# Calcium Ion Coordination: A Comparison with That of Beryllium, Magnesium, and Zinc

**Amy Kaufman Katz,[†] Jenny P. Glusker,*[,†] Scott A. Beebe,[‡] and Charles W. Bock[†,‡]**

*Contribution from the Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111, and Philadelphia College of Textiles and Science, Philadelphia, Pennsylvania 19144*

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**Abstract:** The coordination geometry of divalent calcium ions has been investigated by analyses of the crystal structures of small molecules containing this cation that are found in the Cambridge Structural Database, protein crystal structures in the Protein Databank, and by *ab initio* molecular orbital calculations on hydrated structures of the form $Ca[H_2O]_n^{2+}\cdot mH_2O$, in which there are *n* water molecules in the first coordination shell and *m* water molecules in the second coordination shell (hydrogen bonded to water molecules in the first shell). Calcium ions in crystal structures generally bind to oxygen atoms in ligands (rather than any other element), and their preferred coordination numbers range from 6 to 8. In protein crystal structures the tendency of calcium to bind water molecules is less than for magnesium (1.5 versus 2.2 water molecules on the average per metal ion site, respectively). The ratio of bidentate to monodentate binding of calcium ions to carboxylate groups is similar for small molecules and protein structures in that no bidentate binding occurs if the coordination number of $Ca^{2+}$ is 6, but its occurrence rises to near 20% for coordination numbers 7 and 8. Complexes of the form $Ca[H_2O]_5^{2+}\cdot H_2O$ and $Ca[H_2O]_4^{2+}\cdot 2H_2O$ were found (by *ab initio* molecular orbital calculations *in vacuo*) to be significantly higher in energy than $Ca[H_2O]_6^{2+}$ (by 8.2 and 15.0 kcal/mol, respectively). For $Ca^{2+}$ surrounded by seven or eight water molecules, the differences in energy between $Ca[H_2O]_6^{2+}\cdot H_2O$ and $Ca[H_2O]_7^{2+}$ and among $Ca[H_2O]_6^{2+}\cdot 2H_2O$, $Ca[H_2O]_7^{2+}\cdot H_2O$, and $Ca[H_2O]_8^{2+}$ are extremely small when diffuse functions are included in the basis set. Thus, the net energy penalty for changing the number of water molecules in the first coordination shell between 6 and 8 is small. Molecular orbital calculations also indicate that the effect of a calcium ion on the H−O−H angle to bound water is less (at normal coordination numbers) than that of magnesium, zinc, or beryllium.

## Introduction

Approximately one-third of all proteins require a metal ion for their structure and/or function. Therefore an understanding of the chemical consequences of metal binding is essential to a correct derivation of the mechanism of action of a metalloenzyme (which is often only active if a specific metal ion is bound to it).[1] Some of the factors that contribute to stereoselectivity in metal-binding sites are addressed here, with emphasis on calcium-binding sites, and comparisons with other divalent metal ion-binding sites, such as those involving magnesium.

Calcium, with an ionic radius of 0.99 Å, is the fifth most abundant element on earth.[2,3] It is used to build bone structure in mammals by virtue of the insolubility and the mechanical strength of hydroxyapatite, $Ca_5(PO_4)_3OH$. If calcium is depleted in the body, bones will serve as a necessary source of the ion, thereby becoming brittle. Calcium ions also serve important functions in blood clotting, and by acting as a "second messenger" in signal transduction, in the triggering of muscle contractions, and in the transmissions of nerve impulses.[4,5] In order to maintain the correct concentrations of calcium ions in the extracellular space (∼1.2 mM) and in the cytosol (∼0.1 μM), the body uses calcium pumps. The system is, as a result, able to respond appropriately to signals that occur in the form of sudden changes in the calcium ion concentration. Nature has therefore had to devise specific and selective calcium-binding motifs in proteins that do not interfere with the mineralization of bone, and which are correctly sensitive to these types of biological signals. Such binding motifs in proteins have to selectively bind calcium where and to the extent needed and control the release of the calcium ion when conditions so require. For example, the binding of calcium to the protein calmodulin causes a conformational change that results in the activation of a kinase that phosphorylates glycogen phosphorylase and causes glycogen breakdown to give energy for muscle contraction.[6,7] The binding site in calmodulin is, however, engineered so that calcium can be eliminated when no longer needed. These biochemical reactions occur in response to details in the geometry of the calcium-binding sites.

Three-dimensional coordinates for many crystal structures of proteins are available in the Protein Databank (PDB).[8] In this database the majority of calcium-binding sites in proteins consist of either six or seven metal-bound oxygen atoms, occasionally eight. For example, in the crystal structure of the C-type mannose-binding protein complexed with an oligosaccharide,

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* Corresponding author: Jenny P. Glusker, The Institute for Cancer Research, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111. Tel: 215-728-2220. FAX: 215-728-2863. Internet: jp_glusker@fccc.edu.

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[‡] Philadelphia College of Textiles and Science.

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there are calcium sites with coordination number 7 and with coordination number 8 (including substrate).[9] The calcium binding site in calmodulin involves seven oxygen atoms at the vertices of a pentagonal bipyramid, each ~2.4 Å from the central $Ca^{2+}$ ion; this particular calcium-binding motif is called an EF hand.[10] Not all seven of the oxygen ligands in an EF hand protein are on protein side chains; main-chain carbonyl oxygen atoms and water molecules also take part. In blood clotting, an even stronger calcium chelator is required and, therefore, calcium binding sites involve $\gamma$-carboxyglutamate, which has an additional carboxylate group and hence binds calcium more firmly than do the simpler glutamate or aspartate residues.[11]

In recent studies[12−14] we have examined the nature of metal-ion-binding sites by use of the database of crystal structures of small molecules—the Cambridge Structural Database (CSD)—in conjunction with *ab initio* molecular orbital calculations. We studied the stereochemistry of ligand binding by divalent beryllium, magnesium, and zinc ions and the manner in which these cations bind water. In the case of $Be^{2+}$, with an ionic radius of only 0.34 Å, *ab initio* calculations at several computational levels showed that $Be[H_2O]_4{}^{2+}\cdot 2H_2O$ is at least 12 kcal/mol lower in energy than two different forms of $Be[H_2O]_3{}^{2+}\cdot 3H_2O$, and some 22 kcal/mol lower in energy than $Be[H_2O]_6{}^{2+}$, showing that divalent beryllium ions surrounded by water molecules prefer a tetrahedral environment.[12] Sánchez Marcos and co-workers,[15] using RHF/3-21G calculations, have also shown that $Be[H_2O]_4{}^{2+}$ is preferred to $Be[H_2O]_6{}^{2+}$ in the presence of a solvent reaction field that simulates surrounding bulk water. Comparable *ab initio* calculations on $Mg^{2+}$ show that $Mg[H_2O]_5{}^{2+}\cdot H_2O$, $Mg[H_2O]_4{}^{2+}\cdot 2H_2O$, and $Mg[H_2O]_3{}^{2+}\cdot 3H_2O$, respectively, are approximately 4, 9, and 34 kcal/mol higher in energy than $Mg[H_2O]_6{}^{2+}$. This demonstrates that the optimal binding geometry for a divalent magnesium ion surrounded by water molecules is octahedral and that a significant energy penalty is incurred for altering this environment.[13] Corresponding calculations with zinc show that $Zn[H_2O]_4{}^{2+}\cdot 2H_2O$, $Zn[H_2O]_5{}^{2+}\cdot H_2O$, and $Zn[H_2O]_6{}^{2+}$ differ in energy by less than 1 kcal/mol, suggesting a relatively low energy penalty for altering the immediate environment of divalent zinc cations between coordination numbers 4, 5, and 6.[14] Similar calculations on $Zn[NH_3]_4{}^{2+}\cdot 2NH_3$, $Zn[NH_3]_5{}^{2+}\cdot NH_3$, and $Zn[NH_3]_6{}^{2+}$ also show very small energy differences between these structures.[16] This highlights a major difference between the chemistry of magnesium and zinc ions.

Analyses of calcium−water and calcium−carboxylate interactions in crystal structures have been made by Einspahr and Bugg.[17,18] They found that calcium ions generally lie in the plane of the carboxylate group. We report here the results of an investigation of the coordination geometry of divalent calcium cations by (a) analyses of the crystal structures of small molecules in the CSD that contain $Ca^{2+}$ (now containing many more entries than were present when Einspahr and Bugg carried out their analyses), (b) *ab initio* molecular orbital calculations on a selection of hydrated structures of the form $Ca[H_2O]_n{}^{2+}\cdot mH_2O$ (see Figure 1), and (c) analyses of binding sites of calcium-containing proteins in the PDB.[8] We specifically investigated the manner in which water molecules are arranged around divalent calcium ions, the energy differences between comparable gas-phase clusters, e.g. $Ca[H_2O]_5{}^{2+}\cdot 2H_2O$, $Ca[H_2O]_6{}^{2+}\cdot H_2O$, and $Ca[H_2O]_7{}^{2+}$, and the relationship between water molecules in the first and second coordination shells. The importance of understanding aquated metal ion systems in solution chemistry and bioinorganic chemistry has initiated many recent computational studies of metal cation−water clusters at a variety of calculational levels.[19−28] The results of such studies can provide a guide to the best potential fittings for large-scale molecular dynamics and Monte Carlo simulations of metal-cation hydrations.[29−33]

## Methods

**A. Structural Analyses.** The three-dimensional data on crystal structure determinations contained in the CSD were used as a basis for our structural analyses.[34] This database was searched for all published crystal structures containing divalent calcium ions by use of the program QUEST, and a master file was created of the compounds found in this search. We limited our search to calcium bound to the elements O, N, Cl, Br, and/or S because we were interested in crystal structures relevant to calcium−protein and calcium−nucleic acid interactions in an aqueous environment (Table 1). As a result, 21 entries involving calcium bound to a variety of other chemical elements were eliminated from this analysis (listed in Table 1), and 39 crystal structures (listed in deposited Table 1S) were also eliminated because of disorder or high crystallographic *R* factors (greater than 0.10).

