

Accounts

Demonstration of the Importance of Metal Ion Speciation in Bioactive Systems

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This paper gives insight into chemical speciation. After a brief introduction, the methods available to study solution state of metal ions and the importance of speciation in biology are discussed through different examples. Speciation of toxic Al^{III}, beneficial Cu^{II} and Ca^{II} and potentially medicinal Pd^{II} and VO^{IV} in various biofluids and tissues is discussed in order to describe the solution state of these metal ions in an organism. The relevance of ternary complex formation with endogenous biomolecules is emphasised. The importance of the biospeciation of Al^{III} ion in its transport and involvement in neurological disorders, of the actual chemical form of the insulin-mimetic VO^{IV} complexes in its biological occurrence and Ca^{II} in mineralization/demineralization processes is discussed. The role of time, i.e., the kinetic aspects of biospeciation, is discussed in the case of the transport of Al^{III} and Cu^{II} and some Pd^{II} complexes (models of the anticancer Pt^{II} complexes) in blood serum.

Introduction

The chemical speciation of an element, either essential or toxic, is relevant, because the chemical form, in which an element enters the body, mostly determines its absorption and transport properties, and hence its biological and physiological activities. It is also true that, to some extent, independent of how this essential or toxic element is absorbed (or the form, e.g., a metal ion-containing drug, in which it was administered), it undergoes transformation, because of the possible ligand-exchange reactions with the many potential metal ion-binding biomolecules in the biofluids or tissues.

Definition of Chemical Speciation

The most concise definition of chemical speciation is as follows: composition, concentration, and oxidation state of each of the chemical forms of an element present in a sample. Using chromium as an example, it is not enough to know the chromium content in a biological sample. Its oxidation state is also very important. In the oxidation state of +III, it may be essential for organisms. Cr^{III} is assumed to be involved in glucose metabolism and be present in the so-called glucose tolerance factor (GTF), which, by interacting with the membrane receptor for insulin, optimizes the effect of insulin.[1] However, it has also been found that, in microorganisms, such as yeast cells, GTF does not necessarily contain chromium which, in any case, is an ubiquitous element and hard to specify exactly in ultra-trace quantities in biological samples. Cr^{III} exists mostly as an insoluble hydroxide at neutral pH; its bioavailability is rather limited and can be absorbed by organisms only in complexed forms. At the same time, Cr^{VI} is known to be a mutagenic and even carcinogenic species, and can be absorbed more easily due to its structural similarity with the sulfate ion. It can overcome membrane barriers and reach the cell nucleus, unless rapidly reduced. In the cell nucleus, chromate can oxidatively damage genetically important components.

Substitutionally more labile and stronger oxidizing Cr^{V} or Cr^{IV} intermediates are formed during one-electron reduction steps. The simultaneously produced RS* and *OH radicals can directly attack DNA and cause bond cleavage, cross-linking and, as a consequence, faulty gene expression. Furthermore, the resulting substitutionally inert Cr^{III} can irreversibly bind to phosphate-containing DNA or free nucleotides and thus also affect genetic functions.[2] Due to the ligand-field stabilization for a high-spin d^3 configuration in octahedral symmetry, chromium(III) complexes are generally distinguished by an enormous kinetic stability.

Because of this extreme inertness of chromium(III), the wide-spread use of its $Cr(picolinate)_3$ complex as an essential trace element carrier in various nutrient compositions sold in drugstores mostly is the U.S.A., is rather dubious. Besides, based on the assumed role of Cr^{III} in GTF (see above), this compound has been recommended as an efficient drug in the treatment of diabetes.[3] On the other hand, the time that $Cr(picolinate)_3$ spends in the body is hardly enough to undergo any transformation. In a recent work, the use of Cr^{III} as a nutritional supplement has strongly been questioned.[4] The contradictory status of the essentiality/toxicity of chromium is a

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good example of the problems connected with the bioavailability of the various chemical forms of an element and the uncertainties in the literature results related this question.

Thermodynamic aspects of speciation involve the determination of the stoichiometries (compositions) and stability constants of all species formed with endogenous or exogenous metal ion binder molecules of biological fluids or tissues. In this way, the actual chemical form of an element, how it occurs in a given chemical, ecological, or biological system must be defined. This is usually the way that the term speciation is defined and used in the literature. However, we must not forget that kinetics, which is the time course for the formation of the chemical entities. For example, the ligand-exchange rate of the complexes of various biometals covers almost 16 orders of magnitude; furthermore, the readiness of various potential binding sites to bond a metal ion may differ quite considerably. The rate of binding to low molecular mass (l.m.m.) components is always higher than to large proteins or other biopolymers. This means that saturation or depletion of these various binding sites occurs consecutively i.e., the l.m.m. will bind the metal ion first and also will lose or pass the metal ion to another binder, whereas the same processes at the high molecular mass (h.m.m.) binding sites take place much slower.

In the case of relatively sluggish metal ions, such as Al^{III}, the speciation may change during the time it stays in an organism, because the amount of time may not be enough to reach thermodynamic equilibrium.[5] Moreover, we must keep in mind that living systems are, due to the continuous metabolic links with their environment, in a steady-state equilibrium and never reach real thermodynamic equilibrium.

Methods Used to Describe Speciation in Biological Systems

In biological fluids, a majority of the metal ions are bound to proteins. In fact, only a small proportion of them are bound to l.m.m. compounds, such as amino acids, biophosphates, carboxylates, or hydroxycarboxylates. Free transition metal aqua ions exist in biological fluids only in extremely low concentrations, which cannot play a significant role in physiological processes. In contrast, metals bound to l.m.m. compounds play major roles in many biological processes, such as intestinal absorption, cell absorption, transport, and renal excretion. An understanding of these processes requires an accurate knowledge of the proportions of the l.m.m. and protein-bound fractions of the metal ions and of the delicate equilibria, which determine these proportions.

