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Interaction of proteins with the surface of polymers

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The interaction of proteins such as human γ-globulin with the surface of polymers was studied. The adsorption of the γ-globulin was affected by the hydrophilicity (degree of hydration) of the polymers, in which optimum hydrophilicity appeared to exist for the adsorption of the γ-globulin. This feature was also observed in the adsorption of the γ-globulin on copolymers having various hydrophilic properties. The surface of the polymers was modified by radiation graft polymerization and the addition of a surfactant, and its effect on the interaction with the γ-globulin was studied.

1. Introduction

The adsorption of proteins on the surface of polymers is of interest in the relation to the affinity of proteins for polymeric materials having various properties. Hydrogels have been studied since the early 1960s as blood-compatible polymers and have shown promise for cardiovascular applications [1, 2]. The relationship between the hydrophilicity of polymers and their interaction with proteins have been the subject of much investigation. Specifically with respect to hydrogels, Andrade *et al.* [1, 2], have proposed that an ideally biocompatible surface would demonstrate an interfacial free energy of zero in an aqueous biological environment. The minimum interfacial free energy hypothesis of protein adsorption can only be fully tested with hydrogels. Recently, Bagnall [3, 4] studied the interfacial free energy changes which occur when γ-globulin, fibrinogen, and serum adsorb at the air–aqueous, iso-octane–aqueous, and methylene iodide–aqueous interfaces, and stated that γ-globulin and serum do not distinguish between these interfaces at equilibrium. Okano *et al.* [5] studied the interaction of proteins and microphase separated structure of copolymers, and reported that serum albumin is adsorbed on the hydrophilic domain and avoids the hydrophobic domain, while γ-globulin is selectively adsorbed on the hydrophobic domains.

In this work, we have studied the interaction containing adsorption of human γ-globulin on the surface of polymers having various properties.

2. Experimental procedure

2.1. Materials

Human γ-globulin (I_gG) and peroxidase labelled anti-human I_gG rabbit I_gG were obtained from Japan Immunoresearch Laboratories Co., Ltd.

Hydroxyethyl acrylate (HEA), hydroxyethyl methacrylate (HEMA), tetraethyleneglycol diacrylate (A-4G), nonaethyleneglycol dimethacrylate (P-9G), octyl methacrylate (OMA), and trimethyrolpropane trimethacrylate (TMPT) were obtained from Shin Nakamura Chemical Co., Ltd.

Polyoxyethylene sorbitan monolaurate (Tween 20) was obtained from Tokyo Kasei Chemicals Co., Ltd.

2.2. Preparation of polymers by radiation polymerization

Polymer films were prepared by radiation cast-polymerization using a casting frame as follows. The monomer was charged in a casting frame which was constructed of two glass plates, a silicon rubber gasket, and frame fixing clamps. After charging, the casting frame was irradiated with an irradiation dose of 1 Mrad at room temperature. After irradiation, the polymer films were obtained by releasing the frame fixing clamps.

The films of polyvinyl chloride were grafted by a radiation polymerization technique with HEMA in an nitrogen atmosphere.

2.3. Hydrophilicity

The hydrophilicity of polymers was evaluated by measuring of the degree of hydration, immersing the polymers into distilled water at room temperature for 1 week. The degree of hydration was determined as the ratio of weight to the weight of the polymer at swelling equilibrium at 25° C.

2.4. Adsorption of human γ-globulin

Polymer films (8 mm × 8 mm × 0.2 mm) were immersed in the vessel containing the γ-globulin solution (1.0 mg ml^{-1} in 0.01 M phosphate buffer solution, pH 7.2) for 3 h at 37° C.

2.5. Measurement of adsorbed γ-globulin

The relative amount of the γ-globulin adsorbed on the polymer films was expressed as optical density resulting from the measurment of peroxidase activity after the enzyme reaction of the γ-globulin with peroxidase labelled anti-human I_gG rabbit I_gG as follows. After

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adsorption of the γ-globulin, the polymers were washed with the phosphate buffer solution three times and then immersed in the vessel containing peroxidase labelled anti-human I_gG rabbit I_gG solution (50 μg ml^{-1} in the phosphate buffer solution) and reacted for 1 h at 37° C to form the antigen–antibody complex on the surface of polymers. After reaction, the polymers were washed with the phosphate buffer solution three times and then incubated using the solution containing H_2O_2 and *O*-phenylenediamine for 30 min at room temperature. The optical density of the solution was measured with a spectrophotometer at 492 nm.

