Transport Behavior of Amino Acid across Charged Mosaic Membrane

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Several characteristics on a charged mosaic membrane with parallel arrays of different negative and positive charges were investigated through transport studies. In particular, the transport behaviors of three different amino acids (GluNa, Arg, and Ala), known as amphoteric electrolytes, were examined in contrast to those of the simple electrolytes (KCl and LiCl). From an analysis of the data of the volume flux and salt flux, although preferential amino acid transport across the charged mosaic membrane was observed, the extent of the permeability was less than that of simple salt. Among amino acids, GluNa with almost complete dissociation indicated the maximum permeability. A conductance study indicated that the transport behaviors were dependent on the ionic states in aqueous solution. It was inferred that amino acids with apparent zero net charges have transport behaviors different from a nonelectrolyte, such as sucrose.

Since the synthesis of an ion-exchange membrane by Juda et al. in 1950,[1] charged membranes have been extensively applied in many fields. In particular, cation and anion exchange membranes have been mainly used for salt enrichment from sea water by electrodialysis on the industrial scale. At present, a further development of the charged membrane system with new functional groups is required to increase the efficiency in the desalination of sea water or to recover waste solutions containing heavy metals. Recently, composite charged membranes with unique membrane characteristics, such as a charged mosaic membrane or a bipolar membrane, have been produced, and the membrane properties or membrane characteristics have been discussed compared with ordinary charged membranes.[2—10] The former charged membrane, the charged mosaic membrane, is well known as a membrane containing two different charges within the matrix. The two kinds of charge, anion and cation exchange groups, are arranged in parallel to each other in the membrane and the array of charged groups continuously links from one membrane surface to the other surface.[11—16] A membrane with a charge distribution is supposed to induce concurrent migrations of cations and anions along the respective fixed charges (ion exchange sites), and to reject the nonelectrolyte by the Donnan effect due to the charges in the membrane. Thus, preferential salt fluxes and the resultant separation between the electrolyte and nonelectrolyte can be expected. Such a characteristic feature of a charged mosaic membrane is very attractive for given membrane applications in various fields.

Recently, Nakamura et al. developed a novel charged mosaic membrane using microsphere gels.[17,18] In our latest study[19] using a membrane prepared according to the new philosophy, a separation between the solute and the solvent and the preferential salt transport across the charged mosaic membrane have been reported in the case of simple electrolytes. As a next step, it is interesting to extend this study to the transport behavior of electrolytes having different charge states, such as amino acids in solution. The purpose of this work was to understand the transport mechanism of electrolytes, including amphoteric electrolytes such as amino acids, and to accumulate information about the charged mosaic membrane from a fundamental point of view.

Experimental

Membrane. The charged mosaic membrane used in this study was supplied by Dainichi Seika Industry Co.Ltd. Cationic microsphere gel (4VP/DVB) and anionic microsphere gel (SSNa/DVB) were dispersed in *N*-methyl-2-pyrrolidone, and the matrix resin (polysulfone resin (80Wt%)/polyurethane (20Wt%)) was dispersed in the same organic solvent. The mixed organic solutions were cast and the pyridine groups of 4VP were quaternarized to bear cationic charges.[17,18] In preparing the membrane, although theoretically identical quantities of positive and negative charges were introduced into the membrane matrix, the absolute values have not yet been verified experimentally. The membrane thickness was 50 μm and the water content was 17—22%. The membranes were stored in a 0.1 mol dm^{-3} KCl solution before experimental use. It was already indicated in a previous study that nonelectrolytes did not permeate charged mosaic membranes.[19]

Transport Studies. The experimental cell consisted of two half-glass cells, and the charged mosaic membrane was tightly clamped between the two cells. The temperature was kept at 25 °C by constantly circulating water around the two glass cells during the experiment. Mainly, two kinds of measurements, volume change and salt concentration change, were conducted as a function of time using a graduated capillary and an electrode-type Conductive Cell (TOA, Co.Ltd.), respectively.[19—23]

In a volume flux measurement, two types of experimental systems were made to investigate water transport across the membrane, as described below (M = mol dm^{-3}):

xM NX/Memb./xM NX+ 0.5 M Sucrose System I
Water/Memb. /xM NX System II.

With no electrolyte NX in System I, water transport across the membrane, which originated from only the sucrose, could be observed. When equal concentrations of electrolytes in both solutions were added, an effect of the electrolyte on water transport was expected. The volume flux in System II influences the change in the water flux followed by electrolyte transport. As indicated later, the separation between the solute and the solvent, that is, the semipermeability of the membrane can be estimated from experiments in System II.