The master file of these crystallographic parameters obtained from the CSD was then broken down into smaller files, each containing a calcium ion with a specific coordination number from 3 to 10. Each of these coordination-number files was studied separately. For example, starting with the file of calcium ions with a coordination number of 6, the program GSTAT was used to extract information from the calcium master file on the types of ligand atoms in first coordination sphere of the calcium ion. This was repeated for other coordination numbers

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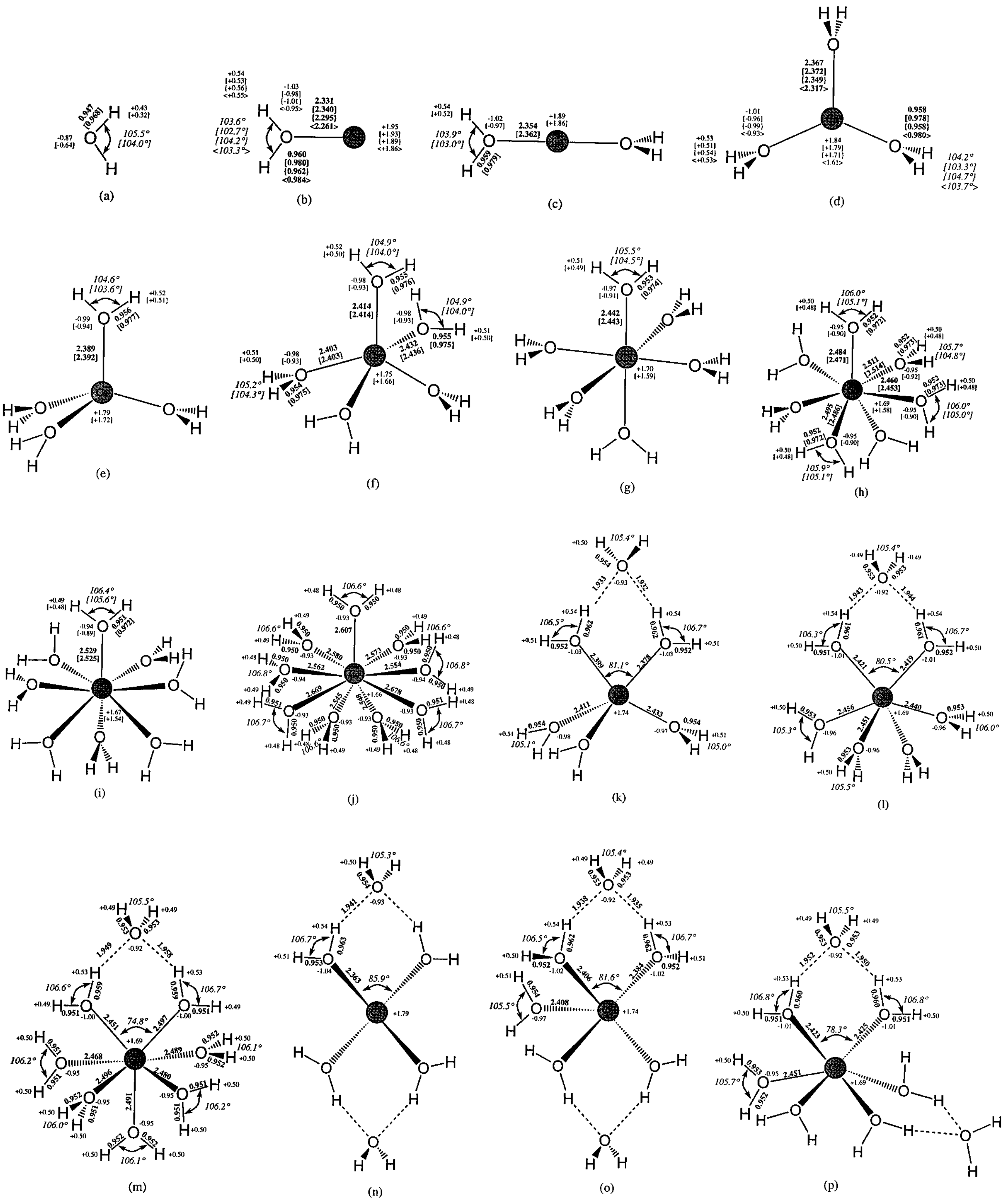

**Figure 1.** Selected geometrical parameters from *ab initio* molecular orbital calculations: (a) $H_2O$, (b) $Ca[H_2O]^{2+}$, (c) $Ca[H_2O]_2{}^{2+}$, (d) $Ca[H_2O]_3{}^{2+}$, (e) $Ca[H_2O]_4{}^{2+}$, (f) $Ca[H_2O]_5{}^{2+}$, (g) $Ca[H_2O]_6{}^{2+}$, (h) $Ca[H_2O]_7{}^{2+}$, (i) $Ca[H_2O]_8{}^{2+}$, (j) $Ca[H_2O]_9{}^{2+}$, (k) $Ca[H_2O]_5{}^{2+}{\cdot}H_2O$, (l) $Ca[H_2O]_6{}^{2+}{\cdot}H_2O$, (m) $Ca[H_2O]_7{}^{2+}{\cdot}H_2O$, (n) $Ca[H_2O]_4{}^{2+}{\cdot}2H_2O$, (o) $Ca[H_2O]_5{}^{2+}{\cdot}2H_2O$, and (p) $Ca[H_2O]_6{}^{2+}{\cdot}2H_2O$. Shown in these diagrams are bond lengths (Å), bond angles (deg), and Mulliken charges (electron units). The solid atoms are the Ca atoms. Values without brackets are at the RHF/HUZSP*(*p*)//RHF/HUZSP*(*p*) level, with square brackets are at the MP2(FULL)/HUZSP*(*p*)//MP2(FULL)/HUZSP*(*p*) level, with curly brackets are at the RHF/SU*(*p*,*d*)//RHF/SU*(*p*,*d*) level, and with angle brackets are at the MP2(FULL)/SU*(*p*,*d*)//MP2(FULL)/SU*(*p*,*d*) level.

and ligand types. In many cases the coordination number had to be checked by a detailed inspection of the molecular structure calculated from the published atomic coordinates using the program ICRVIEW.[35] In other cases the coordination number was evident from the chemical formula drawn by the software provided with the CSD program system.

**B. Molecular Orbital Studies.** *Ab initio* molecular orbital calculations on a variety of hydrated divalent calcium ions of the form $Ca[H_2O]_n{}^{2+}{\cdot}mH_2O$ (where *n* and *m* denote the number of water molecules in the first and second coordination shells, respectively) were carried out on the CRAY YMP computer at the National Cancer

**Table 1.** Occurrences of Ca$^{2+}$−O, Ca$^{2+}$−N, Ca$^{2+}$−S, Ca$^{2+}$−Cl, and Ca$^{2+}$−Br Bonds Found in Structures in the CSD[a]

| | Ca$^{2+}$−O | | Ca$^{2+}$−N | | Ca$^{2+}$−S | | Ca$^{2+}$−Cl | | Ca$^{2+}$−Br | | total no. of bonds | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| coord no. | no.[b] | % | no.[b] | % | no.[b] | % | no.[b] | % | no.[b] | % | no.[b] | % |
| 3 | 0 | 0.0 | 3 | 100.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 0.2 |
| 4 | 6 | 75.0 | 2 | 25.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 8 | 0.4 |
| 5 | 5 | 100.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 5 | 0.3 |
| 6 | 347 | 80.3 | 69 | 16.0 | 0 | 0.0 | 14 | 3.2 | 2 | 0.5 | 432 | 22.1 |
| 7 | 466 | 93.8 | 25 | 5.0 | 0 | 0.0 | 4 | 0.8 | 2 | 0.4 | 497 | 25.4 |
| 8 | 819 | 93.9 | 51 | 5.8 | 0 | 0.0 | 2 | 0.2 | 0 | 0.0 | 872 | 44.6 |
| 9 | 88 | 97.8 | 2 | 2.2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 90 | 4.6 |
| 10 | 50 | 100.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 50 | 2.6 |
| Ca$^{2+}$···X for all ligands | 1781 | 91.0 | 152 | 7.8 | 0 | 0.0 | 20 | 1.0 | 4 | 0.2 | 1957[c] | |

[a] Data for organic crystal structures, extracted from the CSD. Remeasurements of the same crystal structure are not included in the count. [b] Number of Ca$^{2+}$···X bonds. [c] Total number of entries for all elements attached to Ca$^{2+}$ = 309. In 21 entries other elements are bound to Ca; they are (with the number of entries in parentheses) I(1), F(1), Te(2), C(15), H(2), and B(1); their refcodes are listed in deposited Table 1S. In addition, 40 entries (not included in the numbers quoted above) were eliminated because they showed disorder, questionable metal-ion identity, or other crystallographic problems (also listed in deposited Table 1S). The remaining entries are listed in the table above.

