

Ligand design for selective metal-ion transport through liquid membranes

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Abstract - In previous work we have undertaken a program involving the design and synthesis of a series of mixed donor macrocycles, incorporating five donor atoms, for use in metal ion recognition studies. We now report the application of individual ligands from this series to selective extraction and transport studies involving Ni(II), Cu(II), Zn(II), and Cd(II). Initial water/chloroform extraction experiments indicated that, for the present systems, a significant amount of ligand is leached from the organic phase to the aqueous phase; this has been a common problem in previous extraction and transport studies when amine-containing ligands of relatively low molecular weight were used as the complexing agent in the organic phase. Typically, this drawback has been largely overcome by appending long-chain aliphatic groups to the ligand's backbone to increase the lipophilicity of the system. However, in many cases this has not been a trivial synthetic exercise. In the present study a different approach was adopted. It was found that the above difficulty could be alleviated to a significant degree by introducing a lipophilic anion (hexadecanoate) into the organic phase along with the macrocyclic carrier - an observation which could have implications for understanding the biological transport of ions of the present general type.

In a series of experiments, the effect of ligand structure on both metal ion extraction and transport under the above conditions has been investigated. A range of behaviour was documented: for example, the selective transport of Cu(II) in the presence of Ni(II), and Cd(II) in the presence of Zn(II), as well as Zn(II) in the presence of Cd(II) were all observed.

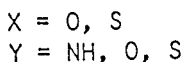
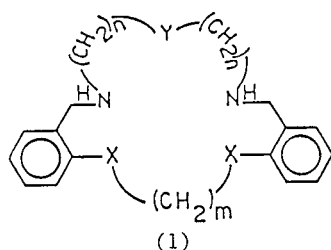
INTRODUCTION

The selective transport of metal ions across lipid membranes is a fundamental process in biology. Biological membranes, such as those surrounding cells, are composed of lipids and proteins exhibiting a partially structured arrangement. Neutral cyclic antibiotics of the valinomycin, enniatin B and monactin classes are all well studied ionophores which aid the selective transport of an alkali metal cation such as potassium across both natural and artificial membranes (ref. 1). A property of these species is that, on metal complex formation, the inside of the molecule is hydrophilic while the outside is hydrophobic. This arrangement is important since the lipid components of biological membranes tend to be orientated such that their polar groups face the membrane surfaces with the non polar hydrocarbon portions occupying the interior of the membrane. The resulting hydrophobic nature of the central region of the membrane will thus tend to inhibit the passage of charged ions in the absence of an ionophore.

There have been many studies involving ligands such as the crown ethers which mimic the ion transport properties of the natural antibiotic ionophores (ref. 2). The majority of such biomimetic studies so far performed have involved the selective transport of metals such as the alkali and alkaline earth ions across liquid membranes. Much less emphasis has been given to the transport of transition and post-transition ions in such studies and a corresponding lack of detailed information concerning the membrane transport of these ions in natural systems is also apparent. Peptide moieties have been demonstrated to facilitate the uptake of metals such as Cu(II) into cells. For example, the plasma tripeptide Gly-His-Lys has been shown to influence the uptake of this ion (ref. 3); in this context, it has long been known that human serum albumin possesses a specific binding site for both Cu(II) and Ni(II) (ref. 4). The selective uptake of Cu(II) [with respect to Zn(II) and Cd(II)] by lymphoid cells has also been demonstrated to occur in the presence of a complexing agent (ref. 5). Analysis of the uptake curve suggested that membrane transport involves both passive and facilitated (that is, carrier aided) diffusion in this case.

