TRANSPORT OF AMINO ACIDS THROUGH CHARGED CATION-EXCHANGE MEMBRANE

Huseyin KARA[1], Zafer YAZICIGIL[2], Fatih DURMAZ[3] and Mustafa ERSOZ[4,*]

Department of Chemistry, Selcuk University, Kampus, 42031 Konya, Turkey; e-mail: [1] hkara@selcuk.edu.tr, [2] zyazicigil@selcuk.edu.tr, [3] fdurmaz@selcuk.edu.tr, [4] mersuz@selcuk.edu.tr

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The sorption and transport of amino acids such as glycine, L-valine and L-leucine through charged polysulfone membranes was investigated as a function of pH lower than 5.6 under three different interfacial conditions. The sorption was characterized by the Langmuir and the Freundlich equations. The highest permeability was obtained if the initial pH on the amino acid source side was 5.6 and that on the opposite side 2.5, the intermediate permeability was obtained if the initial pH on both sides was 2.5, and the smallest permeability was obtained if the initial pH on the amino acid side was 5.6. The obtained results are explained in terms of the interfacial transport based on the chemical reaction (protonation and ion exchange).

Keywords: Amino acids; Transport; Ion-exchange membranes; Sorption; Polysulfones; Permeability.

Amino acids are very important compounds because they take part in major metabolic processes and are manufactured mostly by the fermentation method. Attempts of application of continuous membrane technology to fermentation broth were going on with the recovery, separation and purification of amino acids[1]. Membrane mediated separations have recently become an attractive alternative to most chemical separation methods such as ion-exchange and chromatographic processes for amino acid purification[2–8]. The main advantage, the membrane-based separation processes offer, is the attractive low-energy approach.

The transport of amino acids and other organic molecules through synthetic polymer membranes is of interest as they possess amphoteric properties due to the presence of cationic, anionic and neutral groups depending on the solution pH (refs[9–18]). The amino acid transport through charged membranes depends strongly on pH and the interaction between the membrane and solution interfaces is the rate-limiting step in the process[10]. The

effect of pH on the interfacial transport of amino acids was investigated by Minagawa *et al.*[12–14], who proposed a model which incorporates the dissociation equilibrium equations for the amino acid as well as the rate equation for the interfacial transport based on the interfacial chemical reaction assuming the validity of the Donnan equilibrium relation, and the Nernst–Planck flux equations for the ion transport through the cation exchange membrane.

The charged polysulfone membranes are true microporous ion exchange membranes which are patented for various post-treatment applications; their transport properties must be designed according to the application needs. Glycine, L-valine and L-leucine were used to investigate the transport mechanism of amino acids within a charged membrane and to systematically correlate the results with the literature data. To examine this issue more closely, with special emphasis on the influence of pH, the present experiments are designed for pH below isoelectric points of amino acids in order to show the effect of pH on the interfacial transport.

EXPERIMENTAL

Glycine, L-valine, L-leucine, ninhydrine, HCl and NaOH from Merck, citric acid, $Na_3HPO_4 \cdot 12H_2O$ from BDH Ltd. The charged polysulfone cation-exchange membrane (polyethersulfone) was obtained from Gelman Sciences (Pall Corp.) with sulfonic acid groups as fixed charge groups. The ion-exchange capacity of the membrane is 1.52 mmol/g in the hydrogen ion form, thickness, δ, is 152.4 μm and the water content is 40%. The membranes were conditioned by the equilibration with HCl, distilled water, NaOH and finally with distilled water and then the membrane discs were pretreated with 1.0 M HCl. Amino acid solutions were prepared in buffer solutions (citric acid–phosphate buffer) at different pH values[19]. Ninhydrin solution was prepared in ethanol.

Sorption Experiments

The amount of the sorbed amino acids was calculated from the change in the amino acid concentration in the solution and the weight of the dry membrane used. The amount of amino acids retained in the membrane was determined by stripping amino acid with HCl solution. In all cases the mass balance was confirmed. pH was checked with a pH meter (Orion SA-720) using a combined (Orion 91-02) electrode.

