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Recognition of Metal Cations by Biological Systems

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Recognition of metal cations by biological systems

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Recognition of metal cations by biological systems can be compared with the geochemical criteria for isomorphous replacement. Biological systems are more highly selective and much more rapid. Methods of maintaining an optimum concentration, including storage and transfer for the essential trace elements, copper and iron, used in some organisms are in part reproducible by coordination chemists while other features have not been reproduced in models. Poisoning can result from a foreign metal taking part in a reaction irreversibly so that the recognition site or molecule is not released. For major nutrients, sodium, potassium, magnesium and calcium, there are similarities to the trace metals in selective uptake but differences qualitatively and quantitatively in biological activity. Compounds selective for potassium replace all the solvation sphere with a symmetrical arrangement of oxygen atoms; those selective for sodium give an asymmetrical environment with retention of a solvent molecule. Experiments with naturally occurring antibiotics and synthetic model compounds have shown that flexibility is an important feature of selectivity and that for transfer or carrier properties there is an optimum (as opposed to a maximum) metal–ligand stability constant. Thallium is taken up instead of potassium and will activate some enzymes; it is suggested that the poisonous characteristics arise because the thallium ion may bind more strongly than potassium to part of a site and then fail to bind additional atoms as required for the biological activity.

Criteria for the design of selective complexing agents are given with indications of those which might transfer more than one metal at once.

Introduction

As is well known to biologists there is not a universal explanation for every phenomenon; five legs of an insect will behave one way when given a treatment and the sixth does something else. This is an example of biological variation. Different species may have evolved different methods for solving the same problem; we use an iron complex for the transport of oxygen, some invertebrates use a copper complex. As a chemist, I try to discover principles applicable to all situations but I am aware that the detailed biochemical and biophysical examples apply to particular systems.

One might expect terrestrial plants and animals to have evolved recognition procedures for most of the commonly occurring metal cations and to be able to make use of their specific properties. Table 1 shows a very rough average distribution in the earth's crust and the soil of the metal cations which are major plant nutrients and also a very rough analysis of the relative abundance of these elements in the dry mass of flowering plants. Potassium, magnesium and calcium are taken up in amounts which are reasonably constant for a given plant in adequate or abundant nutrient media whereas sodium may vary quite considerably with nutrient concentration. Aluminium, although abundant, is not taken up and is somewhat poisonous. Titanium is also surprisingly abundant but appears neither useful nor poisonous to plants. While there is an optimum concentration of even major nutrient cations this is not critical and quite an excess

Table 1. Relative concentrations of cations

	lithosphere†	soil‡ (dry)	angiosperms‡ (dry)
	major plant nutrient cations (parts/1000)		
Na^+	28.3	6.3	1.2
Mg^{2+}	20.9	5.0	3.2
K^+	25.9	14.0	14.0
Ca^{2+}	36.3	13.7	18.0
	essential trace elements (parts/1000)		
Fe	50	38	0.14
Mn	1	0.9	0.63
Co	0.04	8×10^{-3}	0.5×10^{-3}
Cu	0.07	0.02	0.01
Zn	0.08	0.05	0.16
Mo	2×10^{-3}	2×10^{-3}	0.9×10^{-3}
V?	0.15	0.10	1.6×10^{-3}
Ti?	4.4	5.0	10^{-3}
	poisonous cations (parts/1000)		
Cr	0.2	0.1	0.2×10^{-3}
Ni?	0.1	0.04	2.7×10^{-3}
Pb	0.016	0.01	2.7×10^{-3}
Tl	0.3×10^{-3}	0.1×10^{-3}	—
Ba	0.43	0.5	1.4×10^{-3}
Al^{3+}	81.3	71.0	0.5

† From Goldschmidt 1954.
‡ From Bowen 1966.
? Essential/poisonous category doubtful.

can be tolerated. For trace elements, however, the optimum is more closely defined and one organism's vital trace element may be another one's poison.

In table 2 are shown the radii of the cations, a most important property in distinguishing between them. The major nutrients have only one oxidation state and a fairly constant cationic radius. Most of the trace elements can have a variable oxidation state, the higher the charge the smaller the radius, and for some of them in a given oxidation state a high field strength of the surrounding atoms can give an effectively smaller radius when the spins of some of the electrons are paired.

