Characteristics of Water in Polypeptide Membrane

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ABSTRACT: In order to elucidate the character of water in polypeptide membranes, sorption, and diffusion of water vapor were measured in membranes of poly(L-leucine) (**I**) and poly(γ -methyl L-glutamate) (**II**). Water sorption in **I** is about 1/5 of that in **II** in a high relative vapor-pressure region and is about a hundred-fold greater than in polyolefines. The apparent diffusion coefficient of the system of water vapor and **I** at low temperature at a relative vapor pressure $p/p_0 = 0.8$ is lower than at $p/p_0 = 0.2$. This is due to the clustering tendency of water molecules in **I**. At a low temperature, the diffusion coefficients of the systems of ions and **I** are smaller than those of ions and **II**. This is also attributable to water clustering in **I**. Shear moduli of water-containing **I** and **II** membranes were measured by the free-damping oscillation method at certain fixed relative vapor pressures. Though the shear modulus of **I** is lower than that of **II** in the dry state, the increase of the former with increasing water content is remarkable, and exceeds the value for **II** by a volume fraction of water of about 0.1. This also reflects a rigid water structure due to clustering in **I**.

KEY WORDS Poly(L-leucine) / Poly(γ -methyl L-glutamate) / Sorption /
Diffusion Coefficient / Shear Modulus / Water Clustering / Rigid Water
Structure /

It has been shown that the permeabilities of poly(L-leucine) (PLL) and poly(γ -methyl L-glutamate) (PMLG) membranes to water are of the same order, but that PLL membranes are more efficient than PMLG membranes for separating water-soluble solutes.¹ It is supposed that the dissolution of water-soluble solute in the PLL membrane is poor because of the hydrophobic nature of the side chain in spite of the rather high diffusivity of water in the membrane. Also, it seems that PLL membranes become somewhat rigid when exposed to water. From these observations, it may be inferred that water in PLL forms rigid clusters which cannot behave like free water. Although the clustering tendency of water in hydrophobic polymers has been reported in many cases,² the effect of this on membrane characteristics is not clear, probably because the usual hydrophobic polymer sorbs too little water to show a change in the membrane characteristics. Since the sorption of water in various polypeptides takes place in the rather large side-chain region between helices,³ the amount

sorbed is expected to be greater than that in ordinary hydrophobic polymers. In this paper, in order to elucidate the behavior of water toward a membrane in regard to the characteristics of the membrane, the sorption and diffusion of water vapor at various relative vapor pressures and temperatures, ionic permeabilities at various temperatures, and the shear moduli of water-containing membranes at various relative vapor pressures are examined using the PLL membrane, and a comparison in these respects with the more hydrophilic PMLG membrane is made.

EXPERIMENTAL

Materials

Poly(L-leucine) (PLL) was prepared by the polymerization of L-leucine *N*-carboxyanhydride (NCA) by triethylamine as an initiator in benzene for one week at 30°C. The viscosity-average molecular weight using trifluoroacetic acid solution is 2.7×10^4 . The PLL membrane was prepared by

casting on the glass plate at 50°C, pouring the 1% benzene solution at 70°C. After the solvent was removed, the residual solvent was substituted by methyl alcohol, and the membrane thus formed was dried and annealed at 80°C for 24 hours under vacuum. The thickness of the membrane was about 2×10^{-2} cm. The density of the PLL membrane observed by the floatation method using toluene-carbon tetrachloride solution was 0.990 g cm^{-3} . Poly(γ -methyl L-glutamate) (PMLG) was supplied by Kyowa Hakko Kogyo Co. Ltd. The molecular weight was about 1.5×10^5 . The PMLG membrane was prepared by the same method as that of PLL except that the original cast solution was 5% ethylene dichloride solution. The thickness of the membrane was also 2.0×10^{-2} cm. The density of the membrane was 1.32 g cm^{-3} . Both the PLL and the PMLG membranes had the α -helical structure, as confirmed by infrared adsorption spectra. The KCl used for the ionic-permeability measurement was of extra pure grade.

