Adsorption of γ-Globulin on Polymer Surfaces Having Various Chemical and Physical Structures

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Synopsis

The adsorption of human γ-globulin on polymer surfaces having various chemical and physical structures was studied. The γ-globulin adsorption on the crosslinked, rigid polymers of polyethyleneglycol dimethacrylate was considerably affected by the length of oxyethylene chains, in which the hydrophilicity of the polymers increased as the oxyethylene chains lengthened. The γ-globulin adsorption on the rigid polymers of tetraethyleneglycol dimethacrylate (or diacrylate) was lower than other soft polymers such as hydrogel, owing to the presence of oxyethylene chains. The polymer having a porous structure appeared to be a high adsorption material for the γ-globulin and the degree of the adsorption varied with pore size.

INTRODUCTION

The interaction of polymers with plasma proteins is of interest because it is related to biocompatibility. Hydrogels such as poly(2-hydroxyethyl methacrylate) are used frequently for biomaterials to improve their biocompatibility. Many hydrogels display good biocompatibility,[1] are reasonably nonthrombogenic,[2] and can be used for drug-delivery systems.[3] Furthermore, the use of contact lenses made of hydrogels is also becoming more and more popular. Hydrated hydrogel structures are of interest as blood and tissue interfacing materials and for other biomedical applications. Knowledge of interfacial interactions of polymers with protein solutions should be important in establishing polymer biocompatibility. Therefore, surface characterization of hydrogels and their protein adsorption have been studied by many workers.[4-6]

Hydrogels being soft polymers have recently attracted considerable attention as candidates for good biocompatible materials, because the interface between the water-swollen gel and blood or tissues may have a very low free energy and thus a very low adverse interaction of the gel surface with biological substances. However, the interaction of proteins with hydrophilic, hard, or rigid polymers, which have various chemical and physical structure has not been studied. In this work, the adsorption of γ-globulin on rigid polymers having various chemical and physical structures has been studied.

MATERIALS AND METHODS

Materials

Human γ-globulin (IgG) and peroxidase labeled antihuman IgG rabbit IgG were obtained from Japan Immunoresearch Laboratories Co., Ltd.

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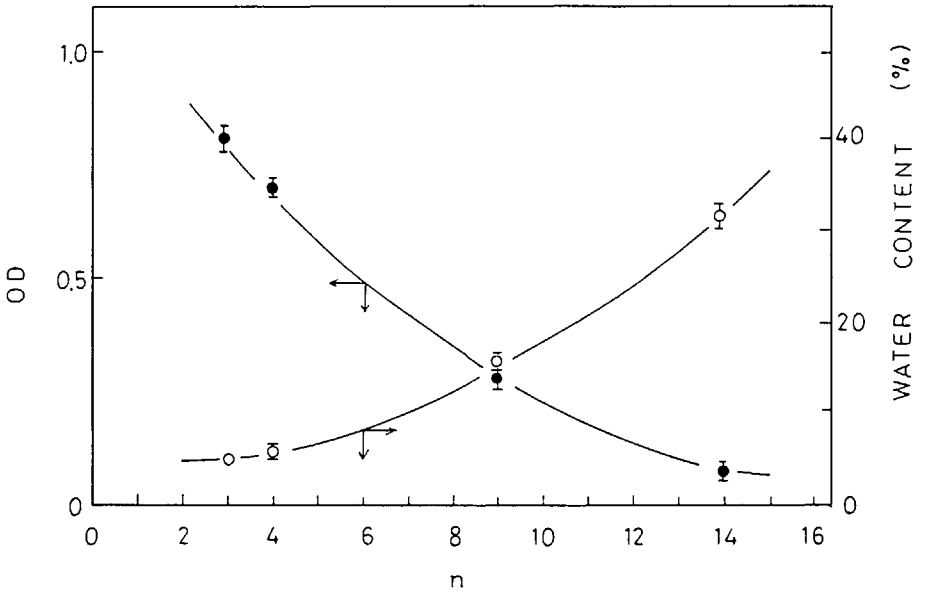

Fig. 1. Relationship between the number (n) of oxyethylene (CH_2CH_2O) units in the monomers and optical density or water content. Monomer: polyethyleneglycol dimethacrylate ($CH_2C(CH_3)COO(CH_2CH_2O)_nCO(CH_3)CCH_2$).

