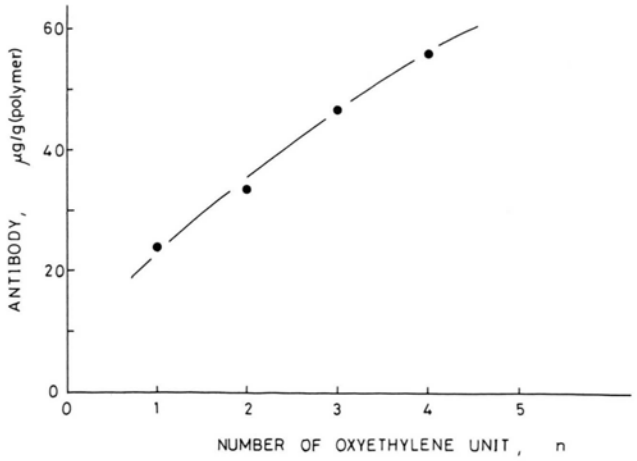

Fig. 2. Relationship between amount of antibody and number (n) of oxyethylene units in polyethyleneglycol dimethacrylate. Polymer dissolved in chloroform: polystyrene. Concentration of polystyrene: 2%, w/v.

polymer such as polarizability relating to molecular weight.

The effect of other polymers on the association of the antibody was investigated in the system dissolving PST. Fig. 2 shows the relationship between amount of the antibody and the number (n) of oxyethylene units ($-OCH_2CH_2-$) in polyethyleneglycol dimethacrylate. Interestingly the degree of the association of the antibody with the polymer increased with increasing length of the molecular chain. This means that the addition of such molecules enhances the association of the antibody with the polymer in organic liquid phase. The molecular weight of polyethyleneglycol dimethacrylate used is not high, it was 338 of the largest molecule (n = 4), which is much smaller than the molecular weight of the antibody and polymers. These molecules are easily dissolved in chloroform and not precipitated by addition of methanol. In polyethyleneglycol dimethacrylate, the hydrophilicity of the molecule increased with the chain length; of course, these molecules were dissolved in the solvent. These molecules having hydrophilic ether bonds and hydrophobic vinyl groups can function as surfactant in the solvent leading to the increase of interaction (association) between solvent, polymer or antibody.

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