Sorption and Permeation of Water Vapor

Sorption and permeation were studied mainly for PLL in this study. Sorption experiments were made by a gravimetric sorption apparatus using a quartz spring balance.⁴ Sorption isotherms were expressed as the relation between the amount of sorbed water mol/(g of residue), n , and relative water vapor pressure p/p_0 . The isotherms were observed at 10, 25, and 40°C. For permeation experiments, the Rouse-type apparatus was used.⁵ On the feed-side or high-pressure side of the membrane, water vapor at a controlled pressure was supplied, by the temperature regulation of water in a trap connected to the cell. The pressure on the permeant-side or low-pressure side cell was essentially zero, and liquid nitrogen was used to trap the permeant vapor. From the slope of the steady-state straight-line portion of the permeation curve (amount permeated *vs.* time relation) the permeability coefficient $\bar{P}$ ($\text{cm}^3 \text{ stp cm}^{-1} \text{ s}^{-1} \text{ cmHg}^{-1}$, the bar denotes concentration average.) was calculated as usual. Since the sorption isotherm does not obey Henry's law, the "apparent" average diffusion coefficient $\bar{D}$ ($\text{cm}^2 \text{ s}^{-1}$) was obtained by $\bar{P}/S$, where S is the solubility coefficient.

Ionic Permeability

The measurement of the permeability of ions through the membranes was carried out in a manner

analogous to that of Tasaka.⁶ The whole apparatus was made by Acrylate resin (Mitsubishi Rayon Co. Ltd., Japan). The membrane was set up with Teflon plate support and silicon rubber packing on the permeation cell. The effective permeation area was 7.07 cm^2 . The feed-side compartment of the cell was filled with an aqueous solution of KCl of initial concentration, $C_1 \text{ mol l}^{-1}$ ($5 \times 10^{-3} \text{ mol l}^{-1}$ for PLL and $10^{-1} \sim 2 \times 10^{-3} \text{ mol l}^{-1}$ for PMLG). The salt concentration of the permeated-side compartment was $C_2 \text{ mol l}^{-1}$ ($5 \times 10^{-4} \text{ mol l}^{-1}$ for PLL and $1.25 \times 10^{-2} \sim 2 \times 10^{-4} \text{ mol l}^{-1}$ for PMLG). The change in the concentration with time was measured with an electroconductivity-meter (Toa Electronics, Ltd., Japan, CN-5B Type). The changes in both the feed and permeant concentration with time were small compared with the initial concentrations. The whole cell was immersed in a water bath whose temperature was controlled to within 0.1°C . The measurement of ionic permeability was carried out at temperatures between $10 \sim 35^\circ\text{C}$ at 5°C intervals. From the steady-state straight line of the relation between conductance of the permeant and time, the flux of J_s (which is equal to the flux of ion in the case of KCl) was calculated. Then, using eq 1, permeability of salt or ions P_s ($\text{cm}^2 \text{ s}^{-1}$) was obtained,

$$P_s = J_s \Delta x / (C_1 - C_2) \quad (1)$$

where, Δx is the membrane thickness. The diffusion coefficient of the system D_s ($\text{cm}^2 \text{ s}^{-1}$) was obtained by P_s/K_s , where K_s is the solubility coefficient of salt. In order to obtain K_s , about 0.08 g of the membrane was immersed in a salt solution of desired concentration, and after the sorption equilibrium was attained, the membrane was removed, rinsed, blotted, and immersed in distilled water. From the value of conductance of the solution at desorption equilibrium, the amount of salt $\bar{C}$ (mol l^{-1} in swollen membrane), which had been present in the membrane, was calculated. The K_s is defined as the ratio of $\bar{C}$ to C (mol l^{-1} on initial solution). In this case, K_s is independent of concentration and temperature.¹³

Shear Modulus of Water-Containing Membrane

In order to obtain the shear modulus of a membrane in which a known amount of water is sorbed, we made the observation for the membrane in a closed system (Figure 1). The usual free oscillating pendulum, consisting a sample mem-

