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Factors Controlling Metal-Ion Selectivity in the Binding Sites of Calcium-Binding Proteins. The Metal-Binding Properties of Amide Donors. A Crystallographic and Thermodynamic Study

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The metal-ion complexing properties of the ligand EDTAM (ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetamide) are investigated as a model for the role of amide oxygen donors in the binding sites of Ca-binding proteins. The structures of the complexes $[Ca(EDTAM)NO_3]NO_3$ (**1**), $[La(EDTAM)(H_2O)_4](NO_3)_3 \cdot H_2O$ (**2**), and $[Cd(EDTAM)(NO_3)]NO_3$ (**3**) are reported: **1** monoclinic, $P2_1/c$, $a = 10.853(2)$ Å, $b = 12.893(3)$ Å, $c = 13.407(3)$ Å, $\beta = 103.28(3)°$, $Z = 4$, $R = 0.0281$; **2** triclinic, $P\bar{1}$, $a = 8.695(2)$ Å, $b = 9.960(2)$ Å, $c = 16.136(3)$ Å, $\alpha = 95.57(3)°$, $\beta = 94.84(3)°$, $\gamma = 98.72(3)°$, $Z = 2$, $R = 0.0394$; **3** monoclinic, $P2_1/c$, $a = 10.767(2)$ Å, $b = 12.952(2)$ Å, $c = 13.273(2)$ Å, $\beta = 103.572(3)°$, $Z = 4$, $R = 0.0167$. Compounds **1** and **3** are isostructural, and the EDTAM binds to the metal ion through its two N-donors and four O-donors from the amide groups. Ca(II) in **1** is 8-coordinate with a chelating NO_3^- group, while Cd(II) in **3** may possibly be 7-coordinate, with an asymmetrically coordinated NO_3^- that is best regarded as unidentate. The La(III) in **2** is coordinated to the EDTAM in a manner similar to that of **1** and **3**, but it is 10-coordinate with four water molecules coordinated to the La(III). The formation constants (log K_1) for complexes of a variety of metal ions with EDTAM are reported in 0.1 M $NaNO_3$ at 25.0 ± 0.1 °C. These are compared to the log K_1 values for en (ethylenediamine) and THPED (*N*,*N*,*N*′,*N*′-tetrakis(2-hydroxypropyl)-ethylenediamine). For large metal ions, such as Ca^{2+} or La^{3+}, log K_1 increases strongly when the four acetamide groups are added to en to give EDTAM, whereas for a small metal ion, such as Mg^{2+}, this increase is small. The log K_1 values for EDTAM compared to THPED suggest that the amide oxygen is a much stronger base than the alcoholic oxygen. Structures of binding sites in 40 Ca-binding proteins are examined. It is shown that the Ca–O═C bond angles involving coordinated amides in these sites are large, commonly being in the 150–180° range. This is discussed in terms of the idea that for purely ionic bonding the M–O═C bond angle will approach 180°, while for covalent bonding the angle should be closer to 120°. How this fact might be used by the proteins to control selectivity for different metal ions is discussed.

Introduction

The selective binding of Ca^{2+} is of importance in a wide range of proteins,[1] where it may act as a "second messenger" and control many biological processes. A few examples[1] are triggering of enzyme action, cell division, vesicle formation, apoptosis (programmed cell death), transmission of neurotransmitters across synapses, and initiation of muscle contraction. An important aspect[2] of the binding of Ca^{2+} is that it should be selective against Mg^{2+}, which is present in concentration in excess over Ca^{2+} in the cytoplasm by a factor of some 10^4. In the resting state, Ca^{2+} is held outside

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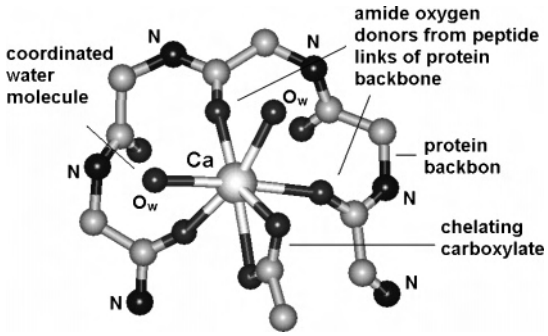

Figure 1. Typical Ca-binding site in annexin A1 (1mcx), which is a type II binding site drawn using coordinates from the reported[11] structure. Side chains of some residues have been omitted for clarity. O_w = oxygen of water molecule.

the cell, or in the cytoplasmic reticulum, and its actions are initiated by the opening of Ca-ion channels, which raises the Ca^{2+} concentration in the cytoplasm. Functions triggered by Ca^{2+} should clearly not be triggered by Mg^{2+}, already in high concentration in the cytoplasm. An examination of the structures of Ca-binding proteins in the Protein Database[3] (PDB) reveals[2] two recurring features in Ca-selective binding sites in proteins. First, in nearly all Ca-selective binding sites, there are chelating carboxylates derived from glutamate or aspartate residues, which form four-membered chelate rings with Ca (Figure 1). The interplay between chelate-ring size and metal-ion size in controlling complex stability has been known for a long time,[4−7] and its relevance for selectivity for the large Ca^{2+} ion over the small Mg^{2+} ion in Ca-selective proteins has been discussed.[2,8,9,10] In brief, the orientation of donor atoms is such that for six-membered chelate rings the lone pairs focus better on small metal ions, while for five-membered chelate rings, they focus better on medium to large metal ions, and for four-membered chelate rings, they focus best on very large metal ions. This can be summarized as[5] the "[decrease] of chelate ring size leads to a greater degree of complex stabilization for larger metal ions than for smaller metal ions". Thus, the binding sites in Ca-selective proteins have carboxylates that are oriented so that they will coordinate to metal ions and form a small four-membered chelate ring, favoring the large Ca^{2+} over the small Mg^{2+}.