Institute and several Silicon Graphics and DEC alpha computers located in Philadelphia. The GAUSSIAN 90, GAUSSIAN 92, and GAUSSIAN 94 series of programs were used throughout.[36-38] Initially, restricted Hartree−Fock (RHF) calculations with gradient optimizations were employed for all the hydrated structures using the standard 6-31G* basis set[39] for the oxygen and hydrogen atoms and a split-valence Huzinaga ⟨43321/43*⟩ basis set for calcium,[40] which includes a p-type polarization function. For convenience we shall refer to this combined basis set as HUZSP*(p), where the *p* denotes the type of polarization function on the calcium atom;[41] *d* functions were used on all the oxygen atoms. This choice of basis set represents a compromise between accuracy and our desire to include a significant number of water molecules surrounding the calcium ion. The effects of electron correlation were included by performing single-point second-order Møller−Plesset (MP2) perturbation calculations[42] at the MP2(FULL)/HUZSP*(p,d)//RHF/HUZSP*(p) level, which includes an additional d-type polarization function on the calcium atoms.[40] In a few instances calculations using the HUZSP*(p,d) basis set, augmented by diffuse functions on all the atoms, were carried out to assess the significance of including such functions on geometrical parameters and energy differences.[43] Higher order MP4SDQ(FULL)/HUZSP*(p,d)//RHF/HUZSP*(p) calculations were also performed for several of the smaller complexes. Vibrational frequencies were obtained from analytical second derivatives calculated at the RHF/HUZSP*(p)//RHF/HUZSP*(p) level in order to verify that the computed structures were indeed local minima and not transition states on the potential energy surface and to provide thermal corrections for reaction energies.[44-47] Many of the structures were reoptimized at the MP2(FULL)/HUZSP*(p)//MP2(FULL)/HUZSP*(p) level, with all orbitals active, to assess the effects of electron correlation on the geometrical parameters. Generalized second-order charge densities were calculated at the MP2(FULL)/HUZSP*(p,d)//RHF/HUZSP*(p) and MP2(FULL)/HUZSP*(p)//MP2(FULL)/HUZSP*(p) levels to study the transfer of charge to the central calcium atom as a function of the number of water molecules in the first coordination sphere. RHF and MP2(FULL) optimizations were also carried out on a few of the smaller hydrated complexes using the more complete ⟨999111111/8811111/22⟩ basis set derived by Sadlej and Urban for calcium[48] but using the same 6-31G* basis set for the oxygen and hydrogen atoms;[39] we shall denote this combined basis set as SU*(p,d). In several cases counterpoise calculations have been used to estimate corrections for the basis set superposition errors (BSSE) to our computed hydration energies, although it is not clear the extent to which such corrections improve the reliability of the results.[49-53]

## Results

**I. Structural Studies.** A list of 309 crystal structures containing calcium from the CSD is given in deposited Tables 2S−6S. The bibliographic references for each are given in deposited Table 7S. Our analysis of these structures involved only those in which divalent calcium is coordinated to oxygen, nitrogen, sulfur, chlorine, and/or bromine atoms, a total of 249 crystal structures (containing 271 calcium-binding sites). No entries were found in the CSD in which calcium is directly coordinated to sulfur. Thus calcium, which is itself hard, prefers the harder anions, as expected.[54] The percentages of the various ligands for each coordination number are listed in Table 1 and shown in deposited Figure A. There is, in the CSD, only one crystal structure with a three-coordinate calcium ion (JOFPID), illustrated in deposited Figure B. Two crystal structures have a coordination number of 4 (JOFPOJ and YALJEA) and one crystal structure has a coordination number of 5 (SICGUG01),

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**Table 2.** Metal Coordination in Crystal Structures Containing $\mathrm{Ca}^{2+}\cdots\mathrm{X}$ (X = O, N, S, Cl, Br) in the CSD[a]

| | | | | |
|---|---|---|---|---|
| Coordination Number 3 | | | | |
| 3N(1) | | | | |
| Coordination Number 4 | | | | |
| 4O(1) | 2O2N(1) | | | |
| Coordination Number 5 | | | | |
| 5O(1) | | | | |
| Coordination Number 6 | | | | |
| 6O(51) | 6N(7) | | | |
| 5O1N(1) | | | | |
| 4O2N(2) | 4O2Cl(1) | 4O2Br(1) | 4N2O(4) | 4Cl2O(1) |
| 3O3N(1) | 3O3Cl(1) | 3Cl2O1N(1) | | |
| 2N2Cl2O(1) | | | | |
| Coordination Number 7 | | | | |
| 7O(53) | | | | |
| 6O1N(8) | 6O1Br(2) | 6O1Cl(1) | 6N1O(1) | |
| 5O2N(5) | | | | |
| 3Cl1N3O(1) | | | | |
| Coordination Number 8 | | | | |
| 8O(83) | | | | |
| 7O1N(6) | 7O1Cl(2) | | | |
| 6O2N(14) | | | | |
| 5N3O(1) | | | | |
| 4O4N(3) | | | | |
| Coordination Number 9 | | | | |
| 9O(9) | | | | |
| 7O2N(1) | | | | |
| Coordination Number 10 | | | | |
| 10O(5) | | | | |

[a] Listed are the ligand atoms around one calcium ion and, in parentheses, the number of individual crystal structures in which these are found. Individual refcodes of the CSD entries and their journal references are given the deposited Tables 2S−6S.

illustrated in deposited Figures C and D, respectively. The general disposition of ligands is indicated in Table 2. Most of the crystal structures containing divalent calcium (93%) have coordination numbers 6, 7, or 8. Those structures with a coordination number of 8 are more common in the CSD than are those with coordination numbers 6 and 7 (109 versus 72 and 71 entries, respectively). The numbers of water molecules and carboxylate groups bound to calcium in this analysis are listed in Table 3. Average $\mathrm{M}^{2+}\cdots\mathrm{O}$ distances from our studies of the CSD, as a function of coordination number, are given in Table 4.

**A. Crystal Structures with 6-Coordinate Calcium Ions.** A total of 72 crystal structures were found in the CSD in which a divalent calcium cation has a coordination number of 6, representing approximately 22% of all the calcium-containing crystal structures used in the present study. Oxygen is the most common ligand (81%) found in these structures, as seen in Table 1. The percentage of structures containing oxygen as a ligand to 6-coordinate metal ions (79%) is essentially the same for calcium and for magnesium ions (80% and 79%, respectively).[13]

It was found that 70% of the entries had six oxygen atoms only (no N, Cl, Br) around the calcium ion. In 35 (48%) of the crystal structures analyzed with a calcium coordination number of 6, at least one water molecule is bound via its oxygen atom to the calcium ion, two water molecules being preferred (Table 3 and deposited Table 8S). When both carboxyl groups and water molecules are calcium ligands, it appears that the carboxyl group(s) bind the metal ion in 6-coordinate complexes in a “monodentate fashion”, that is, with only one of the two oxygen atoms coordinated to the metal ion (see Table 3). The alternative carboxylate binding considered here is “bidentate”, in which both oxygen atoms of the carboxylate group are coordinated to the calcium ion. Bidentate binding is common for calcium ions.[17,55] When no water molecules are present in the crystal structure, the only compounds with carboxyl groups are those for which six carboxyl groups bind, each in a monodentate fashion.

**B. Crystal Structures with 7-Coordinate Calcium Ions.** A total of 72 crystal structures were found in the CSD in which a divalent calcium cation has a coordination number of 7, representing 26% of all the calcium-containing crystal structures used in the present study. Entries containing only seven oxygen atoms bound (no N, Cl, Br) comprise 74% of the total. Out of 73 crystal structures we found that 60 (i.e. 83%) of them contained at least one water molecule in their coordination spheres (Table 3). When carboxylate groups are bound to 7-coordinate calcium ions, 16% of them are bound in a bidentate fashion.

**C. Crystal Structures with 8-Coordinate Calcium Ions.** A total of 108 crystal structures were found in the CSD in which a divalent calcium cation has a coordination number of 8, representing 44% of all the calcium-containing crystal structures used in the present study. Out of 108 crystal structures we found that 83 (i.e. 77%) of them contained at least one water molecule in its coordination sphere (Table 3). It appears that two water molecules are preferred for this calcium coordination number. Our studies also show that when carboxylate groups bind to calcium ions they do so in a bidentate fashion approximately 25% of the time, contrary to the finding of no such examples for coordination number 6. Thus, as the coordination number increases, the tendency of carboxylates to form bidentate complexes increases, in agreement with the results of Einspahr and Bugg,[17,18] who noted that bidentate complexation of calcium is found more often for higher coordination numbers. This is gratifying because their sampling of structures in 1980 was on a smaller database (because there have been so many crystal structures reported in the intervening years).

**II. *Ab Initio* Molecular Orbital Studies.** Selected geometrical parameters from RHF/HUZSP*(*p*) optimizations and, where available, MP2(FULL)/HUZSP*(*p*) and MP2(FULL)/SU*(*p*,*d*) optimizations, of divalent calcium cations surrounded by up to nine water molecules partitioned between the first and second coordination shells are shown in Figure 1. The $x$, $y$, and $z$ coordinates of each of these structures are given in deposited Table 9S. Total molecular energies for each are given at several computational levels in deposited Table 10S. All structures reported here have been verified to be local minima on their respective potential energy surfaces by frequency analyses at the RHF/HUZSP*(*p*)//RHF/HUZSP*(*p*) level.

As can be seen in Figure 1, the primary differences in the calculated geometries for $\mathrm{Ca[H_2O]}_n^{2+}$ ($n = 1-9$) at the RHF/HUZSP*(*p*) and MP2(FULL)/HUZSP*(*p*) levels (where available) are that the length of the O−H bonds increases by approximately 0.02 Å while the H−O−H bond angles are reduced by nearly 1°; these are presumably a result of correlation effects which we find to have a relatively minor effect on the $\mathrm{Ca}^{2+}-\mathrm{O}$ distance using the HUZSP*(*p*) basis set. Since no direct experimental data are available for the individual species of hydrated $\mathrm{Ca}^{2+}$ ions under consideration, it is desirable to establish the degree to which RHF/HUZSP*(*p*) and MP2(FULL)/HUZSP*(*p*) level optimizations adequately describe

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**Table 3.** Number of Entries in the CSD with Water Molecules Bound to Calcium for a Particular Coordination Number (CN#) and Frequency of Monodentate and Bidentate Carboxylate Coordination to Calcium Ions (See Deposited Tables 8S and 13S for Further Details)

(a) Number of Water Molecules in Small-Molecule Crystal Structures (CSD)

| CN# | $1H_2O$ | $2H_2O$ | $3H_2O$ | $4H_2O$ | $5H_2O$ | $6H_2O$ | $7H_2O$ | $8H_2O$ | $9H_2O$ | $10H_2O$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 0 | 0 | 0 | 0 | | | | | | |
| 5 | 0 | 0 | 0 | 0 | 0 | | | | | |
| 6 | 5 | 21 | 4 | 3 | 1 | 1 | | | | |
| 7 | 13 | 16 | 12 | 9 | 5 | 3 | 2 | | | |
| 8 | 14 | 32 | 12 | 21 | 1 | 2 | 0 | 1 | | |
| 9 | 2 | 0 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | |
| 10 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