In a solute flux measurement, System II was used to confirm the electrolyte transport.

In both measurements, the volume or solute concentration changes with time were observed and the fluxes were estimated from the slope of straight lines, taking into account the effective membrane area, 3.14×10^{-4} m^2.[19—23]

Conductance. The electrical conductances were measured using Conductivity Cell (TOA Co. Ltd.) for electrolyte solutions including the amino acids used in the transport studies. Amino acid solutions were prepared without any salts for a buffer adjustment. The pH values of aqueous solutions were 7.0 for GluNa, 11.0 for Arg, and 6.2 for Ala within the concentration range examined, respectively. A plot of the electrical resistances against the concentration was used as calibration curves to determine the quantities transported across the membrane.

Reagents. Chemicals (KCl and LiCl) were procured from KATAYAMA Chemical Co. Ltd. and were used without further purification. Amino acids, sodium glutamate (GluNa · H_2O, MW:187), arginine (Arg, MW : 174), and alanine (Ala, MW:89.1)) were kindly supplied in ultrapure samples from Ajinomoto Co.Inc. Doubly distilled water was used to prepare an aqueous electrolyte solution and others.

Results and Discussion

Conductance. Before studying the transport of amino acids, the electrical conductances of aqueous amino acid solutions along with simple electrolyte solutions (KCl and LiCl) were examined as a function of the concentration, because reliable conductance data about amino acid aqueous solutions could not be found in the literature. The relations between the molar conductance and the square root of the different electrolyte concentrations examined are given in Fig. 1. For acetic acid, the data available in the literature are inserted in Fig. 1.[24] Potassium chloride and acetic acid are well known as typical strong and weak electrolytes, respectively, and the locations of the three amino acid compounds in Fig. 1 suggest the ionic states in aqueous solutions. GluNa in aqueous solution seems to behave as dissociated ions, while Arg and Ala seem to be close to an apparent weak electrolyte. Finally, the fraction of the ionic state about each amino acid was estimated using pK values given in the literature,[25] and the dissociated ionic fractions about Arg and Ala proved to be negligibly small.

Water Transport. One of the most important membrane characteristics is water transport across the membrane. Water in System I is driven by osmotic pressure produced due to the existence of sucrose on one side of the membrane. By applying a phenomenological equation to the volume flux across the membrane, which was obtained from the volume change in both solutions, one can obtain a membrane parameter concerning water movement within membrane.[16] The filtration coefficient (water permeability), Lp, and its dependence of added electrolytes are shown in Fig. 2,[19—23] in which there are two noticeable points. One is the Lp value, which is almost of the same order as that in an ordinary charged membrane.[8] This means that present charged mosaic membrane consists of a dense matrix structure, like that of the usual ion-exchange membrane, at least with regard to the water flow. The other is that the water transport is scarcely affected by added electrolytes.[19] The results give a standard index for material transport, such as amino acid transport. Lp as an average value, 7.07×10^{-14} m^3 N^{-1} s^{-1}, was used to estimate the other membrane parameter, σ.

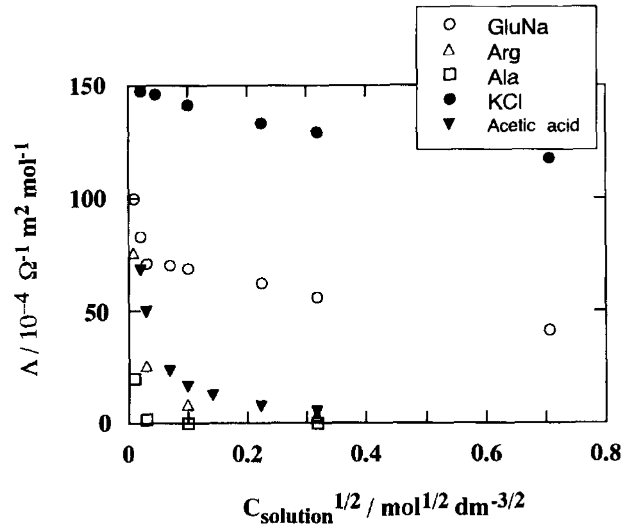

Fig. 1. Relationship between molar conductance and square root of electrolyte concentration. Meaning of each symbol is assigned in the figure.

