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THE SYMBIOSIS OF METAL ION AND PROTEIN CHEMISTRY

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Abstract - The binding of metal ions to biological macromolecules is made extremely complicated by the existence of different compartments in cells, organs and whole organisms. The compartments are not at equilibrium. Apart from considerations of binding constants and rates of combination of metal and ligand with which an inorganic chemist is familiar the distribution of elements within biological systems is controlled by a non-equilibrium partitioning between the compartments. The compartments can differ simply in the sense that they are at different pH or redox values. More complicated situations arise through other energised activities within and between compartments. Particularly important are (i) the controlled syntheses of different proteins and ligands in a given compartment (ii) the pumping of specific ions in and out of compartments (iii) the energised movement of proteins and metalloproteins across membranes. The peculiarities of the function of one metal ion are related to this control over its distribution. Catalytic function of an element can only be understood in the light of this sophisticated fractionation of the elements in space.

INTRODUCTION

This lecture is a continuation of a general analysis of the functional value of the association between metal ions and proteins in biological systems. In summary I consider that during evolution proteins have developed firstly to bind metal ions selectively, both chemically and within specific proteins, then to organise the ensuing metal protein complexes and metalloproteins in space (and maybe in time), and that, as this degree of organisation was being achieved, further improvement of metal ion/protein interactions took place so as to make optimal their joint functions (Ref. 1). The last stage includes the development of mutual strain between metal and protein, the entatic state of Vallee and Williams, i.e. the refinement of energetics (Ref. 2).

In this lecture I shall look firstly at the problem of selectivity of the binding of metal ions in somewhat greater depth than before. This analysis shows that the handling of free iron and manganese ions presents acute chemical problems for living organisms but that these difficulties have been turned to considerable advantage. The problem is most acute in plants and unicellular organisms. The discussion leads naturally to an analysis of the differential, transport, use, and storage of elements in extracellular and in different intracellular spaces and raises not only the difficulties of binding in these parts of space but also of the limited ability of cells to develop functionally valuable compounds of some elements in particular parts of space. The nature of the catalytic site and the entatic state have been discussed at length elsewhere (Ref. 2).

We start the analysis from the generalised picture of protein metal-ion binding shown in Fig. 1. Two types of binding are shown, surface and internal. In each case it has been found that binding of sufficient strength usually needs three ligands at least, though four ligands can be employed. For catalytic sites there must also be a vacant coordination position but this is not necessary, and could be disadvantageous, for carrier, structural, or electron-transfer proteins. We know that proteins have a vast excess of possible ligands, donor side-chains, and it is therefore their folds which generate sites of controlled coordination number and ligand donors, thus selecting the metal ion and leaving the metal open-sided or not as the case may be. The fold also generates a selectivity cavity around the metal. It is the properties of the cavity which will intrigue us finally since it generates substrate specificity.

BINDING CENTRES FOR METAL IONS: STABILITY CONSTANTS AND EXCHANGE

The simplest situation to consider is the binding of the divalent cations of the first transi-

tion series to protein side-chains. The Irving-Williams stability constant series based on model chemistry shows that for these divalent ions there is an almost invariant order independent of ligand.

$$Zn < Cu > Ni > Co > Fe > Mn > Mg$$

(The order is broken by change of spin state but protein side-chains do not cause this change.) The increments between members increases as the donor power of the ligands increases; i.e. O-donors < N-donors < S-donors. We have every reason to suppose that this order holds for proteins as ligands.

In order to make some consequences of stability constants clearer I show in Table 1. the effect of the nature of the protein side chains as organic reagents, together with the effect of ion-pumping which is dependent on the stability constants, upon the free ion concentrations of common metals inside cells (assuming reducing conditions) and outside cells (assuming oxidising conditions). The environmental restrictions on availability have been included in the calculations. Note that the differentials in concentration, inside to outside, of some ions are caused overwhelmingly by pumping, e.g. Mg(II) and Ca(II) and in part this is true for manganese and iron too, while the differentials in others are caused overwhelmingly by chemistry, i.e. redox potentials and binding to specially placed proteins inside or outside cells. As an overall consequence of availability, pumping, and the nature of the synthesis of proteins, free ion concentrations of Cu, Zn, Ni, Co and Mo, are not significant in cells and this distinguishes them from Na, K, Mg, and Ca. Fe and Mn are intermediate in character and we shall have to consider them separately. The functions of the three groups of metals can then be very different.

