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Chemical selection of elements by cells

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Contents

Abstract

The selection of the chemical elements by a particular cell from the environment involves a series of steps, the complexity of which depends upon the organism. There are usually two membranes to be crossed (bacteria) but there may be as many as ten (higher animals which distribute elements from intake fluid, through cells of organs to circulating fluids through a further set of cells to fixed locations in particular parts of space). The individual steps can be thermodynamically controlled or kinetically managed. In the second case energy can be used. The elements may remain in relatively fast exchange in their final condition or in non-exchanging chemical combination. The variety of paths which individual elements follow in any organism adds to the specific character of the organism. Clearly the paths have evolved to create an element distribution which we shall call *the metallome*, to parallel the nomenclature of protein distribution, the proteome. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Biological element selection; Metal equilibria; Protein complexes; Kinetic insertion; Genetic control; Evolution; Metallome.

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1. Introduction

Selection of interaction between elements and organic partners has been studied for more than one hundred years, both by chemists seeking understanding of analytical separations and biochemists searching for explanations of selective associations in cells. One well known *chemical* selection is that successively developed by Fajans, Sidgwick and Chatt which culminated in the 'a' and 'b' classification scheme sometimes generalised in terms of 'hard' and 'soft' [1,2]. Unfortunately the explanation of selection in aqueous solution cannot be based on simple atomic or ionic parameters and must take into account the solvent whence the use of the 'hard' and 'soft' description fails since relative sizes of partners becomes as important as polarity, polarizability or ligand field effects in different solvents [1,2]. For partners with rigid shapes further new factors are introduced. All of these complications carry over into reactions in cells where however the thermodynamic equilibrium thinking of such chemical approaches has to be taken together with kinetic control of the siting of partners and limitations of ligand production based upon genetic control over synthesis [3,4].

In fact recent detailed study of the selection of the chemical elements by cells has revealed an even greater degree of sophistication than is described in conventional biochemical texts. If we start from the element in the environment then selection begins there since cells are able to eject scavenging chelating agents, R, to give selectively bound metals, MR. Examples are given in Table 1 of MR in environmental solutions as well as in extracellular fluids of multicellular organisms. These complexes are often at or close to thermodynamic equilibrium with free ions. We describe such selection in some detail below. The group MR just diffuses in the outer solutions.

The second step involves either M or MR crossing a biological cell membrane so that M enters the main cell fluid, the cytoplasm. This step can use a channel, Ch, a pump, P, or an exchanger, Ex. Now selection can operate by the binding of M or MR to a receptor on the outer surface of the membrane, which may be part of one of these protein units, when again we can consider that the initial selection is made by the magnitude of the thermodynamic binding constant, and then M or MR can enter the cell by diffusion. Alternatively the receptor can act as part of a pump or an exchanger which can aid movement into the cell using metabolic energy. The activation of the pump or exchanger is usually through the recognition of a specific M or MR due not to its strength of binding alone but to the special stereochemistry of M or MR. The exact geometric configuration at the pump or exchanger binding site then allows migration using energy to force M or MR to cross the membrane highly selectively. On the inside the bound M or MR may be released directly to the cytoplasm or it can be transferred to a carrier, Cr. The element itself or bound to its carrier may diffuse freely in the cell and may equilibrate with other binding sites. Alternatively M or MR on a carrier can be delivered to a site where once again energy may be used for pumping across a new membrane or insertion into a shaped centre to increase the selectivity of incorporation, Fig. 1. We label the final site X. Note that at any stage a change of oxidation state can aid selection.