Transport Measurement

Three different experimental conditions were specifically designed to determine the influence of pH on the interfacial transport of amino acids through the charged membrane. The experimental conditions were: (i) pH of solution in cells L and R was the same in the pH ranges 2.5–5.6; (ii) pH in cell L was approximately 5.6 and pH in cell R was varied; (iii) pH in cell L was varied and pH in cell R was approximately 5.6. The concentration of amino acid was 0.05 mol/l in cell L and was initially set to zero in cell R for all experiments. The

flux J_{A^+} and the permeability coefficient P (defined as $J_{A^+} l/C_A$) of amino acids were determined from the concentration changes with time, using the following equation[13]

$$P = \frac{l}{S} \frac{1}{1/V_s + 1/V_r} \frac{1}{\Delta t} \ln \frac{\Delta C_A(t)}{\Delta C_A(t + \Delta t)} , \qquad (1)$$

where l is membrane thickness, S is the active area of the membrane, V_s (V_r) is the volume of source (receiver) solution, $\Delta C_A(t)$ and $\Delta C_A(t + \Delta t)$ are the concentration differences between the receiver solution, $C_{A,r}$, and the source solution, $C_{A,s}$, measured at times t and $(t + \Delta t)$, respectively.

Transport studies were carried out at room temperature in a two-compartment device (made of Teflon) of volume 50 ml separated by the charged membrane, stirred with magnetic stirrers. 1 ml of the solution was sampled from cell R or cell L at chosen times and the concentration of permeated amino acids was measured with a UV-VIS spectrophotometer (Shimadzu UV-160A) at 570 nm. For each concentration measurement, 1 ml was taken from both cells and this volume was not replaced. Each experiment was repeated at least twice; the results were consistent within ±10%. Before measurement, the amino acid solution was pretreated with ninhydrin solution for 24 h. The determination of amino acid with ninhydrin was performed according to the literature[20].

RESULTS AND DISCUSSIONS

Sorption Experiments

The concentration of amino acids in the membrane phase was calculated as

$$q = (C_0 - C) V/W , \qquad (2)$$

where C_0 and C denote the initial and equilibrium concentrations of amino acids in the aqueous phase, V is the volume of the aqueous phase and W is the dry weight of the membrane. Sorption isotherms were obtained by plotting the amount of amino acids sorbed (mmol) per gramm of the membrane *vs* concentration of amino acids remaining in the solution in equilibrium.

The sorption results could be expressed by the Langmuir or the Freundlich isotherms. The sorption constants and correlation coefficients for glycine, L-valine and L-leucine for sorption on the membrane were calculated from the Freundlich and the Langmuir plots; they are given in Table I. The sorption data provided better correlation coefficients in the range of 0.934–0.997 using the Freundlich isotherm, when compared with the Langmuir isotherm as shown in Table I. To test the fit of the data, the Freundlich isotherm equation is written as

$$q = kC^{1/n} \,, \tag{3}$$

where k and n are empirical parameters. The Langmuir isotherm is written as

$$C/q = 1/K_b A_s + C/A_s \,, \tag{4}$$

TABLE I
Parameters of the Langmuir and the Freundlich isotherms for amino acid sorption

Amino acid	pH	Langmuir isotherm			Freundlich isotherm		
		A_s mmol/g	K_b mmol^{-1}	Corr. coefficient	k mmol/g	n	Corr. coefficient
Glycine	2.5	1.753	0.144	0.976	0.315	2.603	0.947
	4.0	1.836	0.109	0.897	0.427	3.445	0.963
	5.6	1.610	0.106	0.953	0.281	2.770	0.997
L-Valine	2.5	1.089	0.081	0.896	0.156	2.520	0.981
	4.0	1.102	0.094	0.905	0.173	2.571	0.945
	5.6	0.771	0.116	0.978	0.116	2.412	0.943
L-Leucine	2.5	0.962	0.109	0.948	0.146	2.448	0.986
	4.0	0.927	0.126	0.968	0.147	2.434	0.934
	5.6	0.677	0.095	0.971	0.079	2.129	0.945