The second recurring theme is that there are usually one to three amide oxygen donors coordinated to the metal ion[1]. These may be derived from peptide linkages in the protein backbone or from side chains of glutamine or asparagine residues. A Ca-binding site of annexin[11] is shown in Figure 1 for the type II binding site of annexin A1. Annexins contain[11] three different types of Ca binding sites (type II, type III, and the AB′ site), but they all have amide donors coordinated to Ca and differ in the sequences in the backbone forming the loop or in the details of the coordination sphere. Type I binding sites are the "EF-hand" sites found in proteins such as calmodulin[1]. Falke and co-workers[12−14] have carried out a series of mutagenesis studies on bacterial Ca-binding proteins aimed at delineating the role of rigidity of the binding site in generating selectivity for Ca^{2+} over Mg^{2+}. These studies suggested that rigidity of the binding site was not a major factor in controlling metal-ion selectivity, which is not surprising considering the situation of these binding sites in exposed loops on the surfaces of the proteins. Dudev and Lim[2] have carried out DFT (density functional theory) calculations aimed at analyzing the role of the amide oxygen donors in the Ca-binding site. These studies suggested that the dielectric constant within proteins might have an effect on the binding of donor groups to the metal ion.

In this work, the aim is to use ligands that can indicate the strength of the interaction of metal ions with amide donors. One wishes to answer questions such as the following: Why does the binding site involve amide oxygen donors, rather than, say, alcoholic oxygens from serine residues? Are the amide oxygens involved in generating Ca/Mg selectivity, separate from the gross geometry of the binding site? If so, what factors contribute to this selectivity? Apart from their intrinsic interest, such questions can be important in efforts to design[15] metal-ion specific binding sites into proteins that do not normally contain them. The amide donor is also of tremendous interest because peptide oxygen donors[16] bind K^+ in K^+-ion channels and the amide donor is probably[17] also present as a donor in Na^+- and Ca^{2+}-ion channels.

The metal-binding properties of the ligand EDTAM are reported here[18] (see Figure 2 for structures of ligands). Both Mg^{2+} and Ca^{2+} have low and similar affinity for the ligand en, so that any marked increases in log K_1 (formation constant) for the EDTAM ligand, which has four amide donor groups attached to an en, can be attributed to an affinity for the amide donors. In addition, the log K values of EDTAM complexes can be compared with those of THPEN, which is similar, but has alcoholic donors in place of the amide donors of EDTAM, to obtain an indication of relative strengths of amide oxygen donors compared to alcoholic oxygens. EDTAM has been used[19] in gold complexes such as $[Au(EDTAM)Cl_2]^+$ or $[Au(EDTAMH_{-1})Cl]^+$ as models of how $[AuCl_4]^-$ might interact with proteins.

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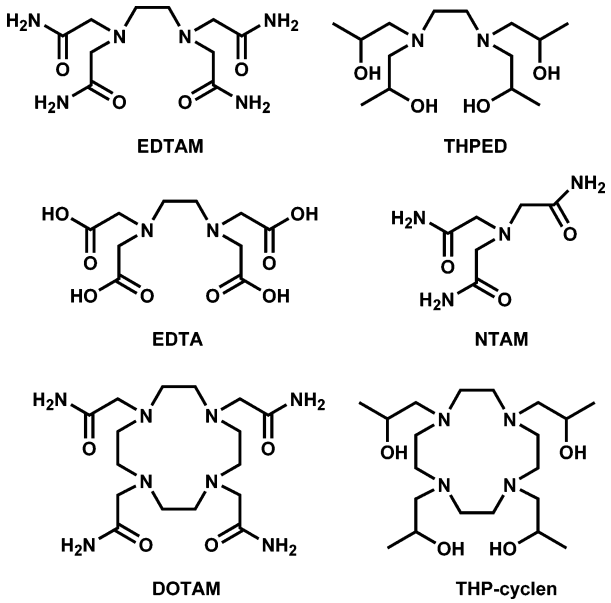

Figure 2. Ligands discussed in this paper.

There are reports[20,21] of metal-ion complexes of EDTAM, but no log K_1 values that would allow for estimation of how strongly it complexes various metal ions. We report here the protonation constant of EDTAM, its log K values with Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}, La^{3+}, Pb^{2+}, Cd^{2+}, Ni^{2+}, and Cu^{2+}, and the structures of its complexes with Ca(II), La(III), and Cd(II).

Experimental

Materials. EDTAM was synthesized following the method of Przyborowski.[22] The complexes, $[Ca(EDTAM)NO_3]NO_3$ (**1**), $[La(EDTAM)(H_2O)_4](NO_3)_3 \cdot H_2O$ (**2**), and $[Cd(EDTAM)(NO_3)]NO_3$ (**3**), were synthesized by dissolving the metal nitrate salts in water, adding 1 equiv of EDTAM, also in aqueous solution, and allowing the water to evaporate slowly in beakers covered with lightly perforated Parafilm, resulting in colorless crystals being deposited. Elemental analyses were satisfactory.