There are further ways of allowing M or MR to enter a cell. M or MR can be captured in a vesicle of the cell by phagocytosis. M or MR may well be captured initially through a special receptor on the outer membrane of the cell which again

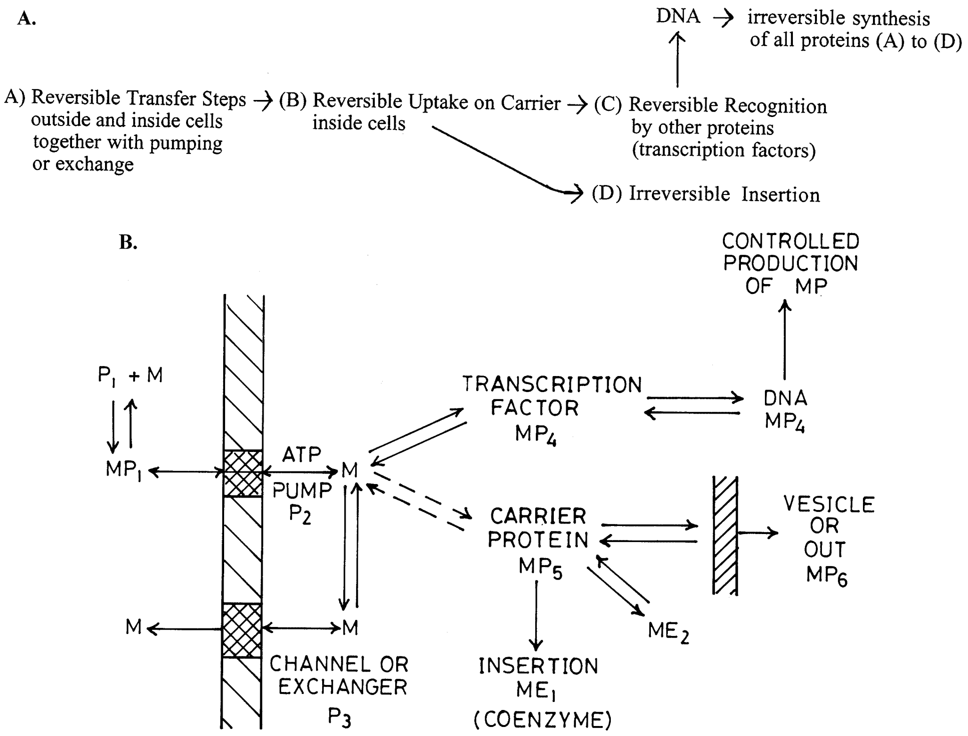

Fig. 1. (A) A general scheme for metal ion uptake [3]. (B) An example of uptake in the case of a metal ion, M [3]. P is a protein and E an enzyme. Certain steps are in equilibrium while others are irreversible, see the text.

Table 1
Selective reagents outside cells [a]

Metal ion	Reagent
Fe^{3+}	Siderophores using hydroxamate or phenolate donors
Fe^{3+}	Transferrin using phenolate donors
Cu^{2+}	Albumin using ionised N-donors
Zn^{2+}	Albumin using N-donors in tetrahedral array
Ni^{2+}	Histidine in plant sap
Ca^{2+}	Phosphoproteins generating 7-coordinate O-donors
Co^{3+}	Vitamin B_{12}
Fe	Porphyrins

[a] Details of all these complexes are given in Refs. [3,12,13].

recognises the stereochemistry of M or MR in a bound condition before phagocytosis. Energy is necessary for phagocytosis. In the vesicle M or MR can be transformed before M(MR) is released into the cytoplasm.

Reagents R, pumps P, channels Ch, exchangers Ex, carriers Cr, and final sites X are all products of cellular metabolism ultimately under the control of the genetic structure. Now for the process to operate with a high degree of selectivity, so that each metal M_1 has a special ligand site, R, and/or in P, Ch, Cr, Ex or X, the organic moieties associated with the site must be selectively produced under the feedback management of the amount of M_1 entering the cell. If this were not the case and considerable excess of a given site were produced then it could well become a binding site for another metal M_2. Thus M_1 and M_2 would not be selectively associated with a particular site. It follows that genes must be regulated selectively by M or by a combination of M and ligand, R, to control production of the sites. Once again the syntheses of the component organic parts of this uptake mechanism associated with phagocytosis must be under genetic control to gain the highest selectivity, and oxidation state changes may be utilised.

