Permeability of Heterogeneous Membranes Based on Methacrylic Acid

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Synopsis

The permeability of H^+, Na^+, insulin, and hemoglobin through porous membranes made of crosslinked poly(methacrylic acid) was investigated at different pH values on both sides of the membrane. It was shown that a change in the charge of the protein during its transport through the membrane might raise the driving force of the process so much that the permeability coefficient of the protein through the membrane would be higher than in solution. In the case reported here, the flow of the protein may be regarded as partial electrophoresis in a porous medium.

INTRODUCTION

The permeability of homogeneous hydrophilic membranes was studied for low molecular weight[1,2] and high molecular weight[3] compounds. It was shown[4] that the introduction of both acidic and basic groups into these membranes raised their permeability.

It is obvious that porous polymers possess a higher permeability than homogeneous polymers of the same chemical composition. Heterogeneous copolymers of methacrylic acid and 1,3,5-triacryloylhexahydrotriazine exhibit high porosity in the swollen state[5] and a high selectivity of sorption of physiologically active compounds, especially of enzymes.[6] The dependence of the permeability of proteins through these sorbents on the pH of solution is particularly interesting. It has been shown earlier[7] that the permeability of serumalbumin through nitrocellulose membranes is pH dependent. This dependence is most pronounced in the range of the isoelectric point of the protein.[7]

It is clear now that the problem of the dependence of permeability of physiologically important compounds through heterogeneous polymers containing the —COOH groups on the degree of ionization deserves attention. This work is dealing with the permeability of low molecular weight cations (H^+, Na^+) and proteins (insulin, hemoglobin) through poly(methacrylic acid) crosslinked with 1,3,5-triacryloylhexahydrotriazine at various degrees of ionization of carboxylic groups and at various pH values on both sides of an ion exchange membrane.

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EXPERIMENTAL

Compounds

Insulin, activity 24.3 IU/mg, and hemoglobin obtained by the Antonini method[9] were used in the work.

Membrane Preparation

The membranes were prepared by solution precipitation polymerization from a 20 wt-% aqueous solution of monomers containing 96 mole-% methacrylic acid (MAA) and 4 mole-% 1,3,5-triacryloylhexahydrotriazine (HHTAT). Ascorbic acid with ammonium persulfate at a 1:1 molar ratio at a concentration of 2.6×10^3 moles of the two compounds per 100 g of the polymerization mixture was used as initiator.[8] A Whatman chromatographic paper, density 40 mg/cm^2, 0.083 cm thick, was used as the reinforcing material. The filter paper was coated with the above mixture of monomers in an amount of 0.05 ml/cm^2 and left to stand in a desiccator at ambient temperature for 48 hr. The reinforced membranes thus obtained were washed from the residues of the monomers and low molecular weight fractions with 0.05*M* NH_4OH and 0.1*N* HCl. The exchange capacity of these membranes was 0.097 meq/cm^2 (analytically determined). The membranes were stored in the H^+ form; prior to the experiment, they were conditioned several days in buffered solutions at pH 5.5, 6.7, and 9.2. In solutions of these pH values the respective degrees of ionization α are 0.3, 0.5, and 0.85. The permeability measurements were performed in an experimental arrangement described earlier[4] with the membrane diameters of 2 and 3.3 cm and with cell volumes of 50 ml.

Analytical Procedures

The Na concentration was determined by means of a sodium electrode ESL-516-04 (at high concentration differences between the cells) or calculated from the composition of the buffer (at small concentration differences). The distribution coefficient of proteins between solution and the polymer phase of the membrane was determined in auxiliary static experiments with a membrane 1 cm^2 in area and 0.085 cm thick, immersed into 10 ml protein solution at a concentration of 1 mg/ml and a constant pH. The distribution coefficient at various pH values was calculated from the difference between the initial and equilibrium protein concentrations.

RESULTS AND DISCUSSION

The membranes contain weakly dissociating carboxylic groups with $pK_{char} = 6.7$. The volume concentration of ionogenic groups is 1.14 meq/ml.

