Convergent Functional Groups. 4. Recognition and Transport of Amino Acids across a Liquid Membrane

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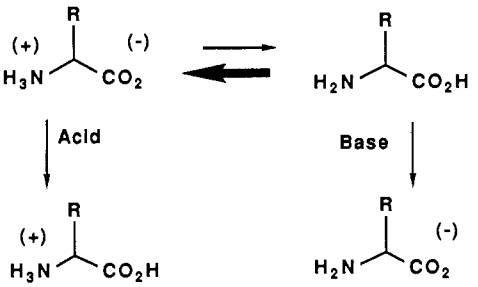
Abstract: A model receptor is described in the form of a molecular cleft that incorporates ionic, hydrophobic, and aromatic domains. These features result in the specific recognition of amino acids bearing aromatic side chains and provide a means by which they can be extracted from water at neutral pH. The selectivity extends to transport experiments, and these trends are compared with transport of amino acids under either basic or acidic conditions.

Molecular recognition is an essential feature of biochemical systems. Structures such as receptors, antibodies, and enzymes must all recognize their reaction partners—often in the presence of quite similar structures—before they are able to proceed with their functions. The ability to discriminate to such a degree was once thought to be an exclusive property of structures the size of biological macromolecules. Recent progress in bioorganic chemistry has shown that many of these functions can be incorporated into smaller, more synthetically accessible structures as model systems. In this paper we examine the progress that has been made in the recognition and transport of amino acids in model systems.

Because of the zwitterionic character amino acids show in solution[1] (eq 1), the likely sites of recognition are either an ammonium function or a carboxylate. The common amino acids



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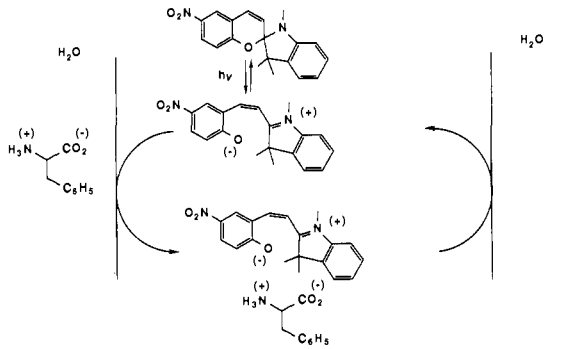
provide a rich variety of side chains as other sites for recognition, but the ammonium function has drawn the most attention. This focus can be traced to Pederson's[2] discovery that ammonium ions are bound by crown ethers; however, even in his original paper, Pederson noted that glycine hydrochloride formed a stoichiometric complex with 18-crown-6 in MeOH whereas glycine itself did not. This reluctance of amino acid zwitterions to form complexes has been encountered frequently in crown ether chemistry. Most recently Lehn[3] showed that the zwitterionic phenylalanine formed only a weak complex with crown ethers that bind simpler ammonium salts quite well. Tabushi[4] has observed similar weak binding with cyclodextrins. The nearby carboxylate thus considerably diminishes the ability of an ammonium ion to form hydrogen bonds to lone pair donors, a feature that has been borne out by theoretical calculations.[5]

The second problem is solubility. In water, where zwitterionic amino acids enjoy their greatest solubility, it is difficult to detect hydrogen bonds, and recent discussions[6] have emphasized that such bonds are rarely created but rather are traded between two sides of an equation. Because of the problems with zwitterions, the recognition of the ammonium acid form as a halide or PF_6^- salt or even as an amino ester hydrohalide has been undertaken by most workers, i.e., complexation under acidic conditions. On the other end of the pH scale, recognition of amino carboxylates as alkali metal salts has also been examined.

Table I. Relative Transport Rates through Toluene with Aliquat 336[8]

Ala	Val	Tyr	Leu	Trp	Phe
1	2	11	14	26	28

The related problem of transporting amino acids across liquid membranes has also attracted considerable interest. The zwitterionic nature of amino acids again comspires against such transport under neutral conditions since desolvation of the double ion is a costly energetic proposition. As in the binding studies, transport has been largely limited to either acidic or basic conditions. A single exception has been provided by Sunamoto[7] who showed that a merocyanine dye would permit the transport of phenylalanine across a liposomal bilayer (eq 2). Since phenylalanine methyl ester hydrochloride is also transported, the selectivity of this intriguing system is unclear. Moreover, with the relatively high concentrations of "carrier" in the bilayers, the photochemical event may be expected to alter the actual structure of the bilayer and its permeability. Both Lehn[8] and Cram[9] have transported the ammonium salts of amino acids or esters under acidic conditions, and asymmetric crown ethers have been successful in chiral recognition under these conditions.[10]