Table 2. Radii of cations/pm

constant

Al^{3+}	50				
Li^+	60	Mg^{2+}	65	Zn^{2+}	74
Na^+	95				
K^+	133	Ca^{2+}	99	La^{3+}	115
Rb^+	148				
Cs^+	169	Ba^{2+}	135	$(Tl^+$	144)

variable

high-spin ≡ low field oxidation state II
Cr^{2+} 84 Mn^{2+} 80 Fe^{2+} 76
Co^{2+} 74 Ni^{2+} 72 Cu^{2+} ~72

low-spin ≡ high field ~10 pm less

high-spin ≡ low field oxidation state III
Cr^{3+} 69 Mn^{3+} 66 Fe^{3+} 64
Co^{3+} 63 Ni^{3+} 62

More is known of the metabolism of trace metals than of the major nutrients, partly because the complexes are more stable and hence will stay still while we look at them, and partly because there are more physicochemical techniques available for looking at them. Features common to the well-studied trace element compounds are: (i) an absorption or uptake and transport; (ii) storage; (iii) use.

All of these involve complexes, not hydrated cations, and the complexes are so strong, that is, they have such high stability constants that addition of chelating agents such as the famous EDTA (ethylenediamine tetraacetic acid) can be tolerated. Not all of the three stages are worked out in detail in all organisms but a very great deal is known and forms the subject of several books.

Optimum concentrations

The optimum concentration is fixed by experiment. For a cation required only in enzymes this may be very small, and because it is very difficult to provide a growth medium absolutely without an element, the number of elements found to be necessary increases as techniques improve.

In 1942 Piper showed that copper was required by plants and that the response was biphasic (or as I put it that there is an optimum concentration) with the maximum growth occurring in a medium containing 0.5 mg/l.

In human blood serum the ionic concentration of copper is maintained at 1 μM by an equilibrium involving free amino acid ligands and albumin, a protein giving quick release, i.e. this is the transport system. However, the albumin only contains 5–10 % of the total copper, the rest is held in another protein, ceruloplasmin, the storage protein. This is an 'anomalous' deep blue copper-protein with the copper not accessible to water and giving spectral properties which cannot be imitated by models. *In vivo* transfer to this protein takes place in the liver. *In vitro* the copper can be removed by reduction.

Although Fe III as a hydrated hydroxide is very insoluble at physiological pH the main constituent of the storage form in man, ferritin, contains a core of about 2000 Fe III in a stoicheiometry of $(FeOOH)_8$ $(FeOPO_4H_2)$ surrounded by protein. Various physical methods give conflicting evidence about the environment of the iron, and some results such as a magnetic moment, 3.5 μ_B (Bohr magnetons), which cannot be reproduced in a model.

In vertebrates the uptake or absorption and transport is regulated by transferrin according to the amount of iron stored in the ferritin. Transferrin in human serum is a glycoprotein of molecular mass about 80000 with a very high affinity (stability constant) for Fe III in two binding sites but low affinity for Fe II; it will also bind other trivalent and some divalent cations. In the exchange between transferrin and ferritin the iron goes through a divalent stage (Llinás 1973). This is clearly a vital recognition step; the extraneous cations will not undergo a change at the same redox potential to give an ion the same radius as Fe II, and the change back on the other side of the transfer again has a discriminating effect.

Competitive uptake

Isomorphous replacement of cations in minerals is fairly simple. As Goldschmidt (1954) pointed out, in a given negatively charged site for cations of the same radius the one of higher positive charge will be preferred. For two ions of the same charge and similar radius the smaller

ion is preferred because the electrostatic attraction is greater. Some of these ideas are still valid in biological systems, for example tripositive lanthanide ions inhibit the uptake of calcium by rat liver mitochondria (Mela 1970) possibly by blocking all the available transport sites on the membrane (the amount of lanthanide required is independent of the calcium ion concentration). Neither smaller cations Zn^{2+}, Al^{3+}, Sc^{3+} nor larger ones Ba^{2+} have an inhibiting effect, the requirement is a radius of about 100 pm.

In biological systems flexible sites are possible and one suggestion for discussion is that flexibility confers greater selectivity. Flexibility here implies that there is a change of shape or conformation of the molecule or site in forming a metal complex.

A series of very effective iron absorption compounds of this sort have been developed by microbes. A nice example is ferrichrome A produced by *Ustilago sphaerogena* if grown in iron-deficient conditions. Like the other ferrichromes this is a cyclic hexapeptide, three adjacent residues consist of *N*-acyl-*N*-hydroxy-L-ornithyl residues, i.e. they are hydroxamic acids, so providing three chelating negatively-charged sites in the anionic form. A neutral complex is formed with Fe III with stability constant of the order of 10^{30} and the iron in a high spin state, with magnetic moment 5.73 μ_B. Studies in solution are consistent with the structure found crystallographically (Zalkin, Forrester & Templeton 1966) for the complex and show that there is a change of conformation and hydrogen bonding when the metal is lost. When functioning properly the desferri ferrichrome transports iron into the cell and is rapidly released. If the chromium complex is put into the medium it is taken up as rapidly as the iron complex but the complexing agent is not released (Leong, Nielands & Raymond 1974). This suggests that the change of conformation does not take place in the cell wall but in the interior of the microbe. It also suggests that chromium is poisonous because it saturates the iron transporter and does not release it, not necessarily because it has a higher stability constant for the preformed site.