The membrane permeability for low molecular weight cations (H^+, Na^+) was measured in unbuffered solutions. A 0.01*N* HCl solution was on the one side of the membrane, while distilled water or a 0.1*N* NaCl solution was on the other. The decrease in pH characterized quantitatively the transport of protons. Figure 1 shows the dependences obtained for water and 0.1*N* NaCl. The differently

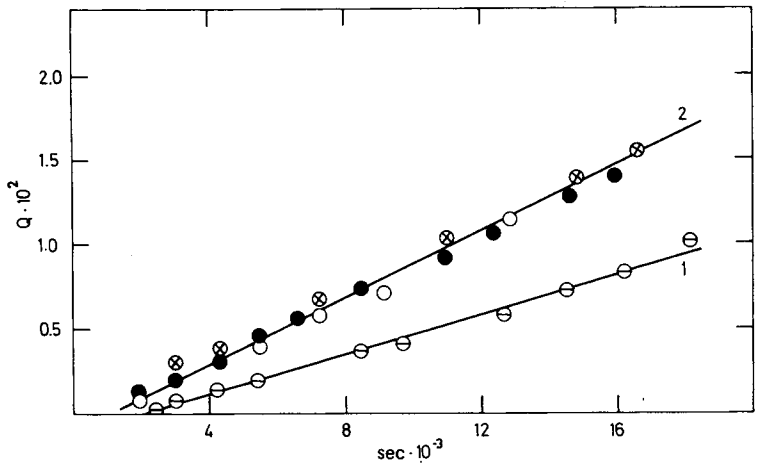

Fig. 1. Flow of protons Q (meq/cm^2) through the membrane in the H^+ form ($\alpha = 0$) into water (1) and into 0.1N NaCl (2).

marked points on the straight line 2 correspond to the respective membranes and express the reproducibility of results. The mean deviation did not exceed 10%.

The higher permeability of the 0.1N NaCl solutions compared with water may be attributed to the participation of Na^+ ions in the ion exchange with protons of the COOH group of the membrane. It should be stressed, however, that the degree of ionization of the membranes used in the H^+ form did not exceed 1%. Unfortunately, the "coupled flow" of Na^+ ions could not be assessed for experimental reasons.

The permeability coefficient was calculated from

$$Q_i = \frac{P_i c_i}{\Delta x}\left(t - \frac{\Delta x^2}{6D}\right) \tag{1}$$

where Q_i is the amount of compound diffused through a unit surface area at a time t (sec), c_i is the initial concentration of compound in the more concentrated cell ($c_i \gg c_i'$; here c_i' is the concentration in the cell with the lower concentration; at a time $t = 0$, $c_i' = 0$), Δx is the membrane thickness, P is the permeability coefficient (cm^2/sec), and D is the diffusion coefficient (cm^2/sec; $P = DK$, where K is the distribution coefficient).

TABLE I
Permeability of H^+ and Na^+ Through the Membranes

Ion	Degree of ionization α of membrane	Solutions on the two sides of the membranes	Permeation coefficient $P \times 10^6$, cm^2/sec
H^+	0	0.01N HCl–H_2O	4.9
H^+	0	0.01N HCl–0.1N NaCl	9.8
H^+	0	0.1N HCl–H_2O	8.9
H^+	0.85	0.01N HCl–0.1N NaCl	7.8
Na^+	0	0.1M NaH_2PO_4–0.1M Na_2HPO_4	1.7
Na^+	0.3	0.1M NaH_2PO_4–0.1M Na_2HPO_4	1.1
Na^+	0.5	0.1M NaH_2PO_4–0.1M Na_2HPO_4	0.9
Na^+	0.85	0.1M NaH_2PO_4–0.1M Na_2HPO_4	0.8

Table I summarizes the permeation coefficients of H^+ and Na^+ at various initial degrees of ionization of the —COOH groups of the membrane.

