HF Reaction. The HF cleavage conditions are not ideal as demonstrated by the activity measurements of native lysozyme after this reaction. Certainly ribonuclease was not affected so severely by the HF conditions. The severity of this reaction undoubtedly varies from protein to protein.

Aspartic Acid. The α-β peptide bond migration of aspartic acid is well known. This reaction has been reported when glycine is C-terminal to aspartic acid.[50] There are three Asp-Gly sequences in lysozyme but unfortunately no simple method exists to determine the extent of cyclization and/or β migration in a peptide of this size.

Asparagine and Glutamine. The deamidation of asparagine and glutamine during HF cleavage has been reported.[51] It is not known how much, if any, deamidation occurs during the chain assembly process. Although asparagine and glutamine are usually used unblocked in the solid-phase procedures, blocking groups for these amino acids are available (for example, ref 52). Nor is it known what effect these groups will have in reducing any HF caused deamidation.

From the results of these two lysozyme syntheses it follows that there is need for milder conditions and a better solid support when attempting a long-term solid-phase synthesis. It has been demonstrated that cleavage conditions milder than HF would be desirable. Milder cleavage conditions, however, necessitate more easily removable side chain blocking groups, which in turn require less severe conditions for α-amino deprotection removal.

If the goal of protein synthesis had been to obtain a lysozyme-like native protein with high specific activity, the purification of the product obtained from this synthesis could have been further pursued. However, the synthesis of the native molecule was only the initial stage of researches leading to the production of synthetic analogs for use in structure–function investigations. We feel that the heterogeneity exhibited by the products obtained from these two lysozyme syntheses (and from other syntheses) underlines the necessity for further development of solid-phase procedures as applied to the synthesis of large molecules before attempts based on analog syntheses can be mounted. In spite of the remarkable success in the syntheses of ribonuclease A[4] and acyl-carrier protein,[53] it seems that the method is, as yet, not reliable enough for general applicability to the synthesis of any large molecule and its significant structural analogs. A problem obviously exists in the purification of analogs (affinity chromatography purification of structural analogs may or may not be of value, depending on their substrate binding properties). At the present time thorough characterizations of the analog are required to establish its nature. For a large molecule with one or two amino acid replacements (the remainder of the molecule being identical with the "native" product) this is a significant undertaking. For this reason it is felt that the development of solid-phase procedures specifically aimed at improving the syntheses of large molecules is crucial.

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(50) E. E. Haley, B. J. Corcoran, F. E. Dorer, and D. L. Buchanan, *Biochemistry*, **5**, 3229 (1966).
(51) A. B. Robinson, J. H. McKerrow, and P. Cary, *Proc. Nat. Acad. Sci. U. S.*, **66**, 753 (1970).
(52) P. G. Pietta and G. R. Marshall, *Chem. Commun.*, 650 (1970).
(53) W. S. Hancock, D. J. Prescott, G. R. Marshall, and P. R. Vagelos, *J. Biol. Chem.*, **247**, 6224 (1972).

Communications to the Editor

Transport of Amino Acids through Organic Liquid Membranes[1]

Sir:

Research in transport phenomena through membranes has reached a very broad development in recent years, above all in the biological field. The study of synthetic thin membranes[2] has also been actively pursued, especially in the case of cation transport using natural or synthetic macrocyclic[2,3] or macrobicyclic[4] carriers. On the other hand, much attention has been directed toward the passive selective and specific transport of inorganic ions through organic bulk liquid membranes[5–7] between two aqueous phases. Transport of sodium cations against their concentration gradient pumped by coupling to a movement of protons in the opposite direction has been described.[8] *Via* studies in cation transport[4] by cryptate[9] type carrier complexes, we have become interested in the general area of transport processes of organic molecules. Indeed, the development of transport systems for organic molecules may have wide consequences in the study of transport