Specific experimental techniques, such as gel filtration,[6] ion chromatography,[7–9] ultrafiltration,[10,11] ultracentrifugation,[12] atomic absorption spectrophotometry,[12–14] gas chromatography-mass spectrometry, liquid-chromatography-mass spectrometry,[15–18] inductively coupled plasma-mass spectrometry, etc., are available[19–22] to separate and determine l.m.m. and h.m.m. bound metals. Fractionation is a very important aspect of the speciation of trace elements in biological samples. This usually involves coupling an analytical separation technique directly to an element-sensitive detection system. However, it is difficult to apply any of the classical structure elucidation techniques, because of the small quantities, usually microgram, of the analyte. Identification is possible only when the fractions can be tested for species thought to be present in the samples and authentic standards are available for comparison.[20] On the other hand, computer programs developed to deal with multiple chemical equilibria can be actively applied not only for speciation calculations of laboratory solutions, but also for the simulation of naturally occurring mixtures of metal ions and ligands in biological samples.[19]

From the trace element composition point of view, biological fluids are generally heterogeneous media that contain suspended or fragmented cells, proteins, or even crystalline particles. In studies of trace element composition of biological fluids, it is often necessary, in addition to the determination of their total content, to obtain information on the chemical forms, in which trace elements occur. Many trace metals are bound to macromolecules (mostly proteins, but also nucleic acids, sugars, etc.) in biofluids. In addition to this quite strong and inert bound fraction, metal ions are also bound to the l.m.m. components of biofluids, such as amino acids, peptides, biophosphates, hydroxycarboxylic acids, inorganic anions, which are much more mobile. Table 1 gives a few examples of applications of the various methods used to describe the distribution of trace element containing species in biological fluids.[19,20] As seen in Table 1, there is no general technique for the determination of most of the transition-metal ions in biological fluids. Each metal ion, biological fluid, and even fraction to be investigated seems to require a specific technique. When the fraction to be studied is quantitatively too small, analytical methods are sometimes are not sensitive enough to estimate it or may induce artefacts.[5] In such cases, computer simulation (vide infra) represents the only technique to assess the proportion of metal bound to each compound.[23]

For monitoring the distribution of metals amongst the l.m.m. binders, the number of experimental techniques is much more limited due to the lability of these metal-binder species. Most of the separation techniques interfere with these equilibria and give false results. Soon after the first computer programs for dealing with multiple chemical equilibria were developed, they were actively applied by coordination chemists, not only for calculations of laboratory solutions but also for simulating naturally occurring mixtures of metal ions and ligands. Such programs could in principle, and today more and more in the practice, too, determine the chemical speciation in very complicated systems. ECCLES is one of the most widely used simulation programs.[23] Computer programs need a rich and reliable database containing stability data for all metal–ligand interactions, which may occur in biological fluids. The most complete database available, e.g., for blood plasma, includes a set of about 10000 complexes of ca. 10 essential and toxic metal ions, over 1000 ligands, and results for plasma conditions. As an illustration, Table 2 shows the distribution of Cu^{II} in plasma calculated by using ECCLES program.

The weak point of such a model calculation approach is the lack of the necessary data. In such cases, the stability constants for the binary and ternary metal ligand systems have to be previously determined, focusing on the most important and strongest binders, and so, a good estimation can be obtained in an easier and simpler way. In ideal case, the calculation results can be confirmed by certain separation methods or structure elucidation techniques.

Table 1. Selected Examples of the Applications of Various Methods and Techniques Used for the Study of the Distribution of Trace Elements-Containing Species in Biological Fluids (Based on Ref. 19)

Sample matrix	Techniques and methods	Detected species	Comments
Blood serum or plasma	Affinity chromatography, GFAAS, Kinetic immunoturbidimetry	Albumin- and globulin-bound Zn	The methods were used to study the relative changes in the distribution of zinc in serum in health and disease.
	GFAAS, SEC	Cu– and Zn–protein complexes	Low-molecular-mass zinc species identified as associated with the retinol-binding protein complex. Copper is thought to be in the form of a thionein complex.
	Computational approach to chemical speciation	Al and Zn distribution	Useful approach to the study of the distribution of elements, particularly those that form relatively weak metal–ligand complexes which are not amenable to laboratory determination.
	Anion-exchange chromatography, electrophoresis nephelometry	Distribution of ^{51}Cr in plasma	The use of radiotracers circumvents a lot of the problems associated with extraneous chemical contamination.
Red blood cells	SEC, GFAAS, Glutathione (GSH-Px) activity	Se-containing fractions in red blood cell lysates	The presence of Se-containing enzyme glutathione peroxidase was detected by measuring its activity. Unequivocal identification of the other species was not possible.
	Selective uptake of chromates: Cr^{VI} instead of Cr^{III} human erythrocytes	Cr^{IV} dissolved in erythrocytes	Novel approach to speciation; an example of the use of the discriminating ability of cell membrane to provide information on chemical species.

Table 2. Calculated Distribution of Cu^{II} in Human Blood Serum (Based on Data in Ref. 23)

Copper(II)	%	Copper(II)	%
Cu(His)(Gln)	19	Cu(His)$(LysH)^+$	4
$Cu(His)_2$	16	Cu(His)(Gly)	4
Cu(His)(Thr)	15	Cu(His)(Asn)	4
Cu(His)(Ser)	8	Cu(His)(Val)	4
Cu(His)(Ala)	5	Cu(His)(Leu)	4

In the following, an insight is given mostly into our departments' recent researches concerning some biospeciation problems relating to toxic (e.g. aluminium), beneficial (e.g. copper and calcium), and medicinal elements (vanadium and palladium). The examples are taken from very different parts of biology and medicine, and it is very difficult to find any links between them (it was not the goal of this paper). They provide, however, good examples to emphasise the importance of knowing the solution state of these metal ions and also to demonstrate how speciation information may be used for a better understanding of the effects of these metal ions in biological systems. Specific bioinorganic chemistry problems concerning each metal ion are not discussed in details in the paper; however, brief descriptions are given in order to clarify how knowing the speciation may help.

Speciation of Al^{III} in Blood Serum

Aluminium has been established as a neurotoxic element.[24] The normal daily load is tolerated by a healthy organism (although the physiological effects of long-term low aluminium load, e.g. from the drinking water are still not clear[5]) due to an efficient defence mechanism, that involves the formation of poorly soluble phosphates and hydroxides in the gastrointestinal tract, which are easily excreted. However, with an abnormally high Al load and/or impaired renal function, an excess of Al^{III} can be absorbed and transported to various target organs, where it may accumulate and exert harmful effects, e.g., osteomalacia in the bones, microcytic anaemia in the red blood cells, or neurodegenerative diseases in the brain. The biological effects of Al^{III} has been reviewed in numerous books and review papers; here, we refer only to a few recent ones.[25–28]

Absorbed Al^{III} is transported by the blood stream, but its speciation in blood serum is still not exactly known. Different speciation models[29–36] have been reported in the literature. As concerns the h.m.m. components, all models show that most of the Al^{III} is bound by transferrin; albumin, the other potential h.m.m. carrier protein, weakly binds to metal ions and is not assumed to be able to compete for a significant amount of Al^{III}.[5] The picture is much more controversial concerning the l.m.m. Al^{III} binders.[29–36] Daydé et al.,[29] Jackson,[30] and Harris[31] have proposed inorganic phosphate as the main Al^{III} binder, whereas Clevette and Orvig,[32] Duffield et al.,[33] and Öhman and Martin[34] have suggested that citrate (Cit) is the only important l.m.m. binder. In a recent paper, Harris et al.,[36] have revised this opinion and have provided evidence for citrate as the primary binder. Our earlier results[35] have led us to conclude that both the phosphato and citrato complexes of Al^{III}

contribute to the l.m.m. fractions in blood plasma, although about 80% of the Al^{III} binds to transferrin.

