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## COMPLEXES OF METAL CATIONS AND LOW-MOLECULAR-MASS COMPOUNDS AND OF METAL CATIONS AND PROTEINS

### 1.1. BIOLOGICALLY SIGNIFICANT METAL CATIONS

Metals, both essential and toxic, are found in the human body, and more than one quarter of the elements known in the periodic table are essential for human life. Of the metals in the human body, six metals are present in the highest concentrations: calcium (1700 g per 70 kg body mass), potassium (250 g per 70 kg), sodium (70 g per 70 kg), magnesium (42 g per 70 kg), iron (5 g per 70 kg), and zinc (3 g per 70 kg). The concentration of all other metals is less than 1 g per 70 kg body mass.

Below is a fragment of the Mendeleev periodic table of the elements that shows the positions of metals playing important roles in biological systems.

Periods	1	2	3	4	5	6	7	8	9	10	11	12
III	Na	Mg										
IV	K	Ca			V		Mn	Fe	Co	Ni	Cu	Zn
V						Mo						
VI						W						

Most of the biologically significant metals are located in the fourth period of the periodic table, except sodium and magnesium, which are in the third period, and molybdenum and tungsten, which are in the fifth and sixth periods, respectively. The electronic structures of some biologically important elements are as follows (metals are set in bold):

He	$1s^2$
Ne	$1s^2 2s^2 2p^6$
Na	<b>[Ne] <math>3s^1</math></b>
Mg	<b>[Ne] <math>3s^2</math></b>
Ar	$1s^2 2s^2 2p^6 3s^2 3p^6$
K	<b>[Ar] <math>4s^1</math></b>
Ca	<b><math>1s^2 2s^2 2p^6 3s^2 3p^6 4s^2</math> [Ar] <math>4s^2</math></b>
Ca <sup>2+</sup>	<b><math>1s^2 2s^2 2p^6 3s^2 3p^6</math></b> (K L $3s^2 3p^6$ )
Mn	<b>[Ar] <math>4s^2 3d^5</math></b>
Fe	<b>[Ar] <math>4s^2 3d^6</math></b>
Co	<b>[Ar] <math>4s^2 3d^7</math></b>
Ni	<b>[Ar] <math>4s^2 3d^8</math></b>
Cu	<b>[Ar] <math>4s^1 3d^{10}</math></b>
Zn	<b>[Ar] <math>4s^2 3d^{10}</math></b>
Kr	$[Ar] 4s^2 3d^{10} 4p^6$
Mo	<b>[Kr] <math>5s^1 4d^5</math></b>

From the viewpoint of modern inorganic chemistry, the biologically significant metals are divided into two groups: non-transition elements (Na, K, Mg, Ca, Zn) and transition elements (Mn, Fe, Co, Cu, Mo, W). Non-transition elements are characterized by constancy of their oxidation state (valency) and formation of ions with completely filled electron shells. In contrast, transition elements are characterized by variable valency (oxidation state) and formation of ions with incompletely filled electron shells. The occurrence of the incompletely filled electron shell is reflected in many physical properties (absorption bands in ultraviolet and visible spectral regions, paramagnetism, and so on), which facilitates their study.

Atoms of alkali elements Na and K possess one *s*-electron on the outer shell in addition to the electron structure of the rare gas atoms, therefore they are characterized by low ionization potentials (5.138 and 4.339 eV, respectively). Their ions have rather large size (0.98 and 1.33 Å, respectively). These atoms have a very weak tendency to form covalent bonds.

The ionization potential of the neutral calcium atom ( $1s^2, 2s^2 2p^6, 3s^2 3p^6, 4s^2$ ) for the first electron is 6.1132 eV, and the ionization potential for the second electron is 11.871 eV. In normal geological and biological conditions, Ca<sup>+</sup> ion does not exist. The completely filled octets of the Mg<sup>2+</sup>, Ca<sup>2+</sup>, and Ba<sup>2+</sup> ions ( $2s^2 2p^6 3s^2 3p^6$ , and  $4s^2 4p^6$ ) have no preferences with respect to the direction of bond formation and can be modeled by spheres with increasing radius (0.65, 0.99, and 1.35 Å, respectively) and decreasing charge density. Close sizes of the ions of calcium, strontium, and lanthanides allow strontium and lanthanides to replace calcium in binding sites.

Transition elements (*d*-elements), significant for biological processes, are iron, copper, cobalt, nickel, molybdenum, tungsten, and manganese. They have incompletely filled *d*-orbitals, therefore they are characterized by variable valences and formation of colored complexes and paramagnetic substances due to unpaired electrons. The transition elements often serve as catalysts of redox reactions.