Examples of synthetic amine carriers selectively transporting Cu(II) in the presence of other transition ions across lipophilic membranes (typically dichloromethane or chloroform) have been reported. For example, the open-chain tetramines 6-hexadecyl-1,4,8,11-tetraazaundecane and 7-hexadecyl-1,5,9,13-tetraazatridecane incorporating pendant cetyl chains have been used in transport experiments (involving two aqueous phases separated by a dichloromethane phase containing one of these tetramines as the carrier) (ref. 6). The transport was driven in each system by the metal concentration gradient between the aqueous source phase and receiving phase. Copper was found to be selectively transported in the presence of equimolar concentrations of Ni(II) and Zn(II). In a related experiment a lipophilic derivative of 2,2-bipyridine has also been demonstrated to preferentially transport Cu(II) through a dichloromethane membrane (ref. 7). As for the previous example, the neutral nature of this carrier results in the necessity for simultaneous transfer of anions with the metal. In this latter case, an active (pH driven) mechanism is involved. In other studies, a long chain derivative of a macrocyclic dioxatetraamine ligand has been utilized as the carrier (refs. 8 and 9). On coordination of a divalent metal the two amide protons are lost to yield an uncharged species. This proton-driven system is quite efficient for transporting Cu(II) through a liquid membrane even against a concentration gradient. Since the carrier is charged, no simultaneous transport of anion with the metal ion is required in this case. This system is quite specific for Cu(II) in the presence of divalent ions such as Fe(II), Co(II), Ni(II), Zn(II) and Pb(II).

We have previously undertaken a program involving the design and synthesis of cyclic reagents of general type (1); these have been found in a number of cases to show



selectivity for particular transition and post-transition ions (ref. 10). We now report an extension of these investigations to include the use of such ligands in comparative extraction and transport studies involving Ni(II) and Cu(II) as well as Zn(II) and Cd(II).

EXTRACTION AND TRANSPORT STUDIES

All extraction and transport studies involved a chloroform organic phase and an aqueous source phase containing 2,6-lutidine-3-sulfonic acid/NaOH buffer. The latter sulfonic acid has poor coordinating properties and is not appreciably soluble in the organic (chloroform) phase. In the transport experiments the aqueous receiving phase was either buffered or contained excess ethylenediaminetetraacetate (edta) (see later). Such experiments were performed in a U-tube apparatus in which all three phases were gently stirred during the course of the experiment (usually 100 hours).

The addition of hexadecanoic acid to the organic phase

In contrast to the situation when crown ethers are used as carriers, cyclic (aliphatic) amine derivatives of low molecular weight tend to be lost from the organic phase through protonation and subsequent solubilization in the aqueous phase(s). Typically, as mentioned previously, this difficulty is overcome by appending long-chain aliphatic groups to the ligand's backbone to increase the system's lipophilicity - a synthetic procedure which is often not straight forward. It is noted that the presence of the lipophilic chain also serves to retard loss of carrier (to the aqueous phase) as its metal complex which, for a neutral carrier, will be ionic.

As expected, preliminary extraction experiments indicated that substantial "bleeding" of particular macrocycles of type (1) occurs from chloroform when this phase is shaken with aqueous solutions in the range pH 2.0 - 5.0. Similarly, attempted extraction of copper from aqueous solution typically resulted in very considerable loss of carrier to the aqueous phase (the latter turns intense blue). Similar leaching effects were also clearly evident in preliminary transport experiments in which the aqueous source and receiving phases were separated by a chloroform carrier phase in a U-tube apparatus.

In view of the above, a different approach was used in the present study in order to inhibit the bleeding of the macrocycle into the aqueous phase(s). Since the bleeding only involves charged species, it was reasoned that the introduction of a lipophilic counter ion (such as a readily deprotonated long chain fatty acid) to the organic phase may impart a greater degree of lipophilicity to these species and also remove the need for a simple counter ion to cross the aqueous/organic interface on metal uptake.

Over a number of extraction experiments, the addition of a long chain fatty acid to the organic phase was found to dramatically reduce the degree of ligand loss in each case and hence considerably enhance the efficiency of metal extraction. Further, the effectiveness of the added carboxylic acid was dependent on the length of its carbon chain, falling in the order hexadecanoic acid > dodecanoic acid > decanoic acid > acetic acid. Hexadecanoic acid was subsequently used for all extraction and transport studies involving the present macrocyclic ligands. As expected, similar benefits were also observed in the respective transport systems. Control experiments confirmed that, by itself, hexadecanoic acid does not promote significant extraction or transport under the conditions used in the present study.

Extraction and transport of Ni(II) and Cu(II)

A number of preliminary extraction experiments were performed using a cross section of ligand types. From these it was established that, for Cu(II), equilibration is reached within 1 hour - the amount extracted is essentially the same at both 1 hour and 40 hours in each case. However, for Ni(II) an extraction time of 1 hour is insufficient for equilibrium to be reached. This is not unexpected due to the greater inertness of this metal relative to Cu(II). Nevertheless, a shaking time of 1 hour was used for all the extraction experiments performed in the present study (at a temperature of 22 ± 2 °C).

