Active and Passive Transport of Amino-acid and Oligopeptide Derivatives by Artificial Ionophore-K^+ Complexes [1]

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Four types of crown ether [the cyclic crown ether (1), the diaza-crown ether (2), the non-cyclic crown ether (3), and the cryptand (4)] have been examined as novel anion transport carriers. They effectively mediated the passive and active transport of amino-acid and oligopeptide derivatives as carboxylate anions, coupled with K^+ ion transport. Their transport efficiences, selectivities, and directions were essentially controlled by the nature of the crown ether used and of the cations present. They provide a new chemical analogue of biological transport systems, as well as further applications for the separation of biologically important anions.

SINCE the membrane transport of amino-acids, oligopeptides, and related substrates plays an essential role in many biological systems,[2] the construction of model systems is important not only for the simulation of biochemical systems, but also for the development of new separation science methodology. Among the artificial carriers previously developed, macrocyclic polyethers and crown ethers have been well-recognized as potential carrier models for the selective transport of cations.[3] In such systems, the rates of cation transport have been significantly influenced by the nature of the anionic species which accompanied the cation–macrocyclic polyether complexes. Recently Lamb *et al.* presented systematic studies of the effects of anions on cation transport phenomena, and revealed that anions with smaller hydration energies allowed faster cation transport.[4] Since other investigators have already demonstrated that picrate and other simple lipophilic anions were effectively transported, together with alkali-metal cations,[5] anionic species of biological importance (*e.g.* amino-acid and oligopeptide anions) may be distinguished by using macrocyclic crown ethers.

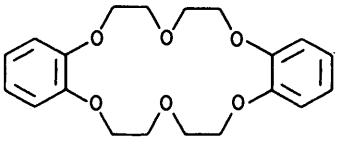
In an earlier communication,[1] we reported that two kinds of crown ether, the crown ether (1) and the diaza-crown ether (2), effectively mediated active transport of some amino-acid anions, coupled with K^+ ion transport. In some biological transport systems, certain univalent cations such as Na^+ and K^+ ions are believed to regulate the amino-acid transport *via* Mitchell's symporter mechanism.[2] By using simple macrocyclic crown ethers, artificial cation-dependent amino-acid transport has, in principle, been achieved. The present article gives details of passive and active transport experiments of a series of biologically important anions. As synthetic ionophores, we examined the non-cyclic crown ether (3) and the cryptand (4) as well as the two crown ethers (1) and (2). Their transport behaviour was found to be largely dependent on the structures of the ionophores and on the nature of the co-transported cations. In particular, the choice of the ionophore employed was an essential factor in determining the efficiency, the selectivity, and the direction of transport.

RESULTS AND DISCUSSION

Liquid Membrane System and Synthetic Ionophores.—A liquid membrane has widely been employed as a potential biomembrane model. For example, in the liquid membrane system depicted in Figure 1, guest anions are transported from one aqueous phase (the source phase: aqueous phase I) to the other aqueous phase (the receiving phase: aqueous phase II) *via* the chloroform ' membrane '. Movement of charged species through a hydrophobic membrane is accomplished by the presence of a suitable artificial carrier, similar to naturally occurring ionophores and transport proteins. The present anion-transport system can be explained by the following, four-step reaction sequence (see Figure 1). (i) At the aqueous phase I/membrane interface, the ionophore selectively complexes the M^+ ion (' cation selection '), thus allowing extraction of the guest anion into the membrane by ion-pairing (' anion-selection '). (ii) The ternary complex of the guest anion–M^+–ionophore thus formed diffuses across the membrane. (iii) At the membrane/aqueous phase II interface, release of the M^+ ion and guest anion occurs. (iv) The empty ionophore diffuses back to the aqueous phase I/membrane interface where the cycle starts again. The present system can be considered, therefore, as an artificial model for some biological symport systems.[2]

Four different types of crown ether whose cation complexation properties are well-understood [6] were chosen as artificial ionophores; they have limited solubility in water which prevents loss of carrier to the aqueous phases. The macrocyclic ionophores (1) and (2) are known to complex effectively with Na^+ and K^+ ions whilst the non-cyclic crown ether (3) can readily adapt to metal ions with differing ion radii such as Li^+, Na^+, K^+, and Cs^+. The cryptand (4) is also a potential complexing reagent for Na^+, K^+, and Ba^{2+}. The characteristics of these crown ethers were expected to offer a variety of different functions in the anion-transport process. Previously the compounds have been widely used as effective cation carriers; in the present article they are applied to an anion-transport system.