Formation Constant Determination. These were determined in jacketed cells under N_2 at 25.0 ± 0.1 °C in 0.1 M $NaNO_3$. The protonation constant of EDTAM and the formation constants of Ca^{2+}, Sr^{2+}, Ba^{2+}, Cd^{2+}, Co^{2+}, La^{3+}, and Pb^{2+} were determined by glass-electrode potentiometry by conventional methods.[23] The log K_1 value for the Mg^{2+} complex was determined from the shift of the 3.40 ppm band of the ^{1}H NMR spectrum of EDTAM in a 10:90 D_2O/H_2O mixture as a function of the Mg^{2+} concentration, in 0.1 M $NaNO_3$ to control the ionic strength. The log K_1 values for the Cu^{2+} and Cd^{2+} complexes was determined by polarography.[24] The pK_a value of the Cu(II) complex of EDTAM was determined by monitoring the band at 650 nm in the UV−vis spectrum as a function of pH. Data analysis was carried out using EXCEL.[25]

Table 1. Formation Constants Determined Here for Complexes of EDTAM (L) in 0.1 M $NaNO_3$ at 25 °C[a]

equilibrium	log K	ref
$H^+ + OH^- \leftrightarrows H_2O$	13.78	28
$L + H^+ \leftrightarrows HL^+$	4.36(2)	this work
$L + Ca^{2+} \leftrightarrows CaL^{2+}$	3.29(5)	this work
$L + Mg^{2+} \leftrightarrows MgL^{2+}$	1.6(1)[b]	this work
$L + Sr^{2+} \leftrightarrows SrL^{2+}$	2.30(5)	this work
$L + Ba^{2+} \leftrightarrows BaL^{2+}$	2.15(5)	this work
$L + Cd^{2+} \leftrightarrows CdL^{2+}$	7.40(5)	this work
$L + Pb^{2+} \leftrightarrows PbL^{2+}$	5.85(5)	this work
$L + La^{3+} \leftrightarrows LaL^{3+}$	5.19(5)	this work
$L + Co^{2+} \leftrightarrows CoL^{2+}$	6.1(1)	this work
$L + Ni^{2+} \leftrightarrows NiL^{2+}$	7.4(1)	this work
$L + Cu^{2+} \leftrightarrows CuL^{2+}$	8.8(1)	this work
$CuLH_{-1} + H^+ \leftrightarrows CuL^{2+}$	6.12(3)[c]	this work[a]

[a] The figures in parentheses after each log K value are estimated uncertainties in the last significant figure. [b] This value of log K_1 was determined using ^{1}H NMR and is to be preferred to the approximate value of 0.6 reported in the preliminary communication on this work,[18] which was obtained from glass-electrode potentiometry, which is less accurate for such small log K_1 values. [c] This refers to protonation of the deprotonated amide N (LH_{-1}), which is coordinated to the Cu above pH 6.12.

Table 2. Crystal Data and Details of Structure Refinement for $[Ca(EDTAM)NO_3]NO_3$ (**1**), $[La(EDTAM)(H_2O)_4](NO_3)_3 \cdot 1.5H_2O$ (**2**), and $[Cd(EDTAM)(NO_3)]NO_3$ (**3**)

	1	**2**	**3**
empirical formula	$C_{10}H_{20}N_8O_{10}Ca$	$C_{10}H_{31}N_9O_{18.5}La$	$C_{10}H_{20}N_8O_{10}Cd$
M	452.42	704.28	524.74
T (K)	173(2)	173(2)	173(2)
cryst syst	monoclinic	triclinic	monoclinic
space group (No.)	$P2_1/c$ (14)	$P\bar{1}$ (2)	$P2_1/c$ (14)
unit cell (Å)			
a	10.853(2)	8.695(2)	10.767(2)
b	12.893(3)	9.960(2)	12.952(2)
c	13.407(3)	16.136(3)	13.273(2)
α (deg)	90	95.57(3)	90
β (deg)	103.28(3)	94.84(3)	103.572(3)
γ (deg)	90	98.72(3)	90
V (Å^3)	1825.9(6)	1367.6(5)	1799.2(5)
Z	4	2	4
ρ_{calcd} (g cm^{-3})	1.646	1.710	1.937
radiation (λ (Å))	0.71073	0.71073	0.71073
μ (mm^{-1})	0.416	1.654	1.287
$[I > 2\sigma(I)]$ R1[a]	0.0281	0.0394	0.0167
wR2[b]	0.1133	0.1091	0.0426

[a] $R1 = \sum||F_o| - |F_c||/\sum|F_o|$. [b] $wR2 = \{\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]\}^{1/2}$.

Formation constants determined here for EDTAM complexes are given in Table 1.

Molecular Structure Determination. Rigaku Mercury (structures of **1** and **2**) and Bruker SMART 1K (structure of **3**) diffractometers, using the ω-scan mode were employed for crystal screening, unit cell determination, and data collection. The structures were solved by direct methods, and refined to convergence.[26] Some details of the structure determinations are given in Table 2, and the crystal coordinates and details of the structure determinations of **1**, **2**, and **3** have been deposited with the CSD (Cambridge Structural Database).[27] A selection of bond lengths and angles for complexes **1, 2**, and **3** are given in Tables 3, 4, and 5.