TABLE II
Physicochemical properties of amino acids

Amino acid	pK_a	Isoelectric pH	Molecular weight	Partial molar volume cm^3/mol	Solubility in water at 25 °C (g/100 g water)
Glycine	2.350	6.064	75.07	57.80	25.00
L-Valine	2.286	6.002	117.10	136.50	3.58
L-Leucine	2.318	6.038	131.20	164.60	2.01

where the parameters K_b and A_s are the sorption binding constant ($mmol^{-1}$) and saturation capacity (mmol amino acid/g dry weight of membrane), respectively. Both isotherm parameters were determined by the least-squares fit of sorption data.

In the experiments reported here, the sorption of amino acids was higher for lower pH. This observation can be attributed to a combination of several factors, such as protonation of amino acids, anchoring to fixed groups of the polymer matrix, polymer entropy effect and a lower permittivity of the membrane matrix. As shown, the sorption must be influenced by pK_a, the values of A_s and K_b are expected to depend on pK_a. Physicochemical parameters of the studied amino acids are listed in Table II. These values are very similar, so it is difficult to interpret the data in terms of the pK_a values. In this experiment, the effect is more pronounced for the hydrophobic CH_2 groups, solubilities and partial molar volumes. Coupled with the fact that different concentrations of solutions were employed, these results may be interpreted as indicating that the sorption and transport processes depend on pH, molecular weight and hydrophobic group under the conditions employed.

Transport

A schematic description of the transport through the charged membrane is shown in Fig. 1. The membrane contains initially an amino acid in the source phase (cell L) and a buffer solution in the receiver phase (cell R). We assume that the amino acid is protonated at pH of the medium which is lower than the isoelectric point of the studied amino acid. Therefore, the total concentration of the amino acid in the source cell is assumed to be equal to that of the protonated form. We are dealing with a membrane having cation-exchange properties. The transport processes through the charged membrane for A^+ (protonated amino acid ions) and H^+ ions are represented by Nernst–Planck equations;

$$J_{A^+} = -D_{A^+}\left(\frac{dC_{A^+}}{dx} + z_{A^+}C_{A^+}\frac{F}{RT}\frac{d\Psi}{dx}\right) \quad (5)$$

$$J_{H^+} = -D_{H^+}\left(\frac{dC_{H^+}}{dx} + z_{H^+}C_{H^+}\frac{F}{RT}\frac{d\Psi}{dx}\right), \quad (6)$$

where ***J***, *D* and *C* are the flux, diffusion coefficient and concentration of ions in the membrane, respectively, *F* is the Faraday constant, *R* the gas constant, *T* temperature, Ψ the electric potential and *x* is the position in the membrane. In the experiment described here, a permeable cationic membrane separates two ionic solutions containing protonated amino acid and hydrogen ions and no external potential is put on electrodes. The fixed charge concentration (*X*) of the membrane is assumed to be higher than the H^+ concentration in the studied pH region. In this work, the pH region was adjusted to 2.5–5.6, so the fixed charge concentration is higher than the H^+ concentration. If only the positive ions can enter and pass through the membrane, the need for the charge balance within both solutions phases and the membrane bulk polymer phase is equal. Moreover, the electric current density ***I*** must be zero through the membrane cross section, which requires to fulfill the following equations