The transfer of the proton is mainly affected by the ionic strength of the salt solution and less by the degree of ionization of carboxylic groups. On the contrary, the permeation of sodium ions is inversely proportional to the degree of ionization α. This may be explained by strong electrostatic interactions between Na^+ and the ionized carboxylic groups of the membrane. The permeability coefficients of H^+ and Na^+ for the membranes under study are much higher than those of homogeneous membranes but lower than the diffusion coefficients in pure solvents.[10]

As is generally known,[11] the measured diffusion (permeability) coefficients exhibit concentration dependence in those cases in which the thermodynamic potential of components is concentration dependent,

$$D = D_0 g$$

where D_0 is the diffusion coefficient, if there are no interactions in the system, and

$$g = \frac{c_i}{RT}\left(\frac{\partial \mu_i}{\partial c_i}\right)_{P,T} \tag{2}$$

where μ_i is the thermodynamic (chemical) potential of the ith component. In the case of diffusion of two components, which is the case under consideration, the Gibbs–Duhem equation

$$g = 1 + \frac{c_1 c_2}{RT}\left[\frac{\partial^2 G^E}{(\partial x_2)^2}\right]_{P,T} \tag{3}$$

may be used; here, G^E is the excess molar thermodynamic Gibbs potential. It follows from eq. (3) that the dependence of the diffusion coefficient of the component on concentration may pass through both a minimum and a maximum depending on the thermodynamic coefficient g. The activity coefficient of Na^+ decreases with increasing α,[12] which leads to a decrease in the effective diffusion, in accordance with eq. (2).

In the case of diffusion of an ampholyte, the dependence of the thermodynamic coefficient g on the degree of ionization is a complicated one. The free energy of binding of the protein on the polyelectrolyte depends on the pH of solution. This dependence is bell shaped,[13] so that depending on pH either formation or dissociation of the protein–polymer complex takes place.

Figure 2 shows the dependence of the distribution coefficient k_i of insulin and hemoglobin on pH. The highest binding of insulin on the membrane can be seen in the pH range of 4.5–5.2; for hemoglobin, the optimal pH range is 6.3–6.8. No binding of these two proteins on the polymer occurs at pH > 9.0. On the contrary, in this range the protein–polymer complex is dissociated owing to the strong repulsion between the negative charges of the two components.

Figure 3 shows the permeability of insulin and hemoglobin, both at the same pH values on both sides of the membrane (cf. Table II) and at pH 3.0 and 9.2 for insulin, or 6.2 and 9.2 for hemoglobin. An increase in permeability can be observed with increasing pH on that side of the membrane to which the protein diffuses. For the above differences in pH, the permeability of insulin is 6.1×10^{-6} cm^2/sec and that of hemoglobin is 4.0×10^{-6} cm^2/sec, which is six times

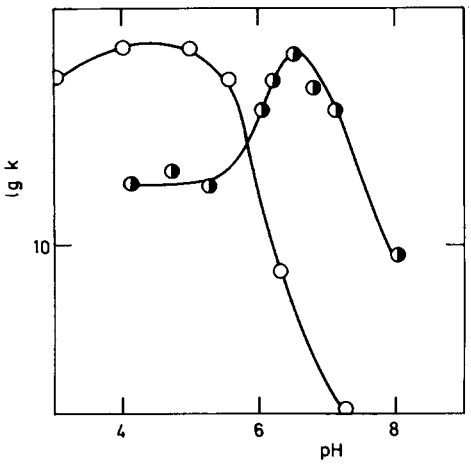

Fig. 2. Dependence of distribution coefficient K of insulin (○) and hemoglobin (◑) on pH of an equilibrium solution in semilogarithmic plot.