Results and Discussion

The log K_1 values for complexes of EDTAM measured here, and reported in Table 1, show that with many metal

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Table 3. Significant Bond Lengths (Å) and Angles (deg) for **1** ([Ca(EDTAM)NO_3]NO_3)

Ca(1)−O(19)	2.3680(11)	Ca(1)−O(17)	2.3830(11)
Ca(1)−O(24)	2.4387(12)	Ca(1)−O(20)	2.4388(12)
Ca(1)−O(18)	2.4417(11)	Ca(1)−O(22)	2.5352(12)
Ca(1)−N(7)	2.5443(14)	Ca(1)−N(4)	2.5901(13)
O(19)−Ca(1)−O(17)	100.80(4)	O(19)−Ca(1)−O(24)	83.82(4)
O(19)−Ca(1)−O(20)	77.75(4)	O(17)−Ca(1)−O(20)	170.52(4)
O(19)−Ca(1)−O(18)	154.05(4)	N(4)−Ca−N(7)	70.10(4)
O(20)−Ca(1)−N(7)	67.04(4)	O(18)−Ca(1)−N(7)	65.97(4)
O(19)−Ca(1)−N(4)	66.68(4)	O(17)−Ca(1)−N(4)	65.81(4)
Ca(1)−O(17)−C(2)	120.48(9)	Ca(1)−O(18)−C(9)	117.10(9)
Ca(1)−O(19)−C(12)	122.03(9)	Ca(1)−O(20)−C(15)	118.70(9)

Table 4. Significant Bond Lengths (Å) and Angles (deg) for **2** ([La(EDTAM)$(H_2O)_4$]$(NO_3)_3$·1.5H_2O)

La(1)−O(3)	2.520(3)	La(1)−O(8)	2.542(3)	La(1)−O(2)	2.545(3)
La(1)−O(4)	2.579(3)	La(1)−O(1)	2.583(4)	La(1)−O(5)	2.586(4)
La(1)−O(7)	2.586(4)	La(1)−O(6)	2.602(4)	La(1)−N(4)	2.838(4)
La(1)−N(2)	2.877(4)				
O(3)−La(1)−O(8)	78.73(11)	O(3)−La(1)−O(1)	71.97(11)		
O(2)−La(1)−O(1)	102.58(12)	O(4)−La(1)−O(1)	167.01(12)		
O(8)−La(1)−O(5)	75.09(12)	O(2)−La(1)−O(5)	69.83(13)		
O(1)−La(1)−O(5)	65.69(12)	O(8)−La(1)−O(7)	68.56(12)		
O(4)−La(1)−O(7)	65.83(11)	O(5)−La(1)−O(7)	129.65(12)		
O(8)−La(1)−O(6)	79.40(13)	O(2)−La(1)−O(6)	84.06(13)		
O(5)−La(1)−O(6)	66.59(12)	O(3)−La(1)−N(4)	60.21(11)		
O(4)−La(1)−N(4)	59.87(11)	O(2)−La(1)−N(2)	59.25(11)		
O(1)−La(1)−N(2)	59.06(12)	N(4)−La(1)−N(2)	62.28(12)		
C(1)−O(1)−La(1)	127.2(3)	C(4)−O(2)−La(1)	122.0(3)		
C(8)−O(3)−La(1)	121.7(3)	C(10)−O(4)−La(1)	126.8(3)		

Table 5. Significant Bond Lengths (Å) and Angles (deg) for **3** ([Cd(EDTAM)NO_3]NO_3)

Cd(1)−O(10)	2.3193(14)	Cd(1)−O(3)	2.3390(14)
Cd(1)−O(4)	2.3511(14)	Cd(1)−O(2)	2.44496(14)
Cd(1)−O(1)	2.4795(15)	Cd(1)−O(9)	2.7167(12)
Cd(1)−N(1)	2.3798(16)	Cd(1)−N(4)	2.4444(16)
O(1)−Cd(1)−O(2)	108.65(5)	O(1)−Cd(1)−O(4)	161.26(5)
O(2)−Cd(1)−O(4)	77.05(5)	O(3)−Cd(1)−O(4)	104.20(5)
O(1)−Cd(1)−O(10)	80.55(5)	O(9)−Cd(1)−O(10)	50.19(5)
O(4)−Cd(1)−N(4)	69.23(5)	N(1)−Cd(1)−N(4)	75.08(5)
O(1)−Cd(1)−N(1)	69.46(5)	O(2)−Cd(1)−N(1)	68.97(5)
Cd(1)−O(1)−C(2)	113.44(13)	Cd(1)−O(2)−C(4)	112.90(12)
Cd(1)−O(3)−C(8)	117.85(12)	Cd(1)−O(4)−C(10)	116.56(12)