Zinc, also very important for biological processes, is not a transition element because it has no empty *d*-orbitals. Zinc ions are very different from the rest of the non-transition metal ions. Whereas the radius of zinc ion is close to the radius of magnesium ion, its ionization potentials are essentially higher than the ionization potentials of calcium and magnesium. The high values of zinc ionization potentials are reflected in its stronger tendency to form covalent bonds.

Atoms with completely filled electron shells have no magnetic moments and degenerate states that could be split by an external magnetic field. Nevertheless, the external magnetic field still induces in the completely filled shells small magnetic moments directed opposite to the field. Such atoms are pushed out of the field and are called diamagnetic. Diamagnetism is characteristic of compounds where all the electrons are paired in the *d*-orbitals. Atoms with unpaired electrons are pulled to the magnetic field and are called paramagnetic. Paramagnetism in the transition elements is caused by the presence of unpaired electrons in the *d*-orbital.

Metal cations in water solution are surrounded by water molecules oriented by the electric field of the ion, and the orientation depends upon the nature of the ion. This is a charge-dipole interaction. The smaller the radius of an ion, the greater the charge density and the stronger its interactions with the dipole moment of water. At least three layers of water molecules are influenced by the central ion. Some ions possess rather a rigid and stable first hydration shell, which can have tetrahedral ( $\text{Li}^+$ ) or octahedral ( $\text{Mg}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ) geometry.  $\text{Zn}^{2+}$  ion can have both tetrahedral and octahedral geometry of the first hydration shell.  $\text{Mg}^{2+}$ , with its +2 charge and small radius, tightly orders six water molecules (the first or inner hydration shell) in an octahedral arrangement. A second and perhaps third layer of water are also organized by the ion charge and contribute to the overall hydration free energy of  $-455 \text{ kcal mol}^{-1}$ .  $\text{K}^+$  is larger and has only a +1 charge; as a result, eight or nine water molecules pack around the ion in a less-well-ordered manner, and the hydration energy is  $-80 \text{ kcal mol}^{-1}$ .

Calcium and monovalent metal cations, except  $\text{Li}^+$ , are characterized by a wide variety of geometries of the first hydration shell. The number of the ion-associated water molecules and the distance between them and the central ion vary and increase with increasing ion size.

## 1.2. STRUCTURES AND PROPERTIES OF THE COMPLEXES OF METAL CATIONS AND LOW-MOLECULAR-MASS COMPOUNDS

A structure containing a metal (usually a metal ion) bonded (coordinated) to a group of surrounding molecules or ions is called a *coordination complex*. Molecules or ions surrounding a metal ion in a complex are called *ligands*. A ligand is a molecule or ion that is directly bonded to a metal ion in a coordination complex. Ligands are

usually anions or polar molecules. In any case, they have at least one lone pair of electrons. The ligand uses the lone pair of electrons (Lewis base) to bind to the metal ion (Lewis acid). A Lewis base is a molecule or ion that donates a lone pair of electrons to make a bond. A Lewis acid is a molecule or ion that accepts a lone pair of electrons to make a bond. The formation of a coordinate complex is a Lewis acid–base reaction. The bond from ligand to metal is covalent (shared pair), but both electrons come from the ligand (coordinate covalent bond).

The central ion in a complex coordinates ligands. The central ion and bound ligands form a *coordination sphere*. The ligand atom bound to the central metal ion in the complex is called a *donor atom*. The number of donor atoms bound to the metal ion is termed the *coordination number*. A ligand with a single donor atom is called a monodentate ligand. There exist ligands with two (bidentate ligand) or more donor atoms located in such a way that they all can be simultaneously coordinated by the metal ion (polydentate ligands or chelators).

In some cases, the formation of bonds between metal ion and its ligands occurs due to just electrostatic interactions between the positive ion and negative ions or negative ends of polar molecules. The ability of metals to form complexes usually increases with the increase of positive charge of the metal ion and with the decrease of its ionic radius. Alkali metals  $\text{Na}^+$  and  $\text{K}^+$  hardly form complexes, whereas two- or three-charged positive ions of transition metals are inclined to form complexes.

Calcium ( $\text{Ca}^{2+}$ ), magnesium ( $\text{Mg}^{2+}$ ), sodium ( $\text{Na}^+$ ), and potassium ( $\text{K}^+$ ) ions are coordinated mainly by oxygen atoms. The interaction is purely electrostatic.  $\text{Ca}^{2+}$  ions prefer a higher coordination number compared with that of  $\text{Mg}^{2+}$  ions. The usual coordination number for magnesium is 6 (octahedral coordination). Calcium demonstrates a lot more variety of coordination numbers, 7 to 9 being the most ordinary coordination numbers. Most crystal structures containing calcium (93% from 271 calcium binding sites) are characterized by coordination numbers 6, 7, and 8 (Katz et al., 1996).

