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Transport and Selectivities of Amino Acids on Periderm and Cuticular Membranes

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ABSTRACT

The transport of amino acids through isolated potato periderm and pear fruit cuticular membranes were investigated. The transport rate depended on the molecular size of amino acids and decreased with an increase in molecular size through extension of the hydrophobic $—CH_2—$ group. The selectivity coefficients of amino acids for the ammonium ion form of periderm and cuticular membranes were also determined, using the mass action law. They were found to be correlated with various physicochemical parameters such as the partial molar volume and the molecular weight.

INTRODUCTION

Amino acids are very important compounds because they participate in a great variety of metabolic processes; their permeation through biological membranes depends on their predominantly hydrophilic character, so that coupling with carrier systems is assumed for their transport (1). For a better understanding of this phenomenon, it is important to build model

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systems capable of demonstrating some aspects pertaining to the mechanism of transport.

The ecotoxicological importance of plant cuticles as a lipophilic sorption compartment has been pointed out (2). The need to know the effect of chemical substances in plant materials, i.e., transport, sorption, and desorption, especially in cuticles, was pointed out, but the idea was not pursued, at least from the ion-exchange, quantitative, physical, and thermodynamic viewpoints. Rates of uptake tend to increase with increasing lipophilicity of compounds (3). This behavior was predicted by transport theory (4), and it is generally observed with biological and synthetic membranes. Charge also plays an important role in the sorption and transport of simple electrolytes in both synthetic and biological membrane systems. In such systems, the mobility of ions is strongly affected by the fixed charge of the membranes.

All nonvolatile compounds reaching the plant surface from the atmosphere must penetrate the cuticle before entering the plant (5). Since plant cuticles are mainly lipid materials, they are likely to sorb and accumulate lipophilic compounds. Therefore, a program was initiated to quantitatively measure the transport and selectivities of amino acids on the ammonium form of periderm and cuticles. The objective is to provide a general description of amino acid transport of potato periderm membranes (PM) and pear fruit cuticular membranes (PFCM) in terms of permeability, and also to establish an empirical relationship between periderm/cuticle and amino acids, and to estimate the relative importance of selectivity coefficients of PM and PFCM.

Description of Selectivity

The ion-exchange isotherm shows the ionic composition of the ion exchanger as a function of the experimental conditions. The ion-exchange reaction between an ion exchanger and counterions in solution is expressed as

$$C_{A^{+n}} + nR_{NH_4^+} \rightarrow R_{A^{+n}} + nC_{NH_4^+} \quad (1)$$

The mass action law is applied to Eq. (1) and the selectivity coefficient, $K^{A}_{NH_4}$, is defined in equilibrium as follows:

$$K^{A}_{NH_4} = \left(\frac{C_{NH_4}}{R_{NH_4}}\right)^n \left(\frac{R_A}{C_A}\right) \quad (2)$$

where R_A and R_{NH_4} represent the equivalent ionic fractions of the counterions in the membrane phase and C_A and C_{NH_4} the corresponding equiva-

lent fraction of these ions in the solution phase. The selectivity coefficients of amino acids were determined using Eq. (2).

EXPERIMENTAL

Materials

The amino acids were analytical grade. Glycine and potassium dihydrogen phosphate were obtained from B.D.H.; NH_4Cl from Hopkin and Williams; DL-methionine, D-alanine, D-valine, DL-cysteine, cellulase, and 9-fluorenylmethyl chloroformate from Sigma; L-alanine and DL-leucine from Biochemical; pectinase from ICN Biomedicals Ltd.; sodium tetraborate from May & Baker; and acetone and acetonitrile from Merck. Spectrapor Dialysis membrane, used for comparison with PM and PFCM, was obtained from Spectrum Medical Industries Inc. Wet cellulose dialysis tubing in 1% sodium azide was composed of cotton cellulose, 0.05 mm thick, with a nominal molecular weight cutoff of 3500.

Amino acid solutions were prepared using deionized water without further purification. 35% (w/v) HCl and ammonium chloride of analytical grade were used as reagents. Each amino acid was dissolved in deionized water to prepare a solution of about 0.1 mol/L, and then this solution was adjusted to a pH around 3.5–4 with dilute HCl, followed by final adjustment of its concentration from 0.01 to 0.05 mol/L with deionized water.