The reason for the contradictory results is the lack of reliable speciation data. In the case of Al^{III}–phosphate (B^{3-}), under model conditions, in the millimolar concentration range, precipitation occurs at pH >3.5, and one is forced to extrapolate from these data to physiological conditions, which are far from the model conditions.[37]

Further, there are rather slow oligomerization reactions in the case of Al^{III}–citrate (A^{3-}), which are strongly concentration dependent. As first reported by Öhman and Sjöberg[38] and Öhman,[39] at mM model concentrations, a trinuclear species $[Al_3(CitH_{-1})_3(OH)]^{4-}$ predominates in a wide pH range, including the physiological pH, in a thermodynamic equilibrium state. This trinuclear species is formed, but in a rather slow process, via the interactions of mononuclear 1:1 and 1:2 complexes, which exist at the beginning of complex formation and also predominate in the presence of an excess of the ligand.[40,41] ESI-MS measurements have proven that the half-life of formation of the trinuclear species is ≈80 min at about 10 μM Al^{III} concentration.[36] It is reasonable to wonder whether this equilibrium approach provides a realistic description of the Al^{III} speciation under physiological conditions. When the Al^{III} concentration rarely exceeds a few μM, the ligand excess for potential binders is at least 1000- to 10000-fold, and, in consequence of the continuous metabolism, biological fluids are open systems, which never reach true thermodynamic equilibrium. The problem of time-dependent speciation is a real challenge in Al^{III} bioinorganic chemistry.[42]

Harris et al.,[36] using difference UV spectroscopy as the basic method and supported by pH-metric and ESI-MS measurements, have reported a model for the biospeciation of Al^{III} in serum at ≈10 μM Al^{III} concentration, which is much closer to the biologically relevant value, of ≈0.1–0.3 μM.[43,44] Their speciation model, given in Table 3, suggests that 93% of the total Al^{III} is bound to transferrin. Of the pool of l.m.m. aluminium, 88% of the Al^{III} would be bound to citrate, 8% to hydroxide, and only ≈2% to phosphate.[36]

Speciation models, reported so far, have ignored the formation of ternary complexes between citrate and phosphate. We have recently studied the Al^{III}–citrate (A^{3-})–phosphate (B^{3-}) ternary system and monitored the time-dependent speciation by pH-potentiometry and multinuclear ^1H, ^{13}C, and ^{31}P NMR spectroscopy.[35] The speciation curves obtained by pH-metry at zero-time (the pH values corresponding to the time of mixing of the components, obtained by extrapolation[45]) and in thermodynamic equilibrium are shown in Fig. 1. It can be seen that the ternary species $[AlABH]^{2-}$, $[AlAB]^{3-}$, and $[AlABH_{-1}]^{4-}$ dominate throughout the whole pH range from mixing until real thermodynamic equilibrium is reached. Time affects only the concentration of the trinuclear species, which forms in a rather slow process from the mononuclear species $[AlAH_{-1}]^{-}$ and OH^-. A possible route for this oligomerization has been suggested on the basis of ^1H, ^{13}C, and ^{31}P NMR studies.[40,41] In the physiological pH range, Al^{III} has been found to be bound in ternary species $[AlAB]^{3-}$ and $[AlABH_{-1}]^{4-}$, and binary species with phosphate, $[AlBH_{-1}]^{-}$, and citrate, $[AlAH_{-2}]^{2-}$. The results are rather surprising as the monodentate phosphate is nearly at least as efficient an Al^{III} binder as the tridentate citrate. Multinuclear NMR measurements were used to confirm this result. From Fig. 2, when phosphate was added in 2-fold excess to a solution of the trinuclear species, the phosphate slowly displaced citrate from the oligomeric complex, and in a few days, the characteristic quartet of citrate was observed in the ^1H NMR spectrum. A comparison of the NMR spectrum with the speciation curves calculated data acquired under the experimental conditions of the NMR measurements indicates practically complete agreement between the speciation results obtained by two independent methods.

Table 3. Speciation of Al^{III} at pH ≈7.4 and 25 °C

	% of Al^{III} bound	
	$c_{Al^{III}}$ = 10 μM[36]	$c_{Al^{III}}$ = 1 mM[37]
High Molecular Mass components		
Albumin	—	—
Transferrin	93	77
Low Molecular Mass components		
Phosphate	0.1	14
Citrate	6	5
Citrate-Phosphate	—	4

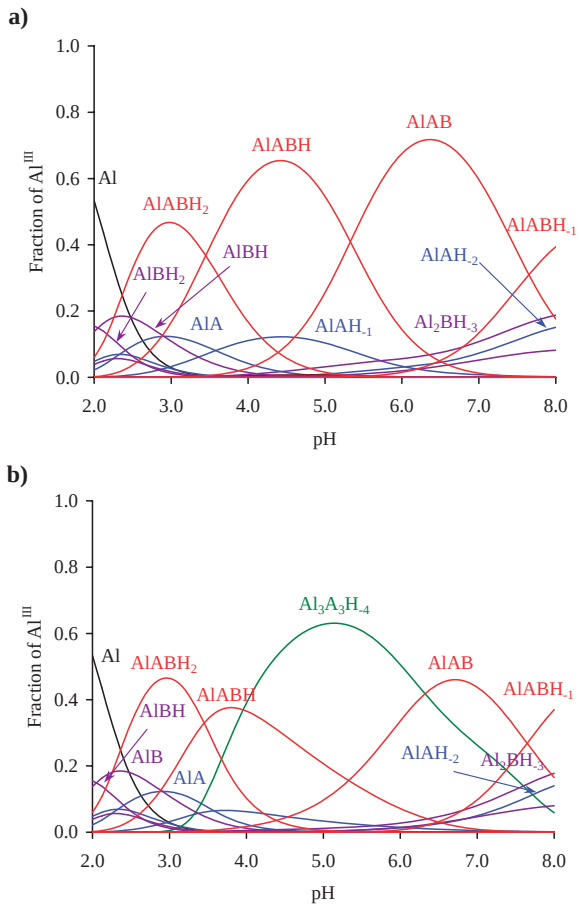

Fig. 1. Time-dependent speciation curves (see text) of Al^{III}–citrate (A^{3-})–phosphate (B^{3-}) system at a 1:1:4 metal ion to ligand ratio $c_{Al^{III}} = 0.04$ mol dm^{-3} and at different times after sample preparation (a) immediately after mixing, (b) at equilibrium (after 30 h) (taken from Ref. 45).