The stability order tells us little about exchange rates. It is here that the protein-fold serves its second major function. The fold energy is such that especially for those proteins with hydrophobic sequences it is difficult to unfold even quite small regions of them. A metal ion which binds internally, Fig. 1, contributes to the fold energy by cross-linking. Locking metal ions into internal sites of hydrophobic proteins then restricts exchange and virtually prevents it if the stability constants are high. For this reason metal-proteins of high stability constant, i.e. Cu(II), Ni(II), Zn(II), Mo(VI), haem, corrin and even chlorin in which the metal is bound internally do not exchange metal ions. Cooperative metal clusters of Fe or Mn also do not exchange readily. However even bound in the internal sites generated by protein side chains Co(II), Fe(II), Mn(II), Ca(II) and Mg(II) which bind increasingly more weakly are increasingly readily exchanged. They do not form truly stable metallo-enzymes. This exchange is the faster the less tightly structured, i.e. more hydrophilic, is the protein itself e.g. calcium trigger proteins (Ref. 3). Exchange is also a required feature of carrier proteins and nearly all metal ions will exchange if they are only bound to protein surfaces of course.

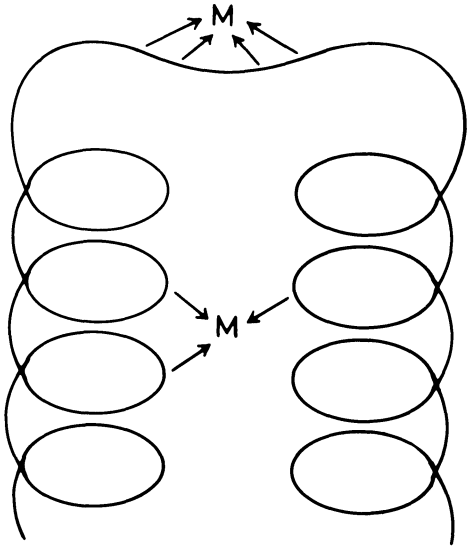


Fig. 1. Two sites at which a protein binds a metal ion. At the top site the metal ion is bound to a surface residue while in the middle site it is bound deeply inside the cross-linked folds of a protein. The first site is suited to exchange while the second is suited for catalysis.

TABLE 1. Approximate molar free cation concentrations ($\log_{10}$ [M])

	Ca^{2+}	Mg^{2+}	Mn^{2+}	Fe^{2+}	Fe^{3+}	Co^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}
Out of cell	-3	-3	-6	-17	-17	< -12	-12	-15	-15
In cell	-8	-3	-8	-8	-17	< -12	-12	-15	-12

N.B. The values for Co, Ni, Cu and Zn are at least as low as shown in most biological compartments. Cobalt is not very available from the environment but the other three cations are strongly retained by proteins. Concentrations in vesicles inside cells may approach the values outside cells for calcium and manganese while for iron(II) they may reach higher values than those inside cell cytoplasm. The differences in different compartments are brought about by pumping (see below).

Coordination partners

The chemistry of individual metal ions with proteins in biology is also restricted by the chemical nature of the coordination partners, protein side chains, to which they are able to bind. There are several factors to consider.

1. Intrinsic stability of binding leads to (a) sulphur and nitrogen binding to Cu, Zn, (Co), (Ni), Fe, Mo but not to (Mn), Mg and Ca and (b) anionic centres using carboxylate are relatively more useful than any other groups as the electronegativity of the metal ion drops or its charge increases, e.g.

RCO_2^- is found associated with Ca(II) > Mg(II) > Mn(II) > Fe(II) > Cu(II) > Cu(I); Fe(III) > Fe(II).

2. Control over protein synthesis limits the production of proteins with high affinity for special metals in accord with cellular requirements in different compartments.

3. The pH and redox potential of the compartment controls ligand availability, especially of thiolate.

The association of a given metal ion with a given protein centre is unique in biology in the vast majority of cases, Table 2. This is a quite remarkable chemical fractionation.

TABLE 2. Mononuclear coordination sites in enzymes

Metal Ion	Coordination No.	Ligands	Function
Zn(II)	4(5) tetrahedral	N, S, (CO_2^-), H_2O	σ-acid
Cu(II), Cu(I)	4(5)	N, S, H_2O	redox, π-base
Ni(II)	6?	N, CO_2^-, H_2O	? σ-acid
Fe(II)	6?	N, CO_2^-, (RS^-), H_2O	redox, control, π-base
Fe(III)	6?	N, (CO_2^-), ϕO^- H_2O	σ-acid, redox
Mn(II)	6	(N), CO_2^-, H_2O	control, redox
Mn(III)	6	N, CO_2^-, H_2O	σ-acid, redox
Mg(II)	6	Oxygen anions	control, trigger
Ca(II)	6-8	CO, CO_2^-	trigger

? Electron transfer

We see that by using the two different binding units of Fig. 1. and by placing different types of coordinating ligands differently in different sequences and therefore in different folds it is possible to separate metal ions into major classes such as (1) Mg(II) and Ca(II) bound only to the surface carboxylates of specific proteins, Fig. 1., and (2) Cu(II), Zn(II), Ni(II), corrin, haem and chlorin bound in internal sites by N, S ligands, but additionally it is possible to fractionate each metal within each class.