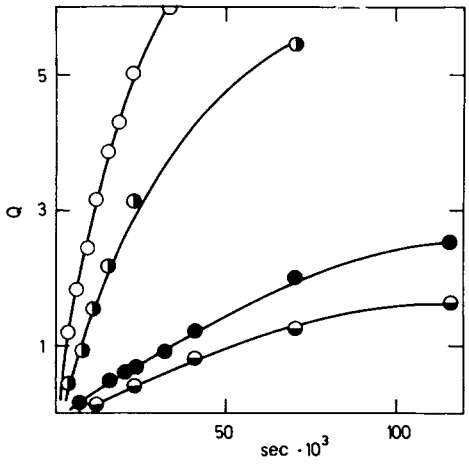

Fig. 3. Flow Q (mg/cm²) of insulin (○, ●) and hemoglobin (◒, ◑) through the membrane in the H^+ form at the same pH on both sides of the membrane (●, ◒) and at a different pH values on the two sides of the membrane (○, ◑). For insulin, ΔpH = 6.2; for hemoglobin, ΔpH = 3.0.

higher than the "self-diffusion" coefficient of these compounds in solution,[14] so that the process under investigation may be regarded as an induced transport of protein through the membrane. This process can be analyzed by means of eq. (3), assuming that the other component is a proton. Assuming a linear gradient of pH through the membrane, it is possible to estimate $\partial^2 G^E/(\partial x_2)^2$ for insulin as +3 and for hemoglobin as +2.3, which agrees with the results obtained within an order of magnitude. Buffer solutions 0.1M were used in our experiments, so that the process involved not only the transport of the protein and proton to the one side but also a simultaneous transport of Na^+ to the other side. Hence, the real pH gradient can be regarded neither as linear nor as time independent.

Table II summarizes measurements of the permeability of insulin and hemoglobin at different pH gradients through the membrane. The permeability of these proteins through the membrane is accelerated with increasing pH gra-

TABLE II
Permeability of Insulin and Hemoglobin Through Membranes at Different pH Values

Protein	pH[a] I	pH[a] II	α[b]	Permeation coefficient $P \times 10^6$, cm^2/sec
Insulin	1.9	2.0	0	0.87
	3.6	6.9	0	0.58
	3.6	7.4	0	2.92
	2.9	9.2	0	6.1
	2.9	9.2	0.5	3.92
	2.9	9.2	0.85	0.99
Hemoglobin	6.7	5.4	0.5	0.2
	6.7	6.9	0.5	0.3
	6.7	7.7	0.5	1.1
	6.2	9.2	0.5	4.0

[a] I, pH of the cell with protein; II, pH of the cell with 0.1M phosphate buffer.
[b] Degree of ionization of membrane.

dient only if pH on the other side (into which the diffusion proceeds) is such as to cause desorption of the protein. An increase in the initial stage of ionization of the membrane, i.e., an increase in the sodium content in the membrane, slows down the flow of the protein; in spite of this, however, in many cases (Table II) the permeability through the membrane is higher than that in solution.

One possible interpretation of the results was pointed out by Katchalsky,[15] who found that chemical reactions (association and dissociation) might accelerate diffusion through the membrane. Another possible analysis consists in an electrochemical description of the sorption and desorption of protein. From the difference in pH on the two sides of the membrane, one may determine the difference of the electrochemical potential

$$\Delta\varphi = 59.1B(\mathrm{pH}_1 - \mathrm{pH}_2) \tag{4}$$

where the parameter B can be determined from the potentiometric characteristic of the membrane.[16] In this case the flow of the protein can be regarded as a partial electrophoresis in a porous medium. At the same time, the energy gain of the process proceeds at the expense of a change in the sign of the protein charge when the pH passes through the isoelectric point. The actual difference of the electrochemical potential of the protein is

$$\Delta\mu = RT \ln \frac{c_1}{c_2} + \mathrm{F}(Z_1\varphi_1 - Z_2\varphi_2) \tag{5}$$

where the indices 1 and 2 correspond to the concentration c, charge Z, and potential of the cation exchanger φ on the two sides of the membrane.

For insulin, the transition from pH 3.0 to pH 9.2 is connected with a change in the charge $Z = +9$ to $Z = -6$ per macromolecule.[17] In this way, during the protein transport in the pH gradient, the driving force ($\Delta\mu$) of the process increases at the expense of a change in the protein charge. If the difference in pH is such that the protein charge Z does not change its sign, the contribution of the electrostatic component to the quantity is less effective.

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