(b) Number of Carboxylate Groups in Small-Molecule Crystal Structures (CSD)

| CN# | | $1H_2O$ | $2H_2O$ | $3H_2O$ | $4H_2O$ | $5H_2O$ | $6H_2O$ | $7H_2O$ | $8H_2O$ | total no. of structures |
|---|---|---|---|---|---|---|---|---|---|---|
| 6 | monodentate | 9 | 9 | 3 | – | 1 | | | | 18 |
| | bidentate | – | – | – | – | | | | | |
| 7 | monodentate | 4 | 20 | 9 | 5 | 2 | 1 | | | 32 |
| | bidentate | 1 | 4 | 2 | 1 | 1 | | | | |
| 8 | monodentate | 27 | 29 | 17 | 12 | 2 | – | – | | 57 |
| | bidentate | 6 | 7 | 3 | 2 | – | – | | | |

(c) Number of Water Molecules in Relatively Precise Protein Crystal Structures (PDB)

| | $0H_2O$ | $1H_2O$ | $2H_2O$ | $3H_2O$ | $4H_2O$ | $5H_2O$ | $6H_2O$ | total no. of structures |
|---|---|---|---|---|---|---|---|---|
| $Ca^{2+}$ | 15 | 24 | 19 | 7 | 3 | 1 | 2 | 71 |
| $Mg^{2+}$ | 1 | 9 | 18 | 11 | 5 | 0 | 0 | 44 |

(d) Number of Carboxylate Groups in Protein Crystal Structures (PDB)

| CN# | | $1H_2O$ | $2H_2O$ | $3H_2O$ | $4H_2O$ | $5H_2O$ | $6H_2O$ | $7H_2O$ | $8H_2O$ | total no. of structures |
|---|---|---|---|---|---|---|---|---|---|---|
| 6 | monodentate | 9 | 30 | 9 | – | 1 | | | | 15 |
| | bidentate | – | – | – | – | | | | | |
| 7 | monodentate | 20 | 32 | 9 | 13 | 2 | 2 | | | 32 |
| | bidentate | 5 | 6 | 2 | 1 | 2 | | | | |
| 8 | monodentate | 31 | 62 | 25 | 25 | 2 | – | – | | 57 |
| | bidentate | 5 | 32 | 4 | 8 | – | – | | | |

**Table 4.** Average $M^{2+}-O$ (M = Ca, Zn, Mg, Be) Bond Lengths[a] (Å) from Crystal Structures in the CSD as a Function of Coordination Number

| coord no. | Ca | Zn | Mg | Be[b] |
|---|---|---|---|---|
| 4 | 2.27[c] | 1.96(1) | 1.99(3) | 1.622(4) |
| 5 | 2.32[c] | 2.04(3) | 2.01(4) | |
| 6 | 2.35(1) | 2.10(1) | 2.073(4) | |
| 7 | 2.41(1)[d] | 2.15(2) | 2.16[c] | |
| 8 | 2.45(1)[d] | | | |
| 9 | 2.48(4) | | | |

[a] Includes all ligand ($H_2O$, carboxyl, carbonyl, hydroxyl, etc.) groups. [b] Be−O distances were only found for coordination number four. [c] Only one entry was found with only oxygen. [d] Einspahr and Bugg[15] give an average value of 2.42 Å for calcium−water interactions for coordination number 7−8.

these hydrated calcium ions. Therefore, in the case of $Ca[H_2O]_n^{2+}$ ($n = 1, 3$), we carried out RHF and MP2(FULL) optimizations using the more complete SU*(*p*,*d*) basis set.[48] The optimized geometries at this level are also shown (with angled brackets) in Figure 1. While there are some differences in the computed geometrical parameters, for example, the $Ca^{2+}-O$ distance is somewhat shorter with the more complete basis set, the *changes* in these geometrical parameters as a function of the computational level as one proceeds from $Ca[H_2O]^{2+}$ to $Ca[H_2O]_3^{2+}$ are generally consistent with each other. The calculated charges on the various atoms are also in reasonable agreement at the various levels although the more complete SU*(*p*,*d*) basis set suggests a slightly larger transfer of charge to the calcium atom.

One significant difference between optimizations using the HUZSP*(*p*) and the SU*(*p*,*d*) basis sets is found for the structure of $Ca[H_2O]_2^{2+}$. RHF and MP2(FULL) optimizations using the HUZSP*(*p*) basis set find that the $O-Ca^{2+}-O$ bond angle is linear in this complex; frequency analyses at both computational levels confirm that such structures are local minima on the potential energy surface. RHF and MP2(FULL) optimizations using the more complete SU*(*p*,*d*) basis set, however, find a local minimum for $Ca[H_2O]_2^{2+}$ in which the $O-Ca^{2+}-O$ angle is severely bent, with angles of 129.5° and 120.9°, respectively; the usual linear structure corresponds to a second-order transition state at this computational level. The energy difference between the linear and bent structures is only 0.6 kcal/mol at the MP2-(FULL)/SU*(*p*,*d*)//MP2(FULL)/SU*(*p*,*d*) level. In order to determine if the difference between these basis sets is primarily a result of the d functions on calcium in the SU*(*p*,*d*) basis set, a single d function was added to the HUZSP*(*p*) basis set and the optimization was repeated at the MP2 level. The resulting structure was found to be bent, with an $O-Ca^{2+}-O$ angle of 129.5°. Including two d functions on $Ca^{2+}$ also resulted in a bent structure with an $O-Ca^{2+}-O$ angle which is even smaller, 124.2° (see Figure 2). Bauschlicher and co-workers[56] have also found the structure of $Ca[H_2O]_2^{2+}$ to have the $O-Ca^{2+}-O$ angle bent, 126°. An explanation for these various findings may lie

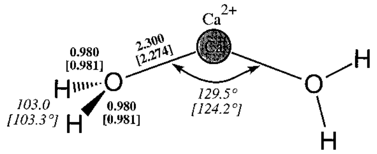

**Figure 2.** Bent $O\cdots Ca^{2+}\cdots O$ angle in $Ca[H_2O]_2^{2+}$ (the dark circle represents the Ca atom). Values without brackets are at the MP2-(FULL)/HUZSP*(*p*,*d*)//MP2(FULL)/HUZSP*(*p*,*d*) level, and values in square brackets are at the MP2(FULL)/HUZSP*(*p*,*2d*)//MP2(FULL)/HUZSP*(*p*,*2d*) level.

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in the greater polarizability of $Ca^{2+}$ ($3.2a_0^3$) compared to that of $Mg^{2+}$ ($0.3a_0^3$), which does not have a bent structure for $Mg[H_2O]_2^{2+}$, and is reinforced by the fact that the inclusion of electron correlation gives an $O-Ca^{2+}-O$ angle of 120.9°, smaller than the corresponding RHF value of 129.5°. Thus, the enhancement in binding due to core polarization is sufficient to compensate for the larger ligand–ligand repulsion in the bent structure. The larger ionic radius of $Ca^{2+}$ compared to $Mg^{2+}$ will also make water–water repulsion less important. It should also be noted that the structure of other calcium compounds, notably $CaF_2$, have proved difficult to determine satisfactorily by molecular orbital methods, and even very high level calculations disagree as to whether this structure is linear or bent.[57–61] Recent calculations by Kaupp and co-workers[61] have shown that d orbitals on calcium contribute significantly to the bending of $CaF_2$. It is interesting, in this context, that $Sr[H_2O]_2^{2+}$ is also calculated to be bent.[56]

We then examined the geometry of binding of three water molecules to a calcium ion. RHF/HUZSP*(*p*), MP2(FULL)/HUZSP*(*p*), and RHF/HUZSP*(*p*,*d*) optimizations all show that $Ca[H_2O]_3^{2+}$ has the calcium ion coplanar with the three oxygen atoms. The MP2(FULL)/SU*(*p*,*d*) optimizations find all three oxygen atoms in a slightly pyramidal arrangement on the same side of the calcium ion, similar to the geometry found by Bauschlicher and co-workers.[56] The sum of the three $O\cdots Ca^{2+}\cdots O$ angles is 355.5°. The energetic consequences of this deviation from coplanarity is, however, minimal; this structure is less than 0.01 kcal/mol lower in energy than the corresponding planar version at this computational level.

**A. $Ca[H_2O]_n^{2+}$ ($n = 1-9$).** We first consider hydrated divalent calcium ions with water only in the first coordination shell. Local minima, as verified by frequency analyses, were found for $Ca[H_2O]_n^{2+}$ ($n = 1-9$) as shown in Figure 1. Nine water molecules represent the largest number of water molecules we could consider with our current computer resources, using the HUZSP*(*p*) basis set. It should be noted that our search of the CSD found a few structures in which calcium has a coordination number greater than 8, but no structures with more than eight water molecules in the primary shell. In general, the hydrated complexes, $Ca[H_2O]_n^{2+}$ ($n = 1-6$), shown in Figure 1, are analogous to the corresponding hydrated structures for divalent beryllium, magnesium, and zinc ions, except that no local minimum could be found for $Be[H_2O]_5^{2+}$. Several attempts to find stable hydrated magnesium and zinc structures with seven water molecules in the primary shell were unsuccessful. When we attempted to find a stable structure with eight water molecules in the inner shell around $Mg^{2+}$, two of the water molecules migrated to the second shell. Cachau and co-workers[62] have previously reported RHF-optimized structures for $Ca[H_2O]_n^{2+}$ ($n = 1-4$, 6) using a somewhat smaller basis set, but no frequency analyses were performed. Although their calculated $Ca^{2+}-O$ bond lengths are shorter and their O–H bond lengths longer than those that we find, the changes in their geometries as the number of water molecules increases are similar to those in Figure 1. Ortega-Blake and co-workers[63] reported *ab initio* pseudopotential calculations on hydrated divalent calcium ions and their interaction with $\gamma$-aminobutyric acid. Earlier calculations on hydrated divalent calcium ions have been reported by Kollmann[64] and by Ortega-Blake[65,66] and their co-workers.