Passive Transport of N-*Benzoylamino-acid Anions.*—The passive transport experiments were conducted at room temperature in an apparatus similar to that described before,[7] which consists of a stirred chloroform membrane separating two aqueous phases I and II.



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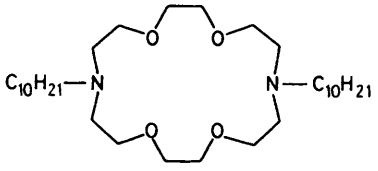

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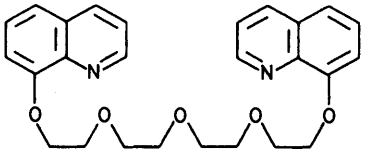

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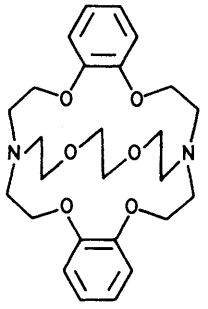

(4)

Aqueous phase I contains the *N*-benzoylamino-acid and a symport cation in alkaline solution (3 ml). Aqueous phase II contains distilled water (9 ml). The chloroform membrane (8 ml) containing ionophore bridges them, and is stirred constantly by a magnetic stirrer. The transport rates shown in Table 1 were obtained from the rates of appearance of the amino-acid anion in the aqueous phase II. We confirmed that no diffusion was detected in the absence of carrier. Typical results are shown in Table 1.

Some artificial ionophores were found to mediate the passive transport of amino-acid anions with high efficiency. When the carrier (1) was employed, the transport rates of *N*-benzoylphenylalanine (Bz-Phe) depended on the nature and concentration of the alkali-metal cation in the aqueous phase I. Thus, an increase in the K^+ ion concentration in the aqueous phase I resulted in a marked acceleration in the transport rate, showing that coupling to the K^+ ion gradient was used to pump the amino-acid anions up. It was also

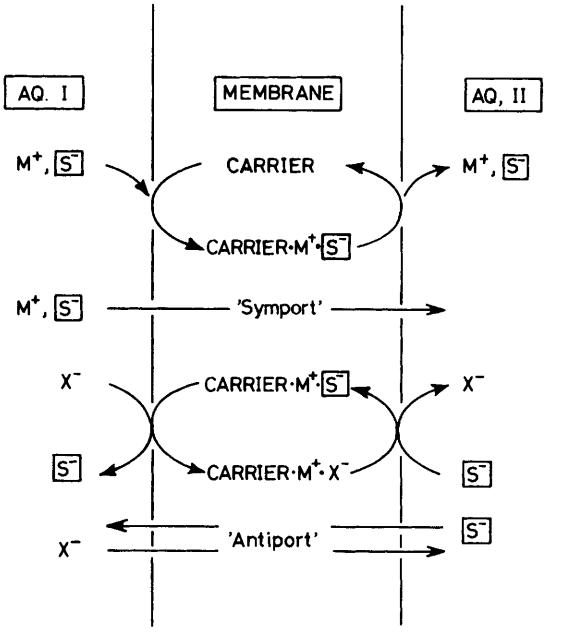

FIGURE 1 Liquid membrane systems for transport of amino acid anions: symport (present carrier) and antiport (transition-metal complex carrier)

demonstrated that the K^+ ion was more effective in promoting amino-acid transport than Na^+ and Cs^+ ions. This trend parallels that of cation-extraction experiments with the crown ether (1),[8] and suggests that the cation-complex formation process is important in determining the transport rates in this system. As is well known, amino-acid or sugar transport is often coupled with alkali-metal cation transport in biological systems.[2]

