Tetrahedron Letters, Vol.22, No.40, pp 3981 - 3984, 1981
Printed in Great Britain

0040-4039/81/403981-04$02.00/0

K^+ ION - DEPENDENT, ACTIVE TRANSPORT OF AMINO ACID ANIONS BY MACROCYCLIC CARRIERS

Hiroshi Tsukube

Department of Chemistry, College of Liberal Arts and Science,
Okayama University, Okayama 700, Japan.

Active and selective transport of amino acid anions was successfully mediated by macrocyclic carriers, coupled with K^+ ion transport.

Synthetic macrocycles have been recognized as model carrier for selective transport of cation across a membrane.[1] In such systems, the rates of cation-transport have significantly been influenced by the nature of anion species which accompanied with cation-macrocycle complexes. Recently Lamb et al.[2] presented systematic studies of the anion effects on the cation transport phenomena, and proposed a possibility that an anion species can be also distinguished by using macrocyclic carriers.[3]

Here we report that amino acid anions were selectively transported by cation-macrocycle complex carrier against their concentration gradients (so-called "uphill transport"). In some biological transport systems, certain monovalent cation such as Na^+ and K^+ ions is believed to regulate the amino acid transport via Mitchell's symporter mechanism.[4] By using simple macrocyclic carriers, artificial cation-dependent amino acid transport is successfully achieved below.

Two macrocyclic carriers, (1) and (2), were examined, which are well-known to be effective in the selective transport of K^+ ion over Li^+, Na^+, Cs^+, and other ions.[5] The active transport of amino acid anions across a chloroform membrane was studied by using a U-tube apparatus similar to those previously

described.[6] The macrocycle in the chloroform was placed in the base of the U, and buffered aqueous solutions of equal amino acid anion concentrations were placed in the arms of the U, floating on the chloroform membrane. The concentrations of amino acid anions in both aqueous phases were monitored spectroscopically, and the concentrations of each amino acid anion of apparently steady state (usually after 24 h) are listed in Table.

For N-benzoyl-phenylalanine as a typical example, the actively transported amounts were confirmed to be enhanced by increasing K^+ ion concentration in the aqueous phase I, showing that coupling to the K^+ ion gradient was used to pump the amino acid anions. The observed transport process can be explained by a fou step reaction sequence (see Figure): (i) At the aqueous phase I / membrane interface, macrocycle selectively complexes with K^+ ion ("cation selection"), allowing that amino acid anion is extracted into the membrane by ion-pairing ("anion selection"); (ii) The formed ternary complex of amino acid - K^+ - macrocyclic carrier diffuses across the membrane; (iii) At the membrane / aqueous phase II interface, releasing K^+ ion and amino acid anion occurs; (iv) The empty macrocycle diffuses back to the aqueous phase I / membrane interface where the cycle starts again.

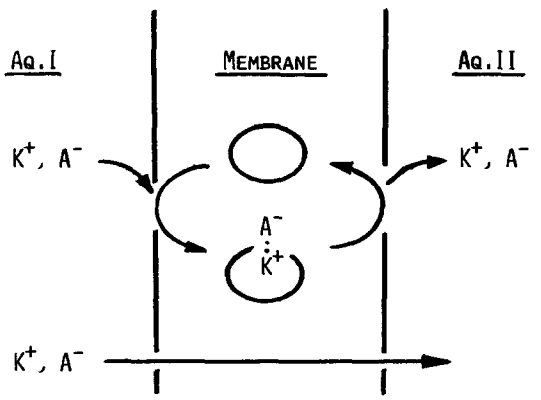
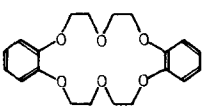
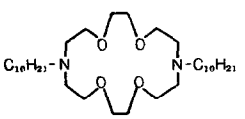


FIGURE. AMINO ACID TRANSPORT MEMBRANE.
A⁻:AMINO ACID ANION, ◯:MACROCYCLE.



