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Volumetric properties of polyols (ethylene glycol, glycerol, *meso*-erythritol, xylitol and mannitol) in relation to their membrane permeability: group additivity and estimation of the maximum radius of their molecules

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The relationship of molecular volume and maximum molecular radii to the ability of some polyols, ethylene glycol, glycerol and *meso*-erythritol, to permeate the *Chara* cell membrane and to the inability of one of the polyols, mannitol, to permeate the *Chara* cell membrane, was examined by measuring the partial molar volumes of the polyols, $\bar{V}_{m(2)}$. Analysis of $\bar{V}_{m(2)}$ at infinite dilution showed that group additivity is maintained for all the groups, i.e., CH_2OH and CHOH, of the polyols tested. However, as the permeability and impermeability could not be related to the geometrical properties of the polyol molecules based only on the thermodynamic quantities, molecular models of the polyol molecules were constructed using the CPK(Corey-Pauling Koltum) molecular model, which is designed to have the van der Waals radius of 1 Å equivalent to 1.25 cm. The results showed that the maximum radius of the water-filled pore (hydrophilic channel) should be 3.2–3.3 Å, and the longer the axial length and maximum radius of the polyol molecule, the lesser was its permeative ability. All the experimental and analytical results and inferences support the idea that water molecules pass across the cell membrane through a narrow pore in a single-file fashion.

Introduction

Theories that cell membranes have capillary or pore structures going through them have a considerably long history in the membrane physiology of plant and animal cells. Recently, however, serious criticism has arisen against one of the theories, the equivalent pore theory, that expresses the capillary or pore radius of originally complex cross sections in terms of equivalent pore radius which is simply determined from the ratio of osmotic of hydrodynamic to diffusive water permeability [1].

The data also support the existence of capillary or pore structures across the cell membrane through which water and solute molecules pass with different permeabilities. Collander [2,3] showed that, among polyols, ethylene glycol whose molecular weight is the smallest (mol. wt. = 62.07 g mol^{-1}), very easily permeates the *Chara* cell membrane; glycerol with a larger molecular weight (mol. wt. = 92.10 g mol^{-1}) permeates with less ease; and *meso*-erythritol with a still larger molecular weight (mol. wt. = 122.12 g mol^{-1}) shows the least ability to permeate the membrane. Recently, Kiyosawa and Okihara [4] showed that molecules of mannitol (mol. wt. = 182.17 g mol^{-1}), one of the polyols, cannot permeate the *Chara* cell membrane. These reports support the idea that, at least, the *Chara* cell membrane has a capillary or pore structure which permits small hydrophilic molecules to pass through it with different degrees of permeability dependent primarily on the molecular weight of the solute and does not permit passage of molecules larger than the capillary or pore radius. A similar relationship between the solute permeability and the molecular weight has been implicitly confirmed with bovine red cells [5].

These facts suggest that the maximum radius of hydrophilic small nonelectrolyte molecules in the homologous series, which have difficulty permeating or cannot permeate the membrane, can be used to determine the 'radius' of the water-filled pore (hydrophilic

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channel) and/or gap among the molecules constructing the cell membrane through which water molecules and/or solute molecules pass (cf. Ref. 6).

The present study was done to check whether or not the membrane permeability of the small hydrophilic nonelectrolyte molecules, i.e., polyol molecules, can be related to their size. First, the partial molar volume of the polyols, which involves the van der Waals volume of the polyol molecules [7–9], was measured and analyzed to find whether or not group additivity (cf. Refs. 10–12) could be confirmed among the partial molar volumes of the polyols. The forms and sizes of the polyol molecules were examined by constructing their molecular models using the CPK (Corey-Paulin Koltum) model, which is designed to have the van der Waals radius of 1 Å equal to 1.25 cm.

Materials and Methods

Ethylene glycol and glycerol used were extra pure reagent grade for amino acid analysis and spectrophotometry, respectively, purchased from Wako Pure Chemical Industries, Ltd. *meso*-Erythritol, xylitol and mannitol used were purchased from Wako Pure Chemical Industries, Ltd. and purified by recrystalization after being dissolved in hot water.