TABLE 1

Passive transport of amino-acid anions with alkali cation

Amino acid	Symport cation (mol/l)	Transport rate × 10^6 (mol/h) (1)	(2)	(3)	(4)
Bz-Phe	KCl (1.0)	13.0	3.1	0.6	3.8
	(0.1)	2.3			
	(0.5)	5.9			
	(2.0)	16.7			
	NaCl (1.0)	1.2	1.1	0.2	9.9
	CsCl (1.0)	1.9	0.4	0.2	7.0
Bz-Leu	KCl (1.0)	3.4	1.6	0.1	5.5
Bz-Met	KCl (1.0)	4.8	1.6	0.2	5.9
Bz-Val	KCl (1.0)	2.0	1.0	0.2	5.2
Bz-His	KCl (1.0)	0.1	0.1	0	3.0
Bz-Ala	KCl (1.0)	0.2	0.7	0	6.6
Bz-Gly-Gly	KCl (1.0)	0	0.4	0	1.5
Bz-Gly	KCl (1.0)	0.1	0.8	0.1	4.0

Initial concentrations: Aq.I; Bz-Amino acid, 0.3 mmol. Salt of symport cation, 0.3—6.0 mmol./0.1N-NaOH, 3 ml. Aq.II; Distilled water, 9 ml. Membrane; Carrier, 0.0372 mmol/$CHCl_3$, 8 ml.

These two substrate ions use a common carrier, and pass through the membrane *via* an amino-acid anion/metal ion/carrier type ternary complex (so-called ' Mitchell's symporter mechanism '). The present system may provide an artificial analogue to this cation-dependent amino-acid transport, and also be considered as a prototype for the design of a specific separation membrane for biologically interesting anionic species.

The passive transport system, mediated by the carrier (1), showed an excellent substrate specificity for the following series of amino-acid anions: Bz-Gly ~ Bz-Gly-Gly ~ Bz-His ~ Ba-Ala ≪ Ba-Val < Bz-Leu < Bz-Met ≪ Bz-Phe. This sequence is different from that previously reported for ' transition-metal complex ' carriers and an ammonium cation surfactant:[9] Bz-Gly > Bz-Ala > Bz-Met > Bz-Val ~ Bz-Leu > Bz-Phe. Since, in the present system, the amino-acid anions with higher hydrophobicities were found to give rise to faster transport rates, the anion extraction process into the membrane by complex formation with K^+-carrier complex could be an important feature. Some investigators have already noted that the transport rates of an alkali-metal cation by crown ether-type carriers are strongly dependent on the anion present.[10] We applied this phenomenon to the selective transport of ' anionic species ' having biological significance.

Although, for a variety of amino-acid derivatives, the cryptand (4) displayed higher transport rates than the other ionophores used, exceptionally low rates were obtained for it with the Bz-Phe anion. It has frequently been observed in related transport experiments[11] that highly hydrophobic species are accumulated in the membrane rather than transported. Probably, the cryptand (4) forms a particularly stable complex with K^+ ion and Bz-Phe anion in the membrane phase, which results in lower transport rates.

The synthetic ionophores (2) and (3) exhibited almost identical transport properties to the ionophore (1), although their transport abilities were modestly suppressed. The transport efficiencies of these four carriers generally increased according in the following order of carriers: cryptand (4) > crown ether (1) > diaza-crown ether (2) > non-cyclic crown ether (3). Since this order parallels that obtained for the complex formation constants between K^+ ion and each of the ionophores,[12] it suggests that effective co-ordination of carrier to the K^+ ion can lead to faster transport of anionic species (see Figure 2).