Now while certain stages of the selection process can be described by thermodynamic equilibrium, those steps involving energy for selective transport or transfer in a defined direction which can isolate the metal from all others, cannot be treated by thermodynamics. Again if the metal is handed to a specific carrier then it is important for selectivity that the carrier does not exchange, M, when equilibration in solution is excluded. There is a drawback to the last procedure in that free M is no longer recognisable by the genetic control system. Only M bound is so recognised. Examples illustrate the different uptake selections. (For a full treatment of all these steps see Refs. [3,4,13] and references cited therein).

2. Equilibrium exchange selection

A binding constant in a biological system

$$M + L \rightleftarrows ML$$

(or for a more complicated equation using more than one ligand of the same kind, several different ligands or several identical metals) as stated above is dependent on the characteristics of M and L. The characteristics include radius, charge, electron affinity and electron donor power of the metal and the donor groups as well as its stereochemical preferences as matched by the stereochemical constraints of the ligand. The only special feature here is the peculiarity of cellular synthesis of binding reagents. A full appreciation of all these factors has been given in a variety of papers [2] and textbooks [5]. Here we wish to stress the metals and ligands to which such selectivity steps apply in organisms and the linkage to the production of ligands, L, through genes.

The major metals which equilibrate between bound and free states certainly include K^+, Na^+, Mg^{2+} and Ca^{2+}. To a degree, similar considerations apply to Mn^{2+} and Fe^{2+}. However they are only applicable to such metals as Co, Ni, Cu,

Table 2
Ring chelate selection [a]

Element	Chelate
K^+/Na^+	Cyclic peptides
Mg^{2+}	Chlorophyll Enolase [a] (cf. EDTA)
Ca^{2+}	EF-hand proteins [a] (cf. EGTA) Phospholipases [a]

[a] Horse-shoe chelation as well as strictly ring chelation is included. Note also haem Fe, vitamin B_{12} (Co) and F-430 (Ni). For details see Refs. [3,12,13].

Zn and Mo to a much lesser extent. It is immediately obvious that it is just to those most weakly bound metals, which as it happens are to be found both in the environment and organisms in the largest amounts, that equilibria apply generally. However, even here the competition for binding sites is biased in several ways. First there are gradients of all the free ions Na^+, K^+, Mg^{2+} and Ca^{2+} across membranes of all cells due to energy applied selectively at pumps. Na^+ and Ca^{2+} are pumped out of cells, K^+ and Mg^{2+} are pumped into cells. The best selective agents in pumps or in solution here are generally formed from weak donor ligands often increased in selectivity by their incorporation in ring chelates. The general idea that any ion could be preferentially retained by a ring chelate as discussed in 1953 [6] was shown to be true by synthetic work some years later [7]. We find such thermodynamic selectivity for these elements also in enzymes and in DNA, Table 2. Typical examples of the association of weak donors with metal ions are Mg^{2+} often with phosphate donors and Ca^{2+} often with carboxylate and even weaker O-donors. One form of selection between these two ions is by mobile linear ligands when binding strength is based on for example their inability to collapse tightly around smaller ions due to steric restraints, Table 2, hence favouring calcium. This is put to very great value in the biological chemistry of a range of Ca^{2+} trigger proteins and pumps in cells, including calmodulins, troponins, annexins and S-100 proteins in cells, ATP-ase pumps in membranes and also outside cells in blood-clotting and bone proteins. In all these examples the selection of one ion over another is very well described by thermodynamic equilibrium binding constants taking into account relative concentrations in particular compartments. The interactions involved in all cases are almost purely electrostatic. The inability of ions such as Ni^{2+} and Cu^{2+} to bind to these sites despite their greater combining ability is due to their relatively low concentration as free ions since they are preferentially removed by stronger binding N- or S-donors from proteins. The proteins in all cases are produced in limited amounts by feedback control, itself dependent upon a permitted level of a given element in a cell.

