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Influence of pH on Skin Permeation of Amino Acids

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Abstract

Skin permeation of amino acids through excised rat skin was measured at various pH values.

The permeabilities varied with the donor pH and amino acid, indicating that each ionic species of amino acid may have a different permeability. The permeability coefficient of each ion was estimated from the permeability-pH profiles using the dissociation constants. The estimated values for mono-cation and uncharged zwitterion were not dependent on the lipophilicity but on the size of the amino acid, suggesting a porous mechanism of transport. The permeability coefficient was highest for di-cation, followed by mono-cation, positively charged, uncharged and negatively charged zwitterions. The electrical potential difference across the skin was too small to affect the permeation of ions.

The permselective property of skin thus seems to be determined by the difference of diffusivity in aqueous pores of skin due to the hydration of ions and other factors.

In the last decade, a great deal of attention has been given to the administration of peptide drugs via the transdermal route. However, it appears impossible to administer these substances in sufficient amounts without skin permeation enhancement because of their high hydrophilicity and large molecular size. Most studies on this subject thus have focused on chemical enhancers and iontophoretic treatment, and limited information is available on passive transport (Hsieh 1987; Liu & Sun 1994).

Amino acids, which make up peptides, are multipolar ions and their polarity is one origin of the hydrophilicity of peptides. They are also known to be components of the natural moisturizing factor of skin and widely applied in the cosmetic industry. To limit systemic absorption of amino acids for cosmetic purposes or to optimize formulation conditions for systemic delivery of peptides, systematic studies on skin permeation of amino acids are important. Although such a study was begun in 1991 by Ruland & Kreuter, some questions remain. Amino acids are always ionized in aqueous solutions but the fraction of ionic species varies depending on pH of the solutions. The relationship between the skin permeability and ionic fraction should be clear.

In the present study, the skin permeability of amino acids was measured at various pH values in-vitro. The permeability of each ionic species of amino acid was then estimated from this data and compared with the physicochemical properties. The electrical potential difference across the skin was also evaluated as this can affect the permeability of ionic species.

Materials and Methods

Materials

L-Alanine, valine, isoleucine, leucine, lysine and aspartic acid were purchased from Nacalai Tesque Co. (Kyoto, Japan). All were reagent grade and were used without further purification.

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^{3}H-Labelled amino acids were obtained from NEN Research Products (Boston, USA) and were used as received. Other chemicals were also reagent grade and used as received.

Skin permeation studies

Rat skin was freshly excised from the abdomen of male Wistar rats (Japan SLC Inc., Hamamatsu, Japan), aged 7 weeks, after being shaven carefully. The skin sample was mounted between two half diffusion cells with a water jacket connected to a water bath at 37°C, each having 3 mL volume and 0·966 cm^2 effective diffusion area. The receiver compartment was charged with pH 7, 0·1 M and the donor compartment with an appropriate phosphate buffer (pH 3 to 7, 0·1 M). The receiver and donor solutions were stirred at 1440 rev min^{-1} with a star-head bar driven by a synchronous motor; a 14-h period of equilibration was allowed. The receiver solution was replaced with fresh buffer, and the donor solution with 10 mg mL^{-1} amino acid buffer solution and radiolabeled amino acid (1·3 μCi mL^{-1}) was added. After 4 min to ensure achievement of a uniform concentration, a sample (200 μL) was taken from the donor compartment and used as an estimation of initial donor concentration. At specified time intervals, samples (200 μL) were withdrawn from the receiver compartment and an equal volume of fresh buffer was added to keep the volume constant. The samples were immediately placed in a vial containing 4 mL scintillation fluid (Atomlighte, NEN Research Products) and the amount of radioactivity was determined using a liquid scintillation counter (Model LS3801, Beckman, Germany). The pseudo steady-state permeation rates permeability coefficients were calculated.

Measurement of transdermal potential difference

The electrical potential difference across skin was measured under similar conditions to those used in the skin permeation experiments. The skin sample was equilibrated with pH 7, 0·1 M phosphate buffer on the diffusion cell for 14 h. After fresh buffer had replaced the used buffer, the transdermal electrical potential difference was sensed between two salt

bridges (3 M KCl in 3% agar) connected via calomel electrodes to a digital oscilloscope (Model 310, Nicolet, Madison, USA). Data collected on the oscilloscope were input to a personal computer (Model PC-9801DA, NEC, Tokyo) for storage and analysis. The buffer in the donor compartment was replaced with buffers over the pH range 3–6, and the potential difference was evaluated. Each buffer required 40 min to reach equilibration.