Many experiments have been carried out on ion interaction in uptake by plants with somewhat conflicting results. For nearly every cation there is in the plasmalemma a selective carrier but antagonism between zinc and copper may be due to their being taken up by the same carrier (Bowen 1969).

Alkali metal cations

Requirement

The uses of alkali metals are partly maintenance of osmotic pressure and partly in the activation of enzymes. Over 60 enzymes are known to require univalent cations (Suelter 1970) in concentrations 0.01 M, much higher than those for the trace elements. For example, potassium activates starch synthetase (Nitsos & Evans 1969) and other enzymes which build from small units (here soluble carbohydrates) to larger ones. It is also required for production of protein from amino acids. Potassium appears to cause the formation of active polymer units from inactive monomers, changes the conformation to the active form and sometimes acts as a bridge between the enzyme and the substrate. Rubidium can carry out all these functions. Ammonium does the polymerization but not the conformational change, and is poisonous in large concentrations.

Selectivity among alkali metals

Transport and the prevention of precipitation are not problems in aqueous systems; the problem is how concentration differentials are maintained across biological membranes. Plants

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take up potassium by processes which can be shown by change of temperature to be chemical in nature. Different plants have different tolerances for sodium and there may be several discriminatory processes in a given plant; no wonder the red blood cell is a more popular experimental material for studying selectivity.

As the ions are invariably unipositive the useful property is the difference in radius. While there are 120 possible permutations of sequences among the five metals, 11 selectivity sequences are found in practice in the fixed sites of glass electrodes and the same 11 in biological uptake and transport. Explanations for this are available (Eisenman 1962; Williams 1970; Morf & Simon 1971). Essentially there is a competition between the site (a vague word which can mean in a silicate glass, on the surface of a membrane or the atoms of a definite molecule) and solvent, particularly water, for the cation. Selectivity depends upon the field strength, both size and electro-negativity, of the site. A factor favourable to loss of water of hydration in the occupation of a fixed site is the resulting increase in entropy.

Our first attempts to make selective compounds followed the logic from this. We used a rigid ligand molecule with two or more atoms suitably placed to chelate the metal and we also varied the nature of the donor atoms, nitrogen, oxygen and sulphur and, by changing substituents to fluorine, the electro-negativity of the donor atoms was increased. The result of this was to produce discrete neutral complexes, and, for the smaller ions, complexes in which the alkali metal was in the centre of an anionic entity.

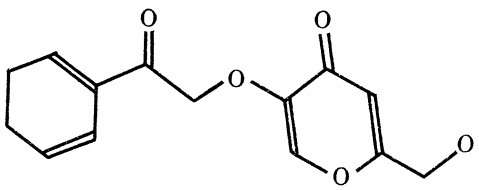

FIGURE 1. The formula of phenacyl kojate

$$PhCOCH_2OC{=}CH{-}O{-}C(CH_2OH){=}CHCO$$

with the double bonds indicated and hydrogen atoms omitted.

A neutral triple chelating agent, phenacyl kojate (PAK), gave complexes with salts of alkali metal cations, other than lithium in a curious stoicheiometry, 1:1 for caesium salts and for rubidium salts of large anions, and 1:2 for the smaller metals (Fenton 1973). This apparently suggested a smaller coordination number for the larger cation. The crystal structure of the potassium iodide $(PAK)_2$ complex showed potassium eight coordinated and not in contact with the anion (Hughes, Phillips & Truter 1974). In the Cs NCS(PAK) complex the cation was eight coordinated by three anions and oxygen atoms from two (PAK) molecules. Sodium iodide gave $NaI(PAK)_2 2H_2O$ with ten possible donor oxygen atoms. In fact the PAK molecules coordinate only a water molecule which is in contact with the sodium ion; octahedral coordination about sodium is completed by water molecules and oxygen atoms of the other type of PAK molecule. This is an example of a fairly general phenomenon, retention by sodium of some water.