While it may be obvious how such selectivity is achieved for Cu and Zn it is not so clear perhaps how a protein can select for Fe(II) or Mn(II) when Zn(II) is present. The Irving-Williams stability series shows that model nitrogen and sulphur donor ligands favour Ni(II), Cu(II) and Zn(II) over Fe(II) and Mn(II) to an overwhelming degree. Biological coordination conforms to this pattern. Carboxylate or phosphate donors alone show little selectivity between say Mg(II), Ca(II), Mn(II) and Zn(II) and in any case they bind too weakly to be useful in the retention of Fe(II) or Mn(II). Combining carboxylate and nitrogen donor ligands in EDDA, ethylenediamine diacetate, the selectivity of ethylenediamine itself is maintained although all stability constants, K, increase by a factor of log $K \simeq 5$, Fig. 2. Thus no simple selection for Mn(II) or Fe(II) appears. Quite strikingly however further increase in the number of carboxylates keeping the ethylenediamine frame to EDTA, ethylenediametetraacetate, increases the stability of the zinc complex by but $\Delta \log K = 4.3$ while it increases that of manganese by $\Delta \log K = 6.3$. The reason for this difference lies in the greater steric repulsion between the larger number of donor groups around the smaller cation.

This is seen again in the log K_{EDTA} - log K_{EDDA} values of 4.2 for magnesium (the same size as zinc) and of 6.0 for calcium (larger but not so much larger than Mn(II)). That steric crowding is responsible for loss of incremental selectivity in the Irving-Williams series is seen clearly by examining the effect of a further increase in ligand denticity to EGTA, 2,2'-Ethylenedioxybis[ethyliminodi(acetate)], Table 3. We observe log K_{EGTA} - log K_{EDTA} is Ca(+0.4), Mn(-1.9), Zn(-3.7), Mg(-3.5). For EGTA log K is almost the same for the cations Mn(II), Fe(II), Co(II), Ni(II) and Zn(II), Fig. 2. The selectivity of the N-donor centres has been lost though all these cations form much stronger complexes than Mg(II). The way Mn(II) or Fe(II) can be selected in model complexes by competition involving a variety of ligands is now clear. Sites of low coordintion number and very strong donor power select Cu, Ni, Zn while those of higher coordination number and poorer donor power are left to take Mn and Fe. We can now turn to the enzymes which bind Cu(II), Zn(II), Mn(II) or Fe(II) through protein side-chains to see if, in fact, they use these principles.

From extensive chemical and crystallographic studies we know that Zn(II) and Cu(II) are usually bound in proteins by three or four N/S donors of coordination number four or five but that Zn(II) can also be held in a rough tetrahedron of two N-donors and one carboxylate, Table 2. From the above model ligand data such sites clearly bind Cu(II) and Zn(II) >> Fe(II) or Mn(II) and therefore lower the relative availability of the first two metal ions to other ligands. In such circumstances Mn(II) and Fe(II) can be taken up selectively by sites such as that provided by EGTA, see Fig. 2. In fact Figs. 3 and 4 show that proteins found in selective combination with Mn(II) and Fe(II) are of this kind. The Mn(II) binding site of concanavillin A is six-coordinate and has one N-donor (Ref. 5). Competition from Mg(II) and Ca(II) is reduced by the N-donor, but it is partly the size of the coordinating sphere of ligands which selects Mn(II) against other metal ions and partly the fact that cations such as Cu(II) and Zn(II) have been preferentially removed at binding sites of lower coordination number and greater donor power. The probable Fe(II) site of hemerythrin, postulated from the known Fe(III) coordination sphere, is shown in Fig. 4. (Ref. 6). Although here the number of N-donors is larger the coordination sphere is also large, six coordinate, and we presume it is

TABLE 3. Some stability constants (log K)

	Ca(II)	Mn(II)	Fe(II)	Zn(II)	Mg(II)
Ethylenediamine	-0.0	2.8	4.3	5.9	0.4
Triethylenetetramine	-0.0	5.4	8.3	12.0	-
2,2',2",2'"-ethylene dinitrilo tetrakis ethylamine	-0.0	9.3	11.0	16.0	-
EDTA	10.6	14.0	14.2	16.2	8.7
EGTA	11.0	12.1	11.9	12.5	5.2
Ethylenediamine N.N'diacetate	4.6	7.7	9.8	11.9	4.5

Data from Ref. 4.

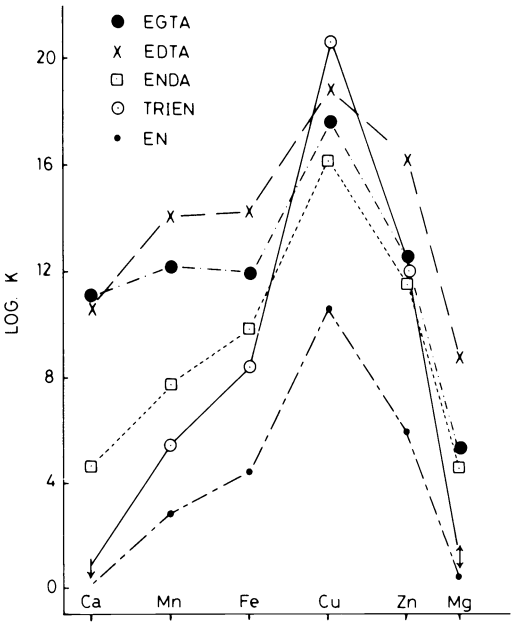


Fig. 2. The stability constants for some divalent ions with ethylenediamine (EN), triethylene tetramine (TRIEN), ethylenediamine diacetate (ENDA), ethylenediamine tetracetate (EDTA), and ethylenedioxybis[ethylimidodi(acetate)] tetracetate (EGTA).

