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# A SYNTHETIC CROWN ETHER CARBOXYLIC ACID IONOPHORE DISPLAYS SYNERGISTIC TRANSPORT OF $Pr^{3+}$ IN CONJUNCTION WITH LASALOCID

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$^{31}P$ Transport of $Pr^{3+}$ across phosphatidyl choline vesicles, as monitored by $^{31}P$ nmr, is second-order in the crown ether carboxylic acid 2, as it is with respect to lasalocid (X-537 A). When the synthetic (2) and the natural (lasalocid) ionophores are incorporated together in ~ 3:1 ratio into the lipidic phase, the transport velocity is markedly enhanced.

We have reported earlier (1) a remarkable synergism in ionic transport which occurs when two different polyether ionophores, such as lasalocid A and etheromycin, cooperate in transporting $Pr^{3+}$ cations across a model membrane (vesicles made from egg yolk lecithin). A plausible explanation for the effect, which is general for polyether ionophores having also a carboxylic acid function, is facilitated back-transport of protons (or sodium ions), which takes advantage of the $\Delta pK_a$ difference between the two moieties A and B in the kinetically-active ($Pr^{3+}$ + A + B) complex (2). We report here a test of this hypothesis, using not natural antibiotic ionophores, but rather synthetic crown ethers also endowed with a carboxylic function (3). The results are gratifying, since these artificial ionophores, in conjunction with a natural ionophore (lasalocid), also display the synergism found earlier. To the best of our knowledge, this is the first example of such synergism in transport when one of the two partners is a synthetic ionophore molecule.

## MATERIALS AND METHODS

The experimental procedure is as previously described (1-2, 4). By monitoring the $^{31}P$ resonances for the inner and outer head groups of the lipid

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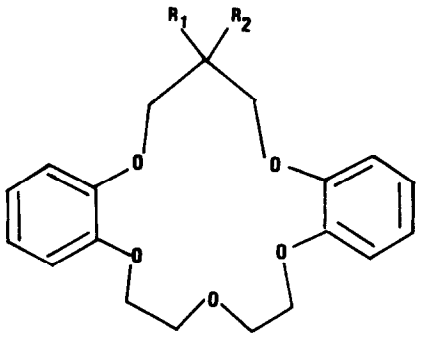
bilayer, the velocity of $Pr^{3+}$ ion penetration inside the vesicles was measured. The choice of $Pr^{3+}$ as the transported species is one of convenience. It provides a distinction between the inside and the outside of the membrane.

The ionophores tested in the present study are the natural lasalocid (X-537 A) and the synthetic crown ether carboxylic acids 1-3 (3).




1 $R_1$ = H; $R_2$ = $OCH_2CO_2H$

2 $R_1$ = H; $R_2$ = O - CH(n-$C_8H_{17}$)$CO_2H$

3 $R_1$ = n-$C_8H_{17}$; $R_2$ = $OCH_2CO_2H$

## RESULTS

The results of a series of kinetic runs for crown carboxylic acids 1-3 are given in Table 1. From these results, it appears that only molecule 2 behaves as a good ionophore. Furthermore, since the transport velocity is second-order with respect to [2], this points to a 2:1 stoichiometry for the $Pr^{3+}$ - transporting complex. Interestingly, neither 1 nor 3 appear to transport $Pr^{3+}$ ions under these experimental conditions. At a larger concentration of 3 (1.78 mM), a weak transport of the lanthanide ion has been detected; but this compound remains ca. 20 times less powerful than 2. It might have been expected that 1, lacking a lipophilic side chain, would not be suited to the lipophilic membrane environment. Thus, it has been observed that in the

TABLE 1

Slope of the linear variation of the internal resonance as a function of time (t = 35°C; [$Pr^{3+}$] = 1.23 mM).

| [Compound], $10^{-4}$ M | slope (Hz.min$^{-1}$, $\pm\sigma$) | Correl. coeff. (no. of points) |
|---|---|---|
| 1 (5.84) | ∿ 0 | - |
| 2 (1.46) | 0.093 ± 0.008 | 0.984 (6) |
| 2 (2.92) | 0.322 ± 0.027 | 0.990 (5) |
| 2 (5.84) | 1.64 ± 0.14 | 0.993 (5) |
| 3 (5.84) | ∿ 0 | - |

solvent extraction of alkali metal cations from aqueous solutions into chloroform, crown carboxylic acid 1 is of insufficient lipophilicity to prevent very serious loss of the crown carboxylate into the aqueous phase (5). On the other hand, in analogous solvent extraction experiments, 2 is sufficiently lipophilic to prevent loss of the corresponding crown carboxylate from the organic layer (6).

However, the findings that 2 transports $Pr^{3+}$ much better than 3 does are quite unexpected. By several criteria, 3 might be expected to provide better transport than 2. Like 2, molecule 3 is sufficiently lipophilic to prevent loss of the corresponding carboxylate from a chloroform phase which is contacted with a highly alkaline aqueous phase (7). For competitive solvent extraction of alkali metal cations from aqueous solutions into chloroform, 3 exhibits greater selectivity for sodium over potassium than does 2 and also exhibits better metal ion loading of the organic phase. Thus, the total metal cation concentration in the chloroform phase is equal to the concentration of 3; whereas with 2, the metal cation concentration is only about 60% that of the crown carboxylic acid (7). For competitive transport of alkali metal cations across a liquid surfactant membrane (8), 3 again shows greater selectivity for transport of sodium over potassium than does 2. The total initial rate of metal cation transport across the liquid surfactant membrane by 3 is nearly three times greater than that with 2 (9). At present, we do not understand the reason for the contrast between 2 and 3 in $Pr^{3+}$ transport ability under our conditions. Clearly, structural details do play an important role. Not only is a lipophilic arm a necessity, it must also be suitably placed. In addition, liquid surfactant membranes are rather unsophisticated membrane models. A closer approximation to biological membranes is represented by lipid bilayers such as the model membranes which are made from egg yolk lecithin, that were used in the present study.

Since 2 was found to be an artificial ionophore (Table 1) with the same 2:1 stoichiometry as a natural ionophore such as lasalocid, we decided to attempt joint transport of $Pr^{3+}$ by 2 and lasalocid (Table 2). Firstly, the

TABLE 2.

Velocities of transport (± σ) by lasalocid alone, or in synergistic association with 2. (t = 35°C; $[Pr^{3+}]$ = 1.23 mM).

| X-537 A, mM | 2, mM | velocity of transport | Correl. coeff. (no. of points) |
|---|---|---|---|
| 0.117 | - | 0.42 ± 0.02 | 0.995 (6) |
| 0.234 | - | 1.43 ± 0.07 | 0.996 (5) |
| - | 1.46 | 1.16 ± 0.02 | 0.999 (5) |
| 0.234 | 0.73 | 6.36 ± 1.12 | 0.985 (3) |

comparison with lasalocid (X-537 A) shows that 2, despite the presence of the ionizable -COOH group which allows the necessary back-flow of protons to compensate for $Pr^{3+}$ entry ("antiport" mechanism for maintenance of electroneutrality), is a poorer ionophore by one or two orders of magnitude.

Second and more interesting is the observation of a positive synergism resulting from the joint use of 2 and (X-537 A). The mixed complex formed by 2 and (X-537 A) transports $Pr^{3+}$ with a calculated velocity which is greater by one order of magnitude than those of the pure 2:1 complexes.

Given the potential use of these observations for metabolic studies, we are actively exploring their generality and scope using other synthetic ionophores prepared in both of our laboratories.

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