3. Results and discussion

3.1. Effect of hydrophilicity on γ-globulin adsorption

The γ-globulin adsorption on polymers was studied as a function of hydrophilicity (degree of hydration) of polymer. The relationship between optical density and degree of hydration is shown in Fig. 1. The optical density corresponding to the amount of adsorbed γ-globulin increased till the degree of hydration of 0.15 and after that decreased with increasing degree of hydration. From this result, it was found that the γ-globulin adsorption on polymers has a maximum at a certain hydrophilicity. This was a striking feature in the adsorption of γ-globulin on polymers. In Fig. 1, as the hydrophilicity of the polymer increased, the adsorption of the γ-globulin clearly decreased. This feature agreed with those reported by Andrade *et al.* [1, 2], and Okano *et al.* [5], giving a low protein adsorption on polymer with lower interfacial free energies. However, low adsorption of the γ-globulin on hydrophobic polymer was a new finding, though Ratner *et al.* [6], have shown that a balance of polar and apolar sites at a polymer surface is important for blood compatibility in an HEMA–ethyl methacrylate copolymer (1 : 1). From the results in Fig. 1, it is proposed that the γ-globulin does not interact with the surface of the polymer with high hydrophobic properties. The hydrophobic polymer would rather act as a reject substance for a hydrophilic γ-globulin molecule. The surface of the polymers of OMA and TMPT is covered with hydrophobic methyl and methylene groups, so that the molecule of the γ-globulin would not interact to be absorbed with the polymers. The molecule of γ-globulin consists of F_{ab} and F_c chains having amino and carboxyl groups, respectively. The F_{ab} chain is the binding site for antigen and it is known that the F_c chain can be bound with various proteins such as complement, to the cells [7]. In the interaction of the γ-globulin with polymers, the hydrophobic sites of the γ-globulin seems to contribute to an adsorption rather than these F_{ab} and F_c chain sites. Recently, it has been shown that the hydrophobic sites of proteins interact with hydrophobic substances to bind and can be utilized for the separation of proteins [7]. However, for adsorption including binding reactions of the γ-globulin on solid substances, it was obvious from Fig. 1 that an optimum condition of hydrophilicity exists. The appearance of a maximum on the adsorption–hydrophilicity curve in Fig. 1 suggests that the surface of the polymer having a degree of hydration of about 0.15 interacts to absorb with the γ-globulin molecule. The A-4G polymer, for which the degree of hydration is 0.15, is a relatively hydrophobic substance having a cross-linked polymer structure. The adsorption of the γ-globulin would not only be related to hydrophobicity but also the chemical structure of the polymer.

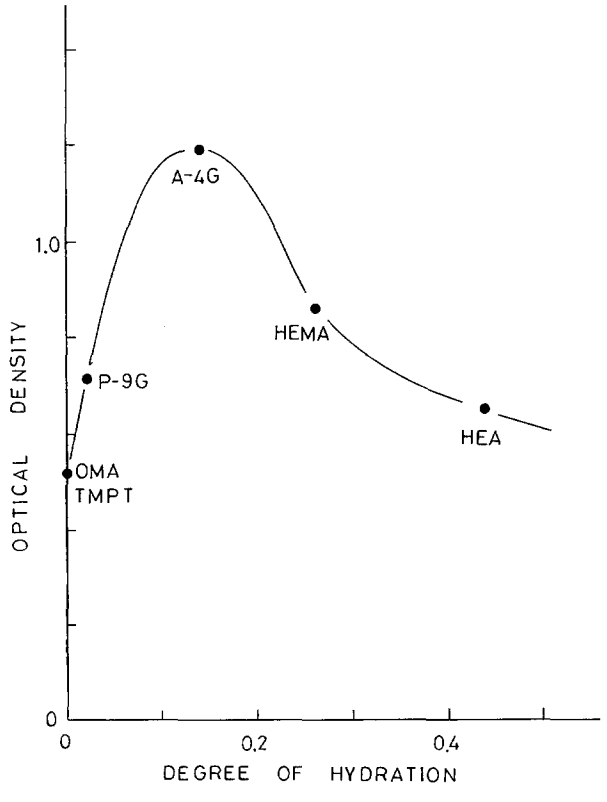

Figure 1 Relationship between optical density and degree of hydration of polymers.

