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NOTES

Insulin Permeation through Amphiphilic Polymer Membranes Having 2-Hydroxyethyl Methacrylate Moiety

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A sustained release system of insulin is useful for the treatment of diabetes because the conventional insulin administration by an injection causes wide fluctuations in blood glucose levels. Many studies about the sustained release system of drugs using polymer materials have been reported, however, only a few studies about the release system of macromolecules such as insulin have been reported.[1] In general, the release rate of drugs from the sustained release system depends on the permeability of the polymer matrix.[2] Since the drug diffuses through the permeable pathway in the polymer matrix, a large size of pathway is necessary for the permeation of insulin.

Poly(2-hydroxyethyl methacrylate) [poly(HEMA)], which is known as a typical hydrogel, swells in water and contains a large amount of water.[3] Therefore, it is useful for the permeation of water-soluble macromolecules.[4] Moreover, poly(HEMA) exhibits low interfacial free energies with aqueous solutions and only a weak tendency to absorb biological species such as blood cells and proteins. As a results, poly(HEMA) shows a good biocompatibility and is strong candidates for a biomedical implant devices.[5]

Winsniewsky and Kim investigated the permeation of water-soluble compounds through poly(HEMA) membrane, and reported that the swelling of the membrane had a great influence on the permeability of the membrane.[6] Thus, in the case of the sustained release system using the polymer membrane with HEMA moiety, the release rate of drugs can be controlled by means of the change in the swelling degree of the membrane.[7] From these points of view, the sustained release system of insulin using the polymer membranes having HEMA moiety has been developed so that the permeation of insulin through the membrane was investigated with attention to the swelling of the membrane. Moreover, in terms of a basic knowledge for its clinical application, the effects of plasma protein such as albumin and γ-globulin on the permeation of insulin through the membrane were investigated.

EXPERIMENTAL

Poly(HEMA) was obtained by homopolymerization of HEMA in 2-propanol for 2 h at 60°C using 2,2′-azobisisobutyronitrile (AIBN) as an initiator. The weight average molecular weight of poly(HEMA) was 3.5×10^5 which

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Table I. HEMA–MMA copolymerization

HEMA mole fraction		Time[a]	Conversion
In monomer	In copolymer	h	%
0.71	0.66	1.0	12.4
0.81	0.76	1.0	12.8
0.86	0.82	0.5	9.8
0.90	0.86	0.5	10.8

[a] [Monomer] = 3.0 M in DMF at 60°C, [AIBN] = 5 mM.

was determined by the viscosity measurement.[8] HEMA–methyl methacrylate (MMA) copolymers were synthesized by the copolymerization of the corresponding monomers in *N,N*-dimethylformamide (DMF)at 60°C. The composition of HEMA moiety in the copolymer was determined by the value of monomer reactivity ratio (r) reported by us [r(HEMA) = 0.66, r(MMA) = 0.86].[9] The results of the copolymerization were listed in Table I. Insulin, albumin (Alb), and γ-globulin (γ-G) were kindly supplied by Dr. T. Okano, Tokyo Women's Medical College.

A 5 ml of DMF solution containing 0.5 g of the polymer was casted on Teflon plate and the solvent was evaporated at 40°C. To remove a trace of remaining solvent, the membrane casted was dried *in vacuo* for 4 days.

Measurement of the water fraction was performed by the ratio of the equilibrium weight of the swollen membrane to predetermined its dry weight. The swollen membrane was quickly blotted to remove excess surface water. The water fraction of the polymer membrane (H) was evaluated as follows:

$$H = \frac{(\text{Wt of swollen membrane}) - (\text{Wt of dry membrane})}{(\text{Wt of swollen membrane})}$$

Four measurements were made and the mean value was used to establish the water fraction of the membrane.

The equilibrium swollen polymer membrane was interposed between two parts of glass cells. A 50 ml of a Tris buffer solution (pH 6.47) containing 8.35 mg of insulin was put at the one side of the cell and a 50 ml of the buffer solution was put at the another side of the cell. These solutions were stirred slowly. Probes of a buffer solution were taken out after given periods of time and the absorbance of the solution at 274 nm was measured to determine the amount of insulin permeated through the polymer membrane. At least two experiments were performed for each membrane.

Permeation experiments of insulin with plasma protein were carried out by adding a known amount of the proteins in both sides of the solution to prevent the permeation of the proteins.

RESULTS AND DISCUSSION

Figure 1 shows the permeation profile of insulin through the HEMA–MMA copolymer membrane. The permeated amount of insulin increased linearly with time. The permeation coefficient (P) of insulin was calculated from the slope of the permeation profile using the following equation.

$$Q = P \cdot (\Delta C \cdot A/1) \cdot t$$

where $Q(g)$ is the amount of insulin permeated, ΔC ($g\,cm^{-3}$) is the concentration difference of insulin between both sides, A (cm^2) is the area of the membrane, l (cm) is the thickness of the membrane, and t (s) is time.