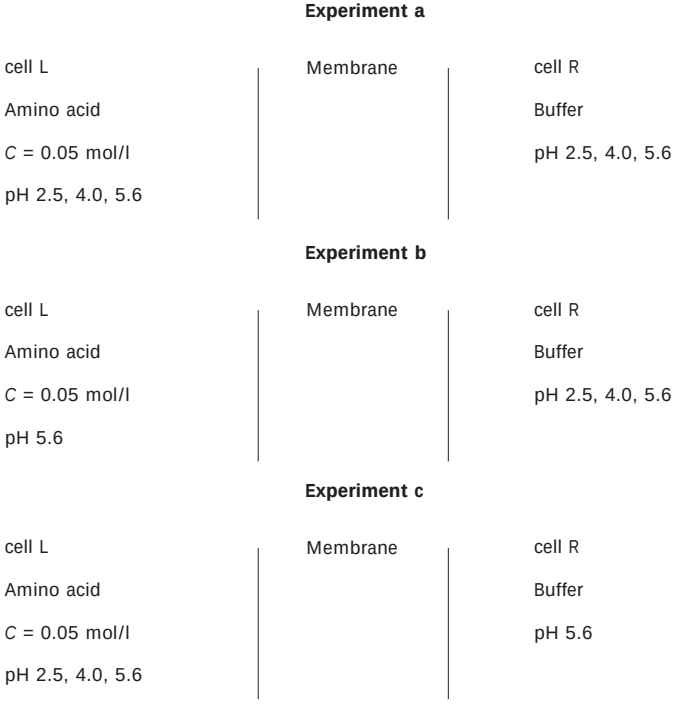

FIG. 1
Schematic representation of experiments a–c for different initial interfacial conditions

$$J_{A^+} + J_{H^+} = 0 \tag{7}$$

$$C_{A^+}(x) + C_{H^+}(x) = X\,. \tag{8}$$

Substitution of Eqs (*6*)–(*8*) into Eq. (*5*) gives

$$J_{A^+} = -\frac{D_{A^+} D_{H^+} X}{(D_{A^+} - D_{H^+}) C_{A^+} + D_{H^+} X} \frac{\mathrm{d}C_{A^+}}{\mathrm{d}x}\,. \tag{9}$$

Boundary conditions are expressed as:

the concentration of amino acids for $x = 0$ (in the source cell) is $C_{A^+,l}$

the concentration of amino acids for $x = l$ (in the receiver cell) is $C_{A^+,r}$,

$$J_{A^+} = -\frac{D_{A^+} D_{H^+} X}{(D_{A^+} - D_{H^+}) l} x \ln \frac{(D_{A^+} - D_{H^+}) C_{A^+,l} + D_{H^+} X}{(D_{A^+} - D_{H^+}) C_{A^+,r} - D_{H^+} X}\,. \tag{10}$$

These results can be expressed for flux of protonated amino acids in the membrane thickness direction. The equivalent quantity of the protonated amino acid which is transferred from the source cell to the receiver cell is equal to the amount of ions transferred from the receiver cell to the left phase according to the mass law conservation. The total concentration of each solution in cell L ($C_{T,l}$) or cell R ($C_{T,r}$) can be expressed as

$$\begin{aligned} C_{A^+,l} + C_{H^+,l} &= C_{T,l} \\ C_{A^+,r} + C_{H^+,r} &= C_{T,r} \\ C_{A^+,l} + C_{A^+,r} &= C_{A^+,T} \\ C_{H^+,l} + C_{H^+,r} &= C_{H^+,T}\,. \end{aligned} \tag{11}$$

The problem consists in the fact that the concentrations involved in the above equations are those in the membrane and we usually know only the concentrations outside the membrane. To solve the problem, the partition coefficients on the surface of the membrane were considered. So, it is assumed that the partition coefficient K on the surface of the membrane in the source cell ($x = 0$) and that in the receiver cell ($x = l$) are defined at the studied pH by the following equations:

$$K_{\mathrm{l}} = \frac{\overline{C}_{\mathrm{A}^+,\mathrm{l}}}{C_{\mathrm{A}^+,\mathrm{l}}} \quad x = 0\,, \qquad K_{\mathrm{r}} = \frac{\overline{C}_{\mathrm{A}^+,\mathrm{r}}}{C_{\mathrm{A}^+,\mathrm{r}}} \quad x = l\,. \tag{12}$$