Phosphate buffer was made of 0.1 M anew for each use. Before making up to solution, the potassium dihydrogen phosphate being used was oven dried for at least 2 days so as to remove all water. The salt was dissolved thoroughly in deionized water and then made up to the desired volume. The newly made phosphate solution was then adjusted to the appropriate pH by adding orthophosphoric acid and recording the pH with a pH meter.

The 9-fluorenylmethyl chloroformate (FMOC-Cl) reagent was freshly prepare on each occasion by dissolving it in acetone to a concentration of 0.01 M.

Isolation of Periderms and Cuticles

The isolation of membrane samples was performed according to the method described previously (6)

Transport

The ion-exchange capacities of the isolated PM and PFCM were determined by measuring the ammonia released. Membranes were converted to the NH_4^+ form by treatment with 0.1 M NH_4Cl, followed by washing with deionized water to remove Cl^- ions, washing with deionized water,

and finally the membranes were treated with 0.1 M HCl to remove adsorbed NH_4^+ ions.

Transport experiments were carried out in a borosilicate cell consisting of two chambers: aqueous solution (donor)/membrane/aqueous solution (receiver). The membrane was laid out in the middle of the two chambers of the cell. Citric acid–Na_2HPO_4 buffer in the pH 6 to 8 range and deionized water were used as the receiver solution in contact with the outer surface of the membrane. Amino acid transport experiments were made with 0.05 M solutions.

This system was used to determine the penetration rates of many different solutes through the same membrane piece. Between solution changes the membrane was rinsed thoroughly with dilute nitric acid (10% v/v) and then with deionized water to remove adsorbed cations. Before starting a new experiment, the membrane was converted into the H^+ form by treatment with 1 M HCl for 45 to 60 minutes followed by rinsing with deionized water to remove all Cl^- ions. Determinations were repeated more than three times for each set of experimental conditions.

Selectivity

Each membrane in the A^+ or NH_4^+ form was usually equilibrated with a mixed amino acid + NH_4Cl solution of 0.05 M total composition. After equilibrium was reached (generally 24 hours), the equilibrated membrane was removed from solution. The selectivity coefficients of amino acids were measured by the batch equilibrium method at a temperature of 25 ± 1°C with various ratios of amino acid cations to ammonium ions. Mass balance was confirmed in all cases. Selectivity coefficients were calculated from the results of these experiments using Eq. (2).

Determination of Amino Acid

Amino acid determinations were carried out using a modification of a high performance liquid chromatography (HPLC) method (7, 8). The method involved gradient Perkin-Elmer Series 400 solvent delivery pumps. The samples were introduced to the column by a Perkin-Elmer ISS-100 autosampler. A Shimadzu Fluorescence HPLC Monitor RF-530 detector was used for detection. The emission light was monitored at 315 nm with an excitation wavelength of 270 nm. The output was recorded by a Perkin-Elmer CCI-100 Integrator. A column (25 cm × 4 mm) packed with APS-3u Hypersil (Shandon) was used for the separations. The eluent was acetonitrile–phosphate buffer (30/70, v/v). A flow rate of 1 mL/min was used throughout the separation. The amino acids were quantified by peak area measurements.

Determination of Ammonium

Ammonium was determined by a modification of the indophenol green method (9) using a complexing reagent to prevent interferences due to the precipitation of hydroxides in the reagent system. The Technicon Auto-analyzer II was used in this study for the analysis of ammonium ion. The system was comprised of a sampler, a pump, a water bath at constant temperature, and a spectrophotometer. Results from the samples were recorded with a single pen chart recorder. The system was connected to a BBC microcomputer which was used for the measurement of peak heights and the calculation of results. The samples were run at the rate of 40/hour, and the color development was carried out in the water bath at 37°C. The color intensity was measured at 650 nm.

RESULTS AND DISCUSSION

The ion-exchange capacities of PM and PFCM in the ammonium form were determined as 0.381 ± 0.043 meq·g^{-1} for PM and 0.205 ± 0.03 meq·g^{-1} for PFCM, respectively. The transport rates of the amino acids in the PM and PFCM were observed in the order Gly > L-Ala ≥ D-Ala > D-Val > DL-Leu > DL-Cys > DL-Met. This is the order of increasing molecular weights of amino acids, and the extension of the hydrophobic —CH_2— group. Steady-state conditions of permeability of amino acids were found to be very variable due to each membrane showing different permeability characteristics. The transport of amino acids across PM, PFCM, and dialysis membranes as a function of molecular weight and partial volume is given Fig. 1.