Figure 1. Schematic diagram of torsion pendulum in a closed system: F, sample membrane; M, mirror; I, inertia rod; EM, electromagnet; W, water flask; Ma, manometer; LS, lamp scale; T, trap; S, switch; VP, vacuum pump.

brane F, mirror M and inertia rod I on both ends of which iron nuts are attached, was set up in an airtight chamber system. Following the evacuation of the whole system at 25°C for 3 to 4 days, water vapor was introduced into the system successively by controlling the temperature of water-supply flask W. The relative water-vapor pressure thus introduced was observed by the manometer Ma. The sorption equilibrium was attained in two days, as could be judged by the pressure change in the manometer. To induce the oscillation, the electromagnet EM was excited instantaneously to produce the initial torsional angle of 10°. After the initiation of the oscillation, the period and amplitude of the free damping oscillation were measured by the lamp scale LS, as usual. The frequency in this case was about 0.22 Hz. The relative modulus G_r , which is a measure of the change of rigidity, is defined as the ratio of the modulus under a certain relative vapor pressure G_i to that in the dry state G_o , and is obtained by eq 2,

$$G_r = (G_i/G_o) = (T_o/T_i)^2 \quad (2)$$

where, T_o and T_i are periods of oscillation in the respective cases. The logarithmic decrement Δ is calculated by the average of the logarithm of the ratio of amplitudes over one cycle (eq 3), and is related to the energy loss resulting from free damping oscillation.⁷

$$\begin{aligned} \Delta &= \ln(A_0/A_1) = \ln(A_1/A_2) \\ &= \dots = \ln(A_{n-1}/A_n) \\ &= \text{const} \end{aligned} \quad (3)$$

where the subscript n on the amplitude A denotes the number of the cycle. The absolute value of a shear modulus G_o was obtained by a torsional free oscillating rheometer (Ohyo Denki Kenkyusho, Co. Ltd., Japan, RD-1 Type), employing dry samples of PLL and PMLG membranes in evacuated state (10^{-2} mmHg). G_o is calculated by eq 4,

$$G_o = L \left\{ \frac{(4\pi^2 + \Delta^2)I}{T^2} - k_o \right\} \quad (4)$$

where, I is the moment of inertia and k_o is the torsion constant of the wire. The values used in this study were $I = 1.33 \times 10^3 \text{ g cm}^{-2}$, $k_o = 0.734 \times 10^2 \text{ dyn cm}^{-1}$. L is the shape factor represented by eq 5.

$$L = \frac{l}{\beta b c^3} \quad (5)$$

where l is the sample length, 5 cm, and b is the sample width, 1 cm, and c is the thickness, 0.008 cm. $\beta = [(1 - 0.63 c/d)/3]$ is the constant, being 1/3, in this case.

RESULTS AND DISCUSSION

Sorption and Permeation of Water Vapor

Sorption isotherms of water in PLL at various temperatures are shown in Figure 2. The effect of the hydrophobic nature of PLL is also shown in this figure. The amount of water in PLL is less than 0.1 mol per residue and is about 1/5 of that in PMLG (dotted line in Figure 2) at high relative pressure at 25°C. However, water sorption in PLL is a hundred-

Figure 2. Sorption isotherms of water by PLL (10, 25, and 40°C) and by PMLG (25°C).

fold greater than that in polyolefines, if comparison is made in the high relative vapor-pressure range. The difference between water solubility in PLL and that in polyolefines is due to the relatively large inter-helix regions of PLL. In strong contrast to the isotherms of ordinary hydrophobic polymer–water systems, those of the PLL–water system depend remarkably on temperature. The isotherms of the PMLG–water system were almost independent of temperature.⁹ Ordinary hydrophilic polymer shows large temperature dependence on water sorption at low relative vapor pressure; but on the other hand, the water-sorption isotherms of PLL at different temperatures deviate from each other at high relative pressures. This result shows that the heat of sorption increases with increasing vapor pressure; that is, water in PLL forms a structure more compact than the liquid-like structure in the high vapor-pressure range. To make clear the size of water cluster in membrane, the concept of cluster function defined by Zimm–Lundberg¹⁰ was applied to the present systems,

$$G_{11}/V_1 = -V_2 \left[\frac{\partial(a_1/v_1)}{\partial a_1} \right]_{p,T} - 1 \quad (6)$$

where v_1 and v_2 are the volume fractions of water and polymer, respectively, V_1 is the molecular volume of water, a_1 is the activity of water vapor,