**Table 5.** Calculated M–O Bond Lengths in $M[H_2O]_n^{2+}$ ($n = 1-8$), M = Ca, Zn, Mg, Be

| *n* | Ca[a] | Zn[b] | Mg[c] | Be[d] |
|---|---|---|---|---|
| 1 | 2.340 | 1.896 | 1.948 | 1.511 |
| 2 | 2.362 | 1.890 | 1.964 | 1.535 |
| 3 | 2.372 | 1.959 | 1.990 | 1.584 |
| 4 | 2.392 | 2.009 | 2.017 | 1.654 |
| 5 | 2.418[e] | 2.075[f] | 2.064[g] | |
| 6 | 2.443 | 2.120 | 2.097 | 1.846 |
| 7 | 2.482[h] | | | |
| 8 | 2.525 | | | |

[a] Calculated at the MP2(FULL)HUZSP*(*p*)//MP2(FULL)/HUZSP*(*p*) level ($Ca^{2+}$). [b] Calculated at the MP2(FC)/HUZSP*(*d*)//MP2(FC)/HUZSP*(*d*) level ($Zn^{2+}$). [c] Calculated at the MP2(FULL)/6-31G*//MP2(FULL)/6-31G* level ($Mg^{2+}$). [d] Calculated at the MP2(FULL)6-31G*//MP2(FULL)/6-31G* level ($Be^{2+}$). [e] Average of the values 2.403, 2.403, 2.414, 2.436, and 2.436 Å. [f] Average of the values 2.039, 2.039, 2.057, 2.120, and 2.120 Å. [g] Average of the values 2.041, 2.042, 2.051, 2.092, and 2.092 Å. [h] Average of the values 2.453, 2.453, 2.471, 2.486, 2.486, 2.514, and 2.514 Å.

In deposited Table 11S, we list the calculated $Ca^{2+}-O$ bond lengths in the various hydrated calcium complexes, and in Table 5 and deposited Figure E, we compare the calculated $M^{2+}-O$ distances for M = Ca, Be, Mg, and Zn. In general, as *n* increases, the $M^{2+}-O$ bond lengths increase, but at different rates. For all values of *n*, the $M^{2+}-O$ bond lengths are longer for calcium than the other metals listed here, which is consistent with the much larger ionic radius of $Ca^{2+}$ (0.95 Å) compared to those of $Be^{2+}$ (0.34 Å), $Mg^{2+}$ (0.65 Å), and $Zn^{2+}$ (0.75 Å).[67,68] Furthermore, the rate of increase in the $Ca^{2+}-O$ distance as *n* increases is smaller than that for the other $M^{2+}-O$ distances. Values for these distances are 0.02–0.12 Å longer than the average values measured in crystal structures in the CSD (see Table 4), but since estimated standard deviations (e.s.d.'s) are 0.01–0.04 Å for the CSD data, differences from *ab initio* values may not be significant.

The changes in enthalpy, $\Delta H^{298}$, for the successive hydration reactions

$$Ca[H_2O]_n^{2+} + H_2O \rightarrow Ca[H_2O]_{n+1}^{2+} \qquad (1)$$

for $n = 0-8$ are given in deposited Table 12S at a variety of computational levels and compared with the corresponding results for beryllium, magnesium, and zinc in Table 6 and deposited Figure F. As can be seen, once correlation effects are included in the calculations at the MP2 level, there is relatively little difference in the hydration enthalpies as higher order perturbation corrections are included in the calculations, which is consistent with what we found for beryllium, magnesium, and zinc. Since there are no direct experimental data for comparison, however, we decided to calculate the enthalpy change for reaction 1 with $n = 0$ using a basis set which includes diffuse functions. The HUZSP*(*p*,*d*) basis set described above was augmented by a set of diffuse sp functions on all the heavy

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**Table 6.** Comparison of Successive Hydration Enthalpies, $\Delta H^{298}$ (kcal/mol), for M = $Ca^{2+}$, $Mg^{2+}$, $Zn^{2+}$, $Be^{2+}$, According to the Equation $M[H_2O]_n^{2+} + H_2O \rightarrow M[H_2O]_{n+1}^{2+}$

| | M | | | |
|---|---|---|---|---|
| *n* | Ca[a] | Zn[b] | Mg[c] | Be[d] |
| 0 | −56.1 | −96.5 | −85.8 | −149.7 |
| 1 | −51.6 | −87.2 | −77.8 | −124.6 |
| 2 | −47.9 | −61.6 | −64.4 | −84.5 |
| 3 | −42.7 | −49.4 | −53.1 | −56.0 |
| 4 | −34.2 | −32.4 | −36.7 | |
| 5 | −31.8 | −30.0 | −34.0 | |
| 6 | −21.4 | | | |
| 7 | −20.3 | | | |
| 8 | −13.7 | | | |

[a] Calculated at the MP2(FULL)/HUZSP*(*p*,*d*)//RHF/HUZSP*(*p*) level using thermal corrections at the RHF/HUZSP*(*p*)//RHF/HUZSP*(*p*) level. [b] Calculated at the MP2(FC)/HUZSP*(*d*)//RHF/HUZSP*(*d*) level using thermal corrections at the RHF/HUZSP*(*d*)//RHF/HUZSP*(*d*) level. [c] Calculated at the MP2(FC)/6-31G*//RHF/6-31G* level using thermal corrections at the RHF/6-31G*/RHF/6-31G* level. [d] Calculated at the MP2(FC)/6-31G*//RHF/6-31G* level using thermal corrections at the RHF/6-31G*//RHF/6-31G* level.

**Table 7.** Net Charge on Central Metal Ion, $M^{2+}$, in M $[H_2O]_n^{2+}$ from the MP2 Charge Density

| *n* | Ca[a] | Zn[b] | Mg[c] | Be[d] |
|---|---|---|---|---|
| 1 | 1.93 (1.93[e]) | 1.79 | 1.76 | 1.40 (1.41[f]) |
| 2 | 1.86 (1.86[e]) | 1.54 | 1.56 | 0.96 (0.97[f]) |
| 3 | 1.78 (1.79[e]) | 1.42 | 1.40 | 0.82 (0.83[f]) |
| 4 | 1.71 (1.72[e]) | 1.36 | 1.29 | 0.77 (0.77[f]) |
| 5 | 1.65 (1.66[e]) | 1.34 | 1.24 | |
| 6 | 1.57 (1.59[e]) | 1.34 | 1.18 | 0.99 (0.93[f]) |
| 7 | 1.56 (1.58[e]) | | | |
| 8 | 1.53 (1.54[e]) | | | |
| 9 | 1.51 | | | |

[a] Calculated at the MP2(FULL)/HUZSP*(*p*,*d*)//RHF/HUZSP*(*p*) level. [b] Calculated at the MP2(FC)/HUZSP*(*d*)//MP2(FC)/HUZSP*(*d*) level. [c] Calculated at the MP2(FULL)/6-31G**//MP2(FULL)/6-31G* level. [d] Calculated at the MP2(FULL)/6-31G**//MP2(FULL)/6-31G* level. [e] Calculated at the MP2(FULL)/HUZSP*(*p*)//MP2(FULL)/HUZSP*(*p*) level. [f] Calculated at the MP2(FULL)/6-31G*//MP2(FULL)/6-31G* level.

atoms and p-type polarization functions and diffuse s functions on the hydrogen atoms. The value of $\Delta H^{298}$ calculated with this enhanced basis set was found to be −50.7 kcal/mol, slightly less negative than that found with the other basis sets, and in reasonable agreement with the results of Floris and co-workers.[69] The exothermicity of successive hydration appears to decrease for all four metals as *n* increases, although for calcium the decrease is much less dramatic (deposited Figure F). It is quite interesting that the values of $\Delta H^{298}$ for the addition of the sixth water to $M[H_2O]_5^{2+}$ for M = Ca, Mg, and Zn are all very similar.

In Table 7 and deposited Figure G, the net charge on the metal ions, $M^{2+}$, of the hydrated complexes $M[H_2O]_n^{2+}$, calculated from generalized MP2 charge densities at similar computational levels, are compared for $Ca^{2+}$, $Be^{2+}$, $Mg^{2+}$, and $Zn^{2+}$, as a function of the number of water molecules in the complex, *n*. It is clear that a significant amount of charge is transferred to the central metal ions for almost every complex. The least amount of charge is transferred to $Ca^{2+}$ for each value *n*. If we compare charges on the metals in the hydrated structures with four to six water molecules, the order of increasing transfer is given by $Ca^{2+} < Zn^{2+} < Mg^{2+} < Be^{2+}$, which is in the order of decreasing ionic radii. Although the charge transferred to the calcium ion generally increases as *n* increases in $Ca[H_2O]_n^{2+}$, the charge transferred is similar for *n* = 6−8.

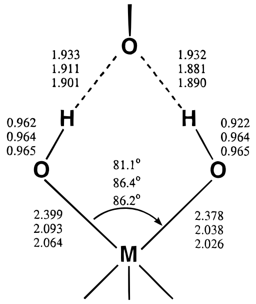

**Figure 3.** Comparison of the calculated geometries of the six-membered rings in $M[H_2O]_5^{2+}\cdot H_2O$ for, from top to bottom, $Ca^{2+}$, $Zn^{2+}$, and $Mg^{2+}$. Computational levels: $Ca^{2+}$, RHF/HUZSP*(*p*)//RHF/HUZSP*(*p*); $Zn^{2+}$, RHF/HUZSP*(*d*)//RHF/HUZSP*(*d*); $Mg^{2+}$, RHF/6-31G*//RHF/6-31G*.

**B. $Ca[H_2O]_n^{2+}\cdot mH_2O$.** Several structures of the form $Ca[H_2O]_n^{2+}\cdot mH_2O$, with *n* water molecules in the first coordination shell and *m* water molecules in the second coordination shell, hydrogen bonded to waters in the first shell, were considered (see Figure 1). In all of these calculations, no symmetry was imposed *a priori* to lessen the chance of optimizing to transition states and all are local minima at the RHF/HUZSP*(*p*)//RHF/HUZSP*(*p*) level, as verified by frequency analyses.

