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COMPLEXES OF CALCIUM AND OTHER CATIONS WITH COMPOUNDS OF LOW MOLECULAR WEIGHT

The structures, kinetics, thermodynamics, and solubilities of metalloorganic and inorganic complexes provide the background for understanding the special properties of calcium binding proteins and metalloproteins in general. In this chapter we survey these low molecular weight complexes and in Chapter 5 consider protein complexes of essential metals, other than calcium, and the biological interactions, often toxic, of nonessential metals.

4.1. CRYSTAL STRUCTURES OF COMPLEXES OF CALCIUM WITH LOW MOLECULAR WEIGHT COMPOUNDS

Examination of the crystal structures of calcium and small molecule complexes led to several conclusions (reviewed by Nelson and Kretsinger, 1976; Einspahr and Bugg, 1984; Katz et al., 1996). Nearly all of the ligands, excluding a few halides, are oxygen atoms. There are no Ca–Ca metal bonds, although a single carboxyl group can bridge two Ca^{2+} ions. Most of the Ca–O distances are 2.3 to 2.5 Å. In contrast, the (biological) transition metals—V, Cr, Mn, Fe, Co, Ni, Cu, and Zn—as well as Mo, Cd, and W (Chapter 3)—"soft metals"—are frequently complexed by nitrogen and sulfur atoms of organic compounds. Further, iron forms clusters with Fe–Fe bonds.

Many of these crystal structures of small-molecule complexes, as well as of calcium binding proteins, incorporate one to three waters of hydration in the primary coordination sphere. The Ca^{2+} ions tend to lie near the plane that

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relates the two hydrogen atoms of a coordinating water molecule. The acute angle between the dipole moment vector of the water and the Ca–O vector is generally between 0 and 60°, although a few Ca^{2+} ions lie at angles as high as 80°.

The coordination of calcium and magnesium can be understood in terms of Mg–O and Ca–O bond distances (2.0 and 2.3 Å) and of van der Waals radii (Mg ~ 0.70 Å, Ca ~1.00 Å, and O ~1.4 Å). The oxygens of hexa-aquomagnesium are at the vertices of an octahedron; the O–Mg–O angle is 90°. With Mg–O 2.0 Å, the O–O distance is 2.84 Å, optimal van der Waals packing. These are happy waters and are reluctant to leave; hence, a k_{off} value of 10^6 s^{-1} is slow. In contrast, the O–O distance in hexa-aquocalcium is $2.3 \times 2^{0.5} = 3.27$ Å. There is room for a seventh oxygen; it is usually accommodated in a pentagonal bipyramid. If the five oxygens about the circumference are in van der Waals contact, 2.8 Å, the Ca–O distance is $(2.8/2 \cdot \sin 36^\circ) = 2.38$ Å. If the distance from Ca to the axial oxygen is 2.3 Å, the $O_{axial}–O_{equatorial}$ distance is $(2.3^2 + 2.38^2)^{0.5} = 3.28$ Å. The equatorial oxygens can get a bit closer to the calcium by moving slightly off equator. Even so, the seven coordinating oxygens have a bit of room to roll about before coming into van der Waals contact. The k_{off} (10^9 s^{-1}) is much faster than for magnesium. In most protein–calcium complexes, the Ca^{2+} ion is seven-coordinate; Mg^{2+} is almost always six coordinate with oxygen.

In a square antiprism the eight oxygens come into van der Waals contact (2.8 Å) when still 2.3 to 2.4 Å from the calcium. Eight coordinate calcium sometimes occurs in organic complexes where the oxygens are less constrained than they would be in a protein.

The Mg^{2+} ion has a strong preference for sixfold (octahedral) coodination; this tends to restrict the Mg^{2+} ion to the dipole axis of water. In crystalline hydrates, Mg^{2+} ions are located within 0.2 Å of this axis. The average Mg–O distance is 2.07 Å.