The transport rate of amino acid in the PM and PFCM depended on the molecular size of the amino acid and decreased with an increase in molecular size through extension of the hydrophobic —CH_2— group. The permeability also decreased through the S— group, and the slopes of the graphs are smaller than unity. The transport-limiting layer in the membranes studied acts both as a mobility and solubility barrier. The preceding discussion shows that the permeability of the membranes cannot be explained quantitatively in terms of the properties of cuticles and compounds. This is due to insufficient information concerning the structural aspects of cutin, soluble lipids, and the cutin/soluble lipid complex.

Many investigations have attempted to correlate cuticular penetration to physicochemical properties of both penetrant and cuticle (10). The permeabilities of cuticles observed so far range from about 10^{-6} to 10^{-10} m/s and is quite large (4). Some workers (11) tried to find a correlation between the water permeabilities of cuticles and their permeabilities to

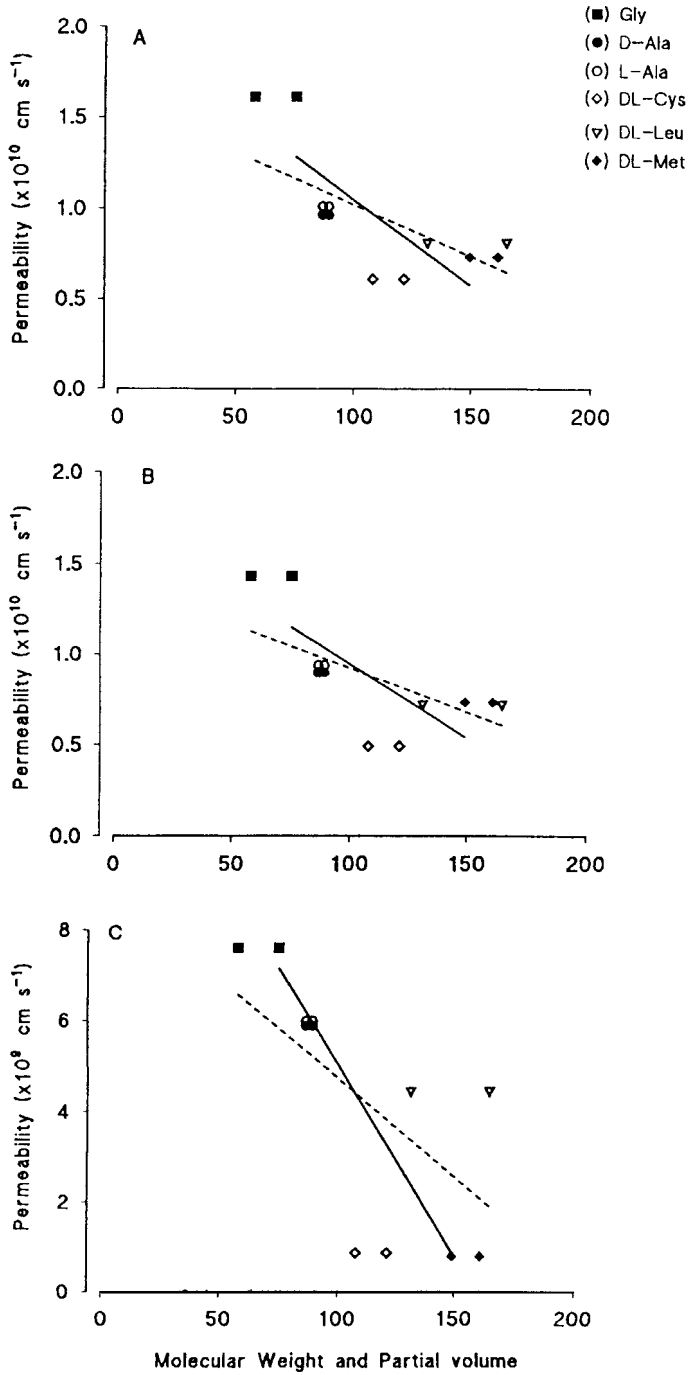

FIG. 1 Relation between permeability and the molecular weights (——) and partial volume (- - -) of amino acids. (A) PM, (B) PFCM, and (C) dialysis membrane.

organic solutes differing widely in lipid solubility. In the case of nonelectrolyte permeability, a correlation has been suggested between lipid solubility of the penetrant and its penetration rate, as observed with a cytoplasmic membrane (12). Most solutes are larger than urea and even larger than the pores of the polymer, and they move mainly or exclusively by diffusion (13).