Artificial Transport of Z-Oligopeptide Anions.—The anion-transport system described here was also applied to the artificial transport of oligopeptide derivatives. Although peptide transport across a biomembrane plays an important role in protein synthesis, in cell growth, in the metabolic cycle, in receptor/acceptor recognition, and in other biological processes, only scattered examples of synthetic carrier models have been reported.[9a,13] Recently we presented the first systematic studies of carrier-mediated oligopeptide transport.[14] It was found that some lipophilic transition-metal complexes could effectively transport a series of dipeptide and tripeptide derivatives as well as simple amino-acid anions. As pointed out in the amino-acid transport system, the crown ether–alkali-metal complex carrier (the present carrier system) generally showed a different transport specificity to that exhibited by a transition-metal complex carrier (previously reported carrier system).

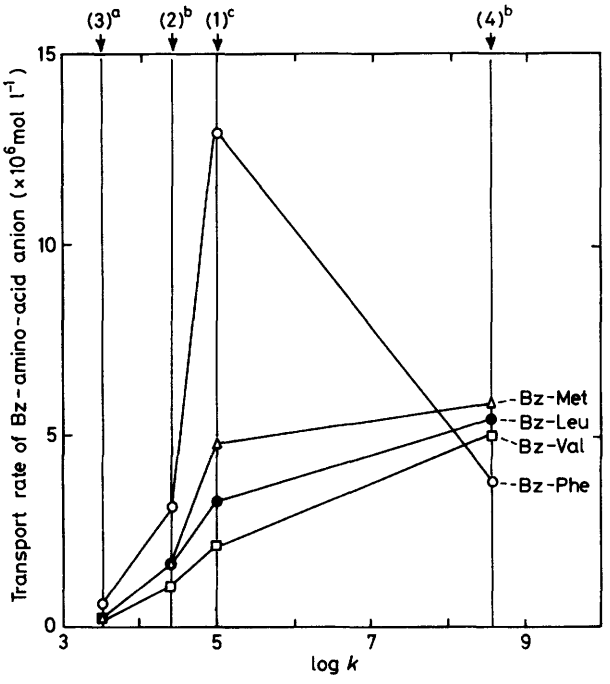

FIGURE 2 Relationships between transport rate of amino-acid anion and K^+-complex formation constant of ionophore (K). (a), (b), (c): see refs. 12*a*, *b*, and *c*

Therefore, the present ' crown ether–alkali-metal complex ' carrier may provide a new and characteristic pattern of behaviour in the oligopeptide transport process. The passive transport experiments were similarly conducted as described before, and typical results are summarized in Table 2, in which corresponding Z-amino-acid anions were also examined as guest anions for comparison.

TABLE 2

Artificial transport of oligopeptide derivatives

Substrate	KCl in Aq.I (mol/l)	Transport rate × 10^6 (mol/h) (1)	(2)	(4)
Z-Gly	1.0	1.3	0.5	1.5
Z-Ala	1.0	3.0	1.1	2.6
Z-Leu	1.0	8.9	5.8	3.6
Z-Phe	0.25	6.0	3.6	1.7
	0.50	7.9	6.4	2.0
	1.0	11.8	8.1	2.9
Z-Gly-Gly	1.0	1.5	0.8	2.5
Z-Gly-Ala	1.0	1.3	0.6	3.5
Z-Gly-Leu	1.0	3.1	1.1	3.7
Z-Gly-Phe	1.0	8.4	3.5	4.2
Z-Gly-Gly-Leu	1.0	1.0	0.3	3.1
Z-Gly-Gly-Phe	1.0	1.4	0.8	4.8

Initial concentrations: Aq.I; Substrate, 0.15 mmol. KCl, 0.75—3.0 mmol./0.05N-NaOH, 3 ml. Aq.II; Distilled water, 9 ml. Membrane; Carrier, 0.0372 mmol/$CHCl_3$, 8 ml.

The crown ether (1) and the diaza-crown ether (2) were found to transport effectively Z-amino-acid and Z-oligopeptide derivatives containing hydrophobic moieties: *Z-Gly* < *Z-Ala* < *Z-Leu* < *Z-Phe*; *Z-Gly-Gly* ~ Z-Gly-*Ala* < Z-Gly-*Leu* < Z-Gly-*Phe*; Z-Gly-Gly-*Leu* < Z-Gly-Gly-*Phe*. These trends were similar to those observed in the Bz-amino-acid anion transport system. It was noted that the crown ethers (1) and (2) showed relatively lower transport rates for oligopeptides, compared with those for amino-acid anions: Z-Gly-Gly-*Phe* < Z-Gly-*Phe* < *Z-Phe*; Z-Gly-Gly-*Leu* < Z-Gly-*Leu* < *Z-Leu*. Hence these types of crown ether appear of little use as carriers of oliogopeptide anions.