Here, the membrane interfacial concentrations at $x = 0$ and $x = l$ are needed. It is difficult to calculate the amounts of A^+ at the membrane-solution interfaces; therefore, in order to estimate $C_{\mathrm{A}^+(0)}$ and $C_{\mathrm{A}^+(l)}$ for amino acids, the sorption equilibria of amino acids on the membrane at different pH values were used. From the equations, the concentrations of protonated amino acids on both surfaces of the membrane are as follows:

$$\overline{C}_{\mathrm{A}^+,\mathrm{l}} = \frac{XC_{\mathrm{A}^+,\mathrm{l}}}{C_{\mathrm{A}^+,\mathrm{l}}(1-K_{\mathrm{l}}) + K_{\mathrm{l}}C_{\mathrm{T,l}}} \tag{13}$$

$$C_{\mathrm{A}^+,\mathrm{r}} = \frac{X(C_{\mathrm{A}^+,\mathrm{T}} - C_{\mathrm{A}^+,\mathrm{l}})}{(1-K_{\mathrm{r}})(C_{\mathrm{A}^+,\mathrm{T}} - C_{\mathrm{A}^+,\mathrm{l}}) + K_{\mathrm{r}}C_{\mathrm{T,r}}}\,. \tag{14}$$

The flux is derived for the positively charged amino acid species. The flux for the neutral amino acid species is not considered here since pH is kept below the isoelectric point of the amino acid. Equation (10) can be rewritten using Eq. (*11*):

$$\begin{aligned} J_{\mathrm{A}^+} &= \frac{D_{\mathrm{A}^+}D_{\mathrm{H}^+}X}{(D_{\mathrm{A}^+} - D_{\mathrm{H}^+})l} \ln \frac{(D_{\mathrm{A}^+} - D_{\mathrm{H}^+})C_{\mathrm{A}^+,\mathrm{l}} + D_{\mathrm{H}^+}X}{(D_{\mathrm{A}^+} - D_{\mathrm{H}^+})C_{\mathrm{A}^+,\mathrm{r}} + D_{\mathrm{H}^+}X} = \\ &= \frac{D_{\mathrm{A}^+}D_{\mathrm{H}^+}X}{(D_{\mathrm{A}^+} - D_{\mathrm{H}^+})l} \ln \left\{ \left[\frac{(1-K_{\mathrm{l}})(C_{\mathrm{A}^+,\mathrm{T}} - C_{\mathrm{A}^+,\mathrm{l}}) + K_{\mathrm{r}}C_{\mathrm{T,r}}}{(1-K_{\mathrm{r}})C_{\mathrm{A}^+,\mathrm{l}} + K_{\mathrm{l}}C_{\mathrm{T,l}}} \right] \right. \\ &\left. x \left[\frac{(D_{\mathrm{A}^+} - K_{\mathrm{l}}D_{\mathrm{H}^+})C_{\mathrm{A}^+,\mathrm{l}} + K_{\mathrm{l}}D_{\mathrm{H}^+}C_{\mathrm{T,l}}}{(D_{\mathrm{A}^+} - K_{\mathrm{r}}D_{\mathrm{H}^+})(C_{\mathrm{A}^+,\mathrm{T}} - C_{\mathrm{A}^+,\mathrm{l}}) + K_{\mathrm{r}}D_{\mathrm{H}^+}C_{\mathrm{T,r}}} \right] \right\}. \end{aligned} \tag{15}$$

Equations (*13*) and (*14*) correspond to the amino acid concentrations in the interfaces, so the membrane interfacial concentration can be calculated. This allows one to calculate the fluxes for each pH value.