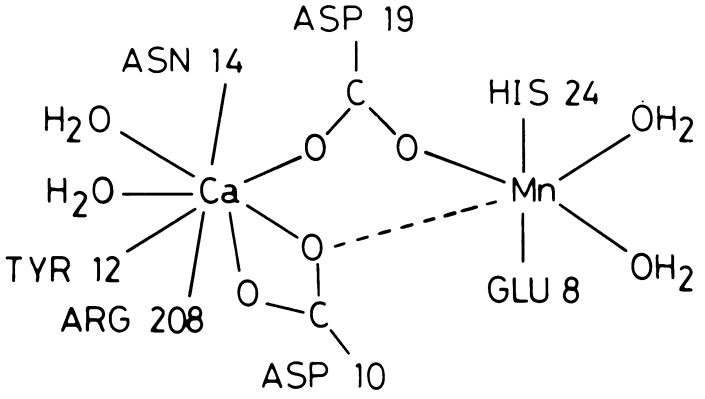
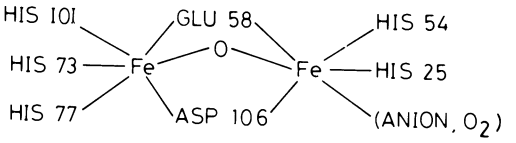


Fig. 3. The structure of concanavillin A.



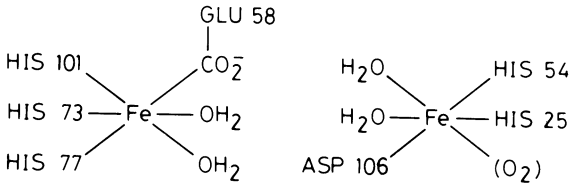


Fig. 4. The structure of hemerythrin, above Fe(III) and below a postulated Fe(II) structure.

again size factors such as are seen for EGTA, Fig. 2. which gives Fe(II) a preferred binding to Zn(II) (which has already been preferentially removed as described above) and the large number of N-donors which facours Fe(II) > Mn(II) > Ca(II). We see that it is only through the fold of the protein and the energetics of that fold that these sites of particular ligand donors, coordination numbers, stereochemistries, and cavity sizes, become so metal ion selective. Now while sites such as those of concanavallin A and of hemerythrin are of value in selecting Mn(II) and Fe(II) they do not confer very high absolute stability. Thus mononuclear complexes formed from protein side-chains which bind these two cations will undoubtedly allow them to exchange. The proteins are then not true metallo-proteins but lie between metallo-proteins and protein-metal complexes. In such situations control over the complexes which form is exerted by the compartments into which the proteins and the free metal ions are pumped. The proteins and the metal ions can be independently placed in such compartments. In contrast in the case of the metallo-proteins once the complex is formed it is the intact complex which is moved into any compartment and the concentration of free ions of the element or of any other complex of the element is of no consequence once the protein complex is formed since it is irreversible. Given these factors the actual distribution of elements in compounds in biology needs careful analysis, Fig. 5., and use becomes related to exchange rates as well as actual binding sites.

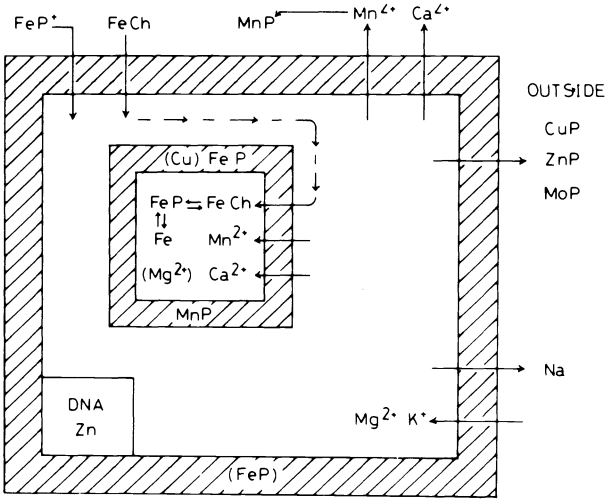


Fig. 5. A schematic diagram of some of the more important ways in which different metal ions are moved into different compartments: P = protein, Ch = small chelating ligand. K^+, Na^+, Mg^{2+}, Ca^{2+} and Mn^{2+} are pumped as ions. Fe, (Mn), Co are transferred as small chelates or in proteins. Zn, Cu, Ni and Mo are frequently transferred as metalloproteins. The last four metallo-proteins can be placed anywhere in an organism without dissociation.