The penetrations of amino acids were found to be very different between both periderm and cuticles, and the fluxes observed also vary extremely among membrane discs. The variation was large and complicated by the fact that almost half of the total membranes were shown to exhibit a permeability characteristic of the amino acids. The failure to adequately describe the permeabilities of PM and PFCM was due to membrane heterogeneity. The purpose of these experiments was to find the appropriate conditions for the transport of amino acids through PM and PFCM rather than to obtain permeabilities and diffusion coefficients.

Studies on membrane permeability should be made under steady-state conditions with constant, well-defined surface concentrations on each membrane surface. Simple relations hold between the flux of a compound under a defined driving force and the parameters of diffusibility and solubility of the compound within the membrane under such conditions. Another disadvantage is that the diffusion coefficient cannot be given quantitatively due to the extreme variability realized at steady state.

The penetration of molecules through plant surfaces is of immense practical importance, particularly as regards pesticide deposition, accumulation, and transport. However, there are many problems inherent in this approach (sometimes not obvious from the literature) such as extreme variability in the data and the permeants used, and differences in experimental design. Most reports on cuticular penetration omit calculations of a single parameter that can be used to compare values. A Student t-test analysis was run on the data and the variation was large; a t-test indicated ($p > .05$) no statistical difference in the permeation of amino acids through PM and PFCM. The variations found between periderm/cuticle discs were also quite large but no statistical differences were observed, so that interpretation of the data was difficult. Plant cuticles are not homogeneous (14), which means that gradients of mobilities and solubilities exist across cuticles (15).

There is a significant correlation between the permeabilities of PM and PFCM and the penetrant molecular weights and molar volumes. The correlation coefficients are $r = -0.78$ and $r = -0.75$ (for molecular weights for PM and PFCM), and $r = -0.71$ and $r = -0.66$ (for partial volume for PM and PFCM), respectively. The coefficients of determinations mean

that molecular size and partial volume are the major determinants for the amino acids tested. A somewhat better result can be obtained when log P is plotted versus the log of the physical parameter.

Amino acid selectivity was studied in solutions of various amino acid/ammonia ratios. Amino acid–ammonium exchanges on isolated PM and PFCM are characterized by ion-exchange isotherms (Fig. 2). Selectivity coefficient isotherms for amino acids–ammonium ion exchange of isolated PM and PFCM at 25 ± 1°C are shown as a function of the equivalent fraction of amino acids in a solution of $A^+ + NH_4^+$ at 0.05 M (Fig. 3). The selectivity coefficient was integrated over all ionic composition at an ionic fraction of 0.5.

In order to clarify the characteristics of the interaction between the exchanger and amino acids, correlation between the selectivity coefficients of amino acids and molecular weights and partial volumes of the respective amino acids were examined. The molecular weights and partial volumes of the amino acids were plotted against $K^{A}_{NH_4}$, as shown in Fig. 4. These results show that the selectivity coefficient value increases with an increase in molecular size of the amino acids through extension of the hydrophobic —CH_2— group.

The selectivity coefficient value increases with an increase in molecular size and partial volume of the amino acids through extension of the hydrophobic —CH_2— group. The study of solute sorption by a solid as a function of the solute concentration can elucidate some of the structural features of the materials on a molecular level (16). Sorption of solutes in cuticles, a reversible process, and desorption can be very rapid (13). Sorption isotherms can be interpreted in terms of sorbent–substrate interactions and the microstructure of the solid phase (17). It has been shown for a large number of liquid/liquid systems that the partition coefficient of a chemical species dramatically decreases upon ionization because the polarity of the molecule is greatly increased (18). More specifically, these data are discussed in terms of the mechanism of the transfer of amino acids into the periderm/cuticle and different sorptive properties.