(1)



(2)

Table. Active Transport of Amino Acid Anions by Macrocyclic Carriers.[a]

Substrate	Salt in Aq. I (mmol)	Equilibrated Substrate Distribution (mmol)[b] [Substrate in Aq.I] / [Substrate in Aq.II] (1)	(2)
Bz-Phe	No	0.235 / 0.235	0.237 / 0.237
	KCl (2.5)	0.157 / 0.336	0.212 / 0.263
	KCl (5.0)	0.117 / 0.376	0.180 / 0.299
	KCl (10.0)	0.063 / 0.436	0.137 / 0.349
	LiCl (10.0)	0.226 / 0.254	0.228 / 0.264
	NaCl (10.0)	0.184 / 0.259	0.171 / 0.305
	CsCl (10.0)	0.207 / 0.278	0.246 / 0.254
Bz-Leu	KCl (10.0)	0.121 / 0.379	0.191 / 0.304
Bz-Met	KCl (10.0)	0.119 / 0.375	0.197 / 0.303
Bz-Val	KCl (10.0)	0.154 / 0.343	0.216 / 0.282
Bz-His	KCl (10.0)	0.243 / 0.257	0.239 / 0.261
Bz-Ala	KCl (10.0)	0.231 / 0.263	0.239 / 0.254
Bz-Gly-Gly	KCl (10.0)	0.245 / 0.255	0.245 / 0.249
Bz-Gly	KCl (10.0)	0.243 / 0.257	0.243 / 0.257

(a) Initial concentrations: Aq.I; substrate, 0.25 mmol/0.05N-NaOH, 5ml. Aq. II; substrate, 0.25 mmol/0.05N-NaOH, 5ml. Membrane; macrocycle, 0.056 mmol/$CHCl_3$, 12 ml. If noted, inorganic salt was added into Aq.I.

(b) The concentrations of substrate in both aqueous phases were determined spectroscopically after 24 h.

This active transport system by using K^+-macrocycle complex carriers showed highly excellent substrate specificity for a series of amino acid anion examined: Bz-Gly $\simeq$ Bz-Gly-Gly $\simeq$ Bz-Ala $\simeq$ Bz-His $<$ Bz-Val $<$ Bz-Met $\simeq$ Bz-Leu $<$ Bz-Phe. This is largely different from those displayed by previously reported "transition metal complex carriers":[6] Bz-Gly $\simeq$ Bz-Ala $>$ Bz-Glu $>$ Bz-Met $>$ Bz-Val $>$ Bz-Leu $>$ Bz-Phe. In the present transport system, the amino acid anions with higher hydrophobicities were found to allow faster transport,

showing that anion extraction process into the membrane by complexation with K^+-macrocycle complex carrier could be importantly operating.

The macrocycle (1) showed higher transport abilities for a variety of amino acid anions, compared to (2). Since some macrocycles containing nitrogen donors[7] have been reported to be less effective in the complexation with alkali metal cations, the transport abilities of these carriers seem to be controlled in the extraction process.

The regulation of the active transport of amino acid is clearly demonstrated by appropriate choice of co-transported cation. The transport phenomena hardly occurred when Li^+, Na^+, and Cs^+ ions were employed as co-transported cation. Therefore, this cation-dependence of transport abilities was determined by cation selectivities of the used macrocyclic carriers.

The present transport system may provide an artificial analog to the biological symport of amino acids, and be considered as a prototype for design of specific anion transport membrane. A number of variations and extensions may be envisaged either as biological models or potential applications.

References and Notes

1. D.J.Cram, "Applications of Biochemical Systems in Organic Chemistry", Part II, John-Wiley, New York, 1976, p815.
2. J.D.Lamb, J.J.Christensen, S.R.Izatt, K.Bedke, M.S.Astin, R.M.Izatt, J.Am. Chem.Soc., **102**, 3399 (1980).
3. J.M.Lehn, J.Simon, A.Moradpour, Helv.Chim.Acta, **61**, 2407 (1978); M.Sugiura, T.Shinbo, Bull.Chem.Soc.Jpn., **52**, 684 (1979).
4. K.Ring, Angew.Chem.Int.Ed.Engl., **9**, 345 (1970).
5. C.F.Reusch, E.L.Cussler, Am.Inst.Chem.Eng.J., **19**, 736 (1973); C.J.Pedersen, H.K.Frensdorff, Angew.Chem.Int.Ed.Engl., **11**, 16 (1972).
6. K.Maruyama, H.Tsukube, T.Araki, Tetrahedron Lett., **22**, 2001 (1981).
7. J.J.Christensen, D.J.Eatough, R.M.Izatt, Chem.Rev., **74**, 351 (1974).

(Received in Japan 19 June 1981)