The apparent molar volume of the polyols, ϕ_v, is related to the densities by the equation

$$\phi_v = \frac{M_2}{d} + \frac{1000}{m}\left(\frac{1}{d} - \frac{1}{d_1}\right) \tag{1}$$

where m is the molal concentration of the polyol solute of molecular weight M_2 (g mol^{-1}), and d (g cm^{-3}) and d_1 (g cm^{-3}) are the densities of the solution to be examined and pure water, respectively [13,14]. The derived ϕ_v values for the polyols exhibit a linear concentration dependence ([15] cf. Results). These results were fitted by the least-squares method to equations of the form

$$\phi_v = \phi_v^0 + a \cdot m \tag{2}$$

where m represents the molal concentration of the polyol and ϕ_v^0 is the apparent molar volume of the polyol at infinite dilution which is equal to the partial molar volume of the polyol at infinite dilution $V_{m(2)}^0$. The partial molar volume of the polyol, $V_{m(2)}$, is related to ϕ_v by equation

$$V_{m(2)} = \phi_v + m\left(\frac{\partial \phi_v}{\partial m}\right)_{T,P} \tag{3}$$

and the partial molar volume of the water of the aqueous polyol solutions, $V_{m(1)}$, is related to ϕ_v by the equation

$$V_{m(1)} = V_{m(1)}^{*} - \frac{M_1 m^2}{1000}\left(\frac{\partial \phi_v}{\partial m}\right)_{T,P} \tag{4}$$

where $V_{m(1)}^{*}$ and M_1 are the molar volume of pure water (cm^3 mol^{-1}) and the molecular weight of water (g mol^{-1}), respectively.

The density of the aqueous polyol solutions, d, was measured with a mechanically oscillating densimeter (Shibayama SS-D-200) at 25.00 ± 0.02°C.

Results

Fig. 1 shows the apparent molar volumes of the polyols, ϕ_v, as functions of the molal concentration m. Clearly, the figure shows the validity of Eqn. 2.

Table I shows the partial molar volumes of ethylene glycol, glycerol, *meso*-erythritol, xylitol and mannitol, $V_{m(2)}$, and those of the water, $V_{m(1)}$, of their aqueous solutions as functions of the molal concentration m.

The partial molar volumes of the polyols at infinite dilution, $V_{m(2)}^0$, were 54.42 cm^3 mol^{-1} for ethylene glycol, 70.84 cm^3 mol^{-1} for glycerol, 86.83 cm^3 mol^{-1} for *meso*-erythritol, 102.12 cm^3 mol^{-1} for xylitol and 119.22 cm^3 mol^{-1} for mannitol. These values are almost equal to those of the respective polyols measured at 24°C by DiPaola and Belleau [15].

The partial molar volumes of the polyols, $V_{m(2)}$, increase with an increase in the molar concentration, suggesting that the solute-solvent interaction, in other words, 'hydration' of the solute molecules changes with addition of polyol molecules, namely, through a change in the solute–solute interaction. The quotation marks around hydration indicate that little is known about the exact nature of hydration, even of nonelectrolyte molecules [16,17].

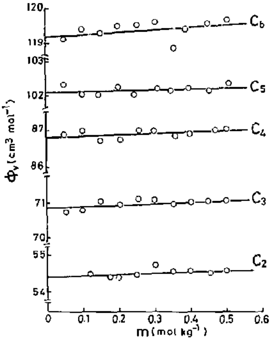

Fig. 1. Apparent molar volumes of the polyols, ϕ_v, as functions of the molal concentration m. C_2, ethylene glycol; C_3, glycerol; C_4, *meso*-erythritol; C_5, xylitol, and C_6, mannitol.

TABLE 1

Partial molar volumes of the polyols, $V_{m(2)}$, and the water, $V_{m(1)}$, of aqueous polyol solutions as functions of concentration m

Partial molar volumes of the polyols at infinite dilution, $V^0_{m(2)}$, are also shown.

$V_{m(2)}$ and $V_{m(1)}$ as functions of concentration	$V^0_{m(2)}$ (cm³ mol⁻¹)
Ethylene glycol	
$V_{m(2)} = 54.42 + 0.8004\ m$ (cm³ mol⁻¹)	54.42
$V_{m(1)} = 18.069 - 0.007210\ m^2$ (cm³ mol⁻¹)	
Glycerol	
$V_{m(2)} = 70.84 + 1.0962\ m$ (cm³ mol⁻¹)	70.84
$V_{m(1)} = 18.069 - 0.009874\ m^2$ (cm³ mol⁻¹)	
meso-Erythritol	
$V_{m(2)} = 86.83 + 0.79416\ m$ (cm³ mol⁻¹)	86.83
$V_{m(1)} = 18.069 - 0.007153\ m^2$ (cm³ mol⁻¹)	
Xylitol	
$V_{m(2)} = 102.12 + 0.59931\ m$ (cm³ mol⁻¹)	102.12
$V_{m(1)} = 18.069 - 0.005398\ m^2$ (cm³ mol⁻¹)	
Mannitol	
$V_{m(2)} = 119.22 + 1.3218\ m$ (cm³ mol⁻¹)	119.22
$V_{m(1)} = 18.069 - 0.011906\ m^2$ (cm³ cm⁻¹)	