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(1) For a recent discussion see: Hughes, D. L.; Bergan, J. T.; Grabowski, E. J. J. *J. Org. Chem.* **1986**, *51*, 2579-2585.
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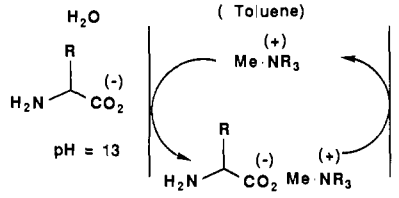
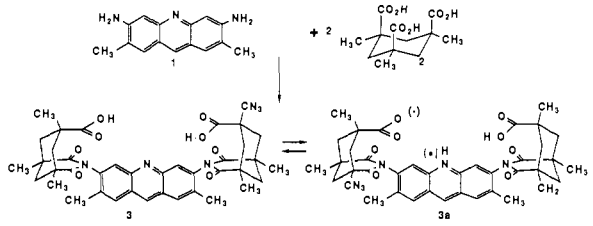
The *transport selectivity* for various amino acids has been examined under either acidic or basic conditions. The relative rates of transport are generally dominated by the nature of the side chain; the lipophilic side chain of, say, phenylalanine is transported much more readily across liquid membranes than the hydrophilic one of serine. For example, Lehn[8] reports the relative transport rates of Table I by using Aliquat 366 in toluene as the membrane phase (eq 3) and an aqueous source phase at pH 13.



(3)

Tsukube[11] also reports transport of copper salts under basic conditions but his N-blocked amino acid derivatives are sufficiently lipophilic that the blocking group may dominate the transport characteristics. With this history it seems fair to say that the complexation and transport of amino acids is not a well-developed area, and the recognition of zwitterionic forms is in its infancy.

We recently introduced[12] a new shape for model receptors which differs from the macrocyclic structures used by others. The new structures feature a molecular cleft, and because of this shape, it is possible to arrange functional groups which converge on smaller structures held within the cleft. It is the *convergence of functionality*, particularly of carboxylic acids, that represents the departure. A typical structure is rapidly assembled from acridine yellow **1** and Kemp's[13a] triacid **2** as shown in eq 4. In methanol,



(4)

the zwitterionic form of this molecule **3a** dominates (as shown by the downfield shift of the NMR signal for H_9), and the interior of the cleft presents a highly polar microenvironment complementary to the zwitterionic forms of α-amino acids. The new structures are quite insoluble in water, a property derived from their lipophilic coatings, and the acridine spacer unit provides a third domain: a large, flat, aromatic surface with a permanent dipole. This feature provides for the recognition of other aromatic structures by stacking forces.

These structural and physical characteristics led us to attempt the extraction of amino acids from their neutral aqueous solutions into chloroform with the new model receptor. A number of common amino acids, including valine, histidine, lysine, methionine, alanine, leucine, isoleucine, phenylglycine, phenylalanine, tryptophan, and tyrosine methyl ether were tested in this capacity. Of these, only the last three showed any interest in forsaking an aqueous environment for the cleavage of the model receptor, but these amino acids did so with great abandon; some 50% of the available receptors became occupied with the aromatic amino acids.[13b] Subsequent experimentation described below established that this figure was no mere accident but actually represents the formation of a ternary complex involving two molecules of the receptor for each bound amino acid. The extractability of the aromatic amino acids was limited only by the solubility of the receptor, which is about 2×10^{-3} M in $CHCl_3$.

The source of the observed binding selectivity appears to be more than simple lipophilicity of the side chains. Wolfenden[14] has determined the water affinity of the side chains of the amino acids with respect to the gas phase, and if the driving force for the extraction process involved the escape of the side chains from water, the expected order of extractability would be leucine = isoleucine > phenylalanine > tryptophan. Direct pairwise competition between tryptophan, phenylalanine, and tyrosine methyl ether in aqueous phases showed that tryptophan was extracted twice as readily as phenylalanine by the receptor. The selectivity observed is thus tryptophan > phenylalanine ≫ leucine. The specific recognition of the aromatic side chains of the amino acids by structural elements within the receptor is responsible for this trend.

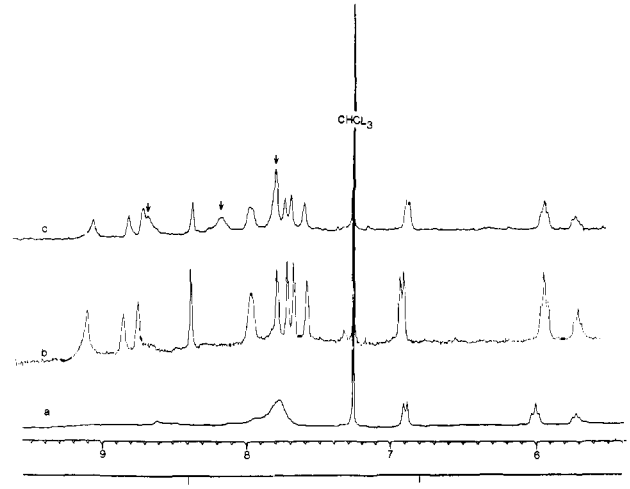


Figure 1. NMR spectra of the downfield region of the complex of **3** with phenylalanine: (a) ambient (25 °C) temperature spectrum; (b) spectrum at −38 °C; (c) spectrum at −25 °C with added **3**; the signals for uncomplexed **3** are indicated with arrows.