**$Ca[H_2O]_n^{2+}\cdot H_2O$ ($n = 5, 6, 7$).** Local minima were found with five, six, and seven water molecules bound to calcium in the first coordination shell and with a single water molecule in the second coordination shell. For all three complexes the water molecule in the second shell is hydrogen-bonded to *two* water molecules in the first shell forming a six-membered ring (see Figure 1). In Figure 3 we compare the calculated geometrical parameters in the six-membered rings in the complexes $M[H_2O]_5^{2+}\cdot H_2O$ for M = Ca, Zn, and Mg. Clearly, the asymmetry in the ring is smallest in the case of $Ca^{2+}$ where the $M^{2+}-O$ and H···O bond distances are the longest. The longer H···O distance in the case of $Ca^{2+}$ suggests that water in the second shell forms a weaker hydrogen bond with water molecules in the first shell. This is confirmed by the enthalpy change for the reactions $Ca[H_2O]_5^{2+} + H_2O \rightarrow Ca[H_2O]_5^{2+}\cdot H_2O$, which is approximately 2 kcal/mol less exothermic than for the corresponding reactions with $Zn^{2+}$ and $Mg^{2\cdot}$ This is probably to be expected since the ionic radius of $Ca^{2+}$ is larger than that of $Mg^{2+}$ and $Zn^{2+}$, which places the second shell waters in a less attractive region of the ion−water potential. At the MP2(FULL)/HUZSP*(*p*,*d*)//RHF/HUZSP*(*p*) level the energy of $Ca[H_2O]_5^{2+}\cdot H_2O$ is 5.0 kcal/mol *higher* in energy than that of $Ca[H_2O]_6^{2+}$, while the energies of $Ca[H_2O]_6^{2+}\cdot H_2O$ and $Ca[H_2O]_7^{2+}\cdot H_2O$ are 2.6 and 3.2 kcal/mol *lower* in energy than $Ca[H_2O]_7^{2+}$ and $Ca[H_2O]_8^{2+}$, respectively, at 298 K. It has been shown recently, however, that including diffuse functions on all atoms in the basis set can alter the energy separation between hydrated metal ion complexes such as $M[H_2O]_6^{2+}$ and $M[H_2O]_4^{2+}\cdot 2H_2O$ (M = Be, Mg) by several kcal/mol.[70] Consequently, additional single-point MP2 calculations were performed using the HUZSP*(*p*,*d*) basis set augmented by diffuse functions on all the atoms.[43] At this computational level, the energy of $Ca[H_2O]_5^{2+}\cdot H_2O$ is 8.2 kcal/mol *higher* in energy than that of $Ca[H_2O]_6^{2+}$, the energy of $Ca[H_2O]_6^{2+}\cdot H_2O$ is 1.4 kcal/mol *lower* in energy than $Ca[H_2O]_7^{2+}$, and $Ca[H_2O]_7^{2+}\cdot H_2O$

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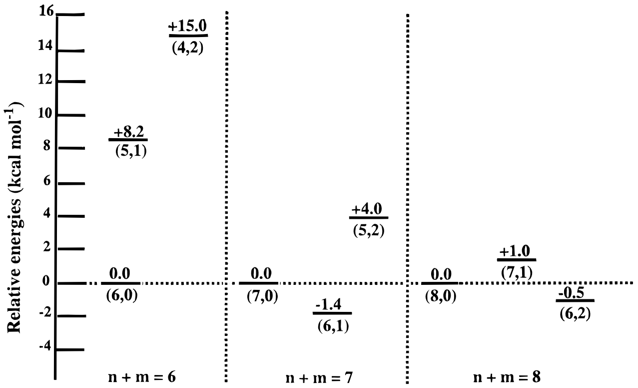

**Figure 4.** Relative energies (kcal/mol) for $Ca[H_2O]_n^{2+}\cdot mH_2O$ ($n + m = 6-8$) calculated at the MP2(FULL) computational level using the HUZSP*(*p*,*d*) basis set augmented by diffuse functions on all atoms.

is 1.0 kcal/mol *higher* in energy than $Ca[H_2O]_8^{2+}$ (see Figure 4). If, in addition to the diffuse functions, p functions are added to all the hydrogen atoms, the energy of $Ca[H_2O]_6^{2+}\cdot H_2O$ is calculated to be only 0.75 kcal/mol *lower* in energy than $Ca[H_2O]_7^{2+}$. Thus, it seems likely that $Ca[H_2O]_7^{2+}$ and $Ca[H_2O]_6^{2+}\cdot H_2O$ are extremely close in energy at 298 K.

**$Ca[H_2O]_n^{2+}\cdot 2H_2O$ ($n = 4, 5, 6$).** Complexes with four, five, and six water molecules in the first coordination shell and with two water molecules in the second coordination shell were also investigated (see Figure 1). The conformers of each of these complexes we considered have both water molecules in the second shell hydrogen bonded to two water molecules in the first shell, forming two six-membered rings. Comparing the $Ca^{2+}-O$ distances in $Ca[H_2O]_4^{2+}\cdot 2H_2O$ and $Ca[H_2O]_6^{2+}\cdot 2H_2O$ with $Ca[H_2O]_4^{2+}$ and $Ca[H_2O]_6^{2+\bullet}$ show clearly that the outer shell water molecules cause the inner shell water molecules to which they are bound to move closer to the calcium atom. Water molecules in a second shell have been observed to have a similar effect in water clusters around carboxylate groups in the sulfide $CH_3SCH_2CO_2^-$ and the sulfonium ion $(CH_3)_2S^+CH_2CO_2^-$, as well as around the sulfur atom in $(CH_3)_3S^+$.[71-73] The structural effects of water molecules in the second hydration shell on water molecules in the first hydration shell surrounding $H_3O^+$ have been investigated by Tunon and co-workers.[74]

The complex $Ca[H_2O]_4^{2+}\cdot 2H_2O$ is calculated to be 11.9 kcal/mol *higher* in energy than $Ca[H_2O]_6^{2+}$ at the MP2(FULL)/HUZSP*(*p*,*d*)//RHF/HUZSP*(*p*) level corrected to 298 K and 15.0 kcal/mol when the HUZSP*(*p*,*d*) basis set is augmented by diffuse functions. Interestingly, $Mg[H_2O]_4^{2+}\cdot 2H_2O$ is 13.2 kcal/mol *higher* in energy than $Mg[H_2O]_6^{2+}$ at the MP2(FULL)/6-311++G** level,[70] while $Zn[H_2O]_4^{2+}\cdot 2H_2O$ differs in energy from that of $Zn[H_2O]_6^{2+}$ by less than 1 kcal/mol, although no diffuse functions were included in the latter calculations.[14] In this sense calcium behaves more like magnesium than zinc. The complex $Ca[H_2O]_5^{2+}\cdot 2H_2O$ is calculated to be only 0.2 kcal/mol higher in energy than $Ca[H_2O]_7^{2+}$ at the MP2(FULL)/HUZSP*(*p*,*d*)//RHF/HUZSP*(*p*) level but 4.0 kcal/mol higher in energy when the diffuse functions are included. At 298 K, $Ca[H_2O]_6^{2+}\cdot 2H_2O$ is found to be 6.6 kcal/mol *lower* in energy than $Ca[H_2O]_8^{2+}$ without the diffuse functions but only 0.5 kcal/mol *lower* in energy when diffuse functions are included (see Figure 4).

It is clear from Figure 4 that, for divalent calcium ions surrounded by six, seven, and eight water molecules, the lower energy configurations consistently have six water molecules in the first coordination shell at the MP2(FULL) level using the HUZSP*(*p*,*d*) basis set, whether or not diffuse functions are included in the basis set. It should be noted, however, that for the seven- and eight-water complexes the local minima with seven and eight waters in the primary shell are only 1.4 (with diffuse functions, 0.7) and 0.5 kcal/mol, respectively, higher in energy than the arrangement with six waters in the inner shell and the remaining water molecules in the second coordination shell. It is not clear how the relative energies shown in Figure 4 for the various conformers of $Ca[H_2O]_n^{2+}\cdot mH_2O$, where $n + m = 6$, 7, and 8, would be altered by including additional water molecules in the second and higher coordination shells. However, at 298 K, the energy difference between $Ca[H_2O]_7^{2+}$ and $Ca[H_2O]_6^{2+}\cdot H_2O$ is 1.4 kcal/mol and the energy difference between $Ca[H_2O]_7^{2+}\cdot H_2O$ and $Ca[H_2O]_6^{2+}\cdot 2H_2O$, in which there is an additional water molecule in the second coordination shell of each complex, is almost the same, 1.5 kcal/mol.

It should be pointed out that X-ray and neutron diffraction studies of various $CaCl_2$ solutions exhibit large variations in the average $Ca^{2+}$ hydration number, with values ranging from 6 to 10.[75-80] In a molecular dynamics study of a 1.1 M solution of $CaCl_2$ using a flexible model for the water molecule, including three-body terms (H−O−H), but only cation−water and water−water pair interactions, Probst and co-workers found an average coordination number of 9.0.[80,81] Using effective pair potentials Floris and co-workers found a value of 8.6.[26] More recently, Bernal-Uruchurtu and Ortega-Blake reported the results of Monto Carlo simulations of both $Mg^{2+}$ and $Ca^{2+}$, using interaction potentials which include polarization and nonadditivity effects.[30] They find the average coordination number for the first hydration shell of $Mg^{2+}$ and $Ca^{2+}$ to be 6.0 and 7.01, respectively, the value for $Ca^{2+}$ being significantly lower than that obtained by Probst and co-workers.[79] The corresponding coordination number for the second shell around $Ca^{2+}$ was reported by them to be 20.[30] Several studies have used a combined discrete-continuum solvation model to extend the *ab initio* cluster calculations to the condensed phase.[15,69,82-85] Recently, Floris and co-workers[69] used both effective pair potentials and *ab initio* calculations with partial optimizations in the framework of the polarizable continuum model to compute the hydration free energies, $\Delta G_{hyd}$, for $Ca[H_2O]_6^{2+}$, $Ca[H_2O]_8^{2+}$, and $Ca[H_2O]_9^{2+}$; the lowest value of $\Delta G_{hyd}$ identified the likely hydration number of $Ca^{2+}$ in solution. Their calculations suggest a hydration number for $Ca^{2+}$ of 8, although the difference in hydration free energies between $Ca[H_2O]_6^{2+}$ and $Ca[H_2O]_8^{2+}$ is only 8.4 kcal/mol and that between $Ca[H_2O]_8^{2+}$ and $Ca[H_2O]_9^{2+}$ is only 0.8 kcal/mol based on their effective