Carboxylate oxygens of Asp and Glu, along with carbonyl oxygens of the main chain, are the primary ligands of calcium in proteins (reviewed by Einspahr and Bugg, 1984; Katz et al., 1996). Ca^{2+} ions in monodentate coordination can be located on any side of the C–O vector; the Ca–O–C angles are 120 to 150°, with the Ca^{2+} ions 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. In bidendate coordination both oxygens coordinate calcium; the Ca^{2+} ion lies near the plane of the carboxylate and near the line of the C–C bond. Ca^{2+} ions in bidentate coordination are restricted to a narrow region equidistant from the two carboxylate oxygens, with Ca–O–C angles of about 90°. Most Ca–O distances in such complexes are between 2.3 and 2.5 Å. These three types of interactions have slightly different mean Ca–O distances: 2.38 Å for monodentate ligands, 2.42 Å for bidentate, and 2.53 Å for carboxylate oxygen in combination with an α-ligand.

Mg^{2+} ions always interact with carboxyl groups in a monodentate manner. If a Mg^{2+} ion is bound by four monodentate carboxyl groups, it still has space for two waters or two other oxygen-containing groups.

In carbonyl interactions the Ca^{2+} ions tends to lie near the plane of the carbonyl group, but this tendency is not as pronounced as in the case of calcium carboxylate interactions. Most of the Ca^{2+} ions lie at Ca–O–C angles between 110 and 150°, but a number of examples lie outside this range. Cyclic peptide examples are tightly clustered in a region nearly collinear with the C–O bond. Most of the Ca–O distances lie between 2.30 and 2.45 Å. Averages of Ca–O distances for the unidentate, chelate, and cyclic peptide categories are 2.36, 2.42, and 2.30 Å, respectively. In general, calcium–carbonyl interactions lack the strong definition of geometrical preferences that was found for calcium–carboxylate interactions.

Ca^{2+} ions are bound to amino acids and peptides primarily through carboxylate and carbonyl groups, and calcium–ligand geometries in the complexes follow the general patterns described above. Phosphate, sulfate, and hydroxyl ions also interact with Ca^{2+} ions through oxygen atoms.

Unlike Mg^{2+} and Ca^{2+}, Zn^{2+} ions prefer "softer" ligands such as Cys and His, although they are also found coordinated to Asp and Glu side chains. The Cys side chains are deprotonated when bound to the metal. The Zn^{2+} ion is octahedrally bound to six water molecules in solution. At the same time, in both Zn finger proteins and enzymes, Zn^{2+} is usually tetrahedrally coordinated, but it can also adopt a five- or six coordinate geometry. The average Zn–ligand distances for a tetrahedral binding site are Zn–N (His) 2.07 to 2.09 Å, Zn–S (Cys) 2.21 to 2.35 Å, Zn–O (Asp/Glu) 1.95 to 2.04 Å, and Zn–O (water) 2.12 to 2.15 Å (reviewed by Dudev and Lim, 2003).

Kirberger et al. (2008) analyzed the calcium binding proteins from the Protein Data Bank to identify structural parameters associated with EF-hand and non-EF-hand calcium binding sites (Chapter 9). Non-EF-hand sites have lower coordination numbers (6 ± 2 vs. 7 ± 1), fewer protein ligands (4 ± 2 vs. 6 ± 1), and more water ligands (2 ± 2 vs. 1) than do EF-hand sites. The orders of ligand preference for non-EF-hands are H_2O (33.1%) > side-chain Asp (24.5%) > main-chain carbonyl (23.9%) > side-chain Glu (10.4%) and for EF-hand sites side-chain Asp (29.7%) > side-chain Glu (26.6%) > main-chain carbonyl (21.4%) > H_2O (13.3%). Fewer negative charges were observed in the non-EF-hand than in the EF-hand binding sites (1 ± 1 vs. 3 ± 1). Over 20% of non-EF-hand sites have formal charge values of zero, due to increased use of water and carbonyl oxygen ligands. The non-EF-hand sites have a broader range of ligand distances and bond angles than do EF-hand sites, possibly due to the highly conserved helix–loop–helix conformation of the EF-hand. Significant differences between ligand types (carbonyl, side chain, bidentate) show that angles associated with each type must be classified separately. The EF-hand side-chain Ca–O–C angles have an unusual bimodal distribution consistent with an Asp distribution that differs from the Gaussian model observed for non-EF-hand proteins.