The time dependences of the concentration of the studied amino acids in cell R are shown in Fig. 2. pH of the amino acid phase was 5.6 and that of the other phase was 2.5, the initial concentration of amino acid being $5 \cdot 10^{-2}$ mol/l and the maximum time 4 h. Time for monitoring was consid-

ered as being sufficient because within 2 h, the concentration changes in the cell R are curved due to the non-steady state behavior. It can be seen that for all pH values the concentration of amino acids increased gradually to a maximum value within 2 h and then the curve started to level off. The observed transport phenomena can be explained by considering the solution pH which is kept below the isoelectric points of the amino acids used. In this pH region, the amino acids are sufficiently positive and partly positively charged to cause strong electrostatic interactions with the negative charges of the sulfonate groups. The amino acids can permeate through the membrane, interact with sulfonate anions in the boundary region in which the membrane swells, and be exchanged for H^+ ion. Thus the total concentration of amino acids in the cell R increases due to pH gradient or their concentration gradient. The highest transport was observed when pH of cell R was kept at 2.5 and that of cell L was 5.6. Therefore, only results obtained under these experimental condition are presented. In the transport system, the experiments were specially designed to determine the role of H^+ ions as the driving force for the interfacial transport of the amino acids. Therefore, pH was adjusted to an acidic value at which amino acids are protonated.

$$H_3N^+–R–COO^- + H^+ \rightleftarrows H_3N^+–R–COOH \qquad (16)$$

The transport of amino acids is promoted by pH gradient between the two aqueous phases. In other words, the solution pH lower than isoelectric point allows the protonation of amino acids which attached them to the sulfonyl groups in the membrane bulk phase. Cell R facilitates the ion-exchange mechanism of amino acids for proton and thus the amino acid transport process can occur.

We were interested here in the influence of pH on interfacial transport of amino acids. The pH gradient of amino acids in the cell L of the membrane – the driving force of the amino acid transport – is generated by the pH difference between both sides. This is the mechanism, in which the driving force is pH as well as the concentration gradient. Parameters such as partition coefficients at interfaces, flux, diffusion coefficient and permeability can be obtained from the experimental data under different conditions. Transport fluxes of amino acids through membranes have been compared under the same conditions.

Transport of glycine and leucine, and glycine–leucine mixture through a charged membrane have been qualitatively explained by Minegowa and Tanioka[12–14] who proposed a theoretical model. In the model, they assumed: (i) the equilibrium dissociation equations for the fraction of amino acids formed at different pH values, (ii) the rate equation for the interfacial transport based on the interfacial chemical reaction between amino acid and hydrogen ion or, alternatively, the Donnan equilibrium, and (iii) the Nernst–Planck flux equations for the amino acid and hydrogen transport through the cation-exchange membrane. In this theory, the fixed charge concentration of the cation-exchange membrane (X, mol per l of water in the polymer membrane) is considered to be much higher than the HCI concentration in the pH range (1–6). Due to the Donnan co-ion exclusion, fluxes of co-ions, chloride or hydroxide were neglected because of negatively fixed groups. The neutral species of amino acids were also considered; however, the equilibrium conditions for the positively charged leucine at the membrane/solution interfaces cannot be assumed if the rate-limiting step is interfacial transport rather than the bulk diffusion in the membrane.

In Table II, physicochemical properties of the studied amino acids are listed. Their molecular structures are very similar and only hydrophobic groups are added which definitely affect the solubility in water. Molecular sizes and partial molar volumes are different. This is one of the important factors for the interpretation of the interfacial transport and the reason why

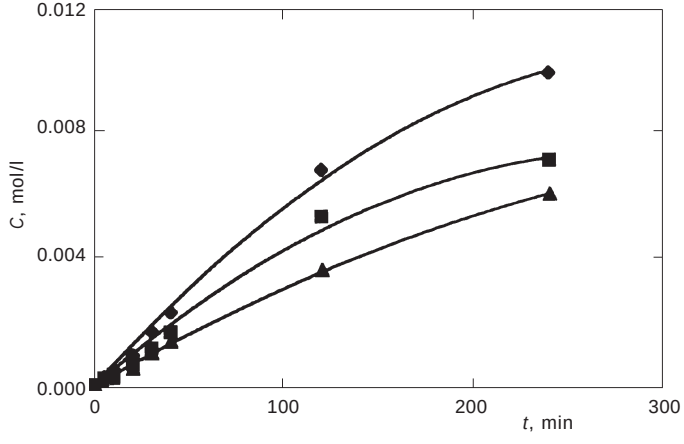

FIG. 2
Time dependence of the amino acid concentration in cell R for experimental conditions b and pH 2.5; ◆ glycine, ■ L-valine, Δ L-leucine

the permeability was found the lowest when the pH of cell R was 5.6. This result is attributed to low concentration of hydrogen ions. Protonation and ion exchange, interfacial chemical reactions at the surfaces in cells L and R, play an important role.