There are two cases in which the need to refer to kinetically controlled transfer is important even for Mg^{2+} and Ca^{2+}. The first concerns the rate of distribution of calcium ions in cells [8]. The calcium concentration in large eukaryote cells is 10^{-8} M and triggering requires free calcium entry into a cell up to say 10^{-6} M when calcium binds several proteins. Unfortunately at these concentrations Ca^{2+} diffuses quantitatively quite slowly. Now there is a calcium binding protein, calbindin, which binds calcium but exchanges it rather slowly and it is in high concentration in many cells, 10^{-3} M, and binds Ca^{2+} strongly. Although its molecular weight exceeds 10 000 it is readily shown that the bound calcium at 10^{-3} M will distribute free calcium by carrier diffusion faster than the 10^{-6} M free Ca^{2+}. The secret lies in the relatively slow release of Ca^{2+} from this protein. Here kinetics controls distribution and biological response. This type of controlled distribution has been described recently under the term 'chaperone' and is particularly related to copper distribution [9,10]. Unfortunately this is a misuse of the 'chaperone' nomenclature related to protein folding.

Another strange situation is the binding of Mg^{2+} in chlorophyll and the further binding of this combination to proteins in membranes. The Mg^{2+} ion is finally trapped in a five-coordinate site where every ligand is an N-donor, whereas Mg^{2+} is invariably six coordinate (octahedral) in chemical combinations, and it is usually bound to oxygen-donor centres only. The Mg^{2+} ion is known to be inserted into the chlorin by employing a chelatase enzyme. Chelatases are also used to insert differentially Fe in porphyrins, Co in cobalamins and Ni into F-430 ring chelates. Now in every case the insertion is of a divalent ion and if thermodynamic reasoning had applied to them all the rings would have contained Ni rather than Co, rather than Fe, rather than Mg. In the presence of copper all sites would have been filled by copper. A mechanism for insertion is required which selects each ion by binding constant or stereochemistry. Taking Mg^{2+} first we have to suppose that due to differences in the free ion levels this ion alone is bound in cells to weak oxygen donor ligands such as ATP. The binding constants for ATP vary only slightly from metal ion to metal ion between 10^4 and 10^6 M^{-1}, Fig. 2. Since the approximate free metal ion concentrations vary from 10^{-3} M (Mg^{2+}) and from 10^{-7} M Mn^{2+} to 10^{-15} M (Cu^{2+}), see Table 3, only Mg^{2+} binds ATP. It is thus possible for the chelatase to accept Mg·ATP (or a comparable Mg complex) and using energy to insert Mg^{2+} into chlorin. Chlorin itself is hydrophobic and goes into membrane proteins where it is fixed in position in part by Mg^{2+} coordination to histidine in an unusual five-coordinate complex.

The assembly of Mg^{2+} chlorin, chlorophyll, illustrates a further feature of biological selection. Very little excess free chlorin or chlorophyll is found in plant cells. One possibility is that free chlorin stops chlorin synthesis and maybe it also increases the production of chlorophyll-binding proteins. The general feature is that feedback of one kind or another prevents excess unwanted ions or small or large molecules (proteins) in cells. As stressed before this control over synthesis greatly increases selectivity. Again even in solutions of high Mg^{2+} concentration such as the sea, organisms stop taking in Mg^{2+} by feedback control over entry once the cellular $[Mg^{2+}]$ reaches 10^{-3} M.

Here we see that Mg^{2+} has two independent selection routes. One is based on pumping Mg^{2+} ions into cells until a level of about 10^{-3} M is reached when feedback from this level stops pumping. The binding sites all equilibrate with free magnesium ions. The reverse pumping is true for calcium which is pumped out of cells until 10^{-8} M is reached in the cytoplasm when a protein which activates the pump, calcium–calmodulin, loses its calcium and the pump no longer functions. The calcium then reaches equilibrium in each compartment but in this case triggering of input channels allows a vast range of novel activity until relaxation to equilibrium. In the second Mg^{2+} selection device into chlorin, Mg^{2+} undergoes enforced incorporation in a non-exchanging site, a kinetic route.