ions, the presence of the amide groups greatly enhances complex stability. This can be seen in Table 6, where the log K_1 values[28] for complexes of a selection of metal ions with en, EDTAM, and THPED are reported, together with the ionic radii[29] (r^+) for the metal ions. A pattern that is apparent is that large metal ions (those with[9] $r^+ \geq 1.0$ Å) such as Ca^{2+} or La^{3+} show moderate increases in log K_1 on addition of donor groups with alcoholic oxygen donors to en to give THPED and much larger increases in log K_1 on passing to the EDTAM complexes. The greater stability of the EDTAM as compared to the THPED complexes supports the general contention that amide donors are stronger bases than alcoholic (and also ether) oxygen donors. One notes that Ca^{2+} experiences a stabilization of about three log units in passing from the en complex to the EDTAM complex, suggesting that a contribution of a little under a log unit is contributed to log K_1 per amide group present. The very positive effect that amide donors have on the binding of Ca^{2+} suggests a reason for their presence in the binding sites of Ca-binding proteins, rather than alternatives such as alcoholic oxygens from serine residues. The selectivity of Ca^{2+} over Mg^{2+} for EDTAM also bears commenting on, although the origin of this may be more complex than a simple preference for amide donors on the part of Ca^{2+}, as discussed below. The high affinity of La^{3+} for EDTAM is also of interest in that lanthanide(III) ions have high affinity[12−14] for the binding sites of Ca-selective proteins and are used as probes of binding to these sites. A general pattern present in Table 6 is that large metal ions show strong increases in log K_1 on passing from the en to the EDTAM complex. This would include Ca^{2+}, Sr^{2+}, Ba^{2+}, La^{3+}, and also the borderline Cd^{2+} ($r^+ = 0.95$ Å[29]). The large Pb^{2+} ion ($r^+ = 1.19$ Å[29]) shows an increase in log K_1 in passing from the en to the EDTAM complex, although it is a little surprising that the EDTAM complex is not more stable than the THPED complex. This may be caused by the lower basicity of the N donors of EDTAM than THPED, as evidenced by the respective protonation constants (pK) of 4.36 and 8.67.[28] Nitrogen basicity will be much less important for metal ions such as Ca^{2+} and La^{3+} that have very little affinity for nitrogen donors in the first place.[9] An additional factor for Pb^{2+} may be the importance of the steric effects of the lone pair, which lead typically to short strong bonds to the N donors, and long weak bonds to the oxygen donors, which are nearer to the lone pair.[31] The contributions of the amide oxygens to the stability of the Pb(II) complex of EDTAM may be diminished by proximity to the lone pair. Evidence for this is seen in the structure[21] of the Pb/EDTAM complex, where the two Pb−N bonds are fairly short, averaging 2.61 Å. In contrast, the Pb−O bonds to the amide donors of EDTAM are as long as 2.69 Å, whereas typically M−O bonds are somewhat shorter than M−N bonds.[31] The Pb−O bond to the coordinated nitrate, which is over the site that appears to be where the lone pair is situated, is very long, at 3.08 Å. This type of distortion of the coordination geometry of Pb(II) is typical[31,32] of the presence of a stereochemically active lone pair and is shown schematically for the Pb(II)/EDTAM complex in Scheme 1.

The small metal ions ($r^+ < 0.8$ Å) Cu^{2+}, Ni^{2+}, Co^{2+}, and Mg^{2+} all show negligible to small increases in log K_1 on passing from the en to the EDTAM complex, and Cu(II) actually shows a decrease. There may be a component to this of the low basicity of the N donors noted above, since Mg^{2+}, which has a low affinity[9,28] for N donors, has a better affinity for EDTAM compared to en than the other small metal ions. In contrast, Cu(II) has by far the highest affinity for N donors of all the metal ions considered here, and it is probably the low basicity of the N donors of EDTAM that leads to a decrease in log K_1 for the EDTAM complex of

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Table 6. log K_1 Values for en, EDTAM, and THPED Complexes of a Selection of Metal Ions, Together with Their Ionic Radii

	Ca^{2+}	Mg^{2+}	Sr^{2+}	Ba^{2+}	La^{3+}	Cd^{2+}	Pb^{2+}	Co^{2+}	Ni^{2+}	Cu^{2+}
r^+ (Å)[a]	1.0	0.74	1.19	1.34	1.03	0.95	1.18	0.74	0.67	0.57
log K_1										
en	0.11	0.37	~0	~0	(1.4)[b]	5.4	1.6	5.5	7.30	10.49
THPED	1.63	~0.3	0.8	~0	2.90	7.98	7.51	6.1	7.45	9.78
EDTAM	3.29	1.6	2.30	2.15	5.19	7.4	5.85	6.1	7.4	8.8

[a] Ionic radius for C.N. = 6, except Cu(II) for which C.N. = 4, from ref 29. [b] Estimated in ref 30.

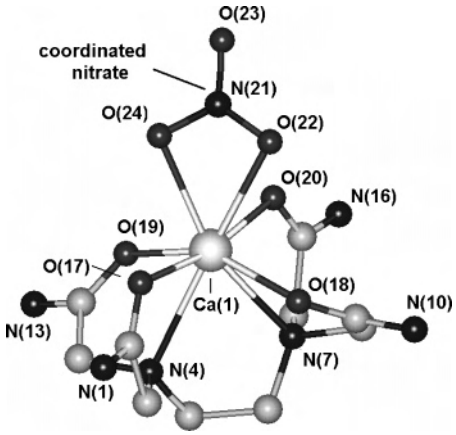
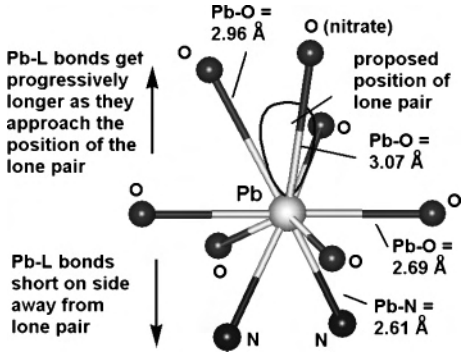


Figure 3. Structure of the $[Ca(EDTAM)NO_3]^+$ complex cation of **1**, showing the numbering scheme of the donor atoms coordinated to the Ca. H atoms omitted for clarity.

Scheme 1. Coordination Sphere of $[Pb(EDTAM)(NO_3)_2]$[21]



Cu(II) as compared to that for the en complex. The same fact should also contribute to the weakening of the Co(II) and Ni(II) complexes of EDTAM. However, it seems likely that factors associated with coordination of amides to small metal ions are also involved, as discussed below.