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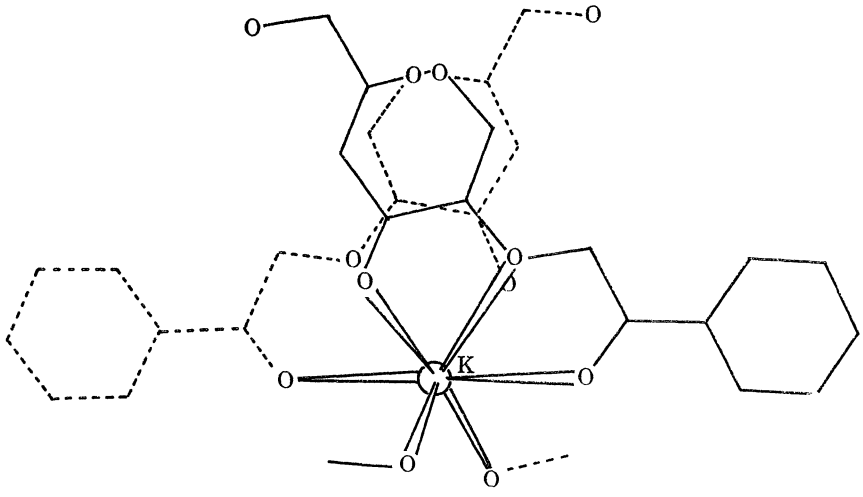


FIGURE 2. The environment of the potassium ion in potassium iodide (phenacyl kojate)$_2$. If the potassium ion is considered to be in the plane of the paper the two phenacyl kojate molecules which hold it by triple chelation are parallel to one another above (full lines) and below (broken lines) this plane, eight coordination is completed by hydroxy groups from two more phenacyl kojate molecules.

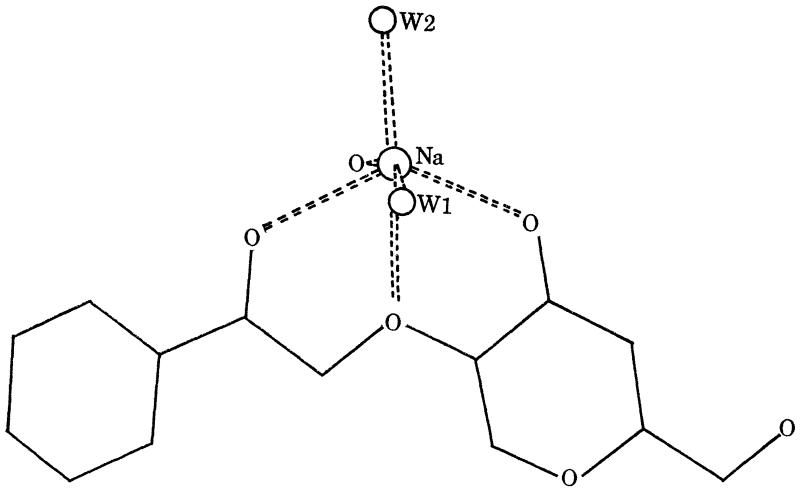
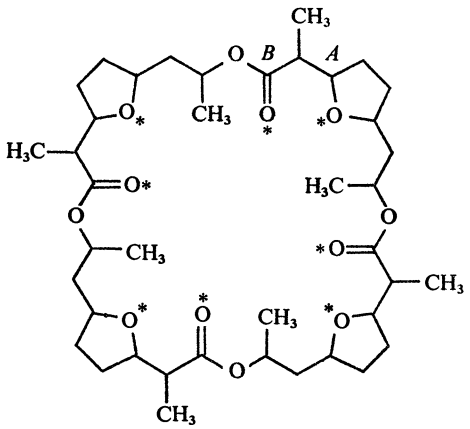


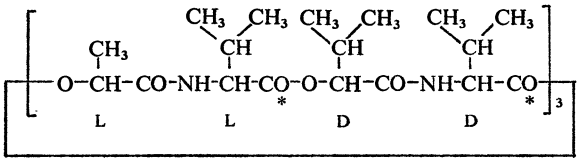
FIGURE 3. The environment of the sodium ion in sodium iodide (phenacyl kojate)$_2$ dihydrate showing triple chelation by one molecule of phenacyl kojate, coordination by two water molecules W1 and W2 and by a hydroxy group from another molecule of phenacyl kojate. Sodium-oxygen contacts are shown by broken lines; there are no sodium-iodine contacts.