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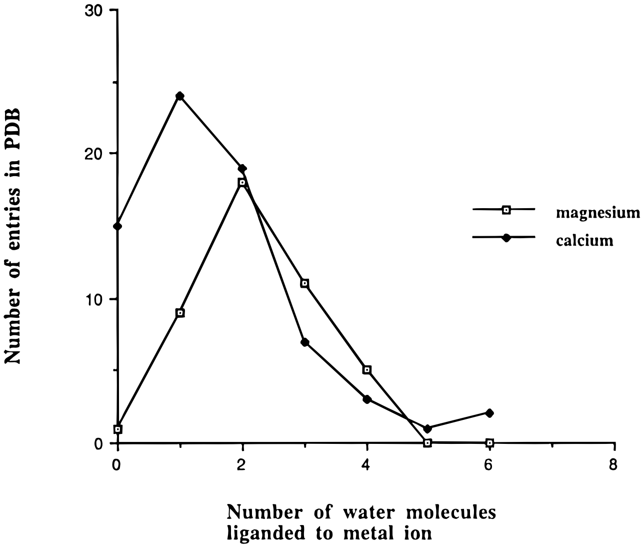

**Figure 5.** Number of entries for protein crystal structures in the Protein Database with differing numbers of water molecules surrounding magnesium (optimal coordination number 6) and calcium ions (optimal coordination number near 7).

pair potentials. We have recomputed $\Delta G_{\mathrm{hyd}}$ using values of $\Delta E$ at the MP2(FULL)/HUZSP*($p,d$)//RHF/HUZSP*($p$) level with diffuse functions on all the atoms (see deposited Table 10S), but all other contributions to $\Delta G_{\mathrm{hyd}}$ were taken directly from Floris and co-workers.[69] The values of $\Delta G_{\mathrm{hyd}}$ for $\mathrm{Ca[H_2O]}_n^{2+}$ ($n = 6, 8, 9$) are $-353.0$, $-357.8$, and $-356.6$ kcal/mol, respectively. While we also find $\Delta G_{\mathrm{hyd}}$ to be the lowest for $\mathrm{Ca[H_2O]_8^{2+}}$, the difference in free energy among the three structures is less than 5 kcal/mol. It should be noted that the basis set we are using here is still relatively small and more sophisticated calculations will be required to confirm the prediction of Floris and co-workers[69] that the hydration number divalent calcium is 8.

**III. Calcium-Binding Sites in Proteins.** The environments of calcium ions in crystal structures reported in the PDB show an average coordination number of approximately 7. This agrees with the data from the CSD presented here and the value of 7.31(5) found from the Inorganic Crystal Structure Database.[86] In some of the protein structures, the metal−oxygen distances are all much longer than an average $\mathrm{Ca^{2+}\cdots O}$ distance of about 2.4 Å; these were eliminated from our analysis on the premise that the metal in these crystalline proteins may not be calcium but some other metal ion extracted from solution during purification or crystallization.

The number of water molecules bound to calcium- and magnesium-containing proteins in the PDB is recorded in Figure 5 (with more detailed information in deposited Table 13S). Some PDB entries were not used because the resolution was too low or the geometry of the metal-binding site was not clear. Many of the protein structures were determined to 1.8 Å resolution or better, and several contained one or more EF hand motifs (which have a coordination number of 7).

We found that calcium-containing proteins often contain no water molecules, while only one example of a no bound water molecule (bacteriochlorophyll a) was found for magnesium-containing proteins. Thus, while the predominant situation for magnesium is two or three bound water molecules that can be displaced to bind substrate or inhibitor, the plot for calcium (Figure 5) is displaced to a lower number of bound water molecules, even though the coordination number of calcium is often higher (6 versus 6 or 7). This may reflect the greater tendency of magnesium rather than calcium ions to be bound in a site with more overall solvent accessibility so that, for enzymes, their substrates have a position to bind on the coordination sphere by displacing water.

Other protein groups that bind calcium in proteins are aspartate and glutamate, the oxygen atom of asparagine or glutamine, occasionally the hydroxyl group of serine or threonine, and often one main-chain carbonyl group. Since calcium carries a positive charge of 2, it would be expected that four carboxyl oxygen atoms (each with a charge of $-0.5$) would be needed to balance this. Four monodentate, two bidentate or a mixture of two monodentate and one bidentate carboxyl groups would satisfy this requirement. This is not always the case, presumably because of the presence of other charged groups in the neighborhood of the calcium ion.

## Discussion

We have shown that calcium ions overwhelmingly prefer to bind oxygen atoms rather than nitrogen, chlorine, or bromine and that they rarely, if ever, bind to sulfur atoms. In some circumstances in small-molecule crystal structures, the choice of ligands is stereochemically constrained, depending on the chemical nature of the ligand molecules. These result in the less common binding modes. Proteins apparently utilize the strong binding affinity of calcium for oxygen atoms. The local charge around the calcium needs to be neutralized,[87] but since the coordination number can be as high as 8, calcium must also bind groups with low charges on oxygen, such as glutamine, asparagine, threonine, serine, main-chain carbonyl groups, and water molecules. The EF-hand motif has been designed specifically to bind calcium ions.

The energetics involved in changing the coordination number of water-bound calcium have been calculated and show that structures of the form $\mathrm{Ca[H_2O]_5^{2+}{\cdot}H_2O}$ and $\mathrm{Ca[H_2O]_4^{2+}{\cdot}2H_2O}$ are significantly higher in energy than $\mathrm{Ca[H_2O]_6^{2+}}$ by 8.2 and 15.0 kcal/mol, respectively; it seems unlikely that this will change as more sophisticated calculations become possible. For $\mathrm{Ca^{2+}}$ surrounded by seven and eight water molecules, the lower energy forms are also found to have six water molecules in the first coordination shell, with the remaining water molecules in the second coordination shell. The differences in energy, however, between the complexes $\mathrm{Ca[H_2O]_6^{2+}{\cdot}H_2O}$ and $\mathrm{Ca[H_2O]_7^{2+}}$ and among the complexes $\mathrm{Ca[H_2O]_6^{2+}{\cdot}2H_2O}$, $\mathrm{Ca[H_2O]_7^{2+}{\cdot}H_2O}$, and $\mathrm{Ca[H_2O]_8^{2+}}$ are extremely small when diffuse functions are included in the basis set. Furthermore, it should be noted that the HUZSP* basis set, even augmented with the polarization functions and diffuse functions we employed, is still quite small and the relative ordering among $\mathrm{Ca[H_2O]_6^{2+}{\cdot}2H_2O}$, $\mathrm{Ca[H_2O]_7^{2+}{\cdot}H_2O}$, and $\mathrm{Ca[H_2O]_8^{2+}}$ must be considered tentative.

In an analysis of the binding of metal ions to carboxyl groups[55] we found that magnesium apparently always binds in a monodentate manner, while calcium can bind in a bidentate manner as well. A similar conclusion has been reached by Einspahr and Bugg.[17,18] Thus, while calcium is somewhat larger than magnesium, it is possible for either metal ion to bind to a site if a magnesium ion can achieve an approximately octahedral arrangement of ligands, and a carboxylate group can rotate to bind calcium in a bidentate manner, but magnesium only in a monodentate manner.[3] We listed in Table 3 the number of monodentate and bidentate carboxyl groups for calcium coordination numbers 6−8. Calcium is larger than magnesium by 0.3 Å (0.65 Å ionic radius for $\mathrm{Mg^{2+}}$, 0.99 Å for $\mathrm{Ca^{2+}}$,[68] and

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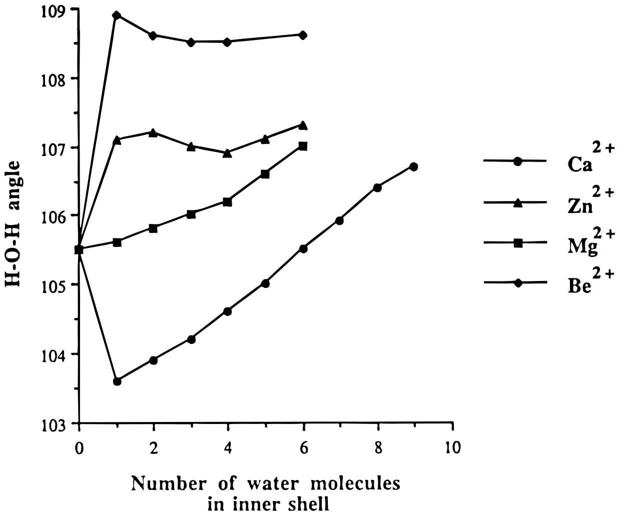

**Figure 6.** H−O−H angles of the first-shell water molecules in the structures $M[H_2O]_n^{2+}$ (M = Be, Mg, Zn, Ca; $n = 1-9$) as a function of the number of water molecules surrounding the metal ion $M^{2+}$. Computational levels: $Ca^{2+}$, RHF/HUZSP*(*p*)//RHF/HUZSP*(*p*); $Zn^{2+}$, RHF/HUZSP*(*d*)//RHF/HUZSP*(*d*); $Mg^{2+}$ and $Be^{2+}$, RHF/6-31G*//RHF/6-31G*.

since the radius of an oxygen atom is 1.40 Å,[67] the average metal−oxygen distances are approximately 2.05 Å for $Mg^{2+}$ and 2.4 Å for $Ca^{2+}$. The surface areas of spheres at these distances are 53 and 72 Å$^2$, respectively, that is, approximately 9 Å$^2$ per bound oxygen atom; thus, on the basis of ionic size, one would expect coordination numbers of 6 and 8, respectively, for magnesium and calcium ions.