(1) Transport in Organic Chemistry. I.
(2) P. Läuger, *Angew. Chem.*, **81**, 56 (1969); *Angew. Chem., Int. Ed. Engl.*, **8**, 42 (1969).
(3) B. C. Pressman and D. H. Haynes in "The Molecular Basis of Membrane Function," D. C. Tosteson, Ed., Prentice-Hall, Englewood Cliffs, N. J., 1969, p 221.
(4) J. M. Lehn and M. Kirch, unpublished results.
(5) H. L. Rosano, J. H. Schulman, and J. B. Weisbuch, *Ann. N. Y. Acad. Sci.*, **92**, 457 (1961).
(6) K. Sollner and G. M. Shean, *J. Amer. Chem. Soc.*, **86**, 1901 (1964).
(7) K. Sollner in "Diffusion Processes, Proceedings of the Thomas Graham Memorial Symposium, University of Strathclyde," Vol. 2, J. N. Sherwood, A. V. Chadwick, W. M. Muir, and F. L. Swinton, Ed., Gordon and Breach, London, 1971, p 655.
(8) J. H. Moore and A. S. Schechter, *Nature (London)*, **222**, 476 (1969).
(9) B. Dietrich, J. M. Lehn, and J. P. Sauvage, *Tetrahedron Lett.*, 2889 (1969); J. M. Lehn and J. P. Sauvage, *Chem. Commun.*, 440 (1971).

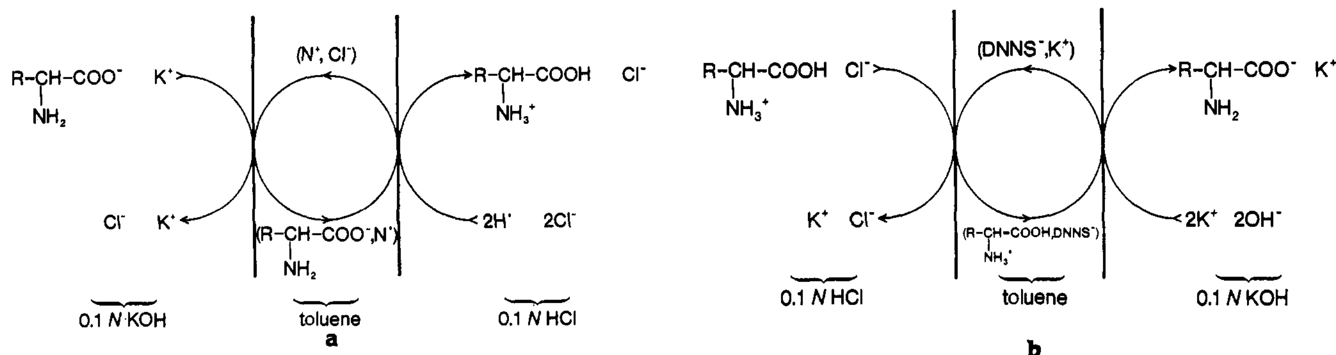

Figure 1. Transport of amino acids through a toluene barrier: (a) from basic to acid aqueous phases using a positively charged carrier (N^+, tricaprylylmethylammonium chloride, Aliquat 336); (b) from acid to basic aqueous phases using a negatively charged carrier ($DNNS^-$, dinonylnaphthalenesulfonate).

mechanisms as well as in applications to separation science. One may hope that owing to the architectural power of organic synthesis it may be possible to build carrier molecules designed to accomplish the selective transport of a given substrate molecule.

In the present communication we report some of our results concerning the transport of amino acids and dipeptides either along but generally against their concentration gradient, through a bulk toluene membrane separating two aqueous phases. The system is in most cases pumped by protonation–deprotonation processes and countertransport of an inorganic ion. Active transport of amino acids in biology[10] is a complex process linked to cation transport[11] and to the cation (H^+, Na^+, K^+) concentration gradients existing across cell membranes.[12,13] Using a simple set-up[14] in which two aqueous phases (L, left; R, right) are bridged by a toluene membrane layer (M), the processes shown in Figure 1 may be effected.