3.2. Adsorption of γ-globulin on copolymers of HEMA and OMA

Effect of the γ-globulin adsorption on the copolymers of HEMA and OMA having various hydrophilic properties was studied. The relationship between optical density and monomer composition in the copolymers is shown in Fig. 2, in which HEMA and OMA are hydrophilic and hydrophobic monomers, respectively. The optical density first increased and then decreased with increasing OMA, indicating that the adsorption of the γ-globulin has a maximum at a certain composition. Thus, γ-globulin adsorption–monomer composition dependence in the copolymers appears to be a convex curve. This feature corresponds to the result which the γ-globulin adsorption has a maximum at a certain degree of hydration as seen in Fig. 1. The maximum of the optical density in Fig. 2 was observed at a rich composition of HEMA, indicating that the degree of hydration at a maximum adsorption is 0.1 to 0.2. The magnitude of the optical density at the maximum in Fig. 2 was smaller in comparison with that in Fig. 1. This difference would be caused by the difference of the chemical structure of polymer. That is, the polymer of A-4G has an oxyethylene unit ($-CH_2CH_2O-$) and the copolymer of HEMA and OMA has a methylene unit ($-CH_2-$).

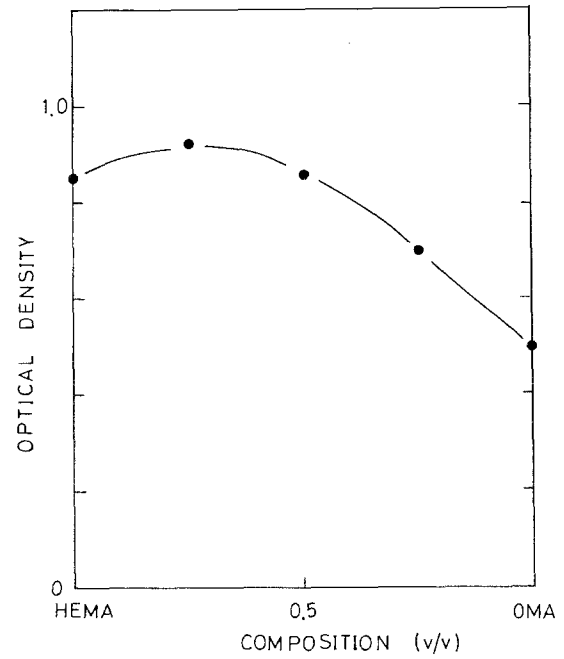

Figure 2 Relationship between optical density and monomer composition in the copolymer of hydroxyethyl methacrylate (HEMA) and octyl methacrylate (OMA).

Thus, the polymer having an oxyethylene unit of four appeared to interact with the γ-globulin by adsorbtion, in which the length of the polymer chain might be suitable for the adsorption of the γ-globulin molecule.

3.3. Interaction of γ-globulin with surface of a grafted polymer

The polymer film of vinyl chloride, which has hydrophobic properties was grafted by radiation polymerization using hydrophilic HEMA, and its effect on the γ-globulin adsorption was studied. Fig. 3 shows