Triethyleneglycol dimethacrylate, tetraethyleneglycol dimethacrylate, nonaethyleneglycol dimethacrylate, and tetradecaethyleneglycol dimethacrylate were used as polyethyleneglycol dimethacrylate monomers. Other monomers, 2-hydroxyethyl methacrylate (HEMA), hydroxyhexyl methacrylate, and tetradecaethyleneglycol diacrylate, obtained from Shin Nakamura Co., Ltd., were used. In this work, monomers were used without purification.

Preparation of Polymers by Radiation Polymerization

Polymer films for examination of the γ-globulin adsorption were prepared by radiation cast-polymerization using a casting frame as follows. The monomer was charged in a casting frame (10 × 20 cm) which was constructed from two glass plates (5 mm in thickness), a silicon-rubber gasket, and frame fixing clamps.[7] After charging, the casting frame was irradiated (irradiation dose: 1×10^6 rad) by γ-ray from ^{60}Co source at room temperature. After irradiation, the polymer film was obtained by releasing of the frame fixing clamps. The glass plates were cleaned by a chemical washing (acid and alkali reagent) prior to casting the films.

Polymer films having porous structure were obtained by radiation cast-polymerization of the monomer aqueous solution at low temperature (−78°C).

Polyvinylchloride film was grafted with tetradecaethyleneglycol dimethacrylate monomer by radiation technique at room temperature, and the graft yield, which was 45%, was determined by a gravimetric method.

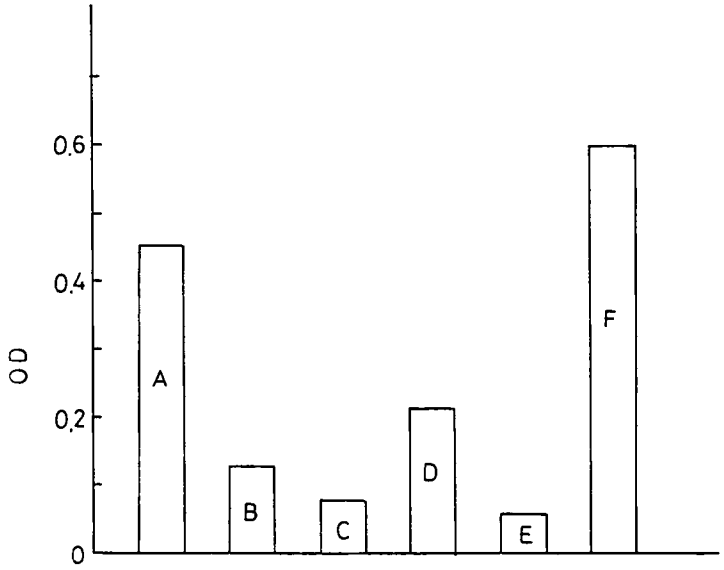

Fig. 2. Adsorption of γ-globulin on various polymers. (A) polymer of vinyl chloride, (B) polymer of vinyl chloride grafted with tetradecaethyleneglycol dimethacrylate, (C) polymer of tetradecaethyleneglycol dimethacrylate, (D) polymer of 2-hydroxyethyl methacrylate, (E) polymer of tetradecaethyleneglycol diacrylate, (F) polymer of hydroxyhexyl methacrylate.

Measurement of Hydrophilicity

The hydrophilicity of the polymer was evaluated by measuring the water content of the polymer. The water content (%) was determined as the ratio of weight of water to the weight of the film, at swelling equilibrium and 25°C.

Measurement of Porosity

The porosity of the polymer was obtained by measuring the average pore diameter in the porous structure using optical microscope.[8]

Human γ-Globulin Adsorption

Polymer films (8 × 8 × 0.2 mm) were immersed in the vessel containing the IgG solution (1.0 mg/mL in 0.01 *M* phosphate buffer solution, pH 7.2) for 3 h at 37°C. After adsorption of the IgG, the films were washed with the phosphate buffer solution three times and then treated with peroxidase labeled antihuman IgG rabbit IgG solution (50 μg/mL in the phosphate buffer solution) for 1.0 h at 37°C to form the antigen-antibody complex on the surface of the film.[9] The relative amount of the IgG adsorbed on the film was expressed as the value of optical density, measuring the peroxidase activity after the enzyme reaction. The enzyme reaction was carried out using H_2O_2 and o-phenylenediamine for 30 min at room temperature. The optical density was measured with a spectrophotometer at 492 nm.