The effect of the metal ion on water molecules was also addressed. The extent of polarization of the water molecules was estimated by examining the H−O−H angle in water molecules that are bound *directly* to the calcium, beryllium, magnesium, or zinc cation. Neutron diffraction studies of some hydrates (in which hydrogen atoms are better located than in X-ray crystal structures) indicated that metal ions can affect the H−O−H angle and that different metal cations do so to different extents. The H−O−H angles calculated in our studies for various calcium coordination numbers are given in deposited Table 14S. An experimental average H−O−H angle for divalent cations bound to water in some careful neutron diffraction studies is 107.7° ($Al^{3+}$, 104.6°;[88] $Cu^{2+}$, 105.2−106.8;[89,90] $Mn^{2+}$, 108.4−109.1°;[91] $Cd^{2+}$, 112.8° [92]). As seen in Figure 6, molecular orbital calculations at the RHF level for $M[H_2O]_n^{2+}$ (M = Ca, Be, Mg, and Zn; $n = 1-8$) confirm that different metals alter the geometries of the water molecules in different ways and to different extents. Interestingly, for all the $Be^{2+}$, $Mg^{2+}$, and $Zn^{2+}$ hydrates, the H−O−H angles are *greater* in the field of the ion than they are in an isolated water molecule when the same basis set is employed. For $Ca^{2+}$, however, the H−O−H angles for $n = 1-6$ are *smaller* in the field of the ion than they are in isolated water using the same basis set. In order to see if this difference in behavior is dependent on the level of the calculations, MP2(FULL) optimizations were carried out for $H_2O$ and $M[H_2O]^{2+}$ (M = Be, Mg, and Zn) using the 6-311++G** basis set and for $H_2O$ and $Ca[H_2O]^{2+}$ using the 6-311++G** basis set for the O and H atoms and the HUZSP basis set augmented by two p-type and two d-type polarization functions as well as diffuse functions on the Ca atom. The results of these optimizations show, together with atomic charges from a natural population analysis,[93] that as the ionic radius increases along the family $Be^{2+}$:$Mg^{2+}$:$Ca^{2+}$ there are clear trends—the H−O−H bond angle, the H−O bond distances, and the charge on the hydrogen atoms decrease, while the M−O bond distance and charge on the metal ion increases. We are currently investigating the effects of other metal ions on the H−O−H angle in $M[H_2O]^{n+}$.

If a magnesium ion is bound by four monodentate carboxyl groups, it still has binding space for two water molecules or two oxygen-containing groups on the substrate. Calcium behaves somewhat differently. It binds, on the average, four carboxyl (monodentate or bidentate) oxygen atoms, leaving three positions available for additional binding. One of these positions is often taken up by a carbonyl group and another by water, leaving (for coordination numbers 7 and 8) another liganding position. The role of calcium as a signaler seems to require that its binding be specific and that its release be unimpeded. Magnesium serves a different function in maintaining groups in a rigid octahedral manner, but its tendency to always bind water in a protein (see Figure 9) implies that nature may use this metal ion to ensure that water is present in the active site. The stronger binding of water by magnesium rather than calcium is highlighted by the averaged values (from deposited Table 13S) of 2.2 water molecules per magnesium site (36 protein crystal structures, 44 sites) versus 1.5 water molecules per calcium site (55 protein crystal structures, 79 sites). This is also expressed in the hydration energies shown in deposited Figure F.

These *ab initio* molecular orbital studies of calcium ion coordination show that it is possible to get local minima on the potential energy surface with at least nine (and perhaps more) water molecules in the first hydration shell around the calcium ion. This result may be compared with our finding that a stable hexahydrate of divalent magnesium had a strong energy minimum, but that we could not obtain a local minimum with seven water molecules in the first hydration shell of a magnesium ion.[13] Beryllium ions (which are much smaller) were able to form a stable structure with six water molecules directly bound to them, but structures with four water molecules in the first coordination shell and two in the second shell were considerably lower in energy.[12] With reference to bulk solvation, however, it should be noted that these are isolated cluster calculations which do not include the effects of bulk water.

For calcium ions, as described in this article, the energies of a variety of water coordination modes $Ca[H_2O]_n^{2+}\cdot mH_2O$ are similar [(7,0), (6,1), (5,2) and (8,0), (7,1), (6,2) for *n* and *m*, respectively], although as shown in Figure 8, a coordination number of six water molecules seems to be marginally preferred. The large energy differences seen in the binding of different numbers of water molecules to the first coordination shell of magnesium ions,[13] with a minimum at six, are not found for calcium ions. The energy difference between the various arrangements of water molecules around a calcium ion may change when more complete basis sets can be used in the calculations. Using the computational results of Floris and coworkers[69] on (6.0), (8,0), and (9,0) (for *n* and *m*, respectively) to estimate contributions due to bulk water suggests that the hydration number for calcium ions is 8, although, again, the energy differences are extremely small. Experimental data from

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the CSD and ICSD suggest that the coordination number can go up to 10 with an average of 7.3 in the ICSD and values from 6 to 8 in the CSD (27% each with coordination number 6 or 7, 41% with coordination number 8). Proteins in the PDB seem to have a general coordination number around 7, and that is the coordination number found in EF hands which are motifs in proteins designed specifically to bind calcium ions.[94]

This information on calcium ion coordination from a variety of sources—crystal structures of small molecules and proteins, and *ab initio* molecular orbital calculations of isolated hydrates *in vacuo*—point to a coordination number of 6 in isolated clusters but suggest that the preferred coordination number lies more in the neighborhood of 7−8 in solution and crystal structure studies. Why this difference? The numerical value of the coordination number probably depends on the partial negative charge on the oxygen atom of the liganding group and its distance from the cation. It could be assumed that the amount of partial negative charge on the oxygen atom of an isolated water molecule is such that six water molecules at a distance of 2.06 Å (average $Mg^{2+}\cdots O$) will locally compensate for the charge of divalent magnesium; this charge compensation may also be aided by a size factor, in that in a hexaaquated magnesium ion the oxygen atoms are at normal contact distances apart (with no space between). Presumably the positive charge on the magnesium ion before hydration is spread over the surface of the $[Mg(H_2O)_6]^{2+}$ unit, on the hydrogen atoms.

Calcium ions are larger than magnesium ions, however. Therefore liganding water molecules must necessarily lie further from the calcium than from the magnesium ion, so that, for hexahydrated calcium ions, the oxygen atoms are no longer in normal contact even though the charge neutralization may be fairly satisfactory. This may explain why the hexaaquated calcium ion is found much less often than the hexaaquated magnesium ion in crystal structures of small molecules (the CSD). Other oxygen-containing functional groups in proteins, such as main-chain carbonyl groups which have a lower partial negative charge on the oxygen atom than does water and carboxylate groups which have a higher partial negative charge, can distribute around the calcium ion and perhaps fill the space better. Indeed the bidentate carboxylate binding mode involves two oxygen atoms 2.2 Å apart, rather than the normal nonbonded oxygen−oxygen distance of 2.6−2.7 Å, and therefore may allow more oxygen atoms to lie in the first coordination shell. Thus, the tendency of a calcium ion to bind oxygen atoms of water molecules is offset by its size which does not present an ideal geometry-versus-charge situation for such binding; if the space is filled by water molecules there is too much charge around the calcium ion. Other functional groups with different partial negative charges from water on their oxygen atoms can better satisfy these dual charge and space-filling requirements.

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**Supporting Information Available:** Figure A, (a) The number of entries in the CSD containing Ca−X interactions (X = N, O, Cl, Br) for coordination numbers 3−10. (b) The number of $Ca^{2+}-X$ first-sphere interactions for coordination numbers 3−10. (c) The percentages of various ligands bound to $Ca^{2+}$ in crystal structures extracted from the CSD; Figure B, Crystal structure of a compound (JOFPID) in which calcium has a coordination number of 3. (Diagrams in deposited Figures A, B, and C are drawn by use of the program ICRVIEW;[18] nitrogen atoms are stippled and oxygen atoms are filled circles.); Figure C, Two crystal structures, (a) JOFPOJ and (b) YALJEA, in which calcium has a coordination number of 4; Figure D, Crystal structure, SICGUG01, in which calcium has a coordination number of 5; Figure E. Variation of the $M^{2+}\cdots O$ bond length (in Å) with coordination number (M = Be, Mg, Zn, Ca) [calculational levels: Be, MP2(FULL)/6-31G*//MP2(FULL)/6-31G*; Mg, MP2(FULL)/6-31G*//MP2(FULL)/6-31G*; Zn, MP2(FC)/HUZSP*(*d*)//MP2(FC)/HUZSP*(*d*); Ca, MP2(FULL)/HUZSP*(*p*)//MP2(FULL)/HUZSP*(*p*)]; Figure F, Variation in enthalpies of hydrates versus number of water molecules in the first hydration shell of $Ca^{2+}$, $Zn^{2+}$, $Mg^{2+}$, and $Be^{2+}$; Figure G, Net charge on the metal cation (M) in the structures $M[H_2O]_n{}^{2+}$ (M = Be, Mg, Zn, Ca; $n = 1-8$) calculated from the MP2 charge density [basis sets: $Be^{2+}$, 6-31G*; $Mg^{2+}$, 6-31G*; $Zn^{2+}$, HUZSP*(*d*); $Ca^{2+}$, HUZSP*(*p*,*d*)]; Table 1S, Entries eliminated from the CSD search; Table 2S−6S, refcodes and metal coordination of calcium-containing crystal structures in the CSD; Table 7S, bibliographic references for crystal structures used (CSD); Table 8S, number of water molecules bound to calcium for a particular coordination number; Table 9S, **Z** matrices from molecular orbital calculations; Table 10S, total molecular energies; Table 11S, calculated Ca−O distances; Table 12S, sequential hydration enthalpies; Table 13S, water bound to metal ion in protein crystal structures (PDB); and Table 14S, computed H−O−H angles (70 pages). Ordering information is given on any current masthead page.

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[94] Ikura, M. *Trends Biochem. Sci.* **1996**, *21*, 14−17.