# Ion Binding Site Structure and the Role of Water in Alkali Earth EDTA Complexes

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#### Abstract:

10 The interactions between molecular hosts and ionic guests and how the chemical environment in aqueous solutions changes these interactions are challenging to disentangle from solution data alone. The vibrational spectra of cold complexes of ethylenediaminetetraacetic acid (EDTA) chelating alkaline earth dications in vacuo encode structural characteristics of these complexes and their dependence on the size of the bound ion. The correlation between metal binding geometry 15 and the relative intensities of vibrational bands of the carboxylate groups forming the binding pocket allows us to characterize changes in molecular geometry by interaction with other molecules. The evolution of these structural markers from bare ions to water adducts to aqueous solution illustrates the role of water for the structure of ion binding sites in chelators. The results show that the binding pocket of EDTA opens up in aqueous solution, bringing the bound ion closer 20 to the mouth of the binding site, and leading to an increased exposure of the bound ion to the chemical environment.

### Introduction

Supramolecular structures binding metal dications are of interest in a wide range of chemical and biological contexts, from chelators and receptors for sensing<sup>1,2</sup> to metal binding proteins in signal transduction and muscle action<sup>3-5</sup