Data analysis

Data analysis was by a non-linear least squares regression program, MULTI (Yamaoka et al 1981), which was run on a personal computer.

Results

Skin permeation of amino acids

The physicochemical characteristics of the amino acids studied here are listed in Table 1. The neutral amino acids have almost the same dissociation constants for carboxyl and amino groups, but differ in the length of their alkyl side chains and lipophilicity. The cationic and anionic amino acids, lysine and aspartic acid, have one more amino and carboxyl group and are thus more polar than neutral amino acids.

The skin permeation of neutral amino acids was first evaluated. Fig. 1 illustrates the permeation profiles of valine at various pH values. A pseudo steady-state permeation was achieved after a short lag time (about 2 h), but the slope of the linear portion, the pseudo steady-state permeation rate, seemed to vary depending on pH.

Table 1. Physicochemical characteristics of amino acids studied.

	Mol. wt	log K_{ow}[a]	pK_{a1}[b]	pK_{a2}[b]	pK_{a3}[b]
Alanine	89·09	−2·89	2·39	9·82	
Valine	117·15	−2·08	2·43	9·50	
Isoleucine	131·17	−1·72	2·42	9·66	
Leucine	131·17	−1·61	2·43	9·60	
Lysine	146·19	−3·31	2·20	8·90	10·28
Aspartic acid	133·10	−3·38	1·88	3·65	9·60

[a]*n*-Octanol/water partition coefficient at isoelectric point (Pliska et al 1981), [b]dissociation constant (Budavari 1989).

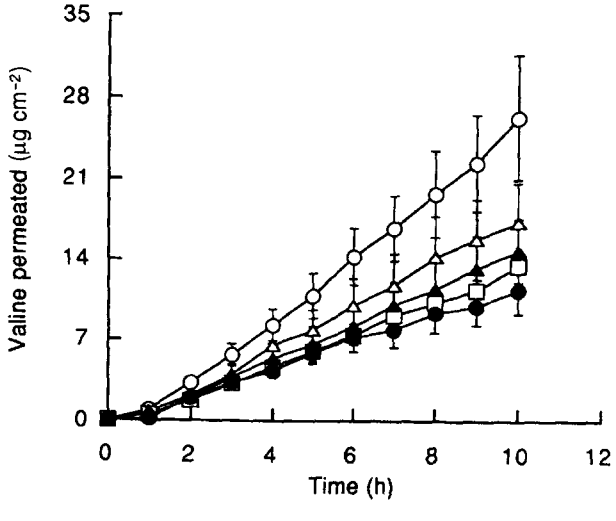

Fig. 1. Skin permeation profiles of valine at pH 3 (○), 4 (△), 5 (□), 6 (●) and 7 (▲). Each point represents the mean ± s.e. of 6 experiments.

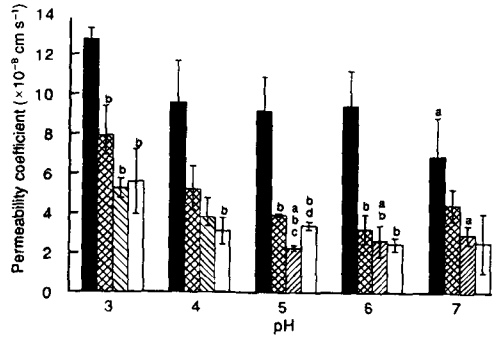

Fig. 2. Permeability coefficients of alanine (■), valine (⊠), isoleucine (▨) and leucine (□) at various pH values. Each column represents the mean ± s.e. of 6 experiments. [a]$P < 0.05$ compared with pH 3, [b]$P < 0.05$ compared with alanine [c]$P < 0.05$ compared with valine, [d]$P < 0.05$ compared with isoleucine.

The permeability coefficients of neutral amino acids at various pH values were calculated from each permeation profile and are summarized in Fig. 2. The permeation coefficient was highest for alanine, followed by valine, isoleucine and leucine, the last two having almost the same value, over the pH range tested. All neutral amino acids showed high permeability at acidic pH, especially pH 3, although there is no significant difference in some of them.