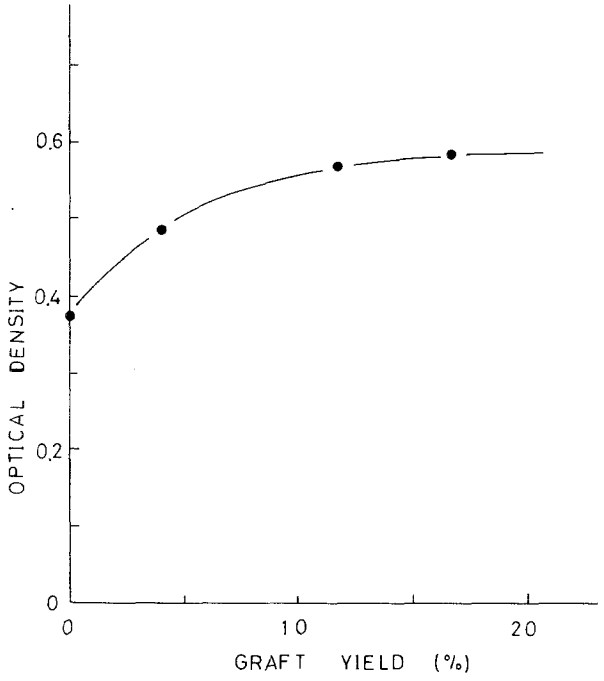

Figure 3 Relationship between optical density and graft yield in the grafted polymer. Polymer film: polyvinyl chloride; graft polymer: poly HEMA.

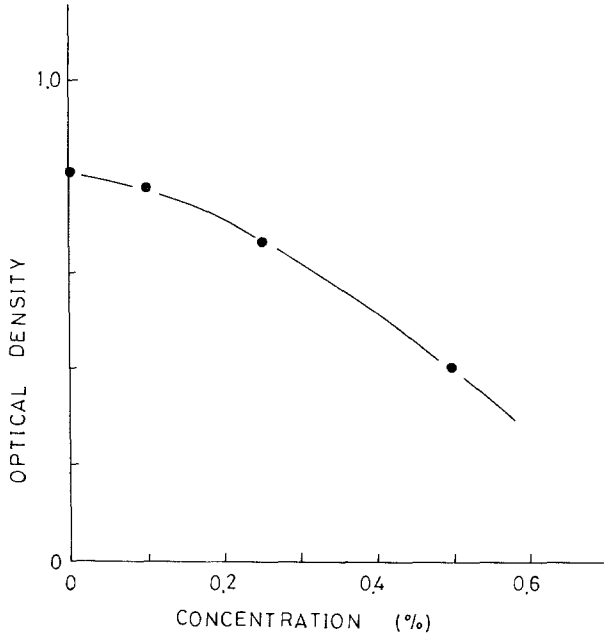

Figure 4 Relationship between optical density and surfactant concentration. Surfactant: Tween 20; polymer film: poly HEMA.

the relationship between optical density and graft yield. The optical density increased with increasing graft yield, indicating that the γ-globulin adsorption increased by grafting of HEMA. In general, the polymer grafted with the hydrophilic monomer becomes a more hydrophilic material, and its hydrophilicity should change by graft yield. From the results in Fig. 3, the surface of the polymer of vinyl chloride grafted with hydrophilic monomer such as HEMA gives a moderate hydrophobicity, in which hydrophilic and hydrophobic parts exist. Such a surface appeared to be slightly more suitable for the adsorption of the γ-globulin than the vinyl chloride polymer itself. The surface of the vinyl chloride polymer had grafted hydrophilic chains consisting of HEMA and its length would be relatively short though the length of the graft chains varies with the graft polymerization condition. The polymer grafted with HEMA gives an uneven surface having the graft chain, so that the γ-globulin molecules are physically entangled and entrapped by the graft chains. As the graft yield is further increased, the surface of the polymer is entirely covered by hydrophilic graft chains, so that the γ-globulin adsorption would be decreased due to the further increase of hydrophilicity.

3.4. Effect of presence of a surfactant on γ-globulin adsorption

Effect of the presence of a surfactant such as tween 20 on the γ-globulin adsorption was examined. Fig. 4 shows the relationship between the optical density and the addition concentration of tween 20. The optical density decreased with increasing tween 20 concentration, indicating that the γ-globulin adsorption decreased by the addition of surfactant. The molecule of tween 20 consists of polyoxyethylene units and it covers the surface of the polymer, so that the adsorption of the γ-globulin might be disturbed. Thus, the adsorption of the γ-globulin appeared to be decreased

by the pre-adsorption of a hydrophilic surfactant. This implies that the γ-globulin does not interact to adsorb hydrophilic substances as observed in the case of hydrophilic polymers.

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