Single metal extraction experiments. The metal ion distribution coefficients, $D_M = \frac{[M^{n+}]_{org}}{[M^{n+}]_{tot}}$, for the extraction of Ni(II) as well as Cu(II) from a solution of their nitrate salts (of concentration 1×10^{-3} mol dm⁻³, pH 4.7) by a range of the macrocycles of type (1) [1×10^{-3} mol dm⁻³ in CHCl₃ containing hexadecanoic acid (4×10^{-3} mol dm⁻³)] have been determined. The respective pH conditions and concentrations of the macrocycle and fatty acid used in these experiments were based on the results from appropriate preliminary experiments.

The extraction of Ni(II) was shown to have a strong dependence on the nature of the donor atom set present with significant extraction only being observed when the macrocyclic ligand incorporated an N₃-donor string in its structure. For the macrocycles incorporating an O₂N₃-donor set, no clear correlation between the complex stabilities and the corresponding D_M values was observed. Such a result is not unexpected as a number of other factors, including the change in lipophilicity along the ligand series, will also influence extraction behaviour. As for Ni(II), the extent of extraction of Cu(II) was again dependent on the nature of the donor at the Y position - in general extraction being favoured when Y = NH over Y = S but with Y = S being superior to Y = O.

Competitive metal ion extraction and transport experiments. In light of the results from the single metal extraction experiments [and particularly because of the apparent slow rate of extraction of Ni(II) mentioned earlier] studies directed at separating Cu(II) and Ni(II) from an equimolar mixture of these ions were initiated. The conditions employed were similar to those used for the single metal experiments. Competitive extractions involving the macrocycles (2), (4), and (5) were undertaken; from the total group of ligands studied, these were the rings which yielded the highest extraction for Cu(II) in the single metal studies. Over the lifetime of the respective experiments (1 hour), virtually no Ni(II) was extracted by any of the above macrocyclic systems. In contrast, the D_M values for Cu(II) follow a similar pattern to those for the corresponding single-metal extractions; under the conditions employed, the D_M values increased in the ligand order (3) < (2) < (5) < (4). Clearly, all these systems are highly selective for Cu(II) in the presence of Ni(II); this discrimination is undoubtedly aided by the retarded extraction rates observed for Ni(II) relative to Cu(II).

Based on the above results, two types of competitive transport experiments involving Cu(II) and Ni(II) were performed using the above macrocycles; the results are summarized in Table 1 under A and B. In the first series (A), equimolar concentrations of Ni(II) and Cu(II) as their nitrate salts (5×10^{-2} mol dm⁻³, buffered at pH 4.7) were used for the source phase. The organic phase was similar to that used for the extraction experiments and consisted of the macrocyclic carrier (1×10^{-3} mol dm⁻³) and hexadecanoic acid (4×10^{-3} mol dm⁻³) in CHCl₃. The receiving phase in these experiments comprised buffer solution (pH 4.7) containing an excess of NaNO₃. As expected from the competitive extraction experiments, no Ni(II) was detected in the receiving phase at the completion of these transport experiments. However, the results also indicated that only a relatively small amount of Cu(II) was transported during the period of these experiments (100 hours).

In a second set of experiments a competing equilibrium was introduced into the receiving phase by the addition of ethylenediaminetetraacetic acid (edta) to act as a "scavenger" for the transported metal. In this case, the compositions of the source and membrane phases were the same as in the previous experiments; however, the receiving phase contained only edta (0.05 mol dm⁻³ adjusted to pH 7.0 by the addition of NaOH). The results of these runs are summarized in Table 1 under B. Once again, no transport of

Ni(II) was detected. However, a significant increase in the transport of Cu(II) was observed. Indeed, under the experimental conditions employed, the transport of Cu(II) by each of the macrocyclic carriers studied exceeded that of Ni(II) by not less than two orders of magnitude.

As for the extraction experiments, it is evident that the addition of hexadecanoic acid in the organic membrane in these transport experiments very considerably reduces the degree of "bleeding" of carrier under the present conditions.