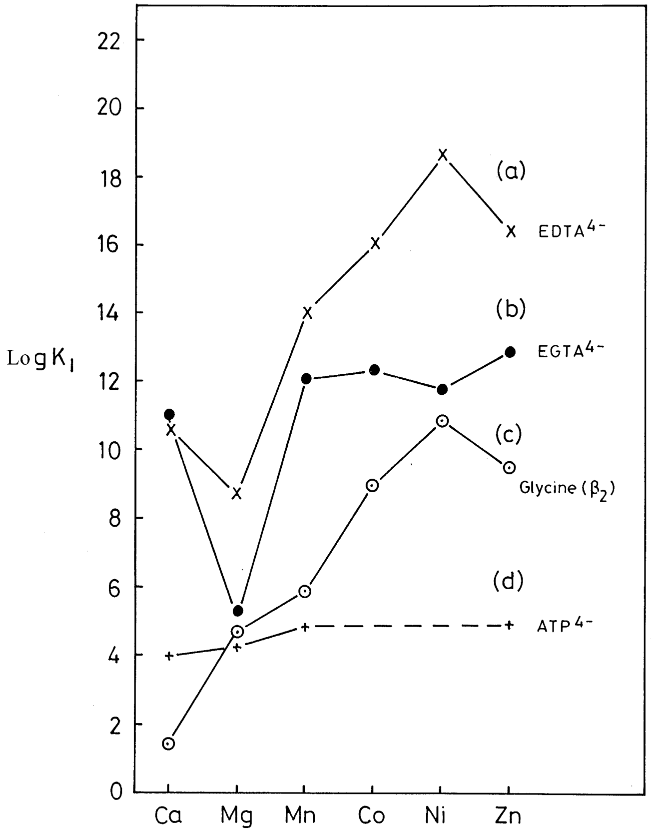

Fig. 2. The binding constants for O-donor ligands showing the weak selectivity which in a competitive system of ligands and metal ions of controlled concentration allows Mg^{2+} binding only to ATP [3].

Table 3
'Equilibrium' concentrations of free metal ions in the cytoplasm of cells, the metallome [a]

	Na^+	K^+	Mg^{2+}	Ca^{2+}	Mn^{2+}	Fe^{2+}	Co^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}
−log[M]	2	1	3	8	7	7	?	?	15	11

[a] The order Mn^{2+} through to Zn^{2+} is likely to be close to that of the Irving–Williams stability series of log K for complex ion formation. The values for the free metal ion concentrations are to be found in the different chapters of Ref. [13].

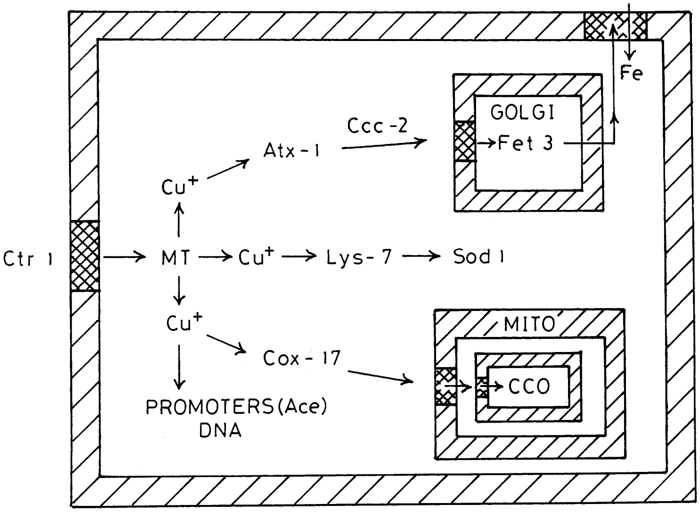

Fig. 3. The scheme for copper uptake [10]: Ctr, a copper transport protein; MT, metallothionein; Atx-1, Lys-7 and Cox-17, copper carrier proteins; Ace, a transcription protein; Sod, superoxide dismutase; Fet, iron transport protein; Ccc-2, copper pump.