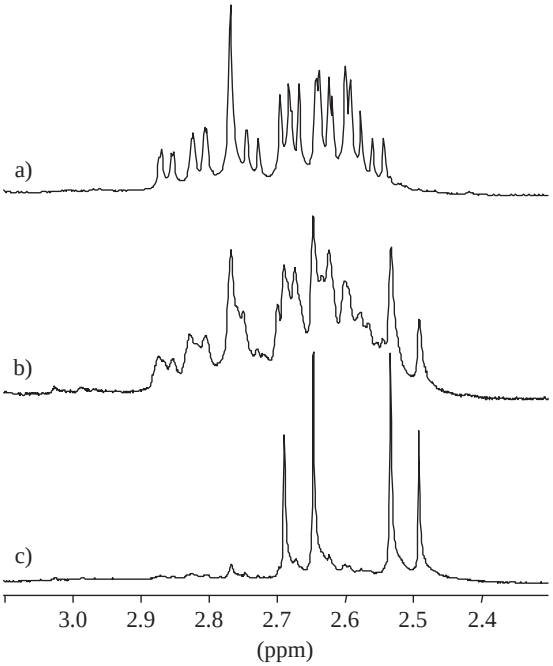

Fig. 2. ^{1}H NMR spectra at pH 7.4 of (a) $[Al_3(CitH_{-1})_3(OH)]^{4-}$, 10 mmol dm^{-3}, (b) 10 h after the addition of phosphate to the sample in 20 mmol dm^{-3} concentration, and (c) 5 days after the addition of the phosphate to the solution (equilibrium state) (taken from Ref. 45).

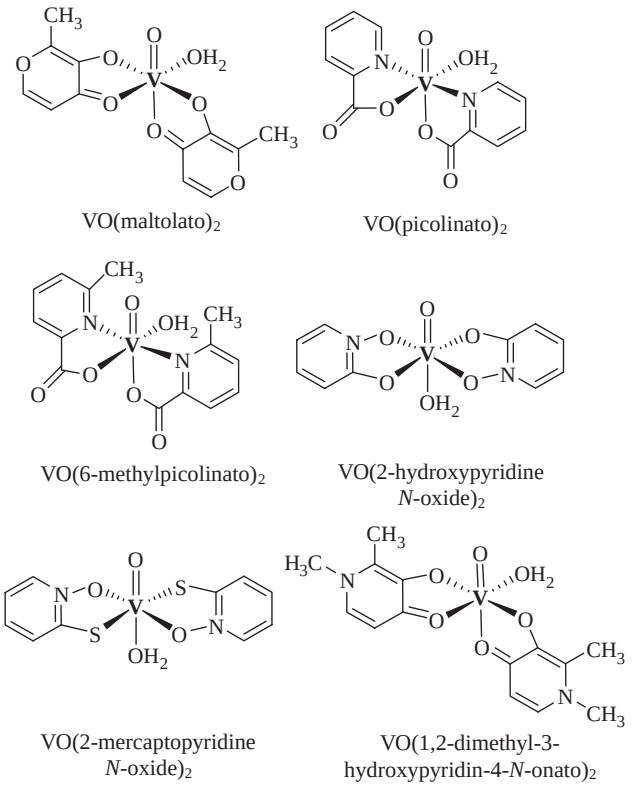

Fig. 3. Several insulin-mimetic VO^{IV} complexes.

Table 3 shows the species distribution of Al^{III} under plasma conditions. At physiological pH, phosphate seems to be a more efficient Al^{III} binder, although citrate-bound Al^{III} also occurs in significant concentration, in accordance with the results of Bell et al.,[46] who detected Al^{III}-bound citrate in native serum by means of ^{1}H NMR. Bantan et al.[44] have studied the speciation of l.m.m. Al^{III} complexes in human serum, which were taken from healthy volunteers, by means of fast liquid chromatography and electrospray techniques. In agreement with our results, they have found that the main l.m.m. Al^{III} species present in the serum is binary Al^{III}–citrate, Al^{III}–phosphate, and ternary Al^{III}–citrate–phosphate complexes. The distribution of these complexes varies from individual to individual.

Biospeciation of the Insulin-Mimetic VO^{IV} Complexes

An interesting feature of VO^{IV} complexes is their insulin-mimetic activity.[47] Since the first report of the insulin mimetic activities of vanadium compounds in vitro[48] and in vivo,[49] great efforts have been made to prepare vanadium(IV) and vanadium(V) complexes of high activity and low toxicity that are readily absorbed.[50–54] Large numbers of complexes with O, N, and S donors have been tested. With regard to the coordination chemistry of VO^{IV}, fairly stable complexes can be expected with O-donor ligands. On the contrary, N or S donor complexes will presumably decompose and liberate most of the ligands when they dissolve after oral administration. Even if they form strong complexes at neutral pH, at the pH of the gastric juice (≈2), they should completely dissociate. Accordingly, other biomolecules, potential metal ion binders present in biological fluids, may partially or completely displace the original ligands from the coordination sphere of the metal.