Trivalent ions

The full expression of selectivity of uptake of metal ions by proteins can not be understood from an examination of the stability constants or the rates of transfer of divalent ions. For example it is clear that the Fe/S proteins which are metallo-proteins are stabilised by the high affinity of iron(III) especially for sulphide. Moreover there are cases of the same coordination sphere appearing for two metals, e.g. four thiolates in rubredoxin (Fe) and in alcohol dehydrogenase (Zn) but the two sites are not confused in metal selectivity. The selection may be a matter of chemical affinities but we must be aware of the possibility that the iron compound, rubredoxin, is found in prokaryotes which have a high cytoplasmic iron level while the zinc compound, alcohol dehydrogenase, is found in the cytoplasm of eukaryotes where the iron is very low.

Apart from differential pumping the fractionation of iron as Fe(III) is obvious in such compartments as plasma, e.g. in transferrin. The restriction of iron movement to an Fe(III) metallo-protein allows the subsequent distribution of iron to be quite unlike that of any other element. This is equally true of the movement of cobalt as vitamin B_{12}. Note this is Co(III). Possibly molybdenum is also handled in this way in a still higher oxidation state.

COMPARTMENTS

In order to understand the observed binding of metal ions to particular ligands we have discussed so far two factors: (1) the intrinsic stability constants of metal ion binding to particular ligand frameworks which have 'designed' structures; (2) the limitations on the concentrations of metal ions imposed by availability and the pumping of different ions into different compartments. We could view the compartments as different phases and the energies applied to pump the ions as ion-specific partition coefficients. It must be remembered that energy can be applied directly to the transfer of an ion across a phase boundary or it can be applied to the whole phase by (3) adjusting the pH or the redox potential of the phase in a compartment. If we take a reference compartment to be at a standard state pH = 7 and at a redox potential the same as the O_2 potential, i.e. +0.8 volts, then roughly speaking the different aquo ion concentrations expected in this phase are those of the sea. As is well-known blood plasma is not too dissimilar from sea water. All other biological compartments are energised relative to this state specifically by ion pumping and by pH control, redox control and in two other ways. The functional value of an element in a compartment depends upon its chemical combinations with different ligands, L, and the distributions of L themselves are energised in the first place in a very similar manner to that of the element, M. For example the state of a reagent carrying two thiol groups can be $(-SH_2)$, $(-S^-)_2$ or -S-S-, i.e. pH and redox control, and the compound containing this sulphur can also be pumped into a particular compartment. However since L is an organic molecule there is an additional possibility — the compound may be synthesised in different amounts in different compartments by cell metabolism. This is only another way of giving an energised distribution of course but now it is of the trapping reagent. The amount formed of ML, where M is a given element and L a given ligand, is now decided by (4) the limitations on the concentration of L in a particular compartment. Finally (5) the standing concentration of ML, metallo-proteins (non-exchangeable), in a compartment depends on the energising of the further movement of ML itself and the subsequent binding of the protein in the cellular organisation.

Given that such a multiplicity of factors controls the siting of a given ML in any biological compartment it seems likely that we cannot deduce where different ML are to be placed but we may be able to work backwards from the knowledge of where the ML complexes are found to the reasons for these dispositions. Tables 4 and 5 outline the major features of element distribution in the compartments of organisms. Note that both metallo-enzymes and enzyme-metal complexes, i.e. metals and ligands separately, are distributed. There are singular striking features. Before discussing the individual elements we need to describe one other feature of biological systems, they have synthesised special small chelating ligands, e.g. corrin (Co), haem (Fe), chlorin (Mg). The association here of a metal with a particular chelate of this kind is almost absolute. The association has two functions. It prevents exchange of a new set of metal ions since the metal complexes themselves exchange only extremely slowly from their final protein partners. Presumably ML is deeply buried, Fig. 1., though during transport some proteins carry ML presumably on their surfaces. Secondly these ligands generate low-spin metal ion states or push the metal ion states very close to the high-spin/low-spin cross-over point. Effectively quite new metal-ion chemistry is generated. Unfortunately these ligands, which can only be made inside cells, are intrinsically reactive and must be protected especially from oxidative attack. Haem, corrin and chlorin enzymes and proteins are almost invariably active inside cells. Finally these ligands and complexes are synthesised in special compartments, e.g. haem in mitochondria.

We turn to the discussion of the observed distribution of individual elements, Tables 4 and 5.