The distance between the central ion and ligand atoms is more variable for calcium than for magnesium. The radius of the coordination sphere for calcium is essentially more than that for magnesium: the distance from the central ion to oxygen atom is 2.0–2.1 Å for magnesium and 2.3–2.6 Å for calcium. Compared with magnesium, calcium forms more loose complexes with large and variable coordination numbers and variable bond length. The bonds have no distinct directions. Generally, magnesium ions require more restricted orientations of corresponding ligand groups.

$\text{Mg}^{2+}$  is a “hard” ion and prefers “hard” ligands with low polarizability, oxygen being the most preferable coordinating atom followed by nitrogen (reviewed by Dudev and Lim, 2003).  $\text{Ca}^{2+}$ , like  $\text{Mg}^{2+}$ , is also a hard ion and prefers hard oxygen-containing ligands as well. In contrast with  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions,  $\text{Zn}^{2+}$  ion and transition metal ions prefer “soft” polarizable ligands such as S and N though they are coordinated also by oxygen atoms.

The results of X-ray studies of about 150 examples of calcium–water interaction in crystal structures of hydrated complexes of calcium allowed the following

conclusions to be made (reviewed by Einspahr and Bugg, 1984; Katz et al., 1996). Calcium ions prefer to locate near the plane bisecting the water molecule. The acute angle between the dipole moment vector of the water molecule and the Ca—O bond is restricted in the range 0 to 60°, though in some cases this angle reaches 80°. Most Ca—O distances are within the range 2.3–2.5 Å with mean distance of 2.42 Å. Usually, in addition to the interaction with calcium ion, water molecule takes part in two or three other interactions. Metal ions can change the H—O—H angle in a water molecule, and different metal cations induce different effects.

Similar to calcium ions, magnesium ions demonstrate a tendency to group near the plane bisecting the water molecule, but they are located much closer to the plane compared with the location of calcium ions. In crystalline hydrates, magnesium ions are located within 0.2 Å of the plane. The mean Mg—O distance is 2.07 Å. The main factor providing the narrow distribution of the Mg—O distances is a strong preference of magnesium ions for ligands located in polyhedrons with six vertices.

Carboxylate oxygens of aspartates and glutamates and also carbonyl oxygens are major ligands of calcium ions in proteins. Studies of crystal structures of about 170 calcium complexes with carboxylates provided the following results (reviewed by Einspahr and Bugg, 1984; Katz et al., 1996). There are three types of calcium interactions with carboxylates. In the most common case of monodentate interaction, only one of two oxygens of carboxylate interacts with calcium ion. In bidentate type of interaction, both oxygens of the carboxylate group chelate calcium ion. The third type of interaction takes place when one more appropriate ligand atom (O or N) is covalently attached to the  $\alpha$ -carbon of carboxylate. In this case, one oxygen of carboxylate and the additional ligand atom are used for calcium chelation. The interactions of calcium ion with one oxygen of the carboxylate group are much more common compared with the bidentate interactions with a couple of oxygens.

Calcium ions are usually located near the plane of carboxylate and tend to group within the rather narrow range of the Ca—O—C angles. Calcium ions bound in a monodentate way can be located on any side of the C—O bond, most calcium ions being within the 120° to 150° range of the Ca—O—C angles on the side directed to the second oxygen of the carboxylate group and within 140° to 160° on the other side of the C—O bond. Calcium ions bound in a bidentate way are restricted by a narrow region equidistant from the two carboxylate oxygens with a Ca—O—C angle of about 90°. In most cases, calcium ions bound to carboxylate oxygen in combination with appropriate  $\alpha$ -ligand are restricted by the Ca—O—C angles range of 110° to 130°. Most Ca—O distances in such complexes are between 2.3 and 2.5 Å. The three types of interactions are characterized by slightly different mean Ca—O distances: 2.38 Å for monodentate type, 2.42 Å for bidentate type, and 2.53 Å for carboxylate oxygen in combination with  $\alpha$ -ligand.

Magnesium ions interact with carboxylates always in monodentate way. Because calcium ion is essentially larger than magnesium ion, it can form approximate octahedral configuration of ligands with bidentate coordination of calcium by carboxylic groups, whereas magnesium ion can do it only in a monodentate way. If a magnesium ion is bound to four monodentate carboxylates, there is enough room in its coordination sphere for two water molecules or for two other oxygen-containing

groups. Calcium ion binds on average four carboxylic oxygens (in a monodentate or bidentate way) and it still has three positions for additional binding. One of these positions is often occupied by a carbonyl group, another one can be filled by a water molecule, and the third one can accept one more ligand (for coordination numbers 7 and 8).