This led us to start a detailed study on the possibility of ternary complex formation between potential insulin-mimetic drugs, such as VO(maltolate)$_2$, VO(picolinate)$_2$, VO(6-Me-picolinate)$_2$, 2-OH-pyridine *N*-oxide, 2-SH-pyridine *N*-oxide, and 3-OH-1,2-diMe-4-pyridone (Fig. 3) and various blood serum containing l.m.m. bioligands, like inorganic phosphate, lactate, oxalate, citrate and h.m.m. bioligands such as transferrin and albumin.[50,55–59] The results of model calculations are shown in Fig. 4 and indicate the following: (i) Only the pyridone derivative is a strong enough carrier to preserve a significant part of VO^{IV} in the original complex; in other cases, the carrier ligands are displaced by serum components. Accordingly, the most important role of the carrier ligand seems to be to facilitate absorption of VO^{IV}. At the same time, it was also found that the pyridone complexes of VO^{IV} were the least biologically active among these complexes, which suggest that there seems to be no direct relationship between the stability of the VO^{IV} complexes and the biological activity. This needs further investigation. (ii) Of the two important h.m.m. binders, transferrin is much more efficient than albumin and will displace 30–70% of the original carrier from the complex (At the biologically more relevant VO^{IV} concentrations (<5 mM) practically all VO^{IV} is bound to transferrin.). Our very recent spectroscopic results suggest that the carrier ligand may be involved in VO^{IV} transport through ternary complex formation with the protein, and thus, it may also reach the cell.[58] (iii) Among the l.m.m. binders, citrate is the only "active" component, able to influence the solution state of these insulin mimetics. At physiological pH, VO^{IV} exists mostly as VO^{IV}–citrate binary complex and as VO^{IV}–ligand A–citrate mixed ligand complex, but in different proportions.

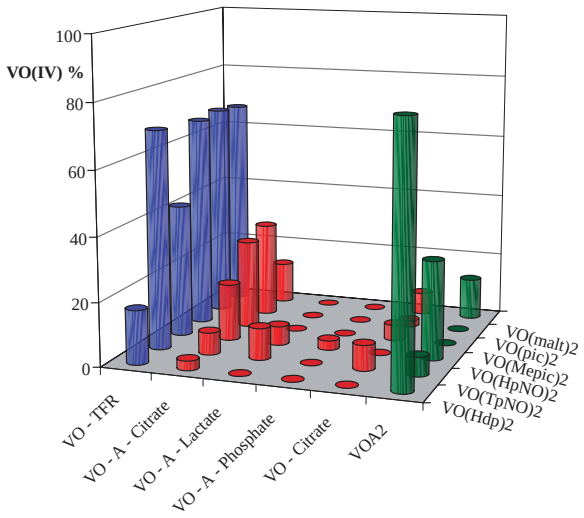

Fig. 4. Speciation of various insulin-mimetic VO^{IV} compounds (100 μM) in serum at pH 7.4 (malt: maltolate, pic: picolinate, Mepic: 6-Me-picolinate, HpNo: 2-HO-pyridine *N*-oxide, TpNo: 2-HS-pyridine *N*-oxide, Hdp:3-HO-1,2-diMe-4-pyridone) (based on data reported in Refs. 55–59).

The results obtained by the above speciation calculation were confirmed by in vivo pharmacodynamical measurements, in which the fate of the metal ion and the carrier ligand was monitored in the case of the $VO(maltolate)_2$. However, no direct relationship was found between the concentration profile of the two components, indicating that shortly after absorption the two components separate during their transport in the body.[60]

Speciation of Ca^{II} in Saliva

The next example is the speciation of Ca^{II} in saliva, which may give some quantitative details about the demineralization–remineralization equilibrium of tooth enamel.[61] Saliva is a rather complex biofluid. It contains anions, cations, nonelectrolytes, amino acids, proteins, carbohydrates, and lipids. The pH of saliva in the absence of the influence of dietary acids etc. varies throughout the day between 5.5 and 7.8, with an average of 6.75.

The major inorganic component of tooth enamel is very similar (or maybe identical) to hydroxyapatite (HAP; $Ca_5(PO_4)_3(OH)$). In practice, however, several different types of calcium phosphates may precipitate in the mouth, including tricalcium phosphate (TCP; $Ca_3(PO_4)_2$), tetracalcium phosphate (TECP; $Ca_4H(PO_4)_3$), and calcium phosphate (CP; $CaHPO_4$). Whenever fluoride is present, the demineralization–remineralization equilibrium is strongly affected by the formation equilibrium of fluoroapatite (FAP; $Ca_5(PO_4)_3F$), which has a much lower solubility and is thus preferentially formed in the remineralization process. Saliva continuously provides calcium and phosphate to surfaces of the teeth at concentrations that not only inhibit the demineralization caused by acids in dental plaque but also can facilitate some remineralization, or at least halt the early stages of carious lesion formation (enamel demineralization). Table 4 shows the major calcium species present in saliva.[62,63] Since the thermodynamically most stable species, HAP and FAP, form rather slowly and by transformation of more readily precipitated precursors, such as TCP, CP, etc, it is convenient to refer to the model that includes HAP and FAP as the long-term model, and the model that omits HAP and FAP as the short term model. The long-term model may be considered to refer to an equilibrium state of the system, whereas in the short-term model, kinetic factors inhibiting the direct formation of HAP and FAP predominate. Computer modelling calculations strongly suggest that CP and TCP are the most likely precursors of HAP.

Table 4. Predicted Ca^{II}–Phosphate Species at Varying pH in Saliva Predicted by Model Calculation[61–63] (See Text)

pH	Long-time model[a)]	Short-time model[a)]
5.00	FAP	Ca^{2+} and $Ca(citrate)^-$
6.00	FAP and HAP	Ca^{2+} and $CaHCO_3^-$
6.75	FAP and HAP	TCP and CP
7.00	FAP and HAP	TCP and CP

a) HAP: $Ca_5(PO_4)_3(OH)$, FAP: $Ca_5(PO_4)_3F$, TCP: $Ca_3(PO_4)_2$, CP: $CaHPO_4$.

Interactions of Pd^{II} Complexes with Nucleotides and Thioether Ligands

Numerous Pt^{II} complexes are widely used anticancer agents. It is now accepted that their antitumour activity is related to the platination of DNA most commonly via the interaction of guanine bases. However, it is also clear from the mechanistic studies that platinum–sulfur interaction may also play a significant role both in the transport and in the nephrotoxicity of Pt^{II}-containing agents. From previous studies on thioether and nucleobase complexes of Pt^{II} species, it can be concluded that the two types of donor sites are opposite in their kinetic and thermodynamic behaviour. The intra- and intermolecular competition between the Pt^{II} complexes and N and S donor biomolecules have been studied by several authors.[64–66] The results have shown that thioether adducts form relatively fast, which results in the formation of various metastable species, whereas in the equilibrium state, they are readily substituted by various nitrogen donors. The very slow formation kinetics of Pt^{II} complexes, however, makes the description of such systems extremely difficult. From this reason, Sóvágó et al.[67] have used the corresponding Pd^{II} systems to study the time dependence of the metal ion speciation. They have studied the thermodynamics and kinetics of the PdL complex with monodentate *N*-alkyl nucleobases (N) and thioether ligands (S). L stands for a tridentate chelating agent, diethylenetriamine (dien) or terpyridine (terpy), which form very stable complexes with Pd^{II}, and leave a single coordination site empty around the metal ion available for further substitution reactions. Thus, the binary complexes PdL can be considered to be ideal "monofunctional" metal ions, which can easily react with monodentate sulfur or nitrogen donor ligands. The reaction scheme of the quaternary Pd(dien)–S–N system is shown in Fig. 5.