Fungal metabolites

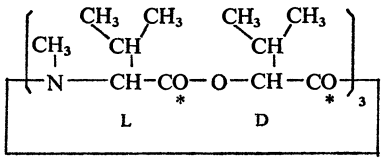
Naturally occurring antibiotics which have been found to complex potassium more strongly than sodium, to cause uptake of potassium by mitochondria, and to transport potassium preferentially through membranes are neutral and cyclic. They are also flexible; nonactin has been shown by Dunitz and co-workers (Dobler, Dunitz & Kilbourn 1969; Dobler 1972; Dobler & Phizackerley 1974) to change from a flattish molecule in the free form to wrap around potassium or sodium as he puts it 'like the seam of a tennis ball'. All studies in solution are consistent with the conformational change being the same as that between the two crystalline



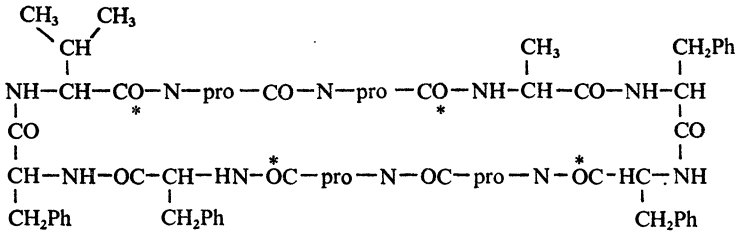
nonactin



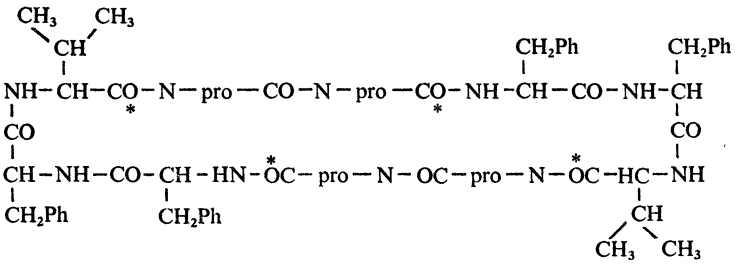
valinomycin



enniatin B



antamanide



antamanide analogue

FIGURE 4. The formulae of selective naturally-occurring complexing agents for alkali metals, with the ligand oxygen atoms marked *. For nonactin one of four pairs of bonds are marked *A* and *B*. Change of torsion angles about the eight bonds results in a change of conformation of the molecule on complex formation.

Valinomycin is held in the complexed form by six hydrogen bonds N—H...O (peptide) and a variety of conformations are found for uncomplexed form in solution. Enniatin B provides an octahedral site of three amide and three ester carbonyl oxygen atoms.

Antamanide and its analogue are decapeptides with all residues L; the coordinating oxygen atoms form the base of a pyramid round the metal and a solvent molecule the apex.

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forms. In this compound potassium is symmetrically surrounded by eight oxygen atoms. In another highly selective compound valinomycin it is symmetrically surrounded by six oxygen atoms (Pinkerton, Steinrauf & Dawkins 1969).