RESULTS AND DISCUSSION

Effect of Chemical Structure of Polymers on γ-Globulin Adsorption

Effect of molecular structure of polymers of polyethyleneglycol dimethacrylate monomers on the γ-globulin adsorption was studied as a function of the

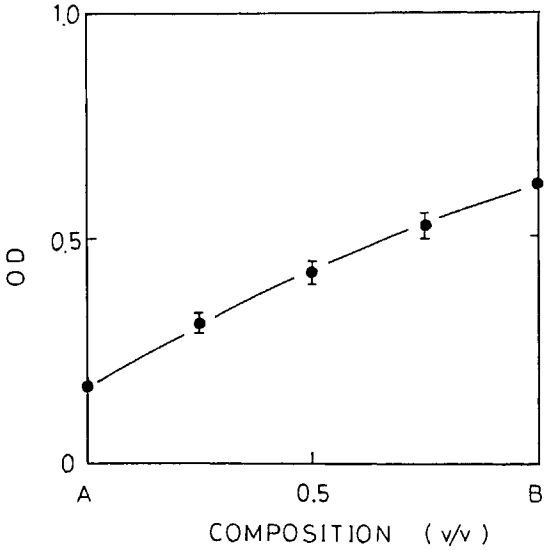

Fig. 3. Relationship between optical density and monomer composition in radiation copolymerization of tetradecaethyleneglycol dimethacrylate and hydroxyhexyl methacrylate. (A) tetradecaethyleneglycol dimethacrylate, (B) hydroxyhexyl methacrylate.

number (n) of oxyethylene ($-CH_2CH_2O-$) units in the monomers. The relationship between optical density and the number of oxyethylene units is shown in Figure 1. The optical density, which corresponds to the magnitude of the γ-globulin adsorption on the polymers, decreased with increasing n. This indicated that the adsorption of proteins, such as the γ-globulin, is considerably affected by the chemical structure of the polymers. The water content of the polymers increased with increasing n as shown in Figure 1. The origin of hydrophilicity in the polymers of polyethyleneglycol dimethacrylate monomers was based on the presence of an ether bond in the oxyethylene units, so that hydrophilicity of the polymers increased with increase of n. The physical nature of the swelled polymers of polyethyleneglycol dimethacrylate monomers was different from that of hydrophilic polymer gels, such as poly-HEMA. The swelled polymers of polyethyleneglycol dimethacrylate and HEMA were rigid and soft, respectively. The rigid property of the polymers of polyethyleneglycol dimethacrylate monomers was due to the crosslinked feature of bifunctional monomer, polyethyleneglycol dimethacrylate, thus the water structure in swollen polymer was affected by polymer structure, though the rigidity of the polymers and the length of oxyethylene chains. The water content (30%) of the polymer with $n = 14$ appeared to be slightly larger than that (26%) of HEMA, but the rigidity of the swollen polymer with $n = 14$ was greater than that of HEMA. The water content of the polymer with $n = 14$ was comparable with that of 2-hydroxyethyl acrylate, which is a more hydrophilic gel than poly-HEMA. In Figure 1, the adsorption of the γ-globulin on the polymers of polyethyleneglycol dimethacrylate monomers appears to be decreased with increasing hydrophilicity whereas γ-globulin adsorption on rigid polymers increased with increasing hydrophilicity. The relationship between the equilibrium water content of a polymer and the interaction with protein has been the subject of much speculation and