A change in ion–water interactions in the exchanger phase might be responsible for the selectivity of this ion-exchange reaction (19). A theoretical treatment based on the Gibbs–Donnan model (20) leads to Eq. (3) for the selectivity coefficients of ion exchange for monovalent ions:

$$\ln K_{A/B} = \ln(r_B/r_A) + \pi(V_B - V_A)RT \qquad (3)$$

where $K_{A/B}$ denotes the selectivity coefficient of A to B, r_B and r_A are the activity coefficients of cation species in the exchanger phase and the

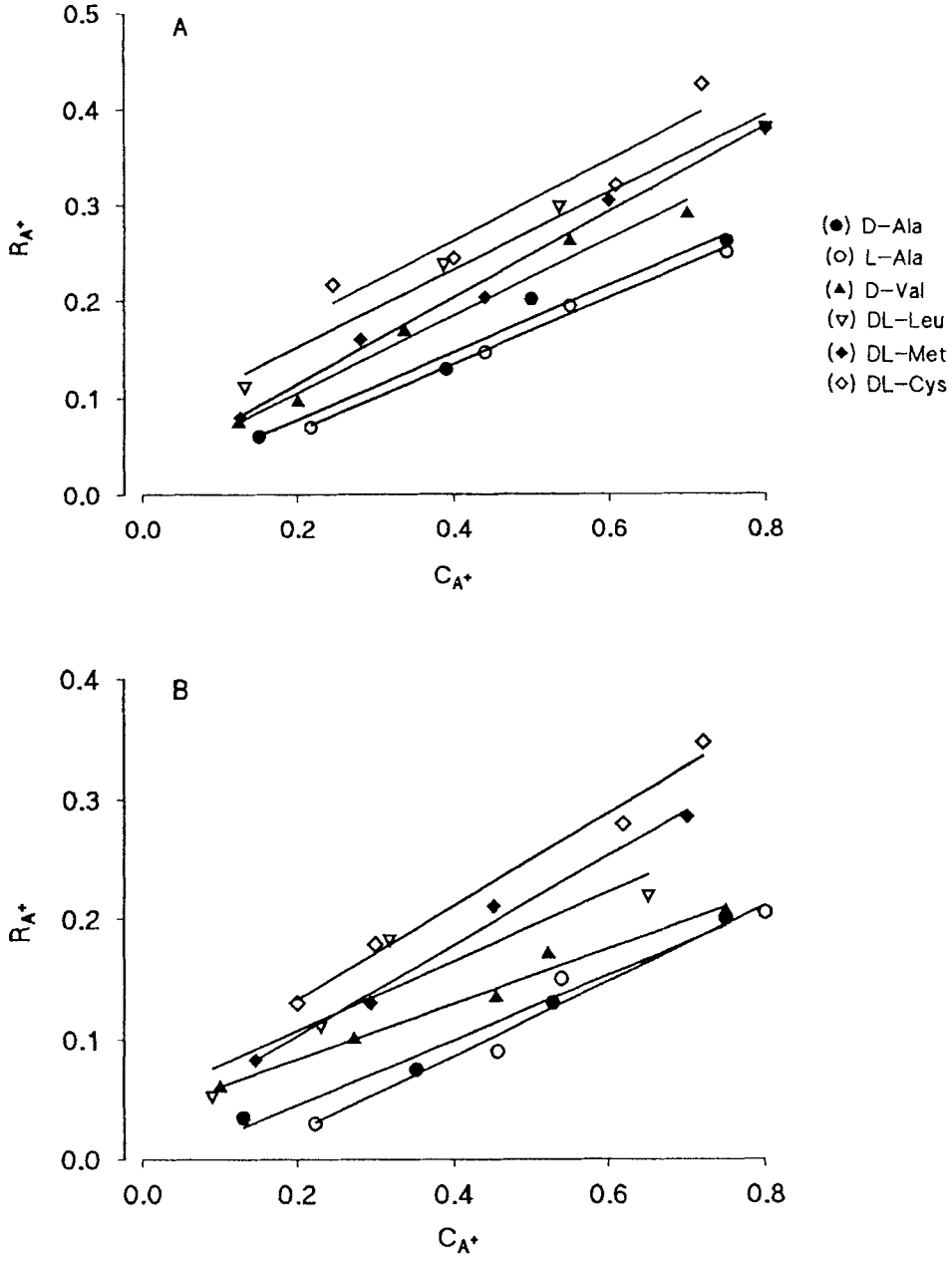

FIG. 2 A^+/NH_4^+ exchange isotherms for amino acids on the (A) PM and (B) PFCM. Total molarity 0.05 M.