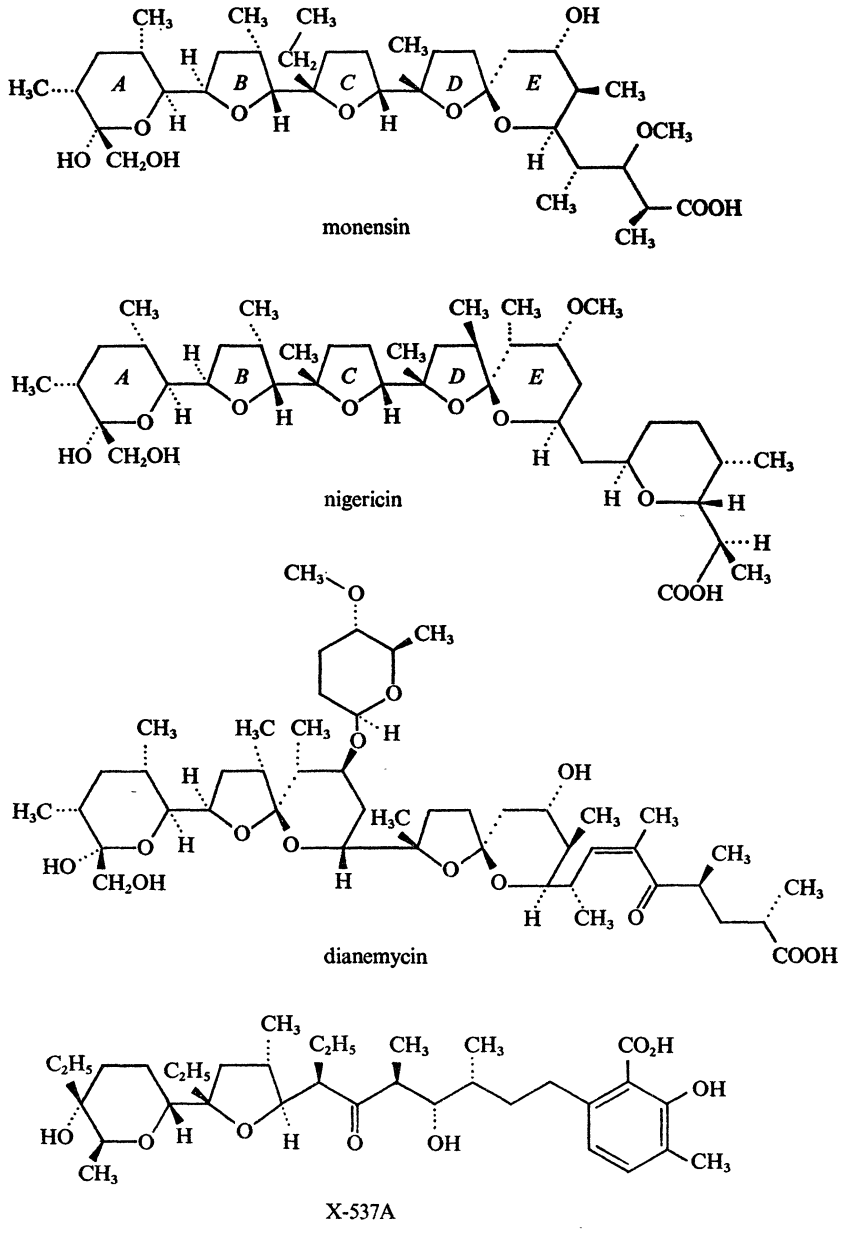

FIGURE 5. Formulae of monobasic acids which neutralize the charge on alkali metal cations giving compounds insoluble in water but soluble in ether; two anions of X-537A form neutral complexes with alkaline earth cations.

By way of contrast the only naturally occurring sodium selective compounds discovered so far, antamanide and its analogues, are also cyclic but the complex has only four atoms in contact with and on one side of the cation and a fifth position occupied by a solvent molecule (Karle 1974). Again we see the effect shown in phenacyl kojate.

An additional feature in biological selectivity appears to be the rapid rate of reaction. Even

small differences in equilibrium stability constants are multiplied by rapid formation and dissociation. This consideration applies equally to a view of the selectivity being the result of many exchanges in a series of more or less fixed sites through a membrane or to a carrier mechanism. The discrete neutral ionophores certainly replace the solvation sphere step by step, with a change of conformation.

Monobasic acids with many oxygen atoms such as monensin and nigericin neutralize the charge on, and complex with, alkali metal cations. In one case, monensin, the crystal structure of the uncomplexed form has been determined (Lutz, Winkler & Dunitz 1971) and found to be similar to that of the complex with water taking the place of the cation. They complex both potassium and sodium strongly but not selectively; this may be because their reaction is metal–water exchange. The reaction rate is much faster than that of nonactin or valinomycin (Winkler 1972).

Thallium as a probe and a poison

Thallium can exist in two oxidation states I and III, the former is achieved by loss of one *p* electron leaving a pair of *s* electrons on a cation of radius 144 pm, intermediate between the values for potassium and rubidium. Some aspects of its chemistry are similar to those of the alkali metals and as it has diagnostic physicochemical properties, fluorescence and an n.m.r. signal, it is in principle a useful probe for potassium in biological systems. It differs from potassium in forming complexes more strongly with chelating anions such as citrate, the ratio of stability constants is about 100:1. Provided that it is applied in low enough concentration < 0.1 mM it will be 'mistaken' for potassium and taken up by red blood cells *in vitro*. It is extremely poisonous *in vivo* to mammals probably because of its efficacy in activating enzymes normally activated by potassium, e.g. pyruvate kinase (Kayne 1971), and ATPase (Britten & Blank 1968), and phytotoxic at 0.2 mM.

Thallium complexes with chelating agents, neutral or anionic, are of two kinds:

(*a*) isomorphous (and isostructural) with sodium, potassium or rubidium;

(*b*) quite different from the alkali metals.

Of the former kind are the complexes of monensin and dianemycin (Steinrauf, Czerwinski & Pinkerton 1971), and synthetic complexes, the cryptates (Lehn & Sauvage 1971, Moras & Weiss 1973). These seem to have a common feature, on complex formation one molecule provides the complete coordination shell.

When the coordination shell consists of several entities the alkali metals and thallium give quite different pictures. If the charge is neutralized by one chelating anion, such as *o*-nitrophenolate or salicylate, and neutral chelating molecules complete the coordination, there is a pyramidal arrangement with thallium at the apex (Hughes & Truter 1972) but an approximately spherical arrangement about the alkali metals (Hughes 1973).