Structures of the EDTAM Complexes. These are seen in Figures 3−5. The Ca and Cd structures appear to be isostructural, and each structure has a coordinated nitrate. The details of the structures do, however, appear to differ. For the Ca complex, the coordination of the nitrate is fairly symmetric, with Ca−O bonds of 2.439 and 2.535 Å, so that this might best be regarded as chelating, with the Ca having a C.N. of 8. In contrast, the Cd−O bonds to the coordinated nitrate are less symmetric, at 2.319 and 2.717 Å, so that it is possibly better to regard the nitrate as unidentate, with a C.N. of 7 for the Cd. These results would then agree with the rather similar structures of the EDTA complexes, where the Ca(II) has C.N. = 8[33] and Cd(II) has C.N. = 7,[34] with waters

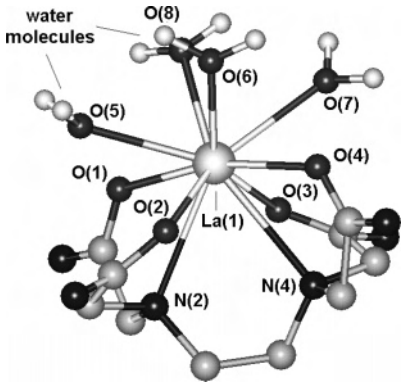


Figure 4. Structure of the complex cation $[La(EDTAM)(H_2O)_4]^{3+}$ from **2**, showing the numbering scheme of the donor atoms coordinated to La. H atoms (except on coordinated waters) omitted for clarity.

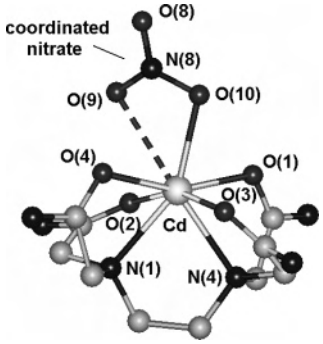


Figure 5. Complex cation $[Cd(EDTAM)NO_3]^+$ from **3**, showing the numbering scheme of atoms coordinated to the Cd. The broken line between the Cd atom and O(9) suggests that this rather long contact may not be a bond, and as discussed in the text, the coordinated nitrate may be effectively unidentate. H atoms omitted for clarity.

completing the coordination sphere in both cases. The distortion of the Pb(II) complex because of the lone pair (Scheme 1) is shown by the comparison of this structure[21] with the structures of the EDTAM complexes reported here. Thus, for Ca^{2+}, the Ca−N bonds average 2.57 Å, longer than the average Ca−O bonds to the amide O-donors of EDTAM, which average 2.408 Å. Similarly, the Cd−N bonds average 2.41 Å, with the Cd−O bonds to the amide O-donors averaging 2.40 Å. For La^{3+}, the La−N bonds average 2.858 Å, while the La−O bonds to the amides of EDTAM average only 2.404 Å. One notes here that the M−N bond lengths are much longer compared to the M−O bond lengths for the "hard" (in the HSAB sense of Pearson[35]) metal ions Ca^{2+} and La^{3+}. By comparison, the "soft" Cd^{2+} ion shows a much smaller difference between the M−N and M−O bond

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lengths. It may be possible in the future to use such bond length differences to draw up a scale of hardness of metal ions.

The La(III)/EDTAM complex has a C.N. of 10 (Figure 4), with four waters coordinated to the La(III). This is higher than the C.N. of 9 in the La(III)/EDTA complex.[36] It seems possible that the higher C.N. of La(III) in its EDTAM complex compared to its EDTA analogue reflects the lesser reduction in positive charge on the La(III) caused by the neutral EDTAM ligand as compared to the negatively charged EDTA. This ability to bind more waters in the La(III)/EDTAM complex as compared to the La(III)/EDTA complex may point to the reason for the differences in numbers of charged groups present in different Ca-binding proteins. Some Ca-binding sites, such as annexins, have[1] mainly neutral amide donors, whereas other sites, such as calmodulins, have several negatively charged carboxylates and only one or two neutral amide donors. The action of annexins appears to involve binding of the Ca^{2+} to phospholipid groups on the surface of lipid bilayers; therefore, a reduced number of charged carboxylates bound to the Ca^{2+} may promote this process. In contrast, the action of calmodulin does not involve binding of external donors to the Ca^{2+}, so that having several charged groups bound to the Ca^{2+} is not a problem. The present structure adds to the growing number of structures of La(III) complexes with neutral saturated amine ligands already known, of which some recent examples are given.[37−40]

An important factor in controlling ligand selectivity for metal ions is the directionality[41] of the M−L bonds, by which is meant the M−L-C bond angles (L= ligand donor atom). In approaches to ligand design, too often total emphasis is placed on obtaining appropriate M−L bond lengths, without paying attention to the correct orientation of the donor atoms, as would be evidenced by correct M−L-C bond angles. The role of correct M−L-C bond angles has already been noted[4−7] as a cause of the chelate ring-size preferences of metal ions as a function of size. Hay et al.[42] have, in a MM (molecular mechanics) study of amide complexes, obtained ideal M−O═C bond angles for a variety of metal ions coordinated to amide oxygens. Thus, we may note that the observed M−O═C bond angles in EDTAM complexes are somewhat smaller than the ideal angles (Table 7), suggesting a certain amount of steric strain in coordinating the amide groups to the metal ions. This raises the question of how well the amides in Ca-selective proteins coordinate to Ca^{2+}, as far as Ca−O═C bond angles involving amide O-donors are concerned. General inspection of Ca^{2+} in binding sites in proteins suggests that the Ca−O═C bond angles involving amide groups from the protein backbone are rather larger than the ideal angles noted above. A survey of 40 representative structures of Ca-binding proteins in the PDB[3], which involve 160 Ca−O═C angles from backbone amides, shows that these are generally rather different from the ideal Ca−O═C angles obtained by Hay et al.[42] The ranges of Ca−O═C bond angles for the Ca-binding proteins are shown in Figure 6. Figure 6 suggests that a majority of Ca−O═C amide donor bond angles in Ca-binding proteins tend to be much larger than the ideal angle of 140°, most falling in the range 150−180°. This fact suggests the possibility that Ca−O═C bond angles involving amide O donors from the protein backbone in the binding sites of Ca-binding proteins achieve Ca/Mg selectivity via the enforcement of very large M−O═C angles on metals that bind to the proteins. Two aspects could be important here. In the first place, the force constants obtained for the Mg−O═C bond angles are[42] nearly twice as large as those for the Ca−O═C bond angles,[42] which fact alone would produce more steric strain for Mg than Ca in the binding site of the protein. The second point is that one has to consider the nature of bonding of metal ions to the oxygen donor of the amide. For more covalent M−O bonding, the M−O═C angle will[42] be close to 120°, the angle expected from a simple VSEPR approach[43] to the bonding. For an ionic approach to the bonding, the M−O═C angle