4.2. DISSOCIATION CONSTANTS OF CALCIUM AND ANALOGS WITH SMALL COMPOUNDS

Simple compounds such as H_2O and NH_3 have a single donor atom (i.e., they function as monodentate ligands). More complex compounds or anions (e.g., NO^{3-}, CO_3^{2-}, $C_2O_4^{2-}$) can coordinate cations as both monodentate and bidentate ligands. Polydentate ligands coordinate cations using several donor atoms, and in the process do not lower the entropy of the system; this results in a drastic increase in the stabilities of the complexes. This effect is governed by both entropic and enthalpic factors. For example, the well-known chelator EDTA (ethylenediaminetetraacetic acid; $C_2H_4[N(CH_2COO)_2]_2^{4-}$) coordinates metal ions by six atoms (four oxygens and two nitrogens) and has very high affinity for Ca^{2+} and other di- and trivalent metal cations.

The most important thermodynamic characteristic of a coordination compound is its stability constant β_n, that is, the equilibrium constant of the reaction

$$M^{z+} + nL \leftrightarrow ML_n \tag{4.1}$$

$$\beta_n = \frac{[ML_n]}{[M][L]^n} \tag{4.2}$$

The stepwise stability constants are defined as

$$K_n = \frac{[ML_n]}{[ML_{n-1}][L]} \tag{4.3}$$

The stabilities of α-amino acids complexed with metal ions change in the series $Ca^{2+} < Mg^{2+} < Mn^{2+} < Fe^{2+} < Cd^{2+} < Co^{2+} < Zn^{2+} < Ni^{2+} < Cu^{2+} < Fe^{3+}$. Without constraints on the free metal ion concentrations in cells and on the steric demands of complicated ligands, calcium would not be bound by any organic molecules in cells. The concentrations of competing metal ions in the cytoplasm are reduced by binding to stronger donors so that the free ion levels are approximately (Williams, 2002)

Ion	Mn^{2+}	Fe^{2+}	Co^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}	Cd^{2+}	Mg^{2+}	Ca^{2+}
$\log[M^{2+}_{free}]$	−7	−8	−9	−11	−15	−12	−15	−3	−8
Ionic radius Å	0.75	0.70	0.68	0.65	0.60	0.65	0.90	0.65	1.0

At these concentrations, none of these ions can bind to the calcium binding sites because the protein structures that hold calcium do not allow a collapse of ligand donor groups to give a smaller cavity than a radius of 1.0 Å, so chelation of the oxygen donors is relatively weak.

The coordination of the Ca^{2+} ion is ionic and spherical without significant directionality. To provide a good calcium site, one needs a nearly spherical

TABLE 4.1. Logarithms of the First Stability Constants for Complexes of Metal Cations with Organic Compounds

	Mg^{2+}	Ca^{2+}	Zn^{2+}	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	4.6	
Aspartate	2.4	1.6	5.8	5.7
Nitrilotriacetate	5.5	6.4	10.7	11.4
EGTA	5.3	10.9	12.6	17.5
EDTA	8.8	10.6	16.4	17.4
Adenosin-5′-diphosphate	3.17	2.86	4.28	
Adenosine-5′-triphosphate	4.22	3.97	4.85	

Source: Data from Martell and Smith (1977).

Conditions: 25°C; 0.1 M ionic strength.

pocket of size appropriate to the coordination number with at least two negatively charged ligands. Table 4.1 contains logarithms of stability constants for some complexes of metal cations with organic substances.

The lanthanide stability constants are always higher than those of calcium. For this reason, one can expect that tripositive lanthanides will displace Ca^{2+} ions from protein binding sites; this agrees with experimental data.

Compared to magnesium, calcium prefers ligands without nitrogen donors (reviewed by Martin, 1984). For example, calcium binds more strongly to tridentate oxydiacetate, but magnesium binds strongly to iminodiacetate. Magnesium also binds to nitrogen donors in chlorophyll, but calcium binds neither. Calcium binds large multidentate anionic ligand groups with higher affinity than that for magnesium. For example, calcium binds 100 times more strongly to EGTA (ethylene glycol tetraacetic acid) than does magnesium since the Ca^{2+} ion fits the steric requirements of EGTA better than it fits the Mg^{2+} ion. In nucleotide phosphates, magnesium binds slightly more strongly than does calcium. Inside a cell, Mg^{2+} is associated with phosphates; whereas, Ca^{2+} is tightly bound by proteins.