Similar contrasts in behaviour are shown with macrocyclic ethers. Thallium salts which are associated, i.e. not 1:1 electrolytes in solution, will not form complexes (Poonia & Truter 1973). Further, when a complex is formed with benzo-15-crown-5, it has the stoicheiometry 1:1 with macrocyclic ethers while potassium, rubidium and caesium are consistently found enclosed in sandwich compounds of ratio metal:crown 1:2.

Use of thallium fluorescence to measure stability constants with ionophores showed that, as expected, they are comparable with potassium for monensin (lower) and also for the neutral compounds valinomycin (lower) and nonactin (higher). An anomalous result was obtained for

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the hexadepsipeptide enniatin B (Cornelius, Gärtner & Haynes 1974), apparently no complex formation, although this, like valinomycin, has potassium in six coordination (Dobler, Dunitz & Krajewski 1969). I suggest the explanation is that the enniatin–potassium complex is a sandwich as deduced by Ivanov *et al.* (1973) on the basis of n.m.r. and lipid bilayer conductivity, and thallium does not form a sandwich and is not strongly held by only half the atoms.

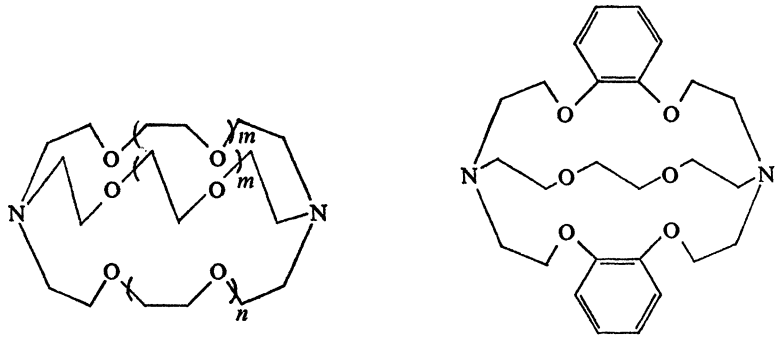

FIGURE 6. General formula of a synthetic 'cryptate' molecule. If $m = n = 1$ the molecule is a [222]cryptate, if $m = 2$ and $n = 1$ the molecule is a [332]cryptate. The cryptates form complexes with alkaline earth metal cations more strongly than with alkali metal cations of the same radius. Within a series the stability constant depends upon the goodness of fit; for the [222]cryptate the maxima are at potassium and barium. Incorporation of more organic groups in the molecule increases the stability of the complex with the univalent cation so that the dibenzo[222]cryptate complexes potassium and barium equally strongly.

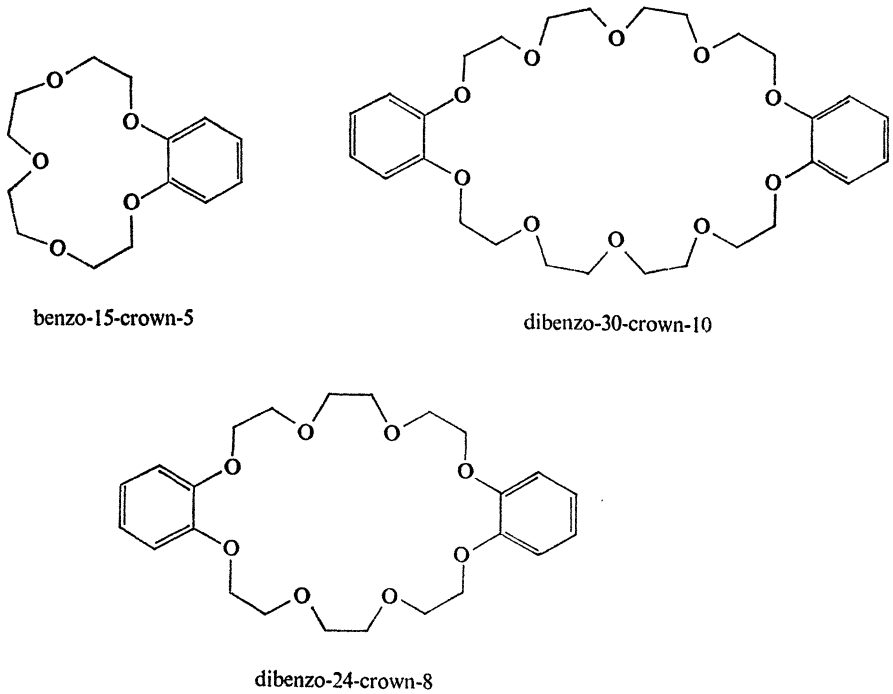

FIGURE 7. Formulae and trivial names of synthetic macrocyclic ethers, 'crown' compounds (Pedersen 1967).