On the other hand, the partial molar volumes of the water of the respective aqueous polyol solutions, $V_{m(1)}$, were almost constant against the molar concentration and equal to the molar volume of pure water, 18.07 cm³ mol⁻¹, suggesting that the solvent (water)–solvent (water) interaction is insignificantly affected by addition of the solute molecules as in the case of aqueous glucose, sucrose and raffinose solution [18].

Discussion

Assuming that the group partial molar volume of both CH_2OH groups of ethylene glycol at infinite dilution are equal to each other, $V^0_{m(CH_2OH)}$ is calculated to be 27.21 cm³ mol⁻¹, which is almost equal to that reported by Harada et al. [19]. Furthermore, if group additivity is held for all group partial molar volumes of the groups of the polyols examined, $V^0_{m(CHOH)}$ is calculated from the equation $V^0_{m(glycerol)} - V^0_{m(ethylene\ glycol)}$ as 16.42 cm³ mol⁻¹. In turn, we can calculate the partial molar volumes of *meso*-erythritol ($CH_2OH \cdot CHOH \cdot CHOH \cdot CH_2OH$), xylitol ($CH_2OH \cdot CHOH \cdot CHOH \cdot CHOH \cdot CH_2OH$) and mannitol ($CH_2OH \cdot CHOH \cdot CHOH \cdot CHOH \cdot CHOH \cdot CH_2OH$) theoretically based only on these values from the equations

$$V^0_{m(erythritol)} = V^0_{m(ethylene\ glycol)} + 2V^0_{m(CHOH)} = 87.26\ \text{cm}^3\ \text{mol}^{-1} \quad (5)$$

$$V^0_{m(xylitol)} = V^0_{m(ethylene\ glycol)} + 3V^0_{m(CHOH)} = 103.68\ \text{cm}^3\ \text{mol}^{-1} \quad (6)$$

$$V^0_{m(mannitol)} = V^0_{m(ethylene\ glycol)} + 4V^0_{m(CHOH)} = 120.10\ \text{cm}^3\ \text{mol}^{-1} \quad (7)$$

Evidently, these calculated partial molar volumes of *meso*-erythritol, xylitol and mannitol are approximately equal to the respective partial molar volumes of the polyols obtained by the present experiments. Here, additivity of the group partial molar volumes has been verified to be held for the groups of the polyols examined. This means that the CHOH and CH_2OH groups of the respective polyol molecules are equal to each other in thermodynamic volumetric property.

Since the polyols examined consist of only two types of groups, i.e., CH_2OH and CHOH, the validity of the group additivity for these groups suggests two possibilities about their molecular structures in an abstract sense. In one possible case, all the polyols should have one and the same maximum radius which should be equal to the van der Waals radius of the largest group, CH_2OH, among the groups of polyol molecules.

In the other possible case, each polyol molecule should have various maximum van der Waals radii because the carbon atoms in the normal saturated hydrocarbons are tetrahedral and the OH-groups do not bind to the respective carbon atoms in a row.

Which case is true cannot be determined only from the thermodynamic quantities obtained in the present experiments and analysis. To solve this problem, I tried to construct molecular models of the polyols in question using the CPK (Corey-Pauling Koltum) molecular model which is designed to have the van der Waals radius of 1 Å equal to 1.25 cm. The CPK models suggested that the surface of each polyol molecule is very rough and the water molecules have access to the surface of each polyol molecule. Therefore, each polyol molecule cannot be assumed to be a sphere or a spheroid of smooth surface as was done for alkyl alcohol molecules [9]. The 'hydrated' volumes of the polyol molecules v_2 can be obtained with some assumptions [9]. However, assuming the polyol molecules to be a sphere or a spheroid and calculating the radius r of a spherical polyol molecule and the minor axis q of a spheroidal polyol molecule from the equations

$$v_2 = \tfrac{4}{3}\pi r^3 \quad (8)$$

$$v_2 = \tfrac{4}{3}\pi \beta q^3 \quad (9)$$

respectively, where $\beta = p/q$ (p: the major axis) obtained by assuming the CPK model of the spheroidal polyol molecules with a caliper, would give somewhat underestimated r and q.