Skin permeability of ionic species of amino acids

Amino acids are always ionized in aqueous solutions but the fraction of ionization varies depending on the pH of the solution. In Fig. 3, the permeability coefficient and fraction of ionic species of valine are plotted as a function of pH. The permeability coefficient decreased as cation fraction decreased and zwitterion fraction increased, and reached a constant level at pH 5 to 7 where only the zwitterion exists. The other neutral amino acids had the same tendency although the levels of permeability coefficient were different. Figs 4 and 5 show the same plots for cationic and anionic amino acids, lysine and aspartic acid. The permeability of lysine decreased as the di-cation fraction decreased and the fraction of positively charged zwitterion increased. The main ionic species of aspartic acid, in contrast, changed from uncharged zwitterion to negatively charged

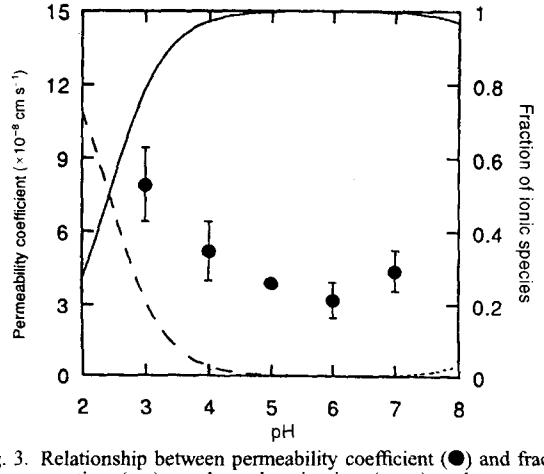

Fig. 3. Relationship between permeability coefficient (●) and fraction of mono-cation (– –), uncharged zwitterion (——) and mono-anion (- - - -) of valine. The permeability coefficient is presented as the mean ± s.e. of 6 experiments. The fraction of ionic species was calculated by using dissociation constants in Table 1.

zwitterion with an increase in pH; the permeability dropped slightly. These results suggest that each ionic species of amino acid has different skin permeability and that the total permeability varies with change in ion fraction dependent on pH.

The permeability coefficient of each ionic species was then estimated. The neutral amino acids exist as mono-cations and uncharged zwitterions at pH 3 to 7 so that the total permeability coefficient can be represented by:

$$P_{tot} = \frac{C_{cat(+1)}}{C_{tot}} P_{cat(+1)} + \frac{C_{zwi(0)}}{C_{tot}} P_{zwi(0)} \quad (1)$$

where P and C are the permeability coefficient and concentration, and subscripts tot, cat(+1) and zwi(0) indicate total, mono-cation and uncharged zwitterion, respectively. Introducing hydrogen ion concentration ($[H^+]$) and dissociation constant of the carboxyl group (K_{a1}) into equation 1, P_{tot} is given by:

$$P_{tot} = \frac{[H^+]P_{cat(+1)} + K_{a1}P_{zwi(0)}}{[H^+] + K_{a1}} \quad (2)$$

Because lysine is present as di-cation and positively charged or uncharged zwitterions in the donor solutions, the total permeability coefficient can be described as the permeability coefficients of these three ions:

$$P_{tot} = \frac{[H^+]^2 P_{cat(+2)} + K_{a1}[H^+]P_{zwi(+1)} + K_{a1}K_{a2}P_{zwi(0)}}{[H^+]^2 + K_{a1}[H^+] + K_{a1}K_{a2}} \quad (3)$$

where K_{a2} is the dissociation constant of amino group and subscript cat(+2) and zwi(+1) mean di-cation and positively charged zwitterion. In aspartic acid, the main ionic species are mono-cation, uncharged and negatively charged zwitterions and the total permeability coefficient is described as follows:

$$P_{tot} = \frac{[H^+]^2 P_{cat(+1)} + K_{a1}[H^+]P_{zwi(0)} + K_{a1}K_{a2}P_{zwi(-1)}}{[H^+]^2 + K_{a1}[H^+] + K_{a1}K_{a2}} \quad (4)$$

where K_{a2} is the dissociation constant of another carboxyl group and subscript zwi(−1) indicates negatively charged zwitterion. The permeability coefficients of ionic species were calculated by fitting the data of each amino acid to equations 2–4 (Table 2). In neutral amino acids, the permeability coefficient of the mono-cation was one order higher than that of uncharged zwitterion. Alanine had the highest value for both ions, and the lowest among neutral amino acids were isoleucine and leucine which were nearly equal. Although the standard deviations of estimation for uncharged zwitterion of lysine and mono-cation of aspartic acid were high due to their small fractions, there was a tendency for ionic species with positive charge to have high permeability.