4.3. SOLUBILITIES OF CALCIUM AND ANALOGS WITH SMALL COMPOUNDS

Solubilities of calcium and magnesium salts vary over a wide range. Calcium is naturally present in water. It may dissolve from rocks such as limestone, marble, calcite, dolomite, gypsum, fluorite, and apatite. Calcium carbonate has a solubility of 14 mg L^{-1}; it is five times higher at normal atmospheric CO_2 partial

pressure. The solubility of calcium phosphate is 20 mg L^{-1}, calcium fluoride 16 mg L^{-1}, calcium chromate 170 g L^{-1}, and calcium hypochlorate 218 g L^{-1}. The solubilities of other calcium compounds lie within this range.

The mineral found in teeth and bone is an impure form of calcium hydroxyapatite, sometimes referred to as "biological apatite." However, a solution of calcium phosphate can give rise to a number of different salts that differ in their calcium/phosphate ratios. Table 4.2 shows the ideal forms of the calcium phosphate salts. Calcium hydroxyapatite is a naturally occurring form of calcium apatite with the formula $Ca_5(PO_4)_3OH$, but is usually written $Ca_{10}(PO_4)_6(OH)_2$ to denote that the crystal unit cell contains two equivalents. Seventy percent of bone consists of hydroxylapatite. Carbonated calcium-deficient hydroxylapatite is the main mineral of which dental enamel and dentin are comprised (Chapter 3).

Note that the large differences in solubility products are not always reflected in the actual solubilities of the various ions concerned. Impurities in biological apatite introduce significant stresses into the crystal structure, which make it much less stable. Biological apatite composition varies widely, and the apparent solubility of the source varies accordingly. The pH of seawater is about 8.4 and is close to saturation in $CaCO_3$. Many organisms in the sea make $CaCO_3$ shells quite easily (reviewed by Williams, 2002). Some freshwater organisms also make $CaCO_3$ shells; this means that the precipitation occurs due to elevated [HCO_3^-], which is produced by the living system. The precipitation of phosphates in the sea is less probable than carbonates because of the low level of phosphate. Vertebrates have an extracellular pH $\sim$7.0 and precipitate calcium phosphate $Ca(HPO_4)_2$ or $Ca_3(PO_4)_2$ before it is transformed into the more insoluble hydroxyapatite [$Ca_5(PO_4)_3OH$].

TABLE 4.2. Solubility Products of Some Calcium Phosphate Salts

Salt	Ionic Composition	Solubility Product	[Me] at Saturation (M)
brushite	$Ca(HPO_4) \cdot 2H_2O$	2.32×10^{-7}	1.08×10^{-6}
calcium phosphate	$Ca_3(PO_4)_2$	2.83×10^{-30}	1.45×10^{-6}
magnesium phosphate	$Mg_3(PO_4)_2$	1×10^{-25}	3.9×10^{-6}
octacalcium phosphate	$Ca_4H(PO_4)_3$	2×10^{-49}	4.8×10^{-4}
hydroxyapatite	$Ca_5(PO_4)_3OH$	2.34×10^{-59}	4.36×10^{-7}
fluorapatite	$Ca_5(PO_4)_3F$	1.16×10^{-60}	3.47×10^{-7}
calcium carbonate	$CaCO_3$	3.8×10^{-9}	6.16×10^{-5}
magnesium carbonate	$MgCO_3$	3.5×10^{-8}	1.87×10^{-4}
calcium hydroxide	$Ca(OH)_2$	5.5×10^{-6}	1.11×10^{-2}
magnesium hydroxide	$Mg(OH)_2$	1.8×10^{-11}	1.65×10^{-4}
calcium oxalate hydrate	$CaC_2O_4 \cdot H_2O$	1.96×10^{-8}	1.4×10^{-4}
magnesium oxalate	MgC_2O_4	7×10^{-7}	8.37×10^{-4}
calcium sulfate	$CaSO_4$	9.1×10^{-6}	3.0×10^{-3}

Source: Data from McDowell et al. (1977).

Bone, which is a composite of polymer and crystals, can be redissolved by making the extracellular fluid acidic. Higher animals have special cells, osteoblasts, for such dissolution. These cells bind to bone, trapping a small aqueous volume between the bone and themselves. They then release acid into this volume to solubilize the phosphate and release enzymes to destroy the biopolymers found in bone.