In marked contrast, the cryptand (4), which was a less effective carrier of amino-acid anions, exhibited excellent transport capability for oligopeptide derivatives: Z-Gly-Gly-*Phe* > Z-Gly-*Phe* > *Z-Phe*; Z-Gly-Gly-*Leu* ~ Z-Gly-*Leu* ~ *Z-Leu*. Probably, the stronger cation co-ordination properties of the cryptand (4) promote the successful binding of the bulky but hydrophobic oligopeptide anions, thus giving rise to efficient transport characteristics.

Active Transport of Bz-Amino-acid Anions.—The active transport of amino-acid anions across a chloroform membrane was studied by using a U-tube apparatus (see Experimental section). The carrier in the chloroform was placed in the base of the U-tube, and buffered aqueous solutions of equal concentrations of the amino-acid anion at the same pH were placed in the arms of the U-tube; they floated on the chloroform membrane. The concentrations of the amino-acid anions in both aqueous phases, monitored spectroscopically, were recorded at steady state, usually after 24 h (see Table 3).

In the absence of an alkali-metal salt such as KCl in the aqueous phase I, the amino-acid anion hardly moved. When the KCl was added as the source of symport cation (K^+), amino-acid derivatives were successfully transported against their concentration gradients by the aid of artificial ionophores. Although a chemical reaction was not linked with this transport process, so-called ' uphill transport ' of amino-acid anions was observed. When the crown ether (1) was employed, the concentration ratio of Bz-Phe anion across the membrane, $[\text{Bz-Phe}]_{\text{aq. II}}/[\text{Bz-Phe}]_{\text{aq. I}}$, rose from 1 (initial value) to 7 (after 24 h) under the same conditions (see Table 3). This observation strongly suggests that a variety of synthetic ionophores can be applied to the enrichment and/or separation of biologically important anions, and that a more practical utilization can be designed by improving the detailed conditions.

TABLE 3

Active transport of amino-acid anions by synthetic ionophores

Substrate	Salt in Aq.I (mmol)	Equilibrated substrate distribution (mmol/mmol) [Substrate in Aq.I]/[Substrate in Aq.II] (1)	(2)	(3)	(4)
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	0.204/0.289	0.199/0.240
	LiCl (10.0)	0.226/0.254	0.228/0.264	0.229/0.258	0.219/0.223
	NaCl (10.0)	0.184/0.259	0.171/0.305	0.223/0.267	0.191/0.246
	CsCl (10.0)	0.207/0.278	0.246/0.254	0.210/0.254	0.232/0.214
Bz-Leu	KCl (10.0)	0.121/0.379	0.191/0.304	0.225/0.274	0.203/0.297
Bz-Met	KCl (10.0)	0.119/0.375	0.197/0.303	0.228/0.263	0.213/0.245
Bz-Val	KCl (10.0)	0.154/0.343	0.216/0.282	0.243/0.257	0.250/0.215 *
Bz-His	KCl (10.0)	0.243/0.257	0.239/0.261	0.235/0.265	0.337/0.163 *
Bz-Ala	KCl (10.0)	0.231/0.263	0.239/0.254	0.247/0.247	0.322/0.161 *
Bz-Gly-Gly	KCl (10.0)	0.245/0.255	0.245/0.249	0.244/0.244	0.289/0.211 *
Bz-Gly	KCl (10.0)	0.243/0.257	0.243/0.257	0.235/0.263	0.298/0.178 *

Initial concentrations: Aq.I; Substrate, 9.25 mmol. Inorganic salt, 0—10.0 mmol./0.05N-NaOH, 5 ml. Aq.II; Substrate, 0.25 mmol./0.05N-NaOH, 5 ml. Membrane; Carrier, 0.056 mmol/$CHCl_3$, 12 ml.