TABLE 4. Enzymes inside cells

Metallo-Enzymes	Metal/Enzyme Complexes
Many Zn-Enzymes	O_2-generation (Mn)?
Cytochrome Oxidase (Cu)	Lysine and Proline Oxidase (Fe)
Fe_nS_n - Enzymes	Superoxide Dismutase (Fe, Mn)
Some Mo-Enzymes	Some Oxygenases (Fe)
O_2-generation (Mn)?	Aconitase (Fe)
Superoxide Dismutase (Cu, Zn)	Many Mg-Enzymes
	Glycosyl-Transferases (Mn)*

Haem, Corrin and Chlorin Complexes are excluded. They are metallo-enzymes.

* In vesicles not in cytoplasm.

TABLE 5. Enzymes outside cells

Metallo-Enzymes	Metal-Enzyme Complexes
Laccase (Cu)	Digestive enzymes (Ca)
Galactose oxidase (Cu)	
Caeruloplasmin (Cu)	Protective devices e.g.
Some amine oxidases (Cu)	Blood clotting (Ca)
Digestive enzymes (Zn)	Complement factors (Ca)
Xanthine oxidase (Mo)	

Haem-enzymes such as catalases and peroxidases which are in cells, but within, vesicles and are then ejected, are excluded. They are metallo-enzymes.

DISTRIBUTION OF INDIVIDUAL METAL IONS

Zinc proteins

Zinc forms metallo-protein complexes. This means that it can be placed in any biological compartment, Table 6., except those which are extremely acidic since its complexes with N, S donor ligands are only stable above pH $\simeq$ 4. In fact zinc is found in all biological compartments performing a variety of catalytic and structural functions. Its concentation is decided by the transfer of the zinc-protein complexes. The movement of the proteins can be timed, by release from vesicles, e.g. in digestion, to avoid constant activity and degradation of the proteins outside the cells. The positioning of the zinc metallo-proteins and indeed all other proteins is therefore decided by recognition at and transport through membranes, an interaction which we do not understand as yet.

TABLE 6. Some zinc enzymes (Ref. 7)

Enzyme	Position in Cell
Alkaline phosphases	Exterior side of cytoplasmic membrane
Proteases	Vesicle for exocytosis
Saccharases	Vesicle for exocytosis
Nucleases	Vesicle for exocytosis
Carbonic anhydrase	?Outer membrane, cytosol
- - - - - - - -	- - - - - - - -
Aldolase	Cytosol
DNA/RNA polymerases	Cytosol
Protein synthesis factor	Cytosol
Alcohol Dehydrogenase	Cytosol

Copper compartments

Copper forms metallo-protein complexes and in principle it like zinc can be transferred as a Cu protein into any compartment, yet Tables 4. and 5. show that it has very limited roles in cells and it is widely used in extracellular proteins. This distribution may well have arisen during evolution because copper is the only metal which gives rise to stable metallo-proteins, Table 7., and to high redox potential couples in proteins, Fig. 6, and so can handle O_2 outside cells. Of the other possible redox-active metals we have seen that iron and manganese can not be retained by proteins irreversibly unless chelating groups such as porphyrin are used but porphyrins are not stable in oxidising atmospheres outside cells.

TABLE 7. The copper proteins associated with oxygen and their spatial distribution (Ref. 8)

Protein	Function	Spatial Position
Cytochrome oxidase	$O_2 \rightarrow H_2O$ H^+ pump	Mitochondrial membrane (High redox potential)
Laccase	$O_2 \rightarrow H_2O$ Phenol oxidation	Outside cell
Caeruloplasmin	$O_2 \rightarrow H_2O$ (Fe(II) Fe(III)?	Blood plasma
Haemocyanin	O_2 carrier	Plasma
Lysine oxidase	Collagen cross-linking	Extracellular
Ascorbate oxidase	$O_2 \rightarrow H_2O$ Ascorbate oxidation	Extracellular(?)
Galactose oxidase	Sugar oxidation	Extracellular
Amine oxidases	Removal of hormones	Extracellular
Blue proteins	Electron transfer	In membranes (High potentials)

Thus it was necessary to place copper enzymes outside cells in order to handle redox chemistry there. For the same reason copper provides the major O_2-carrier outside cells (haemocyanin) and it is intimately involved in the oxidative extracellular protective chemistry e.g. (1) detoxification - removal of amines (2) production of protective polymers - phenol oxidases (plants) and lysine oxidase to give collagen (animals) (3) free radical scavenging - ascorbate oxidase and caeruloplasmin. Inside cells nearly all these roles in oxidative chemistry are taken over by iron with but one or two notable exceptions which concern redox chemistry between +0.3 and +0.8 volts, see below, and one or two cases where flavin is used, e.g. amine oxidases.

Fe(III) and Mn(III) proteins

These proteins are extremely stable metallo-proteins and as such they can be placed either in steady state or by timed release anywhere within a biological organisation. However if these proteins are placed extracellularly in redox proteins the proteins must not undergo redox cycling through the Mn(II) or Fe(II) states which would dissociate. Examples of Mn(III) proteins are given in Table 8. which includes some hydrolytic enzymes and carrier proteins but no redox proteins which require Fe(II) states. Inside cells iron and manganese perform a very different role, see below. Note that acid phosphatases are extracellular Fe(III) or Mn(III) enzymes but extracellular alkaline phosphatases are Zn(II) proteins. (Strictly acid phosphatases are in lysozomes but we shall treat such vesicles and intra-reticulum spaces as extracellular.)