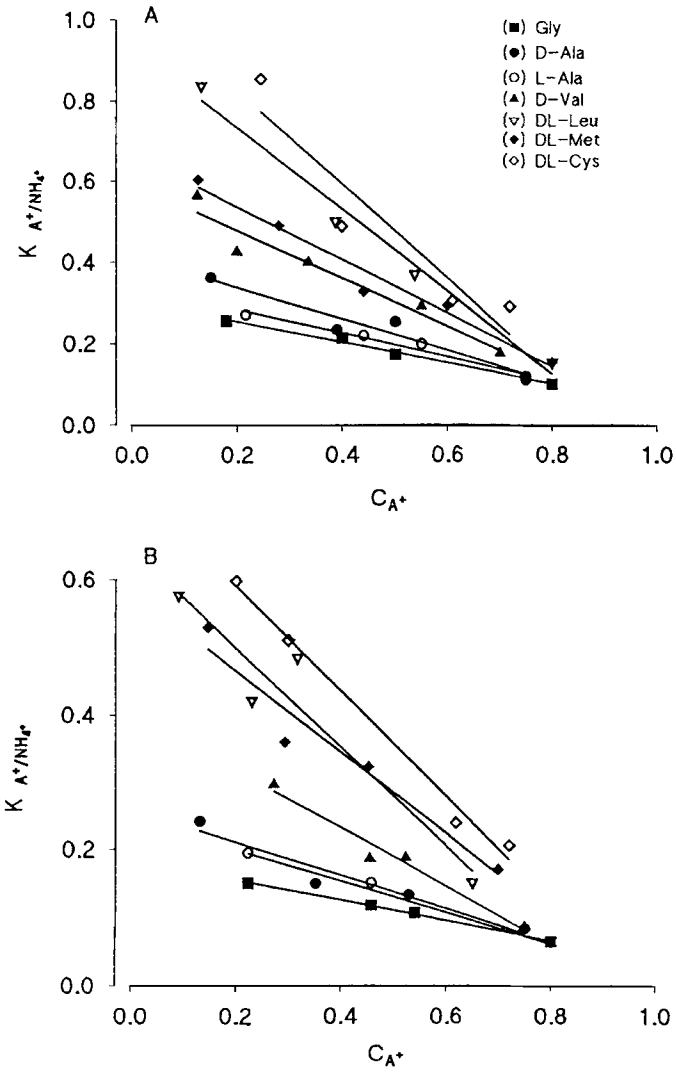

FIG. 3 The selectivity coefficients for (A) PM and (B) PFCM as a function of the equivalent fraction of amino acids in the solution phase at a total concentration of 0.05 M.