Table 7. Ideal M−O═C Bond Angles from MM Studies[42] and Observed M−O═C Angles in EDTAM Complexes (this work)

	Ca^{2+}	Cd^{2+}	La^{3+}	Mg^{2+}
ideal M−O═C angle (deg)[42]	140.5	128.6	144.8	135.1
mean M−O═C angle (deg)	119.6	115.5	124.4	(118.6)[a]

[a] Calculated for $[Mg(EDTAM)(H_2O)]^{2+}$ using the default MM force field in HyperChem.[44]

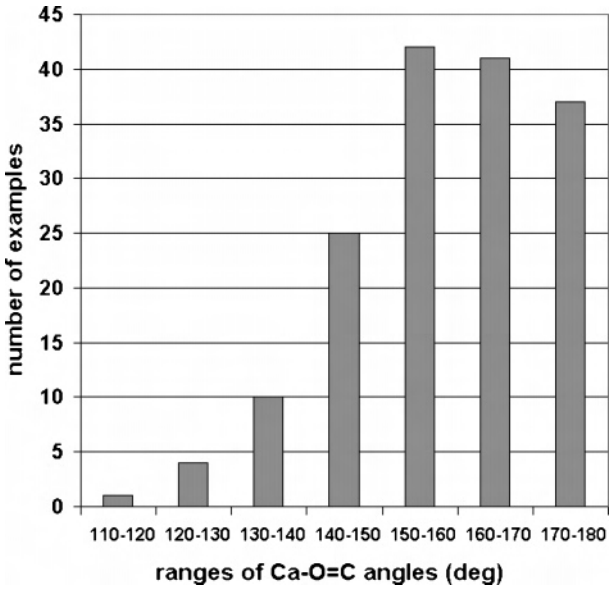

Figure 6. Ranges of Ca−O═C bond angles for coordinated amides derived from the protein backbone, in structures of forty Ca-binding proteins in the PDB.[3] PDB codes for structures used in constructing this chart are 1smd, 1acc, 1alv, 1aui, 1be8, 1a4v, 1b8r, 1e8a, 5chy, 3icb, 1amy, 1ala, 1g4y, 1ctr, 1mcx, 3cln, 1mxe, 1a2t, 1a25, 1bmo, 1dan, 1daq, 1djy, 1dvi, 1dx5, 1edm, 1hqv, 1tcf, 1pex, 2bbm, 2pf2, 3pal, 8tln, 966c, 1aru, 1boi, 1bfd, 9cgt, 1ajj, and 1a2q.

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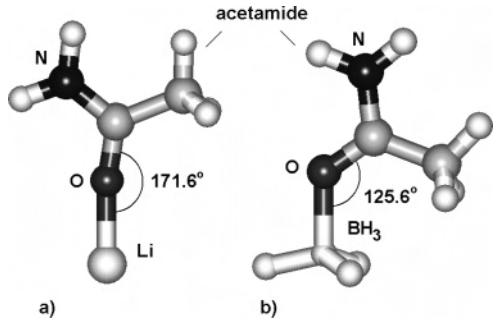

Figure 7. Examples of (a) ionic bonding of acetamide to the metal ion Li^+ and (b) covalent bonding of acetamide to BH_3. Structures generated using HyperGauss function in HyperChem[44] with the 6-31G** basis set.[45]

should be closer to 180°, possibly[43] 154°, although structures examined here suggest a larger value for the most ionic metal ions. One can illustrate these two bonding extremes in Figure 7, where the structures of the highly ionic [Li(acetamide)]$^+$ cation and highly covalent [BH_3·acetamide] complex, calculated using the HyperGauss module of HyperChem[44] at the 6-31G** level,[45] are shown. In support of these calculations, which suggest that with very covalent M−O bonding the M−O═C angle should be small, the M−O═C angles in complexes of the very soft[35] (≈ covalent) metal ions Pd(II)[46] and Hg(II)[47] with unidentate amide ligands are 122.5° and 108.5°, respectively. In contrast, as noted below, with the ionically bound Ca(II), the Ca−O═C angles in unidentate acetamide-like complexes average 161.5°.