* Antiport of amino-acid anions (from aq.II to aq.I) occurred in these systems.

The substrate specificity of the active transport system was similar to those of the passive transport systems (see Table 1): Bz-Phe > Bz-Leu ~ Bz-Met > Bz-Val > Bz-Ala ~ Bz-His ~ Bz-Gly-Gly ~ Bz-Gly. The order of the carrier abilities for active transport was in accord with expectations, based on the results of passive transport systems, except for the case of the cryptand (4); crown ether (1) > diaza-crown ether (2) > non-cyclic crown ether (3) ~ cryptand (4). Therefore, the same factors are believed to control both transport systems.

The cryptand (4) was found to transport amino-acid anions actively in the ' opposite ' direction, *i.e.* from the aqueous phase II to aqueous phase I, suggesting that coupling to the Cl^- anion gradient was used to pump the amino-acid anions up. It also showed ' reversed ' transport selectivities: Bz-Gly > Bz-Gly-Gly ≪ Bz-His ~ Bz-Ala ≫ Bz-Val > Bz-Met > Bz-Leu < *Bz*-Phe.

Earlier,[9] we have demonstrated that some neutral ligand–transition-metal complexes can mediate active transport of some series of amino-acid anions; these display similar characteristics of transport direction and selectivity to those of the cryptand (4). In such systems, very stable cationic complexes of neutral ligand and metal cation can act as a lipophilic cation, and transport anionic species *via* a ' co-ordinated anion exchange mechanism ' (see Figure 1). Since the cryptand (4) is well known to form a very stable K^+ complex, it can act as an effective carrier for transporting amino-acid anions,

in a similar way to the transition-metal complex carriers (so-called ' antiport ').

The results described here clearly demonstrate that substrate specificity, efficiency, and direction of the transport process can, essentially, be controlled by choice of the appropriate transport system.

EXPERIMENTAL

Materials.—The synthetic ionophores (1)—(4) were purchased from Merck Japan Ltd. The *N*-benzyloxycarbonyl-amino acid and oligopeptide derivatives were obtained from Sigma Chem. Corp. *N*-Benzoyl-glycine, -alanine, -valine, and -phenylalanine were purchased from Nakarai Chem. Ltd., and other benzoylamino-acids were received from the Tokyo Chem. Ltd.

All other chemicals were of analytical grade and employed as received.

Transport Procedure.—The passive transport experiments (Tables 1 and 2) were carried out at room temperature in a cell similar to that described before; [7] a cylindrical glass cell (4.0 cm, i.d.) holds a glass tube (2.0 cm, i.d.) which separates the two aqueous phases. The inner aqueous phase I contains substrate in 3 ml of alkaline solution. The outer aqueous phase II includes distilled water (9 ml). The carrier-containing chloroform phase (8 ml) lies below these two aqueous phases, and bridges them. This is stirred constantly by a magnetic stirrer. The transport rates were calculated from the initial rates of appearance of substrate anion into the aqueous phase II, which were determined spectroscopically. Reproducibility was confirmed as ± 15% or better.

The active transport experiments with *N*-benzoylamino-acid anions (Table 3) were performed in an U-shaped glass cell (2.0 cm, i.d.).[9] The carrier in chloroform (12 ml) was placed in the base of the U-tube, and two aqueous phases (5 ml, each) of equal substrate concentration of the same pH were placed in the arms of the U-tube, floating on the chloroform membrane. The membrane phase was stirred with a magnetic stirrer. The concentrations of substrate anion in the aqueous phase II were increased with operation time, and reached at steady state (usually after 24 h). The concentrations of substrate anion in the aqueous phase I were also decreased at corresponding rates. The apparently equilibrated concentrations of each substrate are listed in Table 3.

Similar transport experiments were carried out in the absence of carrier, for reference. It was found that membrane leakages of each substrate were negligible under the employed conditions. The detailed conditions were included in each Table.

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