The transfer of the amino acid from cell L to the bulk membrane phase is controlled by the protonation mechanism and the exit from the membrane to cell R by the ion exchange mechanism. As seen in Table II, pK_a and isoelectric points of amino acids are not very different, the only differences are in the molecular weights, partial molar volumes and hydrophobicity of individual amino acids. They hydrophobicaly affect the amino acid solubility and the interfacial transport which causes the observed differences.

The fluxes of protonated amino acids passing through the membranes were estimated by monitoring their concentration in cell R or cell L as a function of time. The fluxes and permeability values are listed in Table III. The amount of moving ions in the membrane is limited by the fixed charge. In the membranes, the fixed charge assists the solute diffusion by ion-exchange reactions, increasing the solute partition in the membrane. The analysis usually begins with the assumption that at interfaces, the pH gradient as well as the Donnan equilibrium will tend to equilibrate by the

TABLE III
Calculated permeability coefficients and fluxes of amino acids under various experimental conditions

Conditions	pH		Glycine		L-Valine		L-Leucine	
	cell L	cell R	$10^9 \cdot P$ cm/s	$10^6 \cdot J$ mol/cm s	$10^9 \cdot P$ cm/s	$10^6 \cdot J$ mol/cm s	$10^{10} \cdot P$ cm/s	$10^6 \cdot J$ mol/cm s
a	2.5	2.5	3.73	10.5	3.52	7.17	8.15	2.61
	4.0	4.0	3.96	10.0	3.36	6.04	3.96	1.86
	5.6	5.6	3.70	9.54	3.26	5.87	2.37	1.26
b	5.6	2.5	8.74	49.3	5.57	22.5	11.1	3.02
	5.6	4.0	5.84	22.5	4.64	12.5	9.44	2.02
	5.6	5.6	3.70	9.54	3.26	5.87	2.37	1.26
c	2.5	5.6	3.09	20.0	3.00	8.00	3.90	1.77
	4.0	5.6	3.85	17.5	3.39	6.75	3.22	1.86
	5.6	5.6	3.70	9.54	3.26	5.87	2.37	1.26

diffusion. The transport rate is related to proton activity that governs the transport.

The flux and permeability coefficient were obtained in experiments a, b and c as functions of initial pH. Permeability coefficients, *P*, are determined from the linear part of the concentration *vs* time curve in the steady-state regime. In Fig. 3, the permeability coefficient calculated from experiment b is given. The permeability and flux values for experiments a and c do not clearly change; however, *P* increases monotonously with pH in experiment b. The molecular weights and hydrophobic CH_2 groups are supposed to be the reason why the permeability coefficient of leucine is about 10 times smaller compared with glycine and valine. A similar order between glycine and leucine was also reported by Minegawa *et al.*[13,14]. The lowest transport for all experiments was found at pH 5.6. This result can be explained by a low concentration of hydrogen ions in the external solutions. The lack of hydrogen ions has two effects: first, the concentration of protonated amino acid forms is higher than that of H^+ ions, and second, the lack of hydrogen ions forces the amino acid to enter and to exit the membrane by ion exchange or partial deprotonation.