Leaving on one side the equilibrium binding of Na^+, K^+ Mg^{2+} and Ca^{2+}, where differential equilibrium is achieved on both sides of all membranes with energised gradients, we turn to the case of copper, an example of a very different kind of selection.

3. Non-equilibrium selection: copper [9,10]

Copper is taken into higher organisms in the liver and is then distributed further in the extracellular fluids by copper carriers, albumin and histidine maybe closely equilibrated with free ions. It enters cells by uncertain means. Inside cells it is reduced to copper(I) and is carried in linear $-S^--Cu^+-S^--$ complexes by *transport proteins or pumps* to final destinations. The final destinations, Fig. 3, are probably irreversible unless the protein is destroyed since incorporation of copper is in a final step of strong folding of the protein. However the copper pumps and the carriers can be aided in their distribution of copper by direct exchange with metallothionein. There need be no equilibration with free copper ions but the exchanging proteins — carriers, pumps and metallothioneins as well as the transcription factors which control the production of these proteins — all have similar binding constants of 10^{15} M^{-1}. The selective uptake of copper is then due to the following.

1. The peculiar oxidation state of copper as Cu^+ which has very considerable binding to thiolates and an unusual charge to size ratio making it recognisable.

2. The peculiar coordination geometry of Cu^+, two linear bonds or trigonal bonds, allows additional steric control over binding, referred to as constrained binding.
3. Feedback control by Cu^+ proteins limits the production of copper-binding proteins generally so that they are not available to other cations. Note that the feedback by transcription factors must have the same selectivity and binding constants as the pumps and the carriers.
4. The distribution of copper(I) by carriers and pumps and not by simple diffusion.

Notice that in this description of the handling of copper we do not need to refer to the free copper ion concentration although it is useful to have the notional equilibrium level of 10^{-15} M free-copper in mind since it helps to see the degree to which copper is removed from competing freely for ligands other than those to which it is transferred by carriers. The mechanisms of handling copper are probably similar in those for many nickel and zinc systems.

4. Iron selection [3]

We put on one side the iron of haem. Iron in other proteins is selected in two very different centres: the Fe_nS_n, iron/sulfur, proteins and the Fe (O,N) centres of such proteins as the oxidases and hydroxylases. In both cases there are known examples of equilibration between the bound and free iron with a binding constant of around 10^7 M^{-1}. The question arises as to how such centres can avoid competition from Zn^{2+} ions especially. Zn^{2+} binds to almost all known ligands more strongly than Fe^{2+} and while Cu^{2+} (or Cu^+) ions are even stronger competitors we have just shown that free copper ions are virtually absent from cells. Let us consider thermodynamic competition between Zn^{2+} and Fe^{2+} in cells. Provided that the excesses of metal and ligand are kept to a very small concentration then it is possible to bind each metal and ligand in two separate freely exchanging partnerships FeL_1 and ZnL_2. The over-riding criterion is that Zn should bind L_2 much more strongly than L_1 and that it should bind to L_2 much more strongly than Fe^{2+} but only somewhat more strongly to L_1. It is easy to see that for the following binding constants Zn and Fe will be separated as ZnL_2 and FeL_1 under certain conditions

	L_1	L_2
Zn log K	8	11
Fe log K	7	7

The required condition is that little excess of L_2 over Zn concentration is allowed so that both free L_2 and free Zn are controlled when no L_2 can bind Fe. At the same time no Zn can bind L_1. Fe concentration can then be 10^{-7} M and bind.