TABLE 1. The transport of Cu(II) in Ni(II)/Cu(II) competitive metal ion transport experiments^{a,b}

Carrier	"Rate" of copper ion transport ^c	
	A ^d	B ^e
(2)	6.7×10^{-6}	8.9×10^{-5}
(3)	3.3×10^{-5}	8.8×10^{-5}
(4)	-	1.5×10^{-4}
(5)	5.5×10^{-6}	3.8×10^{-5}

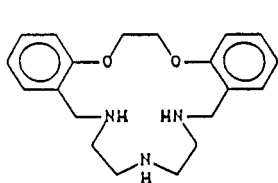
^aAfter 100 hours at $25 \pm 0.1^\circ \text{C}$; all values are the mean from at least two duplicate experiments.

^bIn every case no transport of Ni(II) was detected (that is, the transport rate for this ion was less than $7 \times 10^{-7} \text{ mmol h}^{-1} \text{ cm}^{-2}$).

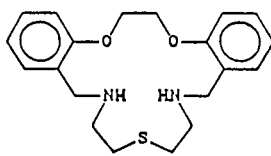
^c $\text{mmol h}^{-1} \text{ cm}^{-2}$ (see ref. 10); estimated error $\pm 15\%$.

^dReceiving phase: $0.1 \text{ mol dm}^{-3} \text{ NaNO}_3$ buffered to pH 4.7.

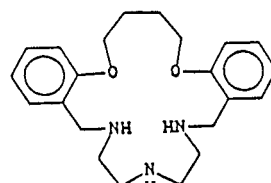
^eReceiving phase: $0.01 \text{ mol dm}^{-3} \text{ edta}$ at pH 7.0.



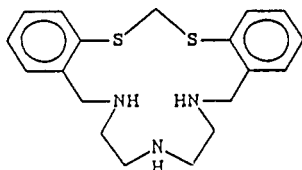
(2)



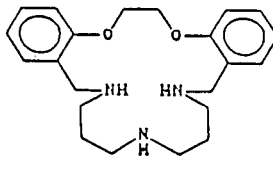
(3)



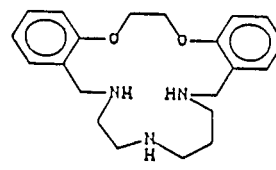
(4)



(5)



(6)



(7)

Extraction and transport of Zn(II) and Cd(II)

Single and competitive metal-ion extraction experiments of the type just discussed have also been performed for Zn(II) and Cd(II). Once again the extraction coefficients are quite dependent on the nature of the donor atom at the Y position in structure (1) with extraction tending to increase when a secondary amine occupies this position rather than an ether or a thioether donor. However, in general, for these metals the degree of extraction follows a similar order to that found for their stability constants in 95% methanol (refs. 12 and 13).

For example, under similar conditions the value of the distribution coefficient for Cd(II) for the 17-membered macrocycle (2) is larger than the corresponding value for Zn(II) but this order is reversed for the complexes of the 19-membered analogue (6). The origins of this behaviour appear to derive (at least in part) from a previously documented "structural dislocation" (see later) which results in the cadmium complexes of (2) and (6) having markedly different structures (ref. 12).

In view of the results from the single-metal extraction experiments just discussed, competitive extraction experiments for Zn(II) and Cd(II) were undertaken using the 17-membered macrocycle (2) and its 19-membered analogue (6) as extractants (at pH 5.6). The results parallel those obtained from the single metal extraction experiments; namely, starting from an equimolar mixture, (2) extracts considerably more Cd(II) than Zn(II) (a Zn:Cd extraction ratio of 7:55 was observed) while the reverse is true for the 19-membered macrocycle (6) (for which the Zn:Cd ratio in the organic phase was 2:1).

Once again, competitive transport experiments [starting from an equimolar Zn(II)/Cd(II) mixture] have been undertaken. The results from the experiments involving (2) and (6) (Table 2) mirror those from the corresponding extraction experiments just discussed.