Thus, to interpret the permeability and impermeability of the cell membrane to each polyol molecule, I tried to relate them to the length of the maximum van der Waals radius of the CPK molecular model because all of these models were approximately spheroid with very rough surfaces which were accessible to water.

TABLE II

Axial length, l, and the maximum radius, r_{max}, of the polyol molecules measured from their CPK (Corey-Pauling Koltum) molecular models

Some variations in the values of l and r_{max} arise from taking into consideration the rotation of the OH-groups around the C-O bond and some differences in the conformations of the polyols.

Polyol	Axial length (Å)	Maximum radius (Å)
$CH_2OH{\cdot}CH_2OH$	5.6– 6.8	2.4–2.5
$CH_2OH{\cdot}CHOH{\cdot}CH_2OH$	6.7– 8.0	2.8
$CH_2OH{\cdot}(CHOH)_2{\cdot}CH_2OH$	8.0– 9.3	3.1–3.2
$CH_2OH{\cdot}(CHOH)_3{\cdot}CH_2OH$	9.6– 9.9	3.2–3.3
$CH_2OH{\cdot}(CHOH)_4{\cdot}CH_2OH$	10.1–10.7	3.4

Table II shows the axial length, l, and length of the maximum van der Waals radius, r_{max}, of the polyol molecules thus measured. The length of the maximum van der Waals radius of the mannitol molecule which cannot permeate the *Chara* cell membrane [4] is 3.4 Å; it is the longest. Thus, the longer the axial length and the length of the maximum van der Waals radius, the less ability of the molecule has to permeate the *Chara* cell membrane, although the permeability of the *Chara* and erythrocyte cell membranes to xylitol has not yet been measured.

The fact that the maximum van der Waals radius of the mannitol molecule, which cannot permeate the *Chara* cell membrane, is 3.4 Å and that of *meso*-erythritol, which has much less permeation ability is 3.1–3.2 Å indicates that the maximum radius of the hydrophilic channel and/or gap among the molecules constructing the cell membrane should be 3.2–3.3 Å. This numerical value of the maximum van der Waals radius of the hydrophilic channel and/or gap among the molecules constructing the cell membrane is just half of that reported recently by Toon and Solomon [20] for human erythrocytes, but is the same as that adopted in computer simulation of diffusion and bulk flows through a narrow pore by Levitt [21]. When exact values of the maximum van der Waals radii of *meso*-erythritol, xylitol and mannitol molecules can be measured in the future, the value of the maximum radius of the hydrophilic channel and/or gap among the molecules constructing the cell membrane should be modified.

The results obtained by the present experiments and analysis support the idea that water molecules pass across the cell membrane through narrow pores in a single-file fashion [1,22–27], because the van der Waals radius of a water molecule is 1.6 Å.

In the *Chara* internodal cell, ions do not pass through the hydrophilic channel together with the bulk water flow [6]. It is very difficult to determine the size of ions both in their aqueous solutions and in the pores or ion channels. Assuming, hwoever, the ion radii of the Li^+, Na^+, K^+, Rb^+, Cs^+, Mg^{2+} and Ca^{2+} are 0.70, 0.98, 1.33, 1.49, 1.65, 0.78 and 1.06 Å [28] in the pore, or ion channel, respectively, and those of the Cl^-, NO_3^- and SO_4^{2-} are 1.21, 1.29 and 2.30 Å [29], respectively, all of these ions should be able to pass through the hydrophilic channel in the *Chara* cell membrane. To explain their inability to permeate a hydrophilic channel whose radius should be larger than the ions, we must assume existence of some Coulombic repulsive force due to highly charged portions at the inlet of the hydrophilic channel as well as steric effects. We may also have to assume different 'chemical affinities' of the inlet of the hydrophilic channel and/or ion channels to the respective ions in quality as well as in quantity. These circumstances also imply that ions should pass through the ion pores, or ion channels, in a single-file fashion (cf. Ref. 30–32).

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