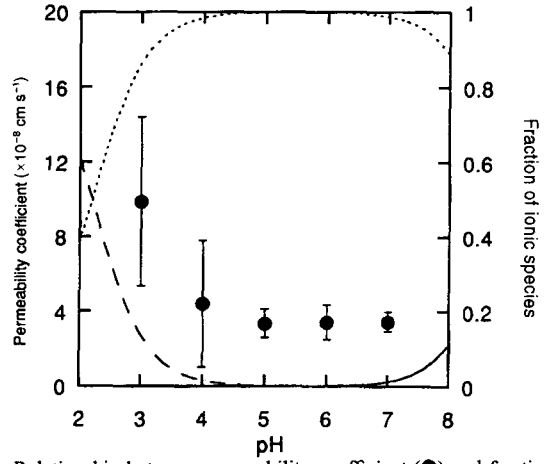

Fig. 4. Relationship between permeability coefficient (●) and fraction of di-cation (– –), positively charged zwitterion (----) and uncharged zwitterion (——) of lysine. The permeability coefficient is presented as the mean ± s.e. of 6 experiments. The fraction of ionic species was calculated by using dissociation constants in Table 1.

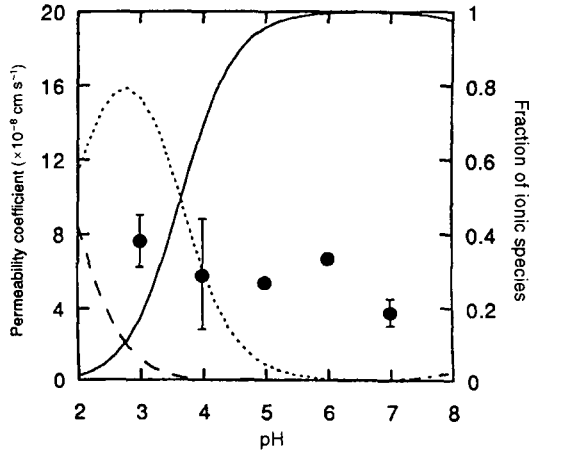

Fig. 5. Relationship between permeability coefficient (●) and fraction of mono-cation (– –), uncharged zwitterion (----), negatively charged zwitterion (——) and di-anion (– · –) of aspartic acid. The permeability coefficient is presented as the mean ± s.e. of 6 experiments. The fraction of ionic species was calculated by using dissociation constants in Table 1.

Table 2. Estimated permeability coefficient of ionic species of amino acids[a].

	Permeability coefficient ($\times 10^{-8}$ cm s^{-1})				
	$P_{cat(+2)}$	$P_{cat(+1)}$	$P_{zwi(+1)}$	$P_{zwi(0)}$	$P_{zwi(-1)}$
Alanine		30·71 ± 8·18		8·46 ± 0·64[b]	
Valine		23·25 ± 4·66		3·93 ± 0·35[b,d]	
Isoleucine		15·57 ± 3·71		2·70 ± 0·27[b,d,e]	
Leucine		16·25 ± 2·73		2·72 ± 0·20[b,d,e]	
Lysine	50·49 ± 1·73		3·45 ± 0·13[c]	2·59 ± 19·19	
Aspartic acid		22·96 ± 81·45		7·01 ± 5·89	5·27 ± 0·90

[a]The values were estimated by computer-fitting of permeation data of amino acids to equations 2–4. Each value represents the mean ± computer-calculated s.d. [b]$P < 0·05$ compared with mono-cation, [c]$P < 0·05$ compared with di-cation, [d]$P < 0·05$ compared with alanine, [e]$P < 0·05$ compared with valine.

Relationship between permeability and physicochemical parameters

The relationship between the estimated permeability coefficients and physicochemical characteristics of amino acids was studied (Fig. 6). The molar volume, calculated by the group contribution method (Fedors 1974), and *n*-octanol/water partition coefficient (Pliska et al 1981) were used as physicochemical parameters. Previously reported data on cephalexin, amino acid-like β-lactam antibiotics were also used in this study (Hatanaka et al 1994). As shown in Fig. 6a the permeability coefficient (P) of mono-cation and uncharged zwitterion decreased exponentially with increase in the molar volume (MV) of amino acid, and each relation could be described by the equations log $P = -6.02 - 0.00605$ MV ($r = -0.986$) for the mono-cation, and log $P = -7.50 - 0.00909$ MV ($r = -0.996$) for uncharged zwitterion. In contrast, no relation could be found between the permeability coefficient and partition coefficient (Fig. 6b). Comparison of ions with similar molar volume revealed the permeability was highest for di-cation followed by mono-cation, positively charged zwitterion, uncharged zwitterion and negatively charged zwitterion; thus, a positive charge favoured skin permeation but a negative one did not.