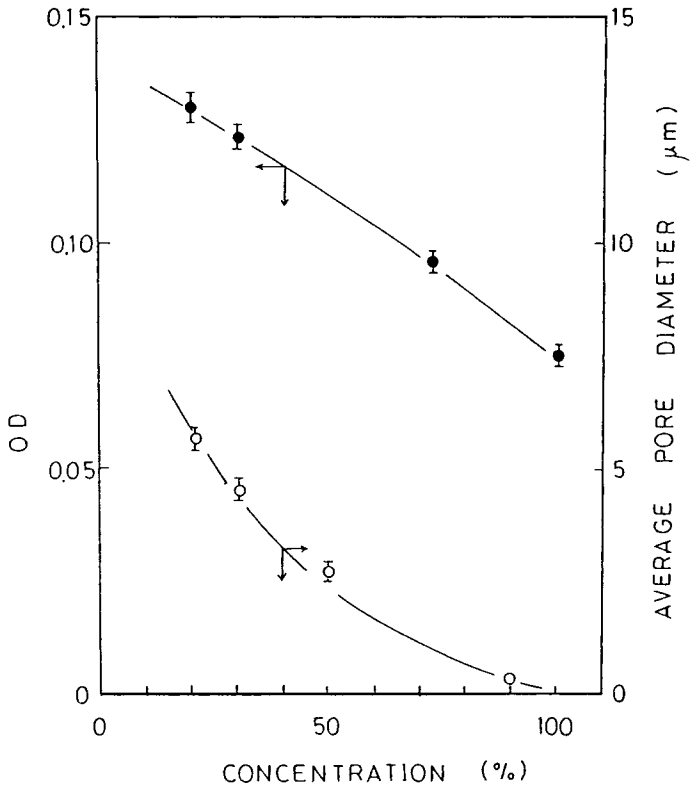

Fig. 4. Relationship between monomer concentration and optical density or average pore diameter. Monomer: tetradecaethyleneglycol dimethacrylate.

study. Andrade et al. have proposed that an ideally biocompatible surface would demonstrate an interfacial free energy of zero in a biological environment.[10] Hydrophilic soft gels can have water contents up to 80% or greater and, as such, should have low interfacial free energy with aqueous systems. Thus, the biocompatibility of hydrophilic polymers was thought to be intimately related to hydrophilicity and the hydrophilic soft surface, wherein the branched polymer segments having OH groups at the polymer surface are loose and can interact with proteins. In this work, it was found that γ-globulin adsorption on the dimethacrylate polymer is similarly related to the hydrophilicity. However, the hydrophilic polymer segments on the surface of the polymers of polyethyleneglycol dimethacrylate are part of the main polymer backbone. Furthermore, these polymers do not have a branched polymer segments having hydrophilic groups, such as OH group. Thus, it is proposed that the interaction of proteins such as the γ-globulin with the polymers with large n would be achieved on the site of main polymer chains consisting of oxyethylene units. Recently, hydrophilic compounds having oxyethylene units such as polyethylene glycol have been used as the hybridizating agent in cell hybridization.[11] This suggests that hydrophilic high molecular weight compounds having oxyethylene units can be interacted with biological substances such as proteins.

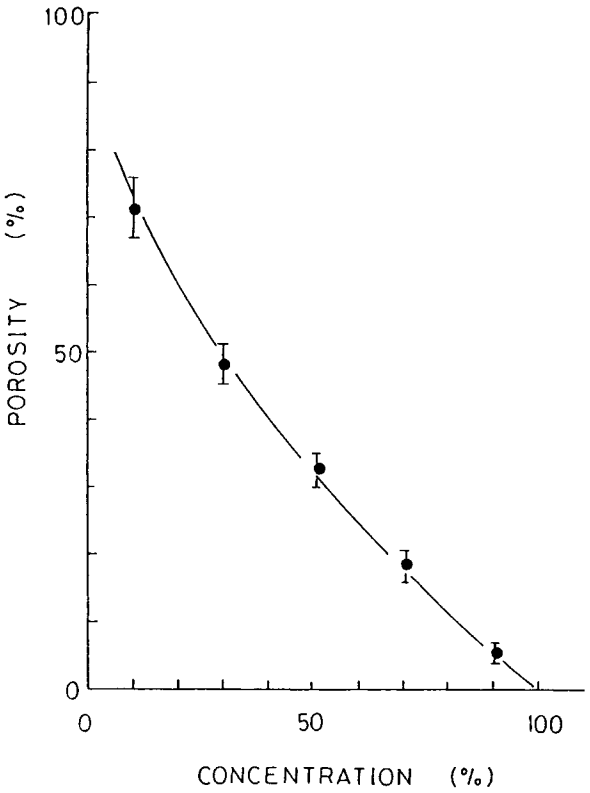

Fig. 5. Relationship between porosity and monomer concentration. Monomer: tetradecaethyleneglycol dimethacrylate.