Figure 2 shows the relation between the permeation coefficient of insulin and the water fraction of the HEMA–MMA copolymer membrane and HEMA mole fraction in the copolymer. It is clear that the permeation coefficient and the water fraction increased with increasing the HEMA composition in the HEMA–MMA copolymer. It is well known that the poly(HEMA) membrane absorb large quantities of water while remaining insoluble.[3] Therefore, it is considered that the increase of the permeation coefficient of insulin was due to

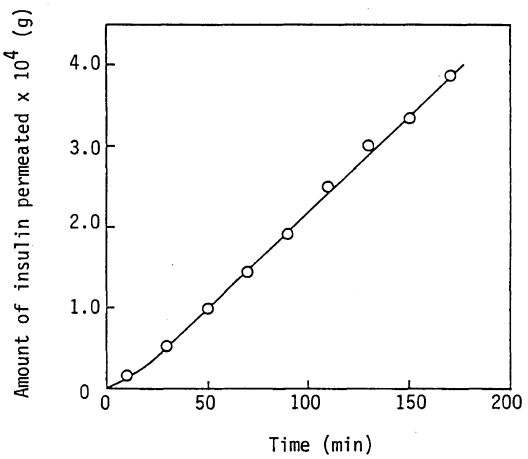

Figure 1. Permeation of insulin through the HEMA–MMA copolymer membrane at 30°C. Mole fraction of HEMA moiety, 0.76; initial concentration of insulin, 0.176 mg ml^{-1}

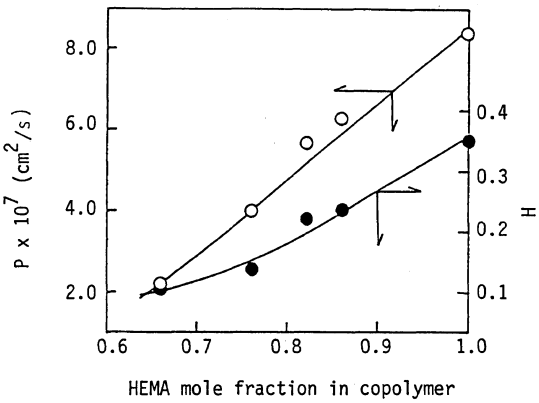

Figure 2. Relation between the permeation coefficient of insulin (P) and the water fraction of the HEMA–MMA copolymer membrane (H) and mole fraction of HEMA moiety in the copolymer at 30°C.

the increase of the permeation pathway for insulin in the membrane because of the increase of the water fraction of the membrane.

According to Yasuda *et al.*, the relation between the logarithm of the permeation coefficient ($\log P$) and the reciprocal of the water fraction ($1/H$) is linear, when the solute permeates through the water region contained in the hydrophilic polymer membrane where no interactions with the polymer chains take place.[10]

Figure 3 shows the relation between $\log P$ and $1/H$ in the HEMA–MMA copolymer membrane system. From this result, according to the theory proposed by Yasuda *et al.* it is considered that insulin permeates through the region of free water in the swollen HEMA–MMA copolymer membrane. These results lead to conclusion that the permeability of insulin through the hydrophilic polymer membrane can be controlled by changing the water fraction of the membrane. Therefore, when the controlled release of insulin using the hydrophilic polymer membrane is attempted, the desired release rate can be obtained by means of the incorporation of apparent hydrophobic groups or cross-linking in the polymer membrane by which the swelling of the membrane

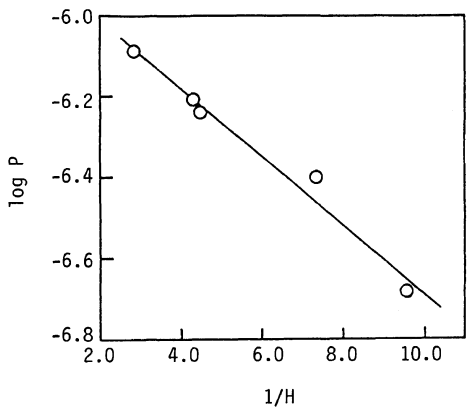

Figure 3. Relation between $\log P$ and $1/H$ in HEMA–MMA copolymer membrane system at 30°C.

was controlled.[7]

When a polymer device containing drug is implanted into the living body, it comes in contact with blood and plasma proteins may be absorbed on the surface of the polymer device.[11] Thus, the effects of the proteins on the drug release are an important factor for the determination of relase profile of drugs in the living body using the polymer matrix.

Table II shows the characteristics of permeation experiment in the system with plasma protein. The permeation coefficients of insulin decreased in the system with plasma protein. For example, in the case of the system with albumin, the ratio of the permeation coefficients with and without albumin was 0.79. However, the water fraction of poly(HEMA) membrane did not change both with and without proteins due to the following reason. On the surface of poly(HEMA) membrane, the amount of proteins adsorbed is smaller than that of other polymethacrylate membrane because of its low surface energy.[5] Though the reason of a decrease in the permeation coefficient of insulin is not evident, it may be due to the complex formation between insulin and proteins.

It is concluded that the amphiphilic polymer membrane having HEMA moiety can be used for the sustained release of insulin because of its high water fraction and the release rate of insulin can be regulated by means of the change in the water fraction of the membrane. Moreover, existence of plasma protein affects the permeability of insulin through the polymer membrane.

Table II. Characteristics of permeation of insulin through poly(HEMA) membrane at 30°C[a]

Condition	Concentration mg dl^{-1}	H	$P \times 10^7$ / cm^2 s^{-1}	P_p/P_0
Tris buffer	0	0.352	8.02	1.00
+Alb	45	0.354	6.41	0.79
+γ-G	16	0.353	6.86	0.85
+Mixture	61	0.357	6.33	0.78

[a] The value of P_p/P_0 represents the ratio of permeation coefficient with protein and that without protein.

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