Transdermal potential difference

The driving forces for the membrane permeation of ions are provided by the gradient of the electrochemical potential. Particularly, electrical potential gradient can foster the permselectivity of membranes as shown in Fig. 6. The electrical potential differences across skin were measured under conditions similar to those used in the permeation experiments and the values are plotted against pH of donor solution in Fig. 7. The potential difference was about 1 mV at pH 3, decreased with increase in pH, changed from a positive to a negative value between pH 4 and 5, and then continued to decrease to pH 6.

Discussion

In this study, we measured the skin permeability of amino acids at various pH values and found pH-dependency due to different permeabilities of ionic species. Some investigators, however, have reported that ionization of amino acids did not significantly influence permeability (Ruland & Kreuter 1991; Sznitowska et al 1993). As in those studies, we found no significant differences of permeability in a narrow pH range. The influence of pH was apparent on comparing the permeability of a variety of amino acids with fractions of ionic species over a wide range of pH. If the fraction of one ion is much smaller than that of another, the contribution of the former to total permeability was not detected. This may be true in the case of neutral amino acids, as reported by Ruland & Kreuter (1991), who measured permeability only at the isoelectric point (pH 6) and physiological pH (pH 7·4). When the difference of permeability between two ions is small, such as positively charged and uncharged zwitterions of lysine, and uncharged and negatively charged zwitterions of aspartic acid (Table 2), it would be difficult to determine the difference from data at only two pH values as reported by Sznitowska et al (1993), because ionic permeants usually produce much more highly variable flux data than neutral ones (Liu et al 1993). A wide range of pH would have to be studied to elucidate the influence of ionization on skin permeability of amino acids. Ruland & Kreuter (1991) observed similar pH dependency in skin permeation of asparagine to that we found by measuring the permeability at pH 2·8 to 10·8.

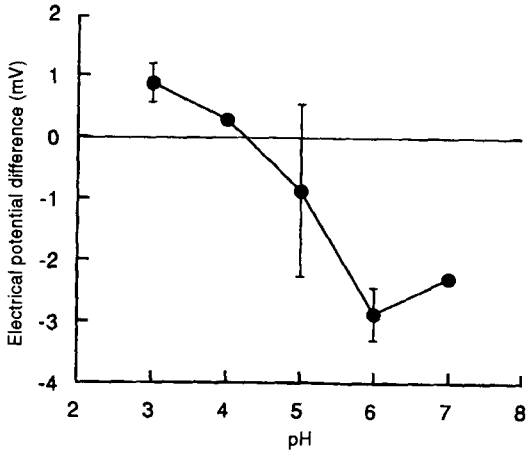

Fig. 7. Transdermal potential difference as a function of donor pH. Each point represents the mean ± s.e. of 3 experiments.

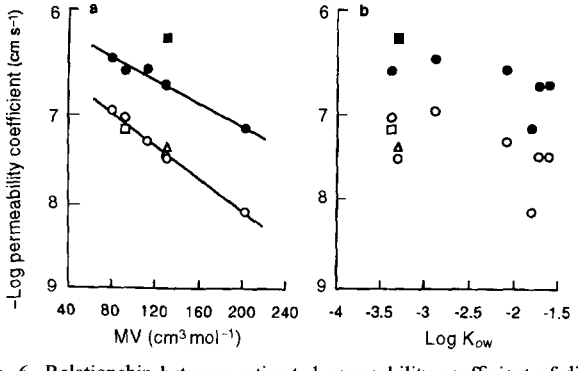

Fig. 6. Relationship between estimated permeability coefficient of di-cation (■), mono-cation (●), positively charged zwitterion (△), uncharged zwitterion (○) and negatively charged zwitterion (□) and physicochemical properties of amino acids. Each point represents the mean value. Solid lines represent the regression lines, log $P = -6.02 - 0.00605$ MV, $r = -0.986$ for mono-cation; log $P = -7.50 - 0.00909$ MV, $r = -0.996$ for uncharged zwitterion. a. Molar volume (MV), b. *n*-octanol/water partition coefficient (K_{ow}).