In line with the idea that M−O═C angles to amide O-donors might be involved in selectivity patterns in Ca-binding proteins, a search of the CSD[27] for Ca complexes involving amide ligands with the unidentate C−C═O−NH fragment coordinated to Ca via the amide oxygen reveals six structures (CSD codes: DURSEO, NMaliD, QESGEA, QOGGEY, VEBGUE, ZOWKIF) that have Ca−O═C bond angles of 161.5 ± 4.5°. By contrast, a similar search for Mg^{2+} reveals 4 structures (DURSAK, MGSACA10, NMaliE, YEMNOT) with Mg−O═C bond angles averaging 146.2 ± 4.9°. These results suggest that Ca(II) may prefer, or at least better tolerate, larger M−O═C bond angles than does Mg(II). It may therefore be able to better tolerate the very large M−O═C bond angles found in binding sites of Ca-binding proteins than Mg(II). One anomaly here is that a search of dimethyl formamide complexes[43] yields somewhat smaller Ca−O═C bond angles. At this point, it seems possible that the large M−O═C bond angles involving coordination to amide O donors, produced by Ca-binding sites, are involved in generating selectivity for Ca(II) over Mg(II). This is the subject of ongoing DFT calculations aimed at resolving the role of M−O═C bond angles in generating metal-ion selectivity in Ca-selective proteins. It should be noted that amide donors bound to Ca^{2+}, derived from glutamine or asparagine residues, are less common than those derived from the protein backbone. In line with the much more flexible situation of these amide donor groups, derived as they are from side chains, the Ca−O═C bond angles are smaller than those involving donors from the protein backbone and give an average Ca−O═C angle of 144°.

A referee has made the helpful suggestion that structures of Ca-binding proteins with Mg in the binding site might be informative. Four such structures were found in the PDB. These showed that the Mg−O═C bond angles involving amide carbonyl groups from the protein backbone were generally large, 153.1 and 158.9° for PDB codes 1B8C (parvalbumin), 161.2° for 4PAL (parvalbumin), 147.1 and 168.4° for 1SBJ (troponin C), and 161.2° for 1IG5 (calbindin). In addition, there is a Mg−O═C angle of 118.2° to the amide side-chain of an asparagine residue in 1SBJ1. A recurring feature in these structures is that the carboxylates coordinated to the Mg(II) are not chelating. These structural features support the idea[8] that Ca(II) selectivity over Mg(II) involves the ability of Ca(II) to form stable 4-membered chelate rings. The fact that the Mg−O═C angles to backbone peptide carbonyls are large can be interpreted as being the result of the protein backbone forcing large M−O═C angles on any metal that binds in the Ca-selective binding site, which Mg(II) is less able to tolerate than Ca(II). It is interesting that the Mg−O═C angle to the side-chain amide donor from the asparagines residue is small in 1SBJ1. This can be interpreted as the Mg−O═C angles being much smaller when not constrained out to a larger value by the protein backbone.

A further important point is the ability, or lack of ability, of the amide group to transfer charge from the metal ion to the solvent. In the case of aquo ions, transfer of charge to the solvent involves the metal ion inducing large positive charges on the H atoms of inner-sphere water molecules. These inner sphere waters in turn H bond to outer-sphere water molecules which have increased, but by a smaller amount, positive charges on their H atoms, until in successive layers of water the charge distributions on the waters is that of bulk water. For metal ions of equal charges, this would be more important for small metal ions than for large metal ions, since small metal ions are more strongly solvated. In the binding site of Ca-selective proteins, contact with the solvent via H bonding is much less than that for a nonbound metal ion. The small Mg(II) ion would be less able to tolerate this lack of H bonding with the solvent. Thus, a relatively poor ability of coordinated amide groups to transfer charge (i.e., H bond) to the solvent might be involved in the preference of larger metal ions for coordination to amide groups seen in EDTAM and in Ca-selective binding sites in proteins.

Conclusions. (1) The amide donors of EDTAM bind strongly to large metal ions such as Ca^{2+} or La^{3+}, as evidenced by large increases in log K_1 for the EDTAM complexes relative to the en complexes. This resembles the metal-ion affinity patterns of binding sites in Ca-selective proteins for large metal ions, where amide oxygen donors are present. Referees have questioned whether one can extend this observation to the preferences of the binding sites of

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Ca-binding proteins for large metal ions. The main objection is the very different geometries of the EDTAM complexes compared to those of the Ca-binding sites in proteins, particularly with regard to the M−O═C angles involving amides. Certainly, this is a reasonable observation, and the factors governing the selectivity of protein binding sites for metal ions are bound to be complex. However, we would argue that the resemblance of the selectivity pattern of log K_1 for EDTAM complexes to the metal-ion affinities of Ca-binding sites in proteins suggests that at least some common factors are operating. The most important one, which is demonstrated by this study of EDTAM complexes, is that amide donors are much stronger donors than alcoholic donors or water itself. The same conclusion can be drawn from an earlier study[48] of DOTAM as compared to THE-cyclen, where the amide donors of DOTAM produce a similar increase in complex stability for large metal ions as compared to the alcoholic oxygen donors of THE-cyclen. In addition, studies in the gas-phase have shown[49] that amide donors are stronger bases toward metal ions than are alcoholic or ethereal oxygen donors. (2) The selectivity of Ca over Mg in Ca-binding proteins may involve the enforcement of large M−O═C bond angles involving the amide O donor, which Ca(II) may be better able to tolerate than Mg(II). In relation to referees queries about the appropriateness of comparing EDTAM to Ca-binding sites in proteins, the selectivity of EDTAM and of these binding sites toward large metal ions such as Ca^{2+} or La^{3+} may be, at least partly, due to the greater tolerance of these more ionically bound metal ions to the distortion of the M−O═C bond angles of the amide donors. In the case of EDTAM complexes, the distortion is toward M−O═C bond angles that are smaller than ideal, while in the protein-binding site the distortion is toward M−O═C bond angles that are too large.

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Supporting Information Available: Crystallographic data files in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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