The geometry of calcium-carbonyl interactions in crystal structures of calcium complexes was studied by the same methods as for study of the geometry of the calcium-water and calcium-carboxylate interactions. In this case, calcium tends to locate near the plane of the carbonyl group, but this tendency is not as clear as for the interactions of calcium with carboxylates. Most calcium ions are located within the range of the Ca—O—C angles of  $110^\circ$  to  $150^\circ$ . Most Ca—O distances are within the range 2.30–2.45 Å in this case. On the whole, the interaction of calcium with carbonyls has no strict geometric preferences characteristic for the interactions of calcium with carboxylates.

Not only do water molecules, carboxylates, and carbonyls interact with calcium ions in biological systems. Phosphate, sulfate, and hydroxyl groups also interact with calcium by means of oxygen atoms. Amino acids, peptides, and proteins bind calcium ions by their carboxyl and carbonyl groups, and the geometry of their binding sites obeys the rules described above.

Transition elements of the fourth period of the periodic table of the elements, which have occupied  $3d$ -orbitals, are usually characterized by maximum coordination number 6. The most common coordination numbers for them are 4 and 6, but 5 is still found rather often. Four-coordinated complexes can have tetrahedral or square planar configuration, and 6-coordinated complexes are usually octahedral. Five-coordinated complexes can have square or trigonal bipyramidal structure. Table 1 shows configurations of complexes of transition elements of the fourth period, which are most significant from the point of view of biology.

The formation of a coordination bond in complexes of transition metals can be considered as a transfer of a lone electron pair from coordinated group or ligand to metal ion. There are several theories of the formation of coordination bonds (see, e.g., Merrel et al., 1968; Hughes, 1983). The *crystal field theory* does not take into consideration covalent interactions between metal ion and ligands and suggests that interactions between them are purely electrostatic. The ligands are modeled by point charges. Strictly speaking, such suggestions are not correct, but in many cases the theory gives good results.

Upon formation of a complex, the energy of metal ion orbitals increases when a negatively charged or polarized ligand approaches the metal ion (“crystal field”). This effect will be different for different  $d$ -orbitals (Fig. 1). For example, in the case of octahedral complexes, the crystal field causes splitting of degenerate  $d$ -orbitals of the free ion into two groups: a set of triply degenerate orbitals and a set of doubly degenerate orbitals. The splitting pattern and magnitude depend upon geometry of the complex formed.

The distribution of electrons in an unoccupied orbital obeys *Hund's rule*, according to which the stable configuration is reached for maximum possible number of unpaired electrons with parallel spins. This situation is complicated by the  $d$ -orbital splitting. Sometimes, it is more energetically favorable to pair electrons than to put them on

**TABLE 1. Configurations and Oxidation Numbers of the Complexes of Some 3d-Elements**

Oxidation Number	Coordination Number	Configuration
Cu(I) ( $d^{10}$ )	2	Linear
	3	Planar
	4	Tetrahedral
Cu(II) ( $d^9$ )	4*	Square planar
	4	Distorted tetrahedral
	5	Square pyramidal
	5	Trigonal bipyramidal
	6*	Distorted octahedral
Ni(II) ( $d^8$ )	4*	Square planar
	4*	Tetrahedral
	5	Trigonal bipyramidal
	6*	Octahedral
Co(I) ( $d^8$ )	4	Square planar
	5	Trigonal bipyramidal
	6	Octahedral
Co(II) ( $d^7$ )	4*	Tetrahedral
	4	Square planar
	5	Trigonal bipyramidal
	5	Square bipyramidal
	6*	Octahedral
Co(III) ( $d^6$ )	4	Tetrahedral
	5	Square bipyramidal
	6*	Octahedral
Fe(II) ( $d^6$ )	4	Tetrahedral
	6*	Octahedral
Fe(III) ( $d^5$ )	4	Tetrahedral
	6*	Octahedral
Mn(II) ( $d^5$ )	4	Tetrahedral
	4	Square planar
	6*	Octahedral

Source: Taken from Hughes (1983).

\*Common state.

orbitals with higher energies. Two major cases are possible: the case of the strong field, when the splitting of  $d$ -orbitals is large and electrons are paired (low spin complexes); and the case of the weak field, when the splitting is small and it is more energetically favorable to have maximum number of unpaired electrons (high spin complexes). If unpaired electrons are absent, the complex is diamagnetic; the presence of unpaired electrons makes it paramagnetic.