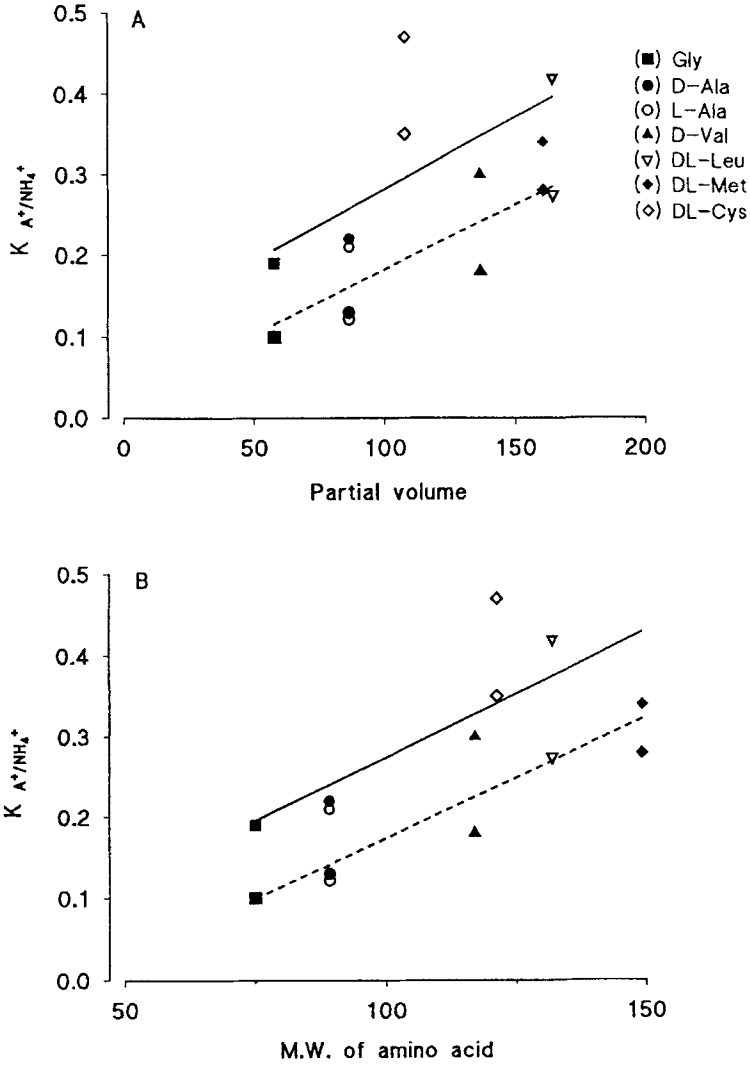

FIG. 4 Relation between selectivity and the partial volume (A) and molecular weights (B) of amino acids. (——) PM and (- - -) PFCM.

solution phase, respectively, and V_B and V_A are the partial molar volumes of the ionic species.

Most theories of ion-exchange selectivity attach key importance to ionic solvation phenomena. Selectivity in the "normal" order is governed by the free energies of hydration of the counterion. A model for predicting the selectivity coefficients from the solvation effect of the exchange ions was proposed (21, 22). The hydration is directly proportional to the charge and inversely proportional to the size of the ion. The electrostatic free energy can be reduced by association between fixed charges and counterions, and by screening neighboring fixed charges (23). It is assumed that the ion-exchange system behaves ideally except for the hydration effect. The activity coefficient terms in Eq. (3) are determined by the hydration effect and can be rewritten by including the hydration effect in the partial molar volume term as follows:

$$\ln K_{A/B} = \pi(V_B - V_A)RT \tag{4}$$

Gregor's model, which is not thermodynamically rigorous, is a very useful approximation for systems in which the hydration effect of ions is a dominant factor (20).

Kawakita et al. (24) studied the selectivity coefficients of amino acids for the ammonium ion on a strong cation-exchange resin. They obtained a good relationship between selectivity coefficients and physicochemical parameters, and they expressed the selectivity coefficients with a regression equation for each amino acid as a function of a physicochemical parameter, viz., partition coefficient, hydration number, and partial molar volume. They pointed out that the selectivity coefficient of amino acids is extensively affected by the hydrophilic interaction, together with the molecular size.

The experimental results were also assessed in terms of either the Langmuir or Freundlich adsorption isotherm. The selectivities of amino acid–ammonium exchange on isolated PM and PFCM resemble a characteristic Freundlich isotherm type (Fig. 5). Sorption of amino acids in isolated PM and PFCM obeys the Freundlich isotherm equation.

Taking periderm and cuticles as the solid phase, their behavior can be expressed by the Freundlich isotherm equation (Fig. 5). Isotherms obeying this relationship could be established for the sorption of amino acids in PM and PFCM. Freundlich isotherms are frequently encountered when solutes interact with heterogeneous substrates (25).

The sorptive capacity of plant cuticles for lipophilic chemicals is high (26). The cuticle acts not only as a transport barrier but also in an ion-exchange capacity. The linearity of the sorption isotherm was demonstrated over the whole range of amino acids. Nonpolar molecules are