Separation between Solute and Solvent. The charged mosaic membrane can not allow the permeation of the nonelectrolyte, but can permeate the electrolyte, as indicated in a previous study.[19] Separation between the solute and the solvent (water) by the membrane is also expected. According to the practical phenomenological equation,[16] the reflection

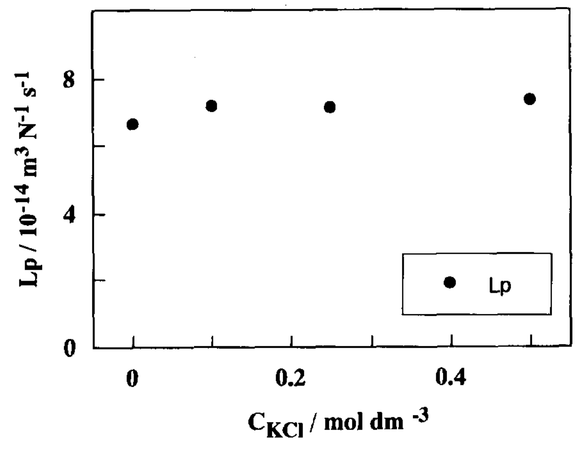

Fig. 2. Dependence of L_p on KCl concentration. L_p values were obtained from volume fluxes in water/sucrose or KCl/KCl + 0.5 mol dm^{-3} sucrose system.

coefficient, σ, can be estimated from the volume flux obtained in System II consisting of water and an electrolyte. The results concering KCl are given in Fig. 3. As can be seen, the values indicate negative signs over the concentration ranges examined in this study. In general, σ is close to unity in a charged membrane, such as ion-exchange membrane, because the counter ion is hard to pass through a charged membrane without electric energy.[8] On the contrary, the negative values of σ in Fig. 3 strongly suggest that the salt flow due to concentration gradient of KCl takes place, the transport rate of salt is faster than the solvent, and thus the separation of the electrolyte from water is possible.[7,8] Based on the information concerning the simple electrolytes mentioned above, the transport behavior of an amino acid through the charged mosaic membrane was investigated. Three kinds of amino acids, which are known as typical neutral and basic compounds, were chosen to investigate the transport mechanism within a charged mosaic membrane. From the volume flux obtained using System II, the values of reflection coefficient for the amino acids were obtained and are given in Fig. 4 along with those of KCl and LiCl for a comparison. The data about amino acids have provided interesting results, that is, the reflection coefficients are larger than the simple salts, but less than unity. This means that the amino acids can permeate through the charged mosaic membrane and can be recovered from aqueous solution by a diffusion dialysis method using this type of membrane. Of these amino acids, GluNa indicated the largest transport because it perhaps completely dissociated under the present pH condition, and the difference for KCl or LiCl may be attributed to their ionic size. The Arg and Ala solutions were kept at the pH values of 11.0 and 6.2, respectively. When the pH values are taken into consideration, together with the corresponding pK values, part of the Arg and Ala concentrations is supposed to be undissociated in aqueous solution. This means that there is no apparent net charge on these species, but it does not mean that the given molecules behave as a nonelectrolyte.

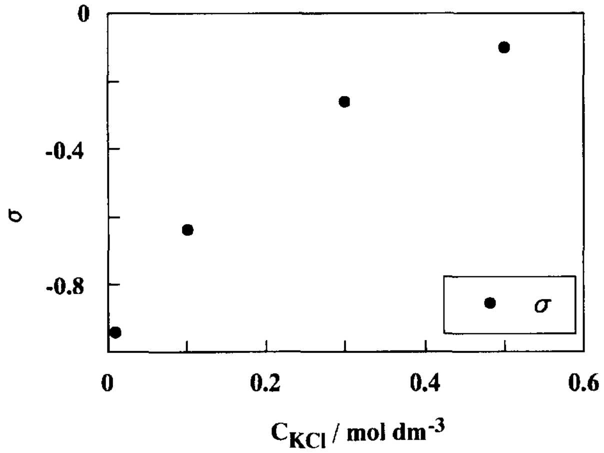

Fig. 3. Dependence of σ on KCl concentration. σ values were obtained from volume fluxes in water/KCl system. The values were calculated by using an averaged L_p, 7.07×10^{-14} $m^3 N^{-1} s^{-1}$.