An amino acid in the carboxylate form $RCH(NH_2)COO^-$ (AA^-) may be transferred from L (0.1 *N* KOH) to M when a positively charged highly lipophilic carrier T^+ (quaternary ammonium salt $T^+ = N^+$; Aliquat 336) is added to M. As the (N^+, AA^-) species reaches R (0.1 *N* HCl) the amino acid is extracted into the acid phase by protonation ($AA^- + 2H^+ \rightarrow AA^+$, $R\text{-}CH(COOH)NH_3^+$); a chloride anion is transported back to the L–M interphase where it is exchanged against AA^- and the process is repeated (Figure 1a) (Table I, entries 1–8). Conversely, starting with AA^+ in L (0.1 *N* HCl) and using a negatively charged carrier T^- (dinonylnaphthalenesulfonate, $T^- = DNNS^-$), transport of AA^+ from L to R (0.1 *N* KOH) *via* (AA^+,$DNNS^-$) in M and back transport of K^+ occur (Figure 1) (Table I, entries 12 and 13).

Most experiments have been conducted along the $AA^- \rightarrow (AA^-,N^+) \rightarrow AA^+$ scheme. The concentration increase of AA^+ in R has been measured as a function of time for various amino acids using spectroscopic methods (uv, nmr) or ninhydrin titration. The results are shown in Figure 2 and the rates of transport are listed in Table I.

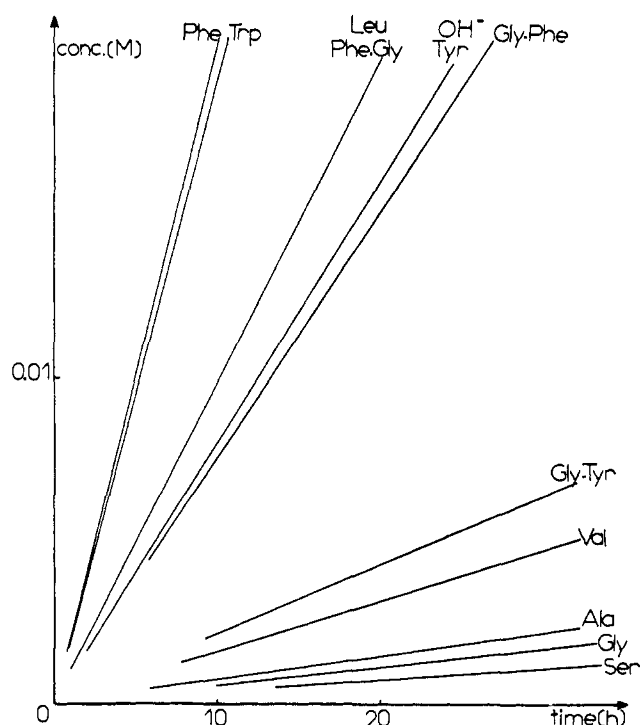

Figure 2. Transport of amino acids and dipeptides (concentration change in R phase *vs.* time) from 0.1 *N* KOH to 0.1 *N* HCl through a toluene barrier containing the quaternary ammonium chloride Aliquat 336 (10^{-2} *M*) (see Figure 1a). This figure shows only the linear part of the plots. Curvatures are observed both at the beginning (before the system reaches a stationary state) and toward the end of the process, when the amino acid concentration in L becomes low. A detailed description will be given in the final account.

In Figures 1a and b, the amino acid is transported against its concentration gradient once half of the quantity initially present in L has been transferred to R. This has been observed in the present experiments; however, the species is AA^+ on one side and AA^- on the other side of the liquid membrane. Strict transport of AA^+ against its concentration gradient pumped by simultaneous back transport of K^+ along its gradient is described in entry 14 of Table I. As one may expect, the rates slow down as the transport goes toward completion.