Ternary complexes of the N donors have been found to have thermodynamic stability constants that are about 3 orders of magnitude higher than those for the thioether complexes, but rate constants of the substitution reactions show that the formation of thioether complexes is the faster reaction by at least

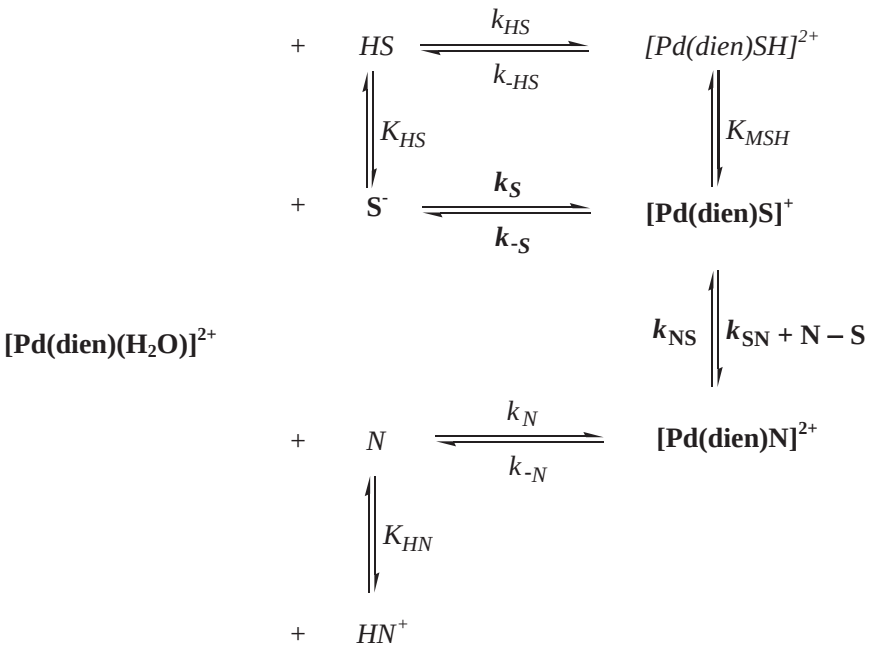

Fig. 5. Possible reaction paths in he ternary Pd(dien)–S–N ligands systems. The most likely reaction route is indicated in bold.

a factor of around 10. The combined application of the thermodynamic and kinetic parameters has afforded the time-dependent speciation shown in Fig. 6. As a consequence, the thermodynamic equilibrium state of this multicomponent system is characterized by the coordination of N-donors, which are formed via the existence of thioether-bonded intermediates. The figure shows that the Pd–S-bonded complexes can be easily detected even in the presence of N donors, supporting the assumption that Pt–S bonds formed by platinum anticancer agents can play a role in the transport and toxicity of platinum drugs.[64–67] Taking into account Fig. 6 and the proposed ratio of the ligand-exchange rate constants of Pd^{II} and Pt^{II} ($\approx 10^5$), the maximum formation of Pt–S-bonded species should occur within a few hours, but the equilibrium should require a few months. Where will be the Pt^{II} compounds such a long period after administration?

Transport of Cu^{II} from Complexes with Histidine-Containing Tripeptides to Cysteine

Cu transport in the blood was extensively studied in the period from 1960 to 80.[68,69] Cu-64 isotope experiments have indicated that most of the Cu bound to ceruloplasmin is not exchangeable, and minor amounts of Cu bound to albumin are exchangeable with low-molecular weight Cu carriers, such as amino acids (AAs). The resultant binary Cu–histidine and ternary Cu–histidine–AA (AA: aspargine, glutamine, serine, or threonine) complexes are considered important as Cu carriers through membranes[70–72] (This coincides with the computer simulation shown in Table 2.[23]):

$$Cu^{II}\text{–albumin} + AA \underset{k_{11-}}{\overset{k_{11+}}{\rightleftharpoons}} Cu^{II}\text{–albumin–AA} \underset{k_{12-}}{\overset{k_{12+}}{\rightleftharpoons}} Cu^{II}(AA)_2$$
$$\Downarrow \text{ membrane}$$
$$Cu^{I} \text{ species.} \quad (1)$$

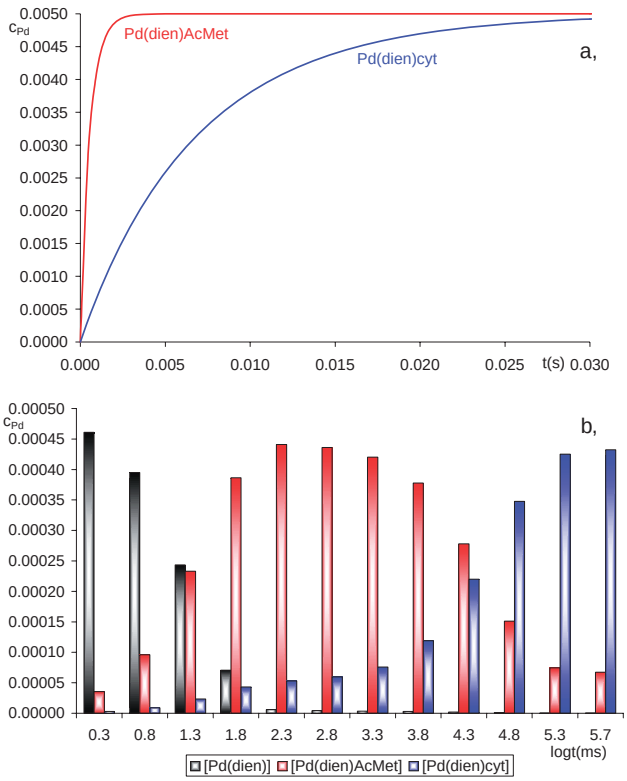

Fig. 6. Concentration of thioether- and nitrogen-bonded adducts as a function of time: (a) binary systems, mixing Pd(dien) and Acetyl-Methionine (AcMet) or cytidine in a 1:2 ratio, (b) ternary system, mixing Pd(dien), AcMet and cytidine in a 1:2:2 ratio.