Adsorption of γ-Globulin on Various Polymers

Adsorption property of the γ-globulin on the polymer with $n = 14$ was compared with that on various polymers as shown in Figure 2. It was found that adsorbing property of the γ-globulin on the polymer with $n = 14$ is lower than that on hydrogel such as the polymer of HEMA as can be seen in Figure 2. Since the adsorption of proteins on the polymer of HEMA is known to be relatively low,[4] it is suggested that adsorption property of the γ-globulin on the polymer with $n = 14$ is considerably lower and its biocompatibility may be superior to other polymers. Furthermore, it is of interest that the adsorption property of the polymer of tetradecaethyleneglycol diacrylate appeared to be lower than that of the dimethacrylate polymer with $n = 14$ as seen in Figure 2. The low adsorption property of this polymer would be caused by the chemical structure of the polymer, consisting of the structure of acrylic acid ester; $-CH_2-CH-COO-$. Such a polymer would give a more hydrophilic and lower surface-free energy than the polymer having the structure of methacrylic acid ester.

The γ-globulin adsorption of polyvinylchloride decreased markedly by the radiation grafting of tetradecaethyleneglycol dimethacrylate monomer as seen in Figure 2. This indicated that the surface of polyvinylchloride is effectively

covered by the hydrophilic polymer layer with $n = 14$, through which its hydrophilicity increased. Ratner et al.[6] have studied blood compatibility/water content relationship for radiation-grafted hydrogels and reported that a balance of hydrophilic and hydrophobic sites at a biomaterial surface may be important for blood compatibility. Their results do not support water content/blood-compatibility hypotheses which have been suggested for hydrogels. In Figure 2, though the water content (35%) of the polymer of tetradecaethyleneglycol diacrylate monomer was comparable with that of tetradecaethyleneglycol dimethacrylate monomer, the γ-globulin adsorption in the polymer of tetradecaethyleneglycol diacrylate monomer was lower than that of tetradecaethyleneglycol dimethacrylate monomer. From this result, the γ-globulin adsorption on polymers appeared not only to depend on water content but also chemical structure.

Adsorption of γ-Globulin on Copolymer of Tetradecaethyleneglycol Dimethacrylate and Hydroxyhexyl Methacrylate Monomer

Adsorption of the γ-globulin on the copolymer of tetradecaethyleneglycol dimethacrylate and hydroxyhexyl methacrylate monomer was studied as a function of monomer composition (Fig. 3), in which the water content of the polymer of hydroxyhexyl methacrylate monomer was 6%. The γ-globulin adsorption in the copolymer decreased with increasing tetradecaethyleneglycol dimethacrylate monomer component, in correlation with the increase of hydrophilicity. In the hydrogel type polymer of hydroxyalkyl methacrylate monomer having OH group, the γ-globulin adsorption appeared to be increased with increase of the length of methylene chains in polymer segments owing to the decrease of hydrophilicity of polymer.

Effect of Porous Structure of Polymer on γ-Globulin Adsorption

The effect of physical structure, such as a pore, on γ-globulin adsorption on polymers was studied using tetradecaethyleneglycol dimethacrylate monomer. The polymer having porous structure was obtained by radiation polymerization of the mixture system of tetradecaethyleneglycol dimethacrylate monomer and water at −78°C. The formation of the porous structure was one of the characteristic features of the polymer matrix obtained by the present method. The mechanism of pore formation was due to small ice particles formed at low temperature (−78°C) in the system at which the radiation polymerization was carried out and subsequent melting as the temperature increased to room temperature after irradiation. In the present method, the pore size in the porous polymer matrix intimately depended on monomer concentration, and the pore shape was cylindrical. The γ-globulin adsorption in the porous polymer varied with monomer concentration is shown in Figure 4. This variation of the γ-globulin adsorption and/or absorption with monomer concentration appeared to be dependent on the pore size in the porous structure as can be seen in Figure 4. That is, the γ-globulin adsorption increased as the average pore diameter increased. The apparent size of γ-globulin molecule is about 100–200 Å, considering its molecular weight (150,000–160,000). It is proposed that γ-globulin molecules are entangled with each other in water. Therefore, the apparent occupation volume of the γ-globulin molecules in water would be large

and these molecules could invade the inner regions of the pore. Since the pore size in the porous structure is larger than the molecular size of the γ-globulin, it is reasonable to consider that the γ-globulin is adsorbed in the inner part of the pore.

The relationship between porosity and monomer concentration is shown in Figure 5. Though the porosity in the porous structure varied with monomer concentration, the porosity in the polymer from low monomer concentrations was large. This implies that the porous polymer matrix from low monomer concentrations gives many adsorption sites for γ-globulin. Furthermore, the inner part of the porous structure in the polymer obtained by the present method is a complex cylindrical structure consisting of various pore sizes, so that adsorbed γ-globulin molecules are not easily leached.

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