Figure 3. Cluster function of water in PLL (10, 25, and 40°C) and in PMLG (25°C).

which is approximated by the relative vapor pressure p/p_0 , and G_{11} is the cluster integral. The calculation result is shown in Figure 3 as a plot of the cluster function against the volume fraction of water in the membrane. Since the present system is composed of helical rods, a rubbery side-chain region and water, the values of Figure 3 may indicate the clustering tendency, and the absolute values should be considered with caution. At all temperatures, the cluster function shows that the water in PLL is segregated below $v_1 \approx 0.006$, where the $G_{11}/V_1 \approx -1$. After some sites have been filled up (corresponding v_1 is independent on temperature), the clustering tendency becomes remarkable. This rapid increase in the cluster function is significant compared with the case of relatively hydrophobic polymers, *e.g.*, poly(vinyl acetate)¹¹ and poly(dimethylsiloxane).¹² The cluster function in the case of PMLG (25°C)⁹ is clearly lower than that of PLL at the same volume fraction of water in the polymer.

The steady-state permeability $\bar{P}$ of water vapor through the PLL membrane *vs.* the relative vapor pressure p/p_0 at various temperatures is shown in Figure 4, together with the steady-state diffusion coefficient $\bar{D}$. Both the $\bar{P}$ and $\bar{D}$ increase with temperature, but the dependency of $\bar{D}$ on relative vapor pressure is somewhat complex. $\bar{D}$ passes through a maximum as the relative vapor pressure increases, and the vapor pressure corresponding to the maximum of $\bar{D}$ shifts to the high relative vapor-

Figure 4. Steady-state permeability coefficient $\bar{P}$ and diffusion coefficient $\bar{D}$ of water in PLL at 10, 25, and 40°C vs. relative vapor pressure p/p_o of water.

Figure 5. Arrhenius plot of the diffusion coefficient of water in PLL (solid line represents the case when relative vapor pressure p/p_o is held to 0.20 at each temperature, broken line represents the case in which p/p_o is held to 0.8) and PMLG (dotted line, p/p_o is held to 0.22).

pressure side as the temperature rises. This may be ascribed to the following two factors: the plasticizing effect of segregated water and the clustering tendency of water, shown in Figure 3. To make a clear assessment of these respective effects, the

values of $\bar{D}$ at each temperature were evaluated at fixed relative vapor pressures, 0.2 and 0.8. Figure 5 shows the results in the Arrhenius plot. At higher temperatures, $\bar{D}$ at $p/p_o = 0.8$ is larger than that of $p/p_o = 0.2$. This suggests that the plasticizing effect of water is rather prominent in this temperature region. At lower temperatures, the clustering tendency and the change in structure of water reduce $\bar{D}$ at high relative pressure. This structural change from free water was confirmed by preliminary IR and broad-line NMR measurements. The details for this will be given elsewhere. The activation energies at 0.2 and 0.8 of p/p_o , are 15.3 and 19.1 kcal mol^{-1} , respectively. For the sake of comparison, the value of $\bar{D}$ of the water-PMLG system, evaluated at $p/p_o = 0.22$,¹ is also shown in the figure. In the latter system, the effect of structural change in water with temperature is considered to be minor, and thus the activation energy of this system, 10.3 kcal mol^{-1} , is lower than that of PLL-water system.

Ionic Permeability

Figure 6 shows the permeability coefficient P_s of KCl through PLL against temperature, in the

Figure 6. Arrhenius plot of permeability coefficient P_s and diffusion coefficient D_s of KCl in PLL (solid line) and PMLG (broken line) membrane.