Recent advances in molecular biology have led to great improvements in the understanding of Cu transport in cells. In particular, findings on many intracellular Cu chaperones have

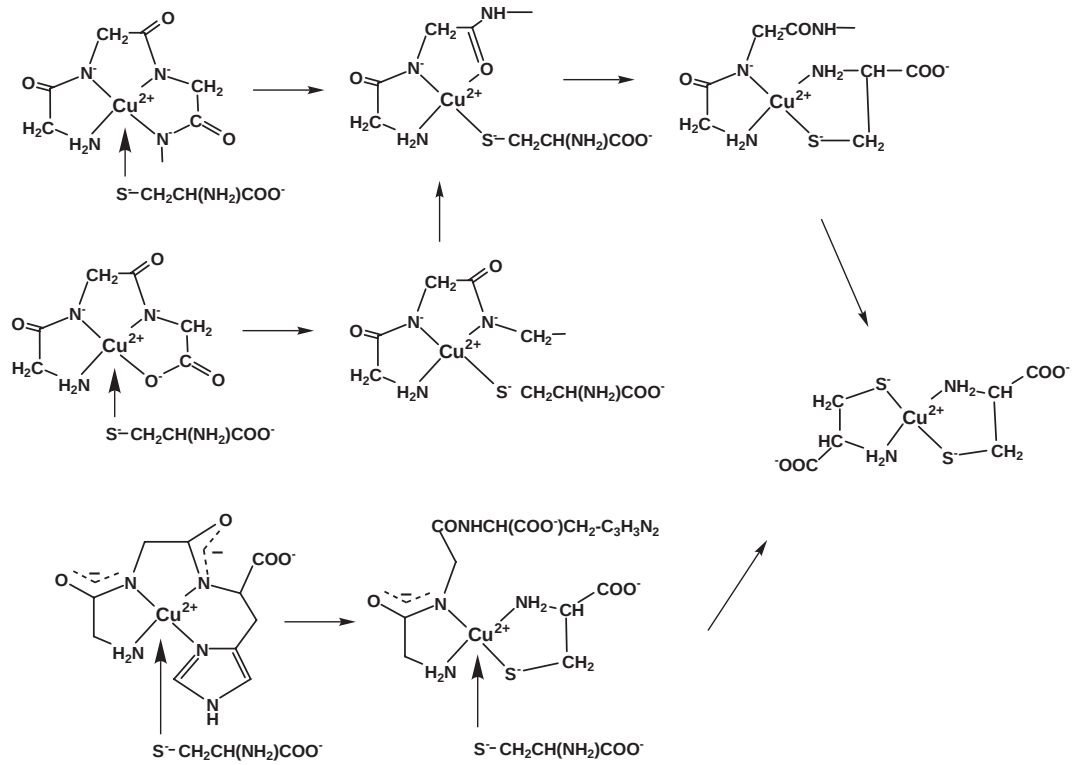

Fig. 7. Suggested reaction paths of formation of the ternary Cu^{II}–L–Cys complex in the case of various tripeptides (L).

clarified the relationships between Cu transport and genetic disorders such as Menkes disease.[73–76] Such studies have demonstrated that there might be pathways through the membranes that involve, not only passive Cu transport, but also active transport as in the presence of ATPase. The Cu inside the cell is Cu^{I}, whereas Cu outside the cell is Cu^{II}. The conversion mechanism involving the redox reaction remains unclear, but Cu^{II} is presumed to be reduced by a thiol compound, such as cysteine, glutathione or Cu transport proteins. As the reduction is probably the force driving the Cu into the cell, its mechanism and speciation are important for an understanding of Cu transport through membranes.

The Cu^{II} in albumin is bound by the N-terminal moiety through NH_2, deprotonated amide-N^-, deprotonated amide-N^-, and histidine imidazole, and forms a stable complex of which λ_{max} of the Cu d–d absorption band is similar as for Cu^{II}–(Gly–Gly–His).[77–79] From this aspect, such a histidine-containing tripeptide is a good model for albumin binding to Cu.[77–80] Kinetic studies on various peptides with cysteine (Cys) by using usual stopped flow methods have shown the following reaction path (H^+ is ignored):[81–85]

$$\begin{array}{l} Cu^{II}\text{–peptide} + AA \underset{k_{11-}}{\overset{k_{11+}}{\rightleftharpoons}} Cu^{II}\text{–peptide–Cys} \underset{k_{12-}}{\overset{k_{12+}}{\rightleftharpoons}} Cu^{II}(Cys)_2 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \Updownarrow \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad Cu^{I}\text{ species.} \quad (2) \end{array}$$

This reaction is similar to ligand substitution reaction (1), but the Cu^{I} complex is formed in solution after formation about the intermediate ternary Cu^{II} complex.

Thus, ternary Cu^{II} complex formation before the reduction of Cu^{II} should be a key reaction to afford information on Cu transport. For the Cu^{II}–(Gly–Gly–Gly) ($CuH_{-2}L$) complex, the cysteine SH first attacks the carboxylate site (the fourth coordination site of $CuH_{-2}L$) (see Fig. 7) and the ternary complex $CuH_{-2}L$–Cys*, in which Cys* is bound monodentate fashion to Cu forms.[86] For the Cu^{II}–(Gly–Gly–Gly) ($CuH_{-2}L$) system, ternary complex formation is very fast, and in fact, a decrease in the ternary complex formation is observed via the spectra. The Cu–donor bond at the fourth coordination site is the key to determining its replacement by S^-. For a strong Cu^{II}–N^- bond in the Cu^{II}–(Gly–Gly–Gly–Gly–Gly) ($CuH_{-3}L$) complex, the replacement reaction is slow, and the spectra of the binary and ternary complexes are observed. Surprisingly, the Cu^{II}–(Gly–Gly–His) complex in the presence of Cys exhibits the spectrum of the binary complex, and no the spectrum of the ternary complex is not observed.[87] A detailed study has indicated that this is due to the relatively slow formation of the ternary complex, i.e., upon formation of the ternary complex, Cu^{II} changes rapidly to the Cu^{I} species (see Fig. 8).