These conditions imply that two proteins other than L_1 and L_2 exist which act as transcription factors binding to DNA and to zinc and iron, equally differentially, and which control the production of L_1 and L_2 to match the Fe and Zn free concentrations. We know these proteins to be FUR and FNR (an N/O binding and Fe_nS_n binding protein) for iron and zinc fingers and metallothionein (an N/S binding and a zinc cluster RS^- binding protein) for zinc [3,4]. Similar sites are found in the cytoplasm of iron and zinc binding proteins and enzymes respectively. The trick of this thermodynamic control lies in the use of *conditional* binding constants with genetic restrictions on synthesis of ligands, not absolute binding constants which almost invariably give the order of divalent cation binding

$$\mathrm{Mg} < \mathrm{Mn} < \mathrm{Fe} < \mathrm{Co} < \mathrm{Ni} < \mathrm{Cu} > \mathrm{Zn}$$

By keeping metal ions such as free zinc as low as 10^{-11} M it is possible to protect weak binding sites exclusively for weaker binding metals while giving the stronger binding metals better donor centres. This is exactly parallel to the use of very weak binding ligands for Mg^{2+} while excluding all the other metals listed above to less than 10^{-6} M. The exclusion is managed by utilising selective binding in the cytoplasm as illustrated above and then binding and removal by pumps of any excess. So we observe separated much manganese, cobalt, nickel, zinc and copper in special vesicles and exported and much iron trapped in ferritin. Of course this implies that the buffer and pump proteins for the metal ions are regulated in production by the same kind of transcription factors that control the production of active proteins in the cytoplasm and the external proteins have parallel selectivities. We know this to be true for iron. Here production of external scavengers, transferrin in higher organisms and siderophores in bacteria and iron receptors in membranes as well as storage proteins are all again under the control of free Fe^{2+} through Fe_nS_n (higher and lower cells) and Fe N/O (bacterial cells) transcription factors. Again we note that because pumps have dual selectivity based on binding constants and stereochemistry enhanced by energised transfer by a factor of say $> 10^5$ it is possible to remove a given metal into a vesicle and by pumping in a special protein produce high selectivity of combination locally. Note especially zinc, copper, manganese and calcium go to selected vesicles and combine there in very usual ways free from competition.

We turn for example to the selective formation of Fe_4S_4 units in the cytoplasm of cells [11]. There is no doubt that the formation of the core unit of Fe_3S_4 in solution is specific for iron due to the binding of sulfide, S^{2-}, by Fe^{3+} ions. The very fine model chemistry of Holm [12] has established this. There are no other M^{3+} ions available in cells. However the final step in a protein

$$Fe_3S_4 + Fe^{2+} \rightleftarrows Fe_4S_4$$

is again open to competition from other M^{2+} ions, e.g. Zn^{2+}, see Fig. 4. That Fe^{2+} and not Zn^{2+} binds to the core follows from the removal of Zn^{2+} by stronger and more selective N, S-donor ligands, the removal of excess Zn^{2+} by pumps of high affinity and the limited formation of the Zn^{2+} binding proteins so that they cannot sequester Fe^{2+}. It is interesting to note in this context that the Fe/S centres just like haem centres are under synthesis control in mitochondria.

In summary the equilibrium binding of all ions largely controls selectivity in the external fluids and in the cytoplasm based upon conditional binding constants. Notional free ion levels are shown in Table 3. These levels are maintained by selective pumps and selective synthesis. Selective removal of a given ion and selective transfer of a given protein can then lead to special associations of elements in specific vesicles. We find different vesicles with high free Ca^{2+}, high free Zn^{2+}, high free Mn^{2+} and high Cu^{+} proteins, while iron and magnesium are rarely exported.