Arrhenius plot. The data of PMLG¹³ is also given in the figure. It has been shown that P_s does not depend on either C_1 or C_1/C_2 in the present concentration range, and so P_s may be considered to be constant.¹³ The activation energies of permeation for PLL and PMLG are obtained as 19 and 14 kcal mol⁻¹, respectively. The solubility coefficients K_s of KCl in PLL and PMLG are obtained as 2.5×10^{-2} and 5.2×10^{-2} , respectively, and these values are independent of ionic concentration and temperature.¹³ Using these values, D_s is calculated, and is shown in Figure 6. Though in the high temperature region, D_s in PLL is higher than that in PMLG, at low temperatures this relation is reversed. Since the amount of water in the membranes remains constant over the temperature range studied, this behavior may be explained by the structural change of water with temperature in the membrane. Particularly in the PLL membrane, it is considered that water makes relatively rigid clusters in the low-temperature range, and that these clusters reduce the diffusivity of ion in the membrane.

From the results of water-vapor sorption and diffusion, and ionic permeability in PLL membrane, it is concluded that the hydrophobic nature of the PLL side chain not only reduces the water content compared with that of PMLG membrane, but also facilitates the clustering tendency of water which leads to a decrease in solute solubility in PLL because of the rigid—*an ice-like*—structure. This slight solubility of solute may be the main reason for the excellent separation of water-soluble solute through PLL membrane.¹

Shear Modulus of Water-Containing Polypeptide Membrane

The structure of water in PLL may be confirmed by the mechanical behavior of water-containing polypeptide membranes. In Figure 7, the relative modulus G_r and the logarithmic decrement Δ of the PLL-water system are plotted against the relative water-vapor pressure p/p_o . The sorption isotherm, which is the volume fraction of water v_1 vs. p/p_o relation, is also shown in the figure. Though the modulus increases with v_1 gradually up to $v_1 \approx 0.004$, a further increase with v_1 is significant, and this may indicate that a cluster of water molecules makes a relatively rigid structure in PLL. Namely, the water cluster may carry out the role of a filler¹⁴ in regard to the modulus. The Δ does not

Figure 7. Relative modulus G_r and logarithmic decrement Δ of water-contained PLL membrane vs. relative vapor pressure p/p_o . The isotherm is expressed by the relation between volume fraction of water in the membrane v_1 and p/p_o .

change appreciably, and this shows that the frictional energy loss in the side-chain region of PLL is not affected by adsorbed water molecules. The results for PMLG membrane are shown in Figure 8. G_r decreases once near $p/p_o = 0.1$. The sorption of water molecules at its initial stage may occur on the relatively polar carboxyl group of the side chain, and enhances the molecular movement of the side chain by the plasticizing effect. However, since the other part of side chain of PMLG is hydrophobic, with a further increase of water in the membrane, the same behavior as that of the PLL membrane is observed. The decrease of Δ shows that the sample becomes elastic by an interaction between side chain and water that is stronger than that in the PLL membrane. The absolute moduli of the dry samples of PLL and PMLG membranes, described in the experimental section, were obtained as $G_o = 1.89 \times 10^{10}$ dyn cm⁻² for PLL and $G_o = 2.41 \times 10^{10}$ dyn cm⁻² for PMLG, respectively. Using these values, in Figure 9, the absolute modulus G is plotted against the volume fraction of water v_1 in the membrane for both PLL and PMLG membranes. In spite of the high modulus of PMLG

Figure 8. Relative modulus G_r and logarithmic decrement Δ of water-contained PMLG membrane vs. relative vapor pressure p/p_0 . The isotherm is expressed by the relation between volume fraction of water in the membrane v_1 and p/p_0 .

Figure 9. Absolute value of shear modulus G vs. volume fraction of water v_1 for water-contained PLL and PMLG membranes.

membrane in the dry state due to the interaction among side chains, the increase in G with v_1 for PLL is remarkable compared with that of PMLG, and the relations for both membranes intersect at about $v_1=0.009$. This behavior agrees well with the rigidity of the PLL membrane in the water and with the various transport phenomena described above, and also gives direct evidence for the rigid structure of water in PLL membrane.

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