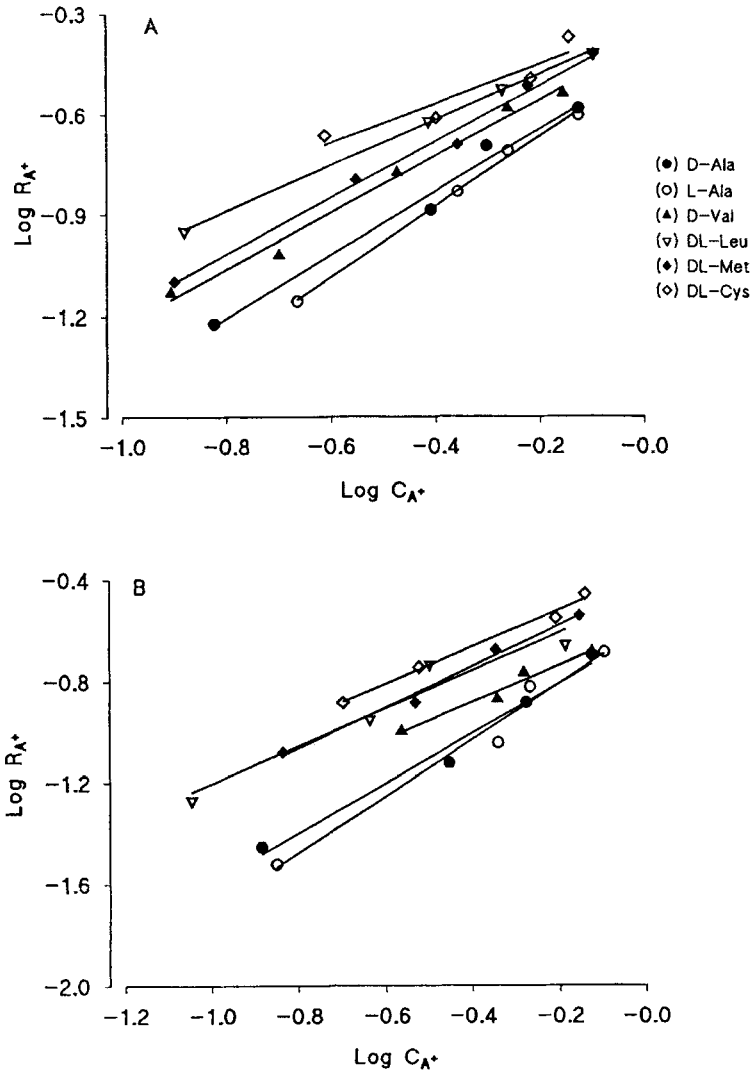

FIG. 5 The Freundlich exchange isotherms of amino acids on the (A) PM and (B) PFCM.

attracted to the aliphatic regions of cutin by London forces, while compounds having additional active substituents like hydroxyl, carbonyl, or amino groups can also form hydrogen bonds with appropriate functional groups of the polymer (16). In terms of sorptive properties, the cuticle behaves like an amorphous solid phase where both apolar and polar sorption sites are present, with the aliphatic ones dominating. The interaction of a solute molecule with the molecular structure of a polymer arrests the molecule for a certain time at a given place and thus increases order. At the same time, it produces a large heat of sorption (13).

The concentration dependence of the partitioning process is best described by sorption isotherms (25). These are plots of the molal equilibrium concentration of solute in the membrane versus the molal concentration in the aqueous solution at given experimental conditions. Straight lines over the whole concentration range are obtained if the two phases behave as ideal solutions (17). In these experiments, straight lines were obtained over the whole concentration range for both PM and PFCM. Thermodynamically, the correlation between the steric properties of amino acids and the configuration of the resin through the use of selectivity coefficient data was discussed previously (26, 27).

A significant correlation was found between the selectivity for PM and PFCM and their molecular weights and molar volumes. The correlation coefficients are $r = -0.77$ and $r = -0.83$ (for molecular weights for PM and PFCM), and $r = -0.67$ (for partial volume for PM and PFCM), respectively. A somewhat better result can be obtained when log $K^{A}_{NH_4}$ versus the log of the physical parameter is plotted. The selectivity coefficients of amino acid can be obtained using molecular weights, partial molar volumes, and hydrophobic groups through extension groups.

CONCLUSION

In these experiments it was shown that permeability and selectivity coefficients of amino acids can be accounted for by using the parameter's molecular size, molar volume, and hydrophobic groups through extension groups. Unfortunately, the practical value of the data is limited by the fact that permeabilities through both periderm and cuticle are extremely variable. The variability between these species is also high. If this is in fact the case, one would expect that the differences in the permeabilities of amino acids between periderm and cuticle should decrease with decreasing polarity of the compounds and increasing molecular size of amino acids.

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