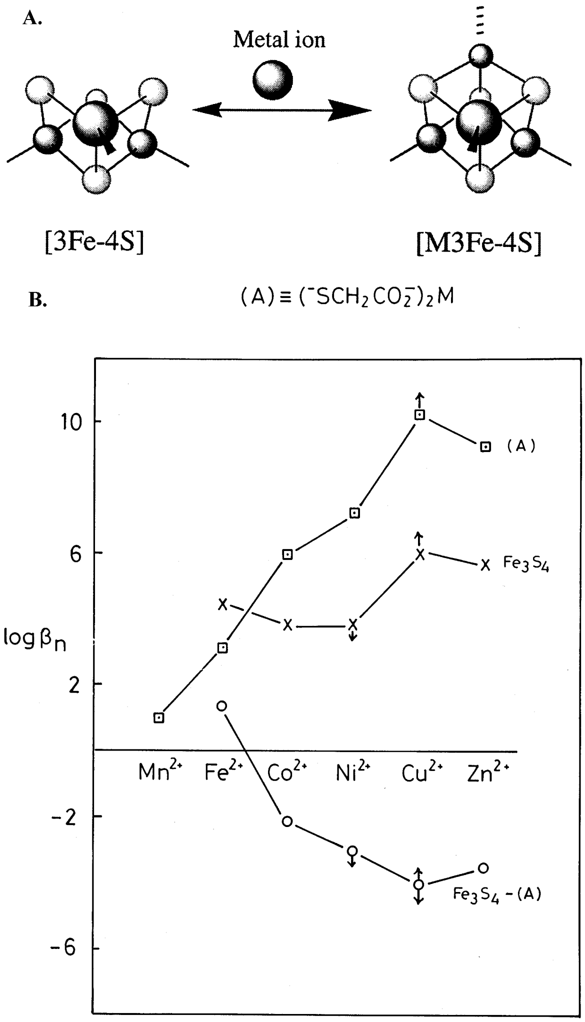

Fig. 4. The competition for Fe_3S_4 sites. (A) The reaction scheme X. (B) The binding constants for the reaction (X), for reaction with bis-thioglycolate □, and the difference (○) showing how Fe^{2+} is preferred in X [3,11].

5. Haem iron selection

Haem iron is prepared by insertion of Fe^{2+} into porphyrin in mitochondria and, as noted before, other metals are inserted in different ligands, e.g. Mg, Co, Ni. The chelate rings are not made in excess of supply of metal ions. The exact level of feedback control is probably at a step in porphyrin synthesis.

6. Zinc, cobalt and manganese in the cytoplasm

There is a mysterious feature of metabolism of peptides and proteins. In extracellular fluids there are numerous zinc peptidases and proteases and no cobalt, manganese or nickel enzymes of this kind. This choice is easily justified on inorganic chemical grounds of effective Lewis acid and exchange properties. Zinc is the best catalyst with no redox properties. In the cytoplasm very few if any hydrolyses are carried out using zinc whereas there are Mn, Co and Fe (no nickel) hydrolases including peptidases. Why? The temptation is to look at evolution since much of cytoplasmic chemistry has had to remain unaltered since cellular life, as we know it, evolved. Under the conditions of early life, anaerobic and high in H_2S, the two most powerful Lewis acids, Cu^{2+} and Zn^{2+}, were virtually unavailable since they have very insoluble sulfides. Thus Lewis acid catalysts had to be chosen from Mg^{2+}, Mn^{2+}, Fe^{2+} and Co^{2+}. We have described the use of Mg^{2+} with phosphate hydrolyses. The choice for stronger catalysis was reduced to that between the other three metal ions. Thus in the final analysis much selective binding of metal ions is based on possibilities which existed billions of years ago since cytoplasmic chemistry once fixed could adjust only very slightly.

7. Conclusion

In the final analysis the selection of elements is opposite function. We must then see how function evolved and what made it possible. This implies that the biological inorganic chemist has to familiarize himself with the subtle nature of cell metabolism, its controls, its relationship with genes, and its origins. However, all such selective organisation has a chemical basis and there are limitations to the availability of elements and to the ways in which metal ions can be allowed to be present as free ions if competition is to be avoided. The cell not only has a characteristic genome in the nucleus but a protein content, a proteome, and a free element content, a metallome, in every one of its compartments. The metallome of the cytoplasm throughout evolution has not changed greatly and follows closely Table 3. The metallome must be recognised as a fundamental feature of a cellular compartment which is linked but not quantitatively to the proteome and the genome since it is related also to environmental availabilities and to energy supply.

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