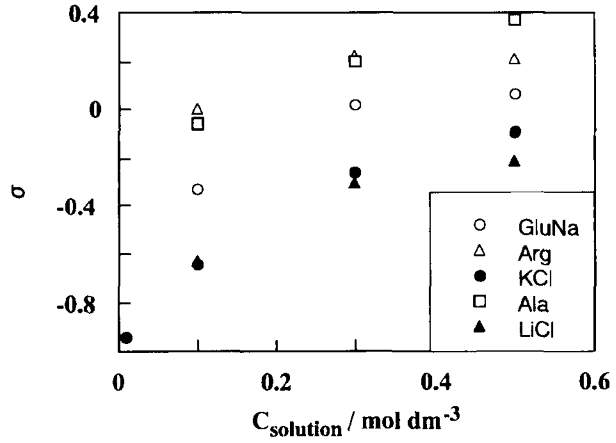

Fig. 4. Dependence of σ on KCl and amino acid concentrations. The values were calculated by using an averaged L_p, 7.07×10^{-14} $m^3 N^{-1} s^{-1}$. Meaning of each symbol is assigned in the figure.

It is imagined that Arg and Ala molecules permeate while forming a kind of ion complex through the charged mosaic membrane. This would be one of the reasons why the reflection coefficients, σ, of amino acids are larger than those of simple salts, but less than that of the nonelectrolyte. Also the molecular size should be considered for the transport of amino acids.

Transport of Amino Acids. The preferential transport of amino acid across a charged mosaic membrane was suggested on the basis of the small reflection coefficient. In order to verify this speculation, a direct observation of the electrolyte flux was made using System II. In the same manner as the procedure in a previous study,[19] the permeability coefficient, ω, was obtained from the solute flux, also, the values of two amino acids, GluNa and Arg, are given along with the ω values of the simple salts in Fig. 5. Unfortunately, the electrical conductance of the Ala solution is too small to detect the reliable change, as can be seen in Fig. 1, and thus a reasonable concentration change across the membrane could

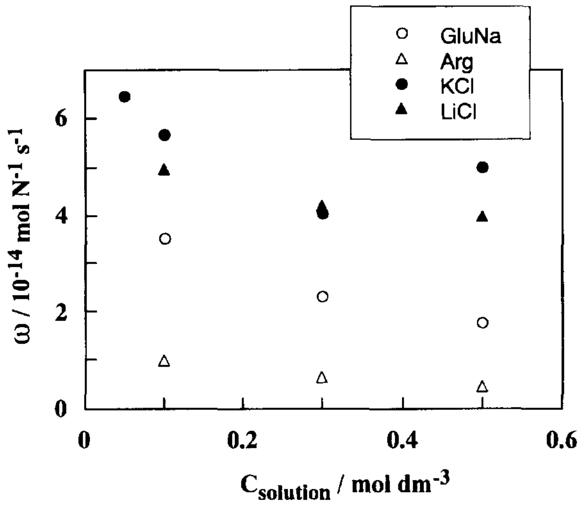

Fig. 5. Dependence of ω on electrolyte concentration. Meaning of each symbol is assigned in the figure.

not be obtained. As can be seen in Fig. 5, an obvious difference in the permeabilities between simple salts and amino acids was observed, and a difference between GluNa and Arg was also recognized. As shown in the data about ω, satisfactorily correlated to σ in Fig. 4, the value of ω increased along with a decrease in solute concentration. The decrease in σ and the increase in ω along with a decrease in the solute concentration may take place as a result of the balance between the osmotic flow and the diffusion flow, which are driving forces in this system. Both σ and ω originate from the identical physical basis. The difference between GluNa and Arg would be caused by the ionic state and a sieve effect recognizing ion size, as discussed above.

Conclusion

The volume flux, J_v, and the salt flux, J_s, were deduced from the volume changes and the concentration changes taking into account the effective membrane area (3.14×10^{-4} m^2), and were analyzed according to a practical linear phenomenological equation presented by Kedem and Katchalsky. As a result, three important membrane parameters (L_p, σ, and ω) were obtained. In a comparative study with the usual charged membrane, like the ion-exchange membrane, L_p, the filtration coefficient of the present membrane, was almost of the same order as that of the ion-exchange membrane. Interestingly, the reflection coefficient, σ, indicated a relatively low value compared with that of ordinary charged membranes. The result was supported by a good correlation with the permeability coefficient, ω, obtained from the salt flux. Three different amino acids indicated transport behavior corresponding to the ionic forms in solution. The order of transport was KCl, LiCl > GluNa > Arg, Ala > Glucose, Sucrose.

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