The poisonous effect of thallium can be seen as a preferential occupation of the first hemisphere of a site normally occupied by potassium followed by a failure to gather the other compounds around (possibly not bridging the enzyme to the substrate) and lack of reversibility because the affinity for the site is too great.

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Polynuclear complexes

'Active' energy-requiring transport takes place with an interesting stoicheiometry, three sodium ions are transported one way for every two potassium ions going the other way (the charge balance is made up by protons), so although no actual carrier has been isolated, there are many theories involving, for example, two binding sites highly specific for potassium on one side of a membrane, then a conformational change to give three sites, one highly specific for sodium on the other (Middleton 1970). While most of the known natural and synthetic complex-forming molecules give mononuclear complexes, i.e. containing one metal and one or more ligand molecules, there are now several well established macrocyclic ether complexes containing two metals. In the complex (potassium isothiocyanate)$_2$ dibenzo-24-crown-8 all the oxygen atoms are approximately coplanar and contact one or both potassium ions, the cations are also bridged by the nitrogen atoms of the isothiocyanate ions one above and one below the plane of the ether ring (Mercer & Truter 1973). Sodium forms compounds with analogous formulae with the thiocyanate anion (in the absence of water) and with a chelating anion such as *o*-nitrophenolate (Poonia & Truter 1973). Crystal structure analysis on this compound (Hughes 1974) has shown that the anion is chelating and bridging so that two anions occupy three coordination sites on each sodium, the other three sites are occupied by oxygen atoms from the cyclic ether so that six of its oxygen atoms are in contact with sodium ions and two oxygens make no such contact. This is the first macrocyclic ether complex in which 'spare capacity' has been found.

At present we are attempting the synthesis of a molecule designed to accommodate three sodium ions. At first sight a possible candidate could be dibenzo-30-crown-10. With sodium salts this gives 1:1 or 2:1 complexes; to make a 1:1 complex a large non coordinating anion, tetraphenylborate, is used and even so water must be rigorously excluded. In the presence of water or coordinating anions 2:1 complexes are formed. We can imagine the dihydrate as containing two entities like the benzo-15-crown-5, aquo, sodium cation in the complex formed between sodium iodide and benzo-15-crown-5 (Parsons, Truter & Wingfield 1975). Knowledge of stoicheiometry is important in the determination of stability constants; it is possible that the reason the ammonium and sodium complexes of dibenzo-30-crown-10 give values (Chock 1972) a hundred times less than those of potassium, rubidium or caesium, is that, as we have found, ammonium thiocyanate, like the sodium salt, forms a 2:1 complex with this ether while 1:1 was assumed in calculating the cation concentration. In general we have found the stoicheiometry of the ammonium salt to follow that of sodium with all the macrocyclic ethers we have tried, despite its radius being similar to that of potassium.

One naturally occurring compound gives binuclear complexes, X-537A, figure 5. Two of the anions neutralize and complex a divalent cation, while the sodium and silver complexes are dimeric. Each sodium ion is pyramidally coordinated, four oxygens from one anion forming the base and one from the other apex (Schmidt, Wang & Paul 1974). This molecule is found free and in various complexes with the same conformation, and raises the further problem of selection between mono and divalent cations.

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Design of selective molecules

Synthetic work in Russia on variations of valinomycin produced compounds some with greater and some with smaller stability constants for complex formation with potassium. All gave slower transport through lipid layers (Ovchinnikov 1974). Similar results were obtained by Lehn and co-workers (Dietrich *et al.* 1973*a*) with the cryptates, that is the one with the highest stability constant did not give the most rapid transport from one aqueous solution to another through a non-polar solvent.

Discrimination between uni- and di-valent ions of the same radius can be achieved by adjustment of the organic part of a molecule, the 'thicker' the greater the preference for a uni-valent ion (Morf & Simon 1971). This has been demonstrated with the cryptates, figure 6; successive introduction of benzene rings increases the affinity for potassium relative to that for barium (Dietrich, Lehn & Sauvage 1973*b*).

Biological systems have evolved recognition procedures for metal cations which make use of changes of oxidation state and of radius where possible, but for cations of invarient charge differences in radii alone are sufficient. This can be achieved by flexible molecules which change conformation on a stepwise replacement of the water round a cation. Potassium selective compounds give complexes with the cation in a symmetrical environment, while for sodium selectivity a less symmetrical environment with some retention of solvent is found. There is a balance between selectivity and speed of complex formation, and also between the stability constant and the rate of transfer of the cation across a lipid barrier.

References (Truter)

General

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