In octahedral complexes there exists only one possible distribution of electrons for configurations  $d^1$ ,  $d^2$ ,  $d^3$ ,  $d^8$ , and  $d^9$ , whereas for configurations  $d^4$  and  $d^7$ , both high- and low-spin states are possible. Similar analysis can be used also for tetrahedral

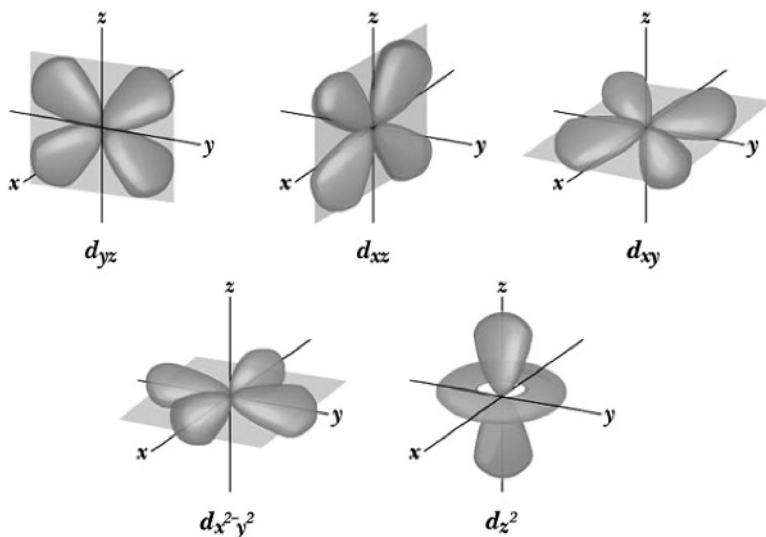


Figure 1. *d*-Orbitals.

complexes. Theoretically  $d^3$ – $d^6$  configurations are able to give both high- and low-spin complexes. In practice, however, we can see only high-spin complexes.

The color of transition metal complexes is a result of electron transitions between *d*-orbitals with different energies. For this reason, the  $d \rightarrow d$  spectra can give information about orbital splitting for various ligands. For octahedral complexes of transition elements of the fourth period, the splitting values are within the range 7500 to 12,500  $\text{cm}^{-1}$  for divalent ions and 14,000 to 25,000  $\text{cm}^{-1}$  for trivalent ions. The splitting value increases from top to bottom of the periodic table groups. The theory of the crystal field provides a qualitative explanation of the  $d \rightarrow d$  transitions in the transition metal complexes.

Interpretation of the spectra of *d*-complexes can be rather complicated because one should take into consideration energy levels of each of the *d*-configurations of free ion. In each particular case,  $d^n$  ( $2 < n < 8$ ), not all electron distributions will have equal energies because electrons repulse each other differently on different orbitals. This results in a situation where each *d*-configuration gives a set of energy levels or *terms*, which are often degenerated. The terms are characterized by total spin moment and total orbital moment, designated by quantum numbers *S* and *L*. One can use the Russell–Saunders scheme of interactions, which suggests that spin and orbital moments do not interact with each other. Terms with *L* values 0, 1, 2, 3, 4, . . . , are designated by letters S, P, D, F, G, . . . , respectively. The classification is supplemented by indication of spin multiplicity ( $2S + 1$ ), which is the number of ways to arrange unpaired electrons.

The crystal field does not split *S* and *P* terms but splits *D* and *F* terms into two and three components, respectively. The same effects are observed with the terms of

Rassel–Saunders irrespective of their  $d$ -configuration. The D-terms of Rassel–Saunders are split into two states by both octahedral and tetrahedral fields.

If all the other factors remain constant, the splitting values depend only upon ligand nature. Arranging ligands in a series according to the splitting value, we can obtain a spectrochemical series reflecting relative ability of ligands to induce splitting of energy levels of  $d$ -orbitals. For some biologically significant ligands, the spectrochemical series looks like that:  $\text{SH} < \text{CO}_2^- < \text{amide} < \text{imidazole}$ .

Evidently, the overlapping of metal ion and ligand orbitals, which is neglected in the crystal field theory, is actually very important. In the ligand field theory, the concept of covalent interactions is not introduced formally but nevertheless used for correction of various parameters. This approach works well for complexes of metals with common oxidation numbers when the orbital overlapping is low. In the *theory of molecular orbitals*, the concept of the overlapping orbitals in bond formation is widely used. Molecular orbitals are formed as linear combinations of atomic orbitals taking into consideration symmetry and energy reasons. Both  $\sigma$ - and  $\pi$ -orbitals can be formed. Only  $\sigma$ -interactions are taken into account first in consideration of the interactions in octahedral complexes.

A transition metal may provide nine orbitals to ligands: five  $3d$  orbitals, one  $4s$  and three  $4p$  orbitals. Among them,  $t_{2g}$  orbitals ( $d_{xy}$ ,  $d_{yz}$ ,  $d_{zx}$ ) are not suited for  $\sigma$ -bonding because they are not directed to the ligand orbitals. Each of the six orbitals provides two electrons. Upon overlapping of each of the six metal orbitals with  $\sigma$ -orbitals of corresponding symmetry, two combinations are possible, which results in formation of bonding and antibonding molecular orbitals.