The nature of the recognition, aryl/aryl stacking interactions, was established by NMR spectroscopy. We have already published[13] the NMR spectrum of the phenylalanine complex; it features dramatic and differential upfield shifts of the phenyl protons that result in the simple, first-order NMR spectrum. Similar shifts are observed in the spectra of complexes of tryptophan and tyrosine methyl ether as described in the Experimental Section. Integration consistently gave a 2:1 ratio for receptor **3** to amino acid.

No readily apparent differences are seen between the UV spectra of the complexes and their components, and we were able to exclude the possibility of charge transfer as a stabilizing force in these complexes by using *p*-nitrophenylalanine. In direct competition with Phe, 6 times as much of the nitro-substituted amino acid was extracted. Thus, charge-transfer interactions cannot be important in the stacking; rather, dipole–dipole interactions may be operating between the aryl–subunits in the complex.

The structure that we originally proposed[13] is inadequate. The termolecular nature of the complex was established by the addition of more of the receptor **3** to a solution of the complexes. At −25 °C, two sets of signals were seen in the NMR spectra; one for the complex and one for the free diacid. Exchange of diacids subunits into and out of the complexes is slow at this temperature with coalescence at about ambient temperature, indicating that the kinetic stability of the complexes is high. Examination of the NMR spectrum of the 2:1 complex at lower temperature (−38 °C) established that at the slow exchange limit the two receptor molecules were different. The low-temperature spectrum is reproduced below (Figure 1) and shows that all ten aromatic signals of the acridine units are unique: the asymmetry of the phenylalanine is imposed on the entire complex. At ambient temperature, a broad spectrum is in accord with the loss of asymmetry and the loss of identity of the two acridine units. A proposed structure

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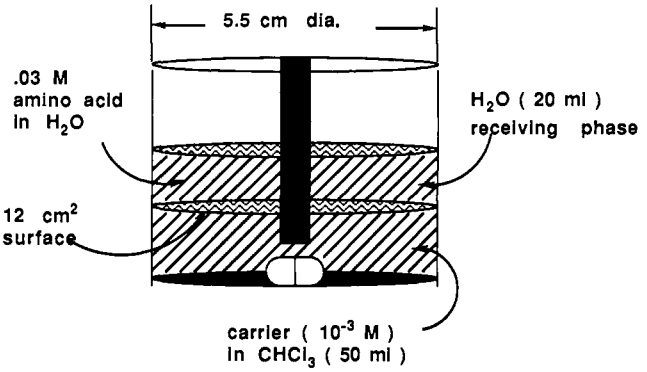
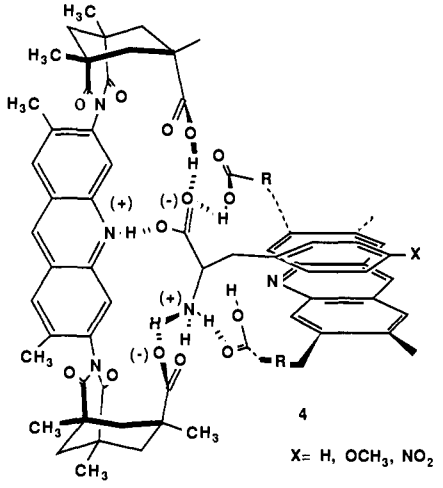


Figure 2. Lehn transport vessel as modified for carrier phase: solvents with densities greater than that of water.

for the complex (which is scarcely more than a restatement of these facts) is given below as **4**.



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The aromatic stacking surface which is the provenance of selectivity for the amino acids has even more to offer in the way of discrimination. For example, neither α-phenylglycine nor γ-phenylbutyrine forms complexes of the sort described above, and only traces of these amino acids are extracted under comparable conditions. For the α-phenyl case, the stacking interactions are not readily achieved while maintaining all of the ionic and hydrogen bonding interactions within the cleft. For γ-phenylbutyrine this may also be the case. In addition, the entropic price of reducing rotations may be too high with so many atoms involved between the ammonium ion and phenyl function.

Transport Experiments

The complexes obtained by extraction could be washed free of their amino acids by adding fresh water, and it seemed likely that transport across a liquid membrane could be observed in a suitably designed system. However, the hydrolytic instability[15] of the carriers prevented transport experiments involving contact with aqueous phases for long times. For the transport studies we used an apparatus described by Lehn[8] but modified for use with liquids with greater density than water. This is shown in Figure 2.