Molybdenum proteins

Molybdenum forms metallo-proteins. It too can be placed in any biological compartment, Table 9. Outside cells it is used as a catalyst for two-electron reactions, e.g. of aldehydes. Inside cells similar reactions are carried out by NAD. Molybdenum is found in milk for example. Molybdenum has special features not common to any other transition metal and therefore finds functions inside and outside cells.

Organic cofactors containing metals

Organic cofactors including haem, vitamin B_{12}, (flavin) and chlorin are stronly held and their proteins are irreversibly formed, compare metallo-proteins. They are made and retained in cells although apparently they could be placed anywhere in view of the fact that they do not exchange or need not be exchangeable. It may well be that since most of the organic molecules in the chelates are vulnerable to oxidation and since the external solutions to cells are at a high redox potential, they are rarely if ever used outside cells. The particular proteins can be placed selectively in special locations within the cellular organisation of course.

We have now described the metallo-proteins and their possible positioning inside or outside cells. They can all be localised much more specifically since they can bind to other proteins in a stable organisation.

TABLE 8. Some iron and manganese proteins: distribution (Ref.9)

Enzyme	Position in Cell
Oxygenases (Fe)	Cytoplasm
Electron transfer (Fe)	Cytoplasm and membranes
Hydroxylases	Cytoplasm and membranes
O_2-binding and reduction	Cytoplasm and membranes
Peroxidases Fe	Vesicles* and outside cells
Catalases Fe	Vesicles* and outside cells
Acid phosphatases (Fe and Mn)	Vesicles* and outside cells
O_2-evolution (Mn)	Membranes
Glycosylation (Mn)	Golgi vesicles* (outside)
Krebs cycle (Mn and Fe)	Mitochondria

*N.B. Vesicular space is effectively outside a cell since the contents are used outside the cell and the vesicle is merely a storage device, see Zinc Proteins.

TABLE 9. Molybdenum proteins (Ref.10)

Protein	Location
Xanthine oxidase	Milk (extracellular)
Aldehyde oxidase	
Nitrogenase	Bacterial cytoplasm
Sulphate/Nitrate reductase	Bacterial cytoplasm

TIMED RELEASE OR ACTIVATION

Many proteins are held in a fixed environment, e.g. haemoglobin. However biological systems must respond to changes in the environment even down to the level of switching on and off digestive and protective devices. This means that some proteins and enzymes associated with metals must be released in response to a signal. Such proteins or their associated metal ions are stored in one compartment to be released into another. For metal/protein complexes which are in fast exchange the metal and protein can be kept apart in the two compartments being brought together only by a trigger. For example calcium and magnesium proteins have many timed activities which arise from the movement of either the metal ion to the protein or of the protein to the metal ion. Activation is the formation of the metal-protein. For metals which can not undergo fast exchange or which do not give rise to a high standing concentration of metal ions this timed activation is not possible. In such cases timed release is of the metallo-protein. The release of zinc enzymes in digestion is one example. Release is from intracellular vesicles to extracellular juices. For iron and manganese the problem is more difficult since only Mn(III) and Fe(III) form metallo-proteins. Thus it is only in the higher oxidation states that these metals are released as metallo-proteins from vesicles. They act both in digestion, phosphatases, and in protection, peroxidases. For obvious reasons these activities are pulsed in time of need only from organelles such as lysozomes and peroxyzomes.

The timed use of manganese and iron enzymes has other facets since these ions are also pumped into compartments. Thus the levels of the free metal ions Mn(II) and Fe(II) in such spaces as vesicles where calcium is found (MnII) and in energy transducing organelles, mitochondria and chloroplasts, both Fe(II) and Mn(II), are open to regulation. The regulation, i.e. control in time over amounts, is not understood but is connected by feed-back to the overall metabolism of the cell. Cells scavenge for iron, and cell multiplication is regulated in major part by its supply. Growth in fact is also regulated by iron supply. The levels of iron in a complex organism require us to go back to examine its storage and distribution. Before doing so we look at one other aspect of spatial distribution.

Localisation within compartments

While the distributions of metallo-proteins inside or outside cells is one part of cell organisation and is brought about by the pumping of the protein units it is also possible to assemble different proteins in organised chains in any one compartment. Examples are the redox chains of the mitochondria, the chloroplasts, and of complex enzymes such as nitrogenase. Although today the specific proteins generate these assemblies many of them are in a sense only spacing devices for metal ions. Obvious examples are the iron that is always at the reducing end of electron transfer chains, the manganese and copper only in the oxidising terminal components of chains, the molybdenum which is also used terminally and at the oxidising end of a chain though at a relatively very low redox potential. Cobalt and nickel are not used in any of the chains. Finally the forms in which iron is used is restricted very largely to multinuclear Fe/S and haem proteins, i.e. in metallo-proteins. There would appear to be a logical necessity lying behind these arrangements and we may be able to discern their advantages from a study of exchange and redox potentials.