TABLE 2. Transport "rates" for Zn(II) and Cd(II) in competitive experiments^a

Ligand	pH 4.7		pH 5.6	
	Zn(II) ^{b,c}	Cd(II) ^{b,c}	Zn(II) ^{b,c}	Cd(II) ^{b,c}
(2)	6.9×10^{-7}	2.6×10^{-5}	2.1×10^{-6}	6.6×10^{-5}
(6)	5.8×10^{-6}	4.3×10^{-7}	3.4×10^{-5}	4.0×10^{-6}

^aAfter 100 hours at $25 \pm 0.1^\circ \text{C}$.

^b $\text{Mmol h}^{-1} \text{cm}^{-2}$.

^cEstimated error $\pm 15\%$.

From table 2 it is seen that at both pH 4.7 and 5.6, the 17-membered macrocycle (2) transports Cd(II) preferentially over Zn(II). Conversely, the 19-membered macrocycle transports Zn(II) in preference to Cd(II). The observed selectivity patterns are once again in accord with the previously determined stabilities for this series of complexes. Although this need not necessarily be the case [since a range of parameters will influence metal ion transport of the present type (ref. 11)]; nevertheless, it does appear that the respective log K values do dominate both extraction and transport under the conditions used for the present experiments. This, in turn, appears to be a consequence of the structural dislocation behaviour postulated to underlie the different affinities of these cyclic systems for Zn(II) and Cd(II). Because of its relevance to the present study, an summary of the nature of the dislocation behaviour is given below.

Dislocation discrimination. A structural dislocation occurs when a gradual variation of properties along a ligand series triggers a sudden change in coordination geometry between adjacent complexes in the series. A process of this type may form the basis for discriminating between metal ions (ref. 10). Several examples of such dislocations have now been documented in the author's laboratory (refs. 12, 14 and 15).

The 17- and 19-membered macrocycles, (2) and (6), constitute the terminal members of the series of 17- to 19-membered O_2N_3 rings whose complexation behaviour with Zn(II) and Cd(II) has been studied in some detail (ref. 11). The respective log K values for the complexes of the series (2), (6), and (7), in 95% methanol are illustrated in Fig. 1.

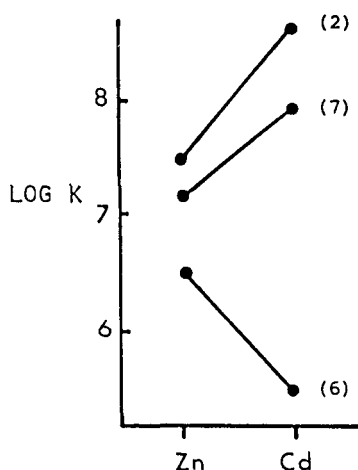


Fig. 1. The relative stabilities of the Zn(II) and Cd(II) complexes of the 17- to 19-membered rings (2), (7), and (6).

Clearly, in the case of the Zn(II) complexes, the stabilities show the expected progressive drop as five-membered chelate rings are replaced by six-membered rings. An X-ray structure of the zinc nitrate complex of (2) indicates non coordination of the ether oxygen donors in the solid and similar behaviour appears to occur in solution for this and the remaining two zinc complexes. In contrast, the higher stabilities of the Cd(II) complexes of the 17- and 18-membered macrocycles relative to their Zn(II) analogues appears to reflect coordination of all five macrocyclic donors in the former complexes; X-ray structural data confirms that this situation occurs in the solid state for the complex of (2). In part, this different coordination behaviour may reflect the larger radius of the Cd(II) ion. However, on passing to the 19-membered ring (6), it is found that the Cd(II) complex is less stable than the corresponding Zn(II) species and now follows the "natural" stability order for simple polyamine ligands. The different thermodynamic behaviour of this larger ring species towards Cd(II) is in accordance with the non coordination of the ether donors in this case. Once again, X-ray data support this postulate: the ether groups do not coordinate to the Cd(II) ion in the solid state.

CONCLUDING REMARKS

The present studies demonstrate that the addition of a long chain (lipophilic) anion to the organic phase can markedly enhance metal ion extraction and transport efficiencies when low molecular weight amine-containing carriers are involved. In the present study, such systems have proved to be effective for the discrimination of particular transition and post transition ions. Finally, when the internal composition of biological membranes is considered, it appears that a related "cooperative" mechanism to that discussed in the present paper may, in particular instances, also aid the selective transport of transition and other heavy metals across lipophilic barriers of this latter type.

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