This slowness of ternary complex formation can be attributed to the strong Cu^{II}–imidazole bond at the fourth coordination site and stabilization of coordination of the deprotonated amide-N^- by the coordinated imidazole. In the presence of the imidazole at the second and third coordination sites, the formation of the ternary complex is not slow. The kinetic constants for the reaction from the ternary complex with Cu^{I} are reported to be similar for each system studied. The pH dependence of the kinetic constant for ternary complex formation suggests that adduct formation occurs with concomitant H^+ transfer. H^+ attachment to the deprotonated peptide bond is probably a limiting factor in the ternary complex $CuH_{-1}L$–

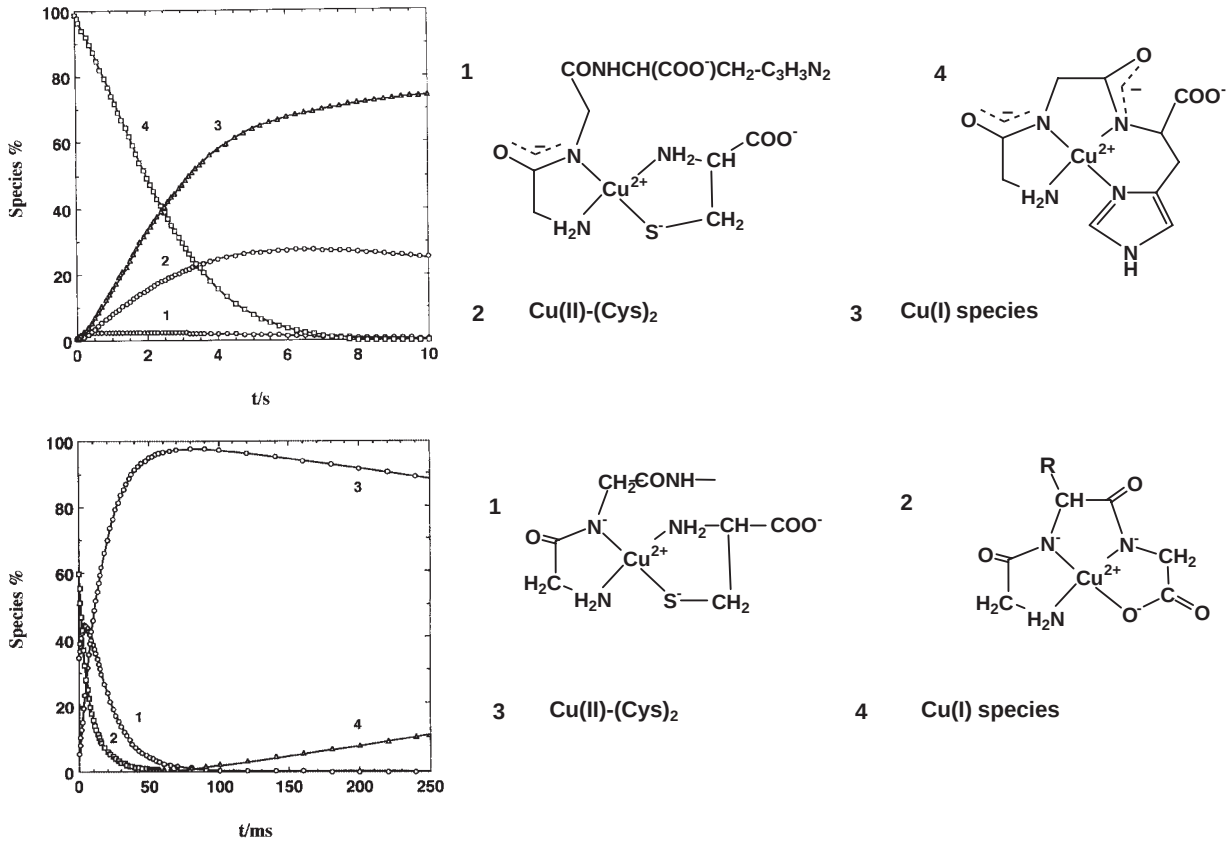

Fig. 8. Time-dependent distribution of the copper species formed in the reactions of Cu(H_{-2}GlyGlyHis) (above)[83] and Cu(H_{-2}Gly-GlyGly)(below)[84] with Cys at pH 8.7.

Cys formation. The Cu^{II}–(Gly–Gly–His) ($CuH_{-2}L$) complex is considered to be a poor H^+ acceptor, because two deprotonated amide-N^- are stabilized by two strong anchors, NH_2 and imidazole. The resultant coordination mode of the ternary Cu^{II}–(Gly–Gly–His)–Cys complex is probably different from that for other peptides, and the less favoured formation is considered to be convenient for protection against a free radical as a side-product of the redox reaction. Accordingly, Cu is transported from the model albumin peptide to Cys through a ternary complex. The Cu-exchange reaction is closely related to many factors, such as the coordination structure, the stability constant, etc., but further studies will hopefully result in an understanding all of the details of Cu transport.

Conclusion

In recent years, synthetic chemistry seems to dominate the research in bioinorganic chemistry. Large numbers of low molecular mass structural or functional models of metalloproteins, metalloenzymes have been prepared and investigated in order to mimick their activity and obtain structural or mechanistical details about their biology. These works certainly have provided a lot of valuable details for a better understanding of their function and of widening our knowledge of biological chemistry. We should mention, however, that sometimes these studies have been performed under conditions, which are scarcely relevant to biology. For example, the complexes may not be soluble in water or in water–organic solvent mixture of low permittivity mimicking cell or membrane conditions, and sometimes the solubility of the model complexes in biologically relevant solvents has not been checked. As well, it is not known if they remain in solution without serious decomposition. Fortunately, this is not the usual case, and numerous review papers, books and chapters of books, e.g., "Metal Ions in Biological Systems" give a complete description of the various metal ions and biologically important metal complexes in biological environment, covering synthetic studies, solid-state structures, and structural models as well as properties in solution.

In this paper, we have examples from different fields of bioinorganic chemistry to demonstrate the importance the solution state and solution speciation of metal ions or metal complexes. In some cases, the results obtained by modeling calculations could be confirmed by analytical separation techniques. In most of the examples interactions of metal ions with high molecular mass bioligands have been studied, and often, the role of time on speciation has also been considered, i.e., solution equilibrium and kinetics have been investigated. We believe that the complex approach of synthetic modelling together with the solid state and solution characterization of the model may offer a complete knowledge of the system studied.

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