Distribution of metal/protein complexes

The concentration of these enzymes depends on the product [M] [protein]. We need to notice that both the proteins and the metals can be localised in specific compartments and that the protein can be trapped in an organisation. Enzymes for glycosyl-transfer (Mn) are in the Golgi vesicles, possibly in membranes, enzymes for the Krebs cycle (Fe) are in mitochondria. The required protein and the required metal are pumped into these spaces by synthetic and transport devices.

The nature of metal carrier complexes

We have described how metal ions are move in biological systems by the following mechanisms

(i) Diffusion of simple ions in all aqueous phases and selective pumping across membranes.

(ii) Diffusion of small chelates, ML, in special aqueous phases and selective pumping across membranes.

(iii) Diffusion of irreversibly formed metallo-proteins in the compartment of synthesis to sites of recognition and selective pumping of their proteins across membranes.

These processes determine the local compartment of given metal systems, ML. It is easy to see how selectivity for metals arises even in transport properties (i) through the use of channels lined with oxygen atoms, the channels selecting ions on the basis of size and charge from K^+, Na^+, Mg^{2+} and Ca^{2+}. Under (ii) we note that many organisms and in some cells Fe(III) for example is transported and recognised as Fe(III) citrate. Here the association/dissociation reactions of the chelate are fast and transport depends on the concentration of free L, here citrate in a compartment as well as upon receptors for the transport of ML. The situation is very different for Fe. porphyrin. No free porphyrin is found except within the compartment of synthesis. The fact that Fe. porphyrin and not some other metal porphyrin is formed depends on a chelatase enzyme but it could equally well depend upon the fact that porphyrin is made in a compartment into which only Fe is discharged.

We have also shown how Fe^{3+}, Zn^{2+}, Cu, Ni^{2+}, Mo, corrin, haem and chlorin in proteins could be selectively transported under (iii) in separate metallo-proteins using the principles of Table 2. In fact many of these irreversibly formed metallo-proteins are known. If they have special lead-sequences movement through membranes can be due to specific recognition of the protein due to structure far from the metal site. The metal ions can be discharged into special cells or compartments since a receptor may be used. The handling of iron is a particularly difficult but instructive example. It is moved in the blood as an Fe^{3+} metallo-protein and desposited differentially in cells according to the number of receptors. Once in the cell iron is released and mitochondria take it up using anions such as citrate as carriers. It is then incorporated into haem and on passing out of the mitochondria the haem is locked in a new metallo-protein or enzyme irreversibly. This protein is put into position.

A final method of bringing protein and metal together in a particular part of space is to pump M and the protein separately into the space or to pump the metal and synthesise the protein in one part of space. Provided that the reaction is reversible the control over protein or metal concentration which manage movement generates an overall control over an activity. There is no fixed concentration of either M or L in the compartment. Especially important here are the ions Mg, Ca, Mn and Fe. We can return now to Fig. 1 and to the discussion of

the ways in which the carrier proteins differ from metal enzymes. One major problem which remains is control over supply of the elements and this problem has been partially solved by the storage of required elements.

Storage

There is good reason to suppose that although selectivity of a storage device can be based on the same type of chemistry as used in enzyme site or carrier selectivity the need to be able to call on the store makes for important distinctions. The stability of the store should be under metabolic control. It is possible to store metals together with anions in solids so that we need to know the control over pH, redox, protein synthesis, and anion concentration in the compartment. For example iron is stored in ferritin as FeO(OH) essentially but this storage needs high pH, oxidising conditions, apoferritin, and some phosphate. Effectively apoferritin forms a compartment. Storage is usually in some special compartment regulated by metabolism. Release is often under metabolic control, e.g. the release of iron is due to redox reactions.

Whereas storage can be in the form of a small polymerised unit it is also possible to store metal ions in monomeric protein sites. Metals such as Zn, Cu, (Cd) can be stored in compartments where a special N/S donor protein is made, e.g. metallothionine. Release of the metal may demand destruction of the protein which in this case is relatively simple since the protein is a random coil in the absence of metal ions. This type of protein is unsuitable for the generation of a metallo-enzyme.

SUMMARY

In this paper I hope to have shown that the biochemistry of metal ions was made extremely complicated by the evolution of compartments. As organisation increased so the role of metal ions developed and the control over their use also increased. Thus metal ions can act in functions as diverse as those found for organic molecules, e.g. as current carriers, as triggers (hormones), structurally, as catalysts, as vitamins and so on. Each metal ion has its chemical peculiarities and this chemistry has been used almost optimally, not just in sites but in the whole of the biological space and time. To avoid confusion and to safeguard supply biological systems have also developed complex feed-back (homeostatic) mechanisms to look after these elements which are so essential for life.

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