Since the membrane is in the H^+-form and hydrogen concentration in cell R allows the amino acids to exit from the membrane in the protonated form following the ion exchange with hydrogen ion. In Fig. 3, the perme-

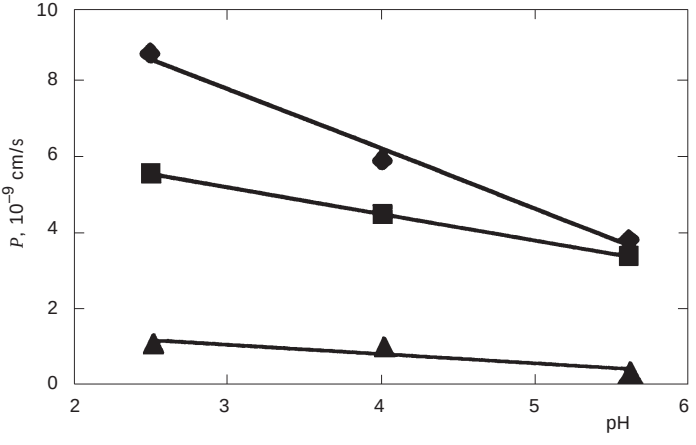

FIG. 3
Permeability coefficients, *P*, obtained in experiment b as a function of pH; ◆ glycine, ■ L-valine, Δ L-leucine

ability as well as fluxes in experiments b and c increase while in experiment a, they remain nearly constant. From the theoretical point of view, the leucine permeability should remain constant like in experiment a as observed previously[12]. There is a good agreement with the work of Glugla and Dindi[10], who obtained similar results when their source side has no supporting electrolyte and the receiver side had pH 1. The observation corresponds to experiment b. The lowest permeabilities were observed when both sides had no supporting electrolyte, which corresponds to experiment a at pH 5.6.

The flux of amino acids through the charged membrane for experiment b is given in Fig. 4. The fluxes do not linearly increase with concentration difference imposed at pH 5.6, which indicates a tendency to saturation in transport through the membrane. This is in agreement with Sikdar's result[9] where the diffusion of the protonated amino acid was assumed to occur along the negatively charged sulfonate groups of the charged membrane. The saturation of the flux at high solute concentrations is characteristic for a facilitated diffusion. Theoretical approach can explain the experimental trends which were observed for the pH dependence of interfacial transport. A more quantitative agremeent between theory and experiment could probably be obtained by multiparameter fitting the theoretical equations to the treated experimental data.

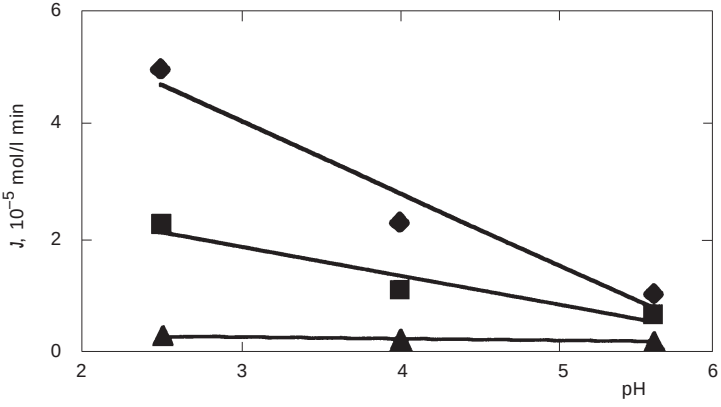

FIG. 4
Flux obtained in experiment b as a function of pH; ◆ glycine, ■ L-valine, Δ L-leucine

CONCLUSIONS

In experiment a, the entrance of the protonated amino acid from cell L into the membrane bulk phase is controlled by the ion-exchange mechanism, and the exit from the membrane to cell R by the ion-exchange mechanism at lower pH and partly by the deprotonation mechanism at higher pH. In experiment b, the entrance of protonated amino acid from cell L in the membrane bulk phase is mainly controlled by the protonation and the exit from the membrane to cell R by the ion-exchange mechanism. In experiment c, the entrance of the protonated amino acid from cell L in the membrane bulk phase is largely controlled by the protonation and partly by the ion-exchange mechanism, and the exit from the membrane to cell R mainly by the deprotonation.

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