The pH-dependency in skin permeation can also be caused by alteration of the barrier properties of skin. It is well known that alkaline solutions destroy the skin membrane (Scheuplein 1965). We previously investigated the skin permeation of cortisone and D-mannitol, typical lipophilic and hydrophilic permeants, in which each of compound's permeability coefficients did not change over the pH range 3 to 7 (Hatanaka et al 1994). Further, the permeability coefficients treated here were within the range $0.5–13 \times 10^{-8}$ cm s^{-1}, and these values were in agreement with those reported by others (Wearley et al 1990; Ruland & Kreuter 1991; Sznitowska et al 1993). Therefore, the pH dependency observed here would not be due to skin damage.

It seems reasonable that pH dependency in skin permeation of electrolytes might result from different permeability of these species. Skin permeation by passive diffusion is known to be greater for un-ionized species than for ionized species (Swarbrick et al 1984). Iontophoretic examinations have also shown that the permselective properties of skin allow positively charged molecules to permeate better at physiological pH (Burnette & Ongpipattanakul 1987). The permselectivity of

skin in iontophoretic transport is caused by electroosmotic flow due to the skin's negative charge; that is, both the electrical volume force effect, resulting from the interaction of ion atmosphere (positive charge) and the electric field (negative potential gradient), and an induced osmotic pressure effect, produce volume flow in the same direction as counterion (cation) flow through skin (Pikal 1990). The current results for passive permeation were similar to the iontophoretic data (Fig. 6). However, because the transdermal potential gradients in the present study were positive at acidic pH and negative at neutral pH (Fig. 7), they may produce a volume flow which is opposite to that of the main species, positively charged ions at acidic pH and negatively charged at neutral pH. Moreover, the potential differences seem too small to affect skin permeation of ions; the electrodiffusion is often described using the Nernst–Planck equation:

$$J = -ucRT\left(\frac{d \ln c}{dx} + \frac{zFd\phi}{RTdx}\right) \quad (5)$$

where J, u, c and z are the flux, mobility, concentration and valency, respectively; R is the gas constant, T is the absolute temperature, F is Faraday's constant, and ϕ is the electrical potential. To evaluate the contribution of concentration gradient and electric potential gradient to the flux, $\Delta \ln c$ and $zF\,\Delta\phi/RT$ were calculated. The calculated $zF\,\Delta\phi/RT$ values were significantly smaller than the $\Delta \ln c$ values even when the greatest contribution of electric potential gradient was expected ($\Delta \ln c = -2{\cdot}189$, $zF\,\Delta\phi/RT = 0{\cdot}033$ for alanine at pH 3; $\Delta \ln c = -2{\cdot}590$, $zF\,\Delta\phi/RT = 0{\cdot}085$ for aspartic acid at pH 7). The mechanism of permselectivity of skin in passive transport thus seems different from that in iontophoretic transport.

As shown in Fig. 6, the skin permeability of ionic species of amino acids did not depend on the lipophilicity but on molecular size. A similar phenomenon has been observed in iontophoresis of amino acids (Green et al 1992). These results are consistent with a porous mechanism of transport. Two types of pathways for skin permeation have been proposed: pore and lipid pathways. Potts & Guy (1992) even postulated skin permeation without the existence of aqueous pores in the skin. In contrast to their theory, the aqueous pore pathway appears more common in skin transport of amino acids and other ionic drugs (Mazzenga et al 1992; Morimoto et al 1992; Sznitowska et al 1993; Ruland et al 1994). The present results also tend to support a porous mechanism. However, these were obtained under hydrated conditions, which is different from clinical conditions, and then do not permit a definitive conclusion.

As mentioned above, one of the most important parameters affecting permeability is the diffusion coefficient of permeant in pores filled with water under the porous mechanism, and the diffusion coefficient is closely related to permeant size. In the present study, the permeability coefficients of mono-cation and uncharged zwitterion decreased exponentially with increase in molar volume. However, the permeability coefficients differed among ionic species even though the amino acids were the same. Ions are generally hydrated in aqueous solution by the iondipole interaction with water (Frank & Wen 1957; Samoilov 1957). Carboxylic ions more than double the effective diffusion radius of solutes by hydration. On the other hand, ammonium ions yield negative hydration, which destroys the water structure and then makes the movement of solutes in water easy, thereby cancelling out the decrease in diffusivity caused by the increase in size. The ionic mobility of cation is higher than that of anion for glycine and alanine (Kiso 1972). The difference in diffusivity among ionic species of amino acids due to hydration may be one reason for permselectivity of skin. although there may be other reasons. Wearley et al (1990) suggested binding of amino acids in skin, and the possibility of self-association of amino acids cannot be ignored. Further studies are required to understand ion-selectivity in skin permeation of amino acids.

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