(10) K. Ring, *Angew. Chem.*, **82**, 343 (1970); *Angew. Chem., Int. Ed. Engl.*, **9**, 345 (1970).

(11) E. Heinz, Ed., "Na-linked Transport of Organic Solutes," Springer-Verlag, New York, N. Y., 1972.

(12) A. A. Eddy and J. A. Nowacki, *Biochem. J.*, **122**, 701 (1971).

(13) A. A. Eddy, *ibid.*, **127**, 52, 53, 54P (1972).

(14) The experimental set-up consists of a cylindrical glass cell (6 cm o.d.; height 5 cm) containing a central glass wall which separates the two aqueous phases (40 ml each). The organic layer (50 ml of toluene) lies above these phases and bridges them across the central separation. All layers are stirred at constant speed and the cell is maintained at 20 ± 0.5°. In order to assure internal consistency of the results despite the numerous factors on which such experiments depend[7] the same set-up has been used for all experiments.

Table I. Rates of Transport of Amino Acids and Other Molecules through a Liquid Membrane[a]

Entry	Substrate	Rate of transport, 10^8 mol/(hr cm^2)	Entry	Substrate	Rate of transport, 10^8 mol/(hr cm^2)
1	Phenylalanine	500	9	Glycyltyrosine	52
2	Tryptophan	475	10	Glycylphenyl-alanine	190
3	Leucine	250	11	Phenylalanyl-glycine	250
4	Tyrosine	200	12[b]	Tyrosine	370
5	Valine	37	13[b]	Tryptophan	220
6	Alanine	18	14[c]	Tyrosine	100
7	Glycine	14	15[d]	Acetylcholine	3000
8	Serine	8	16[e]	OH^-	200

[a] Except when otherwise stated the experiments follow Figure 1a, with 10^{-2} *M* N^+ = Aliquat 336 (methyltricaprylylammonium chloride) in toluene and a starting concentration of amino acid in L of 5×10^{-2} *M*. Reproducibility is within 5%. [b] Experiment following Figure 1b, with 10^{-2} *M* $DNNS^-$ (dinonylnaphthalenesulfonate) in toluene and 5×10^{-2} *M* starting concentration of amino acid in L. [c] L = 3×10^{-2} *M* Tyr in 0.1 *N* HCl; M = 10^{-2} *M* $DNNS^-$ in toluene; R = 3×10^{-2} *M* Tyr and 1 *M* KCl in 0.1 *N* HCl. [d] L = 0.87 *M* acetylcholine bromide in water; R = 1 *M* KBr; M = $DNNS^-$ 0.1 *M* in toluene. [e] L = 5×10^{-2} *N* KOH; R = 5×10^{-3} *N* HCl; M = 10^{-2} *M* Aliquat 336 in toluene.

Measurement of distribution coefficients of AA^- between 0.1 *N* KOH (L) and toluene containing N^+ (M) showed that the relative rates of AA^- transport from L to R follow the order of the distribution coefficients. Thus, the specificity of the process is controlled by the thermodynamic interphase equilibrium between L and M phases. The kinetically important steps, *i.e.*, L → M and M → R transfer, seem to be sufficiently similar for the various amino acids so that only the concentration of (AA^-,N^+) in M appears to differentiate the various substrates. The same result holds for T^- transport (Table I, entries 12, 13, and 15). A case which may lead to interesting further development is the transport of acetylcholine against K^+ (Table I, entry 15).