In order to permit comparisons between transport runs, the stirring rate was set at 500 rpm (as measured by a tachometer), and the source phase concentration of the amino acids was constant at 0.03 M. Aliquots were removed and assayed by ninhydrin analysis, and spot checks of the $CHCl_3$ phase revealed the presence of the 2:1 complexes. Even with these precautions, reproducibility was only fair and induction periods were noted.

The results (Table II) do reveal a significant selectivity for the β-aryl amino acids; as anticipated by the extraction experiments, neither α-phenylglycine nor γ-phenylbutyrine is transported to a significant degree. The transport experiments also reflect the relative extraction selectivities for the β-aryl amino acids.

Table II. Transport of Amino Acids by **3**[a]

Phe	Trp	Leu	Tyr methyl ether	γ-phenyl-butyrine
8.5	42	<0.1	18	0.2

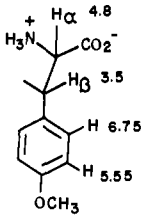
[a] Transport rates: mmol transported, h^{-1}, cm^{-2}, $[carrier]^{-1}$.

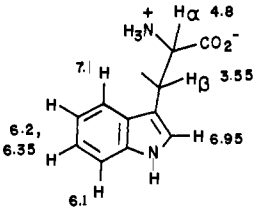
Conclusions

The new system described in this article represents the most effective means of recognition, complexation, and transport of neutral amino acids bearing aromatic side chains. It should be possible to alter the lining of these molecular clefts to recognize other amino acid side chains and even peptides. Recently, we have discovered that the complexation reactions of **3** are general for β-arylethylamines such as dopamine and tryptamine. We will report on these developments in due course.

Experimental Section

I. Extraction of Amino Acids. An excess of amino acid in 2 mL of H_2O and about 1 mg of **3** in 2 mL of $CHCl_3$ were shaken together for about 1 min at room temperature. The phases were separated, and the $CHCl_3$ was filtered through Na_2SO_4 and evaporated. The residue was taken up in $CDCl_3$ for NMR spectroscopy. The relevant portion of the spectrum for the L-Phe complex has been published,[13] and the downfield part of the spectrum at low temperature is reproduced in the text: for Figure 1 (parts a and b) diacid **3** = 2.5×10^{-3} M, L-Phe = 1.25×10^{-3} M; for (c) **3** = 2.5×10^{-3} M; L-Phe = 8.3×10^{-4} M. The chemical shifts (in ppm) of the protons indicated for the L-Trp and L-Tyr-*O*-methyl ether are given in the figures below. The other β-H signal is obscured by the signal for the receptor at 2.5–3 ppm.





In competition experiments, saturated solutions of the two amino acids were extracted with $CDCl_3$ solutions of **3** as above. Results for two separate determinations were averaged to give

Tyr-OMe:Phe	Tyr-OMe:Trp	Trp:Phe
1.8:1	1.7:1	2.8:1

Attempts with phenylglycine under these conditions showed only traces of the amino acid was extracted; in competition experiments Phe:PhenylGly > 10:1.

II. Transport. A solution of L-phenylalanine, 3.4×10^{-2} M, was prepared in 20.0 mL of water which had been purified by filtering through an organic removal cartridge (Fisher no. 09-035-61) and then distilled. A 1.0×10^{-3} M solution of the acridine diacid **3** was prepared in 50.0 mL of chloroform. The $CHCl_3$ solution was transferred to the transport vessel, Figure 2. The receiving phase, 20.0 mL of purified water, was carefully added to one side of the vessel, and the amino acid solution, 20.0 mL, was added likewise to the other side.

The carrier phase was then stirred at 500 rpm (as measured by a digital tachometer). An aliquot (1.0 mL) of the receiving phase was removed with a volumetric pipet prior to stirring to serve as a blank. Each hour thereafter, 1.0 mL of the receiving phase was removed and transferred to a 20.0-mL scintillation vial. At least once during each run, a 1-mL aliquot of the carrier phase was removed, dried (Na_2SO_4), and then evaporated, and the NMR spectrum of the residue was recorded in $CDCl_3$. After 6 h (when up to ~1% of the amino acid was transported), the aliquots were analyzed for the presence of amino acid. To each of the scintillation vials was added 1.0 mL of ninhydrin reagent.[16] The vials were capped, shaken, and placed in a bath of boiling water for 15 min. After the heating, each vial was shaken throughly. The solutions were then transferred to 10-mL volumetric flasks and diluted to 10.0 mL. Then their absorbance was measured at 570 nm.

Acknowledgment. We are grateful to the National Institutes of Health for support and to Dr. John Trend of the 3M Company for advice.

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