Many transition metals are characterized by charge transfer absorption bands in UV and visible spectra (reviewed by Yatsimirsky, 1976; Hughes, 1983). The appearance of these bands is due to electron transitions from an orbital of one atom to an orbital of another atom. The charge transfer can proceed between ligand atoms, but most often it occurs between ligand and metal atom. The position of these absorption bands depends upon the nature of the metal and ligand and especially upon their relative redox properties.

Two oxidation numbers are common for copper: Cu(I) and Cu(II). Whereas Cu(III) is suggested to exist, it seems to be unlikely that this oxidation state has some biological significance, as biological ligands cannot stabilize a +3 oxidation state of copper. Because redox potential of Cu(II)/Cu(III) is high, one-electron oxidation of protein ligands takes place. Cu(II) is characterized by coordination numbers 4 (square planar), 5 (trigonal bipyramidal or square pyramidal), or 6 (octahedron). Stable Cu(II)—N bond is usually inert, whereas the bonds with oxygen are more labile.

Cu(I) prefers coordination numbers 2, 3, or 4 (tetrahedral geometry) and is stabilized by soft ligands. Coordination number 5 is also possible (square pyramidal). The complexes of Cu(I) (configuration  $d^{10}$ ) are characterized by charge transfer Cu(I)  $\rightarrow$  ligand bands in the visible region up to 650 nm. The complexes of Cu(II) (configuration  $d^9$ ) also give charge transfer bands but of Cu(II)  $\leftarrow$  ligand type.

Ligands with sulfur donor groups bind Cu(I) stronger than they bind Cu(II), as well as all unsaturated ligands phenanthroline and bipyridyl. Both nitrogen and sulfur

ligands bind Cu(I) and Cu(II) stronger than do ligands with donor oxygen atoms. Stereochemical effects prevail in the case of polydentate ligands as the behavior of Cu(I) and Cu(II) depends upon their coordination numbers and stereochemical features. Cu(I) often forms linear bicoordination complexes but can increase its coordination number up to 4, and in this case the complex geometry becomes tetrahedral. In contrast, Cu(II) prefers 6-coordinated structure, and most of its 4-coordinated complexes have a distorted tetragonal geometry.

The complexes of iron, Fe(II) and Fe(III), also demonstrate charge transfer bands in electron absorption spectra. The  $d^6$ -complexes of Fe(II) in octahedral configuration have a single transition. In stronger fields, the singlet state can be lower in energy than the  $^5D$  state, and this results in an appearance of a new energy level. Low-spin complexes of Fe(II) are diamagnetic. For  $d^5$ -complexes of Fe(III) with the major term  $^6S$ , the spin-forbidden transitions occur only in the absence of any other sextet states, therefore the intensity of the  $d \rightarrow d$  transitions will be low. The complexes of Fe(II) and Fe(III) have predominately octahedral structure.

### 1.3. DISSOCIATION CONSTANTS FOR COMPLEXES OF METAL CATIONS AND LOW-MOLECULAR-MASS COMPOUNDS

Simple ligands ( $H_2O$ ,  $NH_3$ ,  $NR_3$ , and others) contain a single donor atom (i.e., they are monodentate ligands). More sophisticated ligands ( $NO_3^-$ ,  $CO_3^{2-}$ ,  $C_2O_4^{2-}$ , and others) can be coordinated in both monodentate and bidentate ways. There exist polydentate ligands that coordinate ions by several donor atoms. For example, the well-known chelator ethylenediamine tetraacetate ion  $C_2H_4[N(CH_2COO)_2]_2^{4-}$  can coordinate metal ions by six atoms (four oxygens and two nitrogens). Formation of cycles of polydentate ligands results in a drastic increase of stability constants of complexes, the effect being governed by both entropic and enthalpic contributions.

The most important thermodynamic characteristic of a coordination compound is its stability constant  $\beta_n$  (i.e., equilibrium constant of the reaction):



$$\beta_n = [ML_n]/([M][L]^n) \quad (2)$$

where  $M^{z+}$  is a metal ion with  $z+$  charge;  $L$  is a ligand; and  $n$  is the number of ligands in the reaction. Along with the overall stability constant, one can recognize stepwise stability constants:

$$K_n = [ML_n]/([ML_{n-1}][L]). \quad (3)$$

One can find in the literature numerous data on stability constants for metal complexes of various biologically active substances, including amino acids. It is of interest that in the case of  $\alpha$ -amino acids, complex stabilities change in the series  $Ca^{2+} < Mg^{2+} < Mn^{2+} < Fe^{2+} < Cd^{2+} < Co^{2+} < Zn^{2+} < Ni^{2+} < Cu^{2+} < Fe^{3+}$ .

Table 2 contains logarithms of stability constants for some complexes of metal cations with organic compounds. Calcium ions strictly prefer oxygen ligands. Nitrogen-containing ligands, such as 1,2-diaminomethane, bind calcium very weakly so that their stability constants are hardly measurable. Because calcium coordination occurs due to ionic interactions and has no directionality, to create a good calcium binding site one should create a spheric cavity with a diameter corresponding with coordination number and with at least two negatively charged donor groups.

Stability constants of the complexes with lanthanides are always higher than those for the complexes with calcium. For this reason, one can expect that positively charged lanthanide ions with the charge +3 will easily displace calcium ions from the protein-binding sites, which has indeed been found experimentally.

$\text{Ca}^{2+}$  is larger than  $\text{Mg}^{2+}$ , therefore it is more suited for binding by large multi-dentate anion ligand groups. For example,  $\text{Ca}^{2+}$  is bound to ethylene glycol-bis( $\beta$ -aminoethyl ether)- $N,N,N',N'$ -tetraacetic acid (EGTA)  $10^{5.6}$  times stronger than the binding of  $\text{Mg}^{2+}$  to EGTA, as calcium ion better satisfies the steric requirements of EGTA.

Nucleotide diphosphates bind magnesium ions slightly better than they bind calcium ions. For this reason, in a cell magnesium ions are bound mainly by

**TABLE 2. Logarithms of the First Stability Constant of Complexes of Some Metal Cations and Organic Compounds (25°C, Ionic Strength 0.1 M)**

Organic Compound	Metal Cation						
	$\text{Mg}^{2+}$	$\text{Ca}^{2+}$	$\text{Zn}^{2+}$	$\text{Mn}^{2+}$	$\text{Cu}^{2+}$	$\text{Fe}^{2+}$	$\text{Gd}^{3+}$
Acetate	0.5	0.5	1.1				2.0
Lactate	0.9	1.1	1.9				2.9
Citrate	3.4	3.5	5.0				7.8
Oxydiacetate	1.8	4.4	3.6				5.4
Iminodiacetate	3.0	2.6	7.2				6.7
Alanine	2.0	1.2	5.21	3.02	8.5		
Aspartate	2.4	1.6	5.8	3.74	9.32	4.34	5.7
Glutamate	1.9	1.43	5.45	3.3	7.85	4.6	
Histidine	—	—	6.63	3.58	10.5	5.85	
Cysteine	—	—	9.86	4.1	—	6.2	
Nitrilotriacetate	5.5	6.4	10.7				11.4
Adenosine-5'-diphosphate	3.17	2.86	4.28	4.16	4.50	—	
Adenosine-5'-triphosphate	4.22	3.97	4.85	4.52	5.02	—	
EGTA (Ethylene glycol-bis( $\beta$ -aminoethyl ether)- $N,N,N',N'$ -tetraacetic acid)	5.3	10.9	12.6				17.5
EDTA (Ethylenediamine tetraacetic acid)	8.8	10.6	16.4				17.4

Source: From Yacimirsky (1976) and Martell and Smith (1977).

phosphates, whereas calcium ions are tightly bound by proteins.  $Mg^{2+}$  binds to nitrogen donors in chlorophyll, whereas  $Ca^{2+}$  does not bind to chlorophyll.

#### 1.4. SOLUBILITIES OF COMPLEXES OF CALCIUM AND OF SOME OTHER METAL CATIONS

Solubilities of calcium and magnesium salts vary in very wide limits. Some calcium and magnesium salts play very important roles in biological systems. The mineral part of teeth and bones consists of calcium phosphate. Solution of calcium phosphate contains many salts differing by the calcium:phosphate ratio. Some of them may also be contained in teeth and bones. The main salt is calcium hydroxyapatite with impurities, which is sometimes called *biological apatite*. The solubility of calcium phosphate in human bone is approximately  $7 \times 10^{-5}$  M at physiologic pH and temperature. Table 3 shows properties of ideal forms of calcium and magnesium salts.

It should be noted that large differences in solubility products are not always reflected in large differences in solubilities. The comparison should be carried out in conditions where relative contributions of ions of a salt are the same.

Impurities in biological apatite cause significant tensions in crystal structure, which make it less stable. Composition of biological apatite varies so much that it is almost senseless to measure its solubility product. It is usually assumed that biological apatite is more soluble than hydroxyapatite. The concentration of free calcium in the plasma

**TABLE 3. Solubility Products of Some Calcium and Magnesium Phosphates**

Salt	Ionic Composition	Solubility Product	Calcium Concentration in Equilibrium (M)
Brucite	$Ca(HPO_4) \cdot 2H_2O$	$2.32 \times 10^{-7}$	$1.08 \times 10^{-6}$
Calcium phosphate	$Ca_3(PO_4)_2$	$2.83 \times 10^{-30}$	$1.45 \times 10^{-6}$
Magnesium phosphate	$Mg_3(PO_4)_2$	$1 \times 10^{-25}$	$3.9 \times 10^{-6}$
Octocalcium phosphate	$Ca_4H(PO_4)_3$	$2 \times 10^{-49}$	$4.8 \times 10^{-4}$
Hydroxyapatite	$Ca_5(PO_4)_3OH$	$2.34 \times 10^{-59}$	$4.36 \times 10^{-7}$
Fluoroapatite	$Ca_5(PO_4)_3F$	$1.16 \times 10^{-60}$	$3.47 \times 10^{-7}$
Calcium carbonate	$CaCO_3$	$3.8 \times 10^{-9}$	$6.16 \times 10^{-5}$
Magnesium carbonate	$MgCO_3$	$3.5 \times 10^{-8}$	$1.87 \times 10^{-4}$
Calcium hydroxide	$Ca(OH)_2$	$5.5 \times 10^{-6}$	$1.11 \times 10^{-2}$
Magnesium hydroxide	$Mg(OH)_2$	$1.8 \times 10^{-11}$	$1.65 \times 10^{-4}$
Hydrate of calcium oxalate	$CaC_2O_4 \cdot H_2O$	$1.96 \times 10^{-8}$	$1.4 \times 10^{-4}$
Magnesium oxalate	$MgC_2O_4$	$7 \times 10^{-7}$	$8.37 \times 10^{-4}$
Magnesium sulfate	$CaSO_4$	$9.1 \times 10^{-6}$	$3.0 \times 10^{-3}$

Source: From McDowell et al. (1977).

and extracellular fluid is more than  $10^{-3}$  M. This can be explained in part by the fact that the concentration of the  $\text{PO}_4^{3-}$  ion is very low in plasma and that most of the phosphate is in the form of the  $\text{HPO}_4^{2-}$  ion (reviewed by Talmage and Talmage, 2006).

In water,  $\text{Fe}^{2+}$  is spontaneously oxidized by molecular oxygen to give  $\text{Fe}^{3+}$  with formation of  $\text{Fe}(\text{OH})_3$ . Therefore, the maximum solubility of iron in an oxidative environment, such as intracellular liquids, is restricted by the solubility product of  $\text{Fe}(\text{OH})_3$ . At pH 7.0, maximum solubility of  $\text{Fe}^{3+}$  is as low as  $10^{-17}$  M, whereas the solubility of  $\text{Fe}^{2+}$  is as high as  $10^{-1}$  M. Because of low solubility of iron in the presence of oxygen, the organisms were forced to create special proteins to bind  $\text{Fe}^{3+}$  and to keep it thermodynamically stable but kinetically accessible for biological processes. In vertebrates, the functions of the binding and transportation of extracellular  $\text{Fe}^{3+}$  are fulfilled by plasma protein transferrin possessing two  $\text{Fe}^{3+}$  binding sites with binding constant about  $10^{22}$   $\text{M}^{-1}$ .

In living organisms, copper has two oxidation forms:  $\text{Cu}^+$  and  $\text{Cu}^{2+}$ .  $\text{Cu}^{2+}$  is fairly soluble, whereas the solubility of  $\text{Cu}^+$  is somewhere in the submicromolar range. In biological systems, copper is usually in the  $\text{Cu}^{2+}$  form, as  $\text{Cu}^+$  is easily oxidized to  $\text{Cu}^{2+}$  in the presence of oxygen and other electron acceptors.

The most important oxidation number for zinc is 2+. Zinc forms stable hydroxides. Elementary zinc does not react with water molecules. The ion does form a protective, water-insoluble  $\text{Zn}(\text{OH})_2$  layer with dissolved hydroxide ions according to the following reaction:  $\text{Zn}^{2+} + 2\text{OH}^- \rightarrow \text{Zn}(\text{OH})_2(\text{s})$ . Zinc reacts with  $\text{H}^+$  ions according to the following reaction:  $\text{Zn}(\text{s}) + 2\text{H}^+ \rightarrow \text{Zn}^{2+}(\text{aq}) + \text{H}_2(\text{g})$ . Zinc salts cause a milky turbidity in water in higher concentrations. The solubility of zinc depends on temperature and pH of the water in question. When the pH is fairly neutral, zinc is insoluble in water. Solubility increases with increasing acidity. Above pH 11, solubility also increases. Zinc dissolves in water as  $\text{ZnOH}^+(\text{aq})$  or  $\text{Zn}^{2+}(\text{aq})$ . Anionic  $\text{ZnCO}_3$  has a solubility of 0.21 g/L. Examples of solubility of zinc compounds are zinc chloride ( $\text{ZnCl}_2$ ) 4320 g/L and zinc oxide ( $\text{ZnO}$ ) or zinc vitriol ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ) 580 g/L.