It has also been found that transport of OH^- itself is competing with AA^- transport (Figure 2; Table I, entry 16). Finally, three dipeptides have been included in our study. Interestingly, about a 20% difference in transport rate is observed between phenylalanylglycine and glycylphenylalanine (Table I; entries 10 and 11).[15]

The present results lead to certain conclusions as well as to prospects for further investigations. (1) Transport of amino acids against Cl^- or K^+ has been demonstrated. (2) Transport against the concentration gradient, pumped by chemical energy, has been observed. (3) With the present carriers the specificity of the process is thermodynamically controlled by the distribution equilibrium between the starting aqueous phase and the membrane. (4) Steric effects in the substrate–carrier species on transport specificity should be observable. (5) Carrier design *via* organic synthesis may allow controlling transport specificity. (6) Kinetic effects on transport specificity should be observable as is the case in cation transport.[4] (7) *Chirospecific* transport, allowing separation of racemic mixtures, may be envisaged using either an optically active membrane phase or a chiral carrier. (8) Similar experiments may be performed on various types of molecules. A deeper understanding of transport processes of organic molecules as well as applications in separation science may result from such studies. Research along these lines is being actively pursued.[16]

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(15) The mechanistic details will be discussed in the full account of this work. However, some results may be mentioned here. In the absence of a carrier the rates of transport (leakage) are reduced by a factor of more than 10^5. The transport shows *saturation* kinetics with respect to carrier concentration. The carrier is located in the organic phase (not detected in the aqueous layers; see also ref 6 and 7 and references therein). The chemical nature of the carrier–substrate complex in the membrane phase is thought to be an ion pair in analogy with results on inorganic ions.[6,7] Control experiments confirm the back transport of Cl^- or K^+ ions. Slow changes in pH are observed since (i) amino acid protonation–deprotonation processes occur; (ii) OH^- transport competes with AA^-. The transport of amino acid goes asymptotically to completion in the conditions described here. Finally, the nature of the kinetically important steps, transfer across the interfaces, rests on previous results obtained with inorganic ions (ref 6 and 7 and references therein). We have also shown that changes in surface area at the interfaces lead to proportional changes in transport rates. The formation and dissociation of the ion-pair carrier–substrate complex should be a very fast process.[7] The synthesis of new carriers may shed light on the possible importance of these steps in the overall kinetics. Additional investigations of these mechanistic and kinetic aspects are under way and will be described in detail in the full paper.

(16) Note Added in Proof. In anology to the above results on transport selectivity, it has recently been shown that the permeability of phospholipid vesicles to amino acids is proportional to the hydrophobicity of the latter (P. D. Wilson and K. P. Wheeler, *Biochem. Soc. Trans.*, **1**, 369 (1973)).

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The Electron Paramagnetic Resonance Spectra of 1,1-Dialkylhydrazyl Radicals in Solution[1]

Sir:

Although triarylhydrazyl radicals are among the most stable radicals known[2] there is very little information available concerning alkylhydrazyl radicals. The epr spectra of three 1,1-dialkylhydrazyls trapped in an adamantane matrix have been reported very recently.[3] The uv spectra of the radicals formed by reaction of hydroxyl with 1,1- and 1,2-dimethylhydrazines in aqueous solutions have also been reported recently.[4] Since this work was completed data on some trialkylhydrazyls[5] and 1,1-dialkyl-2-benzenesulfonylhydrazyls[6] have appeared.

We wish to report that 1,1-dialkylhydrazyl radicals can be readily produced in solution by photolysis of solutions containing 1,1-dialkylhydrazines and di-*tert*-butyl peroxide. Photolysis in the cavity of a Varian E-3 epr spectrometer yielded strong, well-resolved spectra for a number of 1,1-dialkylhydrazyls (see, *e.g.*,

(1) Issued as NRCC No. 13419.
(2) For a leading review see: A. R. Forrester, J. M. Hay, and R. H. Thomson, "Organic Chemistry of Stable Free Radicals," Academic Press, New York, N. Y., 1968, p 137.
(3) D. E. Wood, C. A. Wood, and W. A. Latham, *J. Amer. Chem. Soc.*, **94**, 9278 (1972).
(4) E. Hayon and M. Simic, *ibid.*, **94**, 42 (1972).
(5) S. F. Nelsen and R. T. Landis, II, *ibid.*, **95**, 2719 (1973); *ibid.*, in press.
(6) A. T. Balaban and R. Istratoiu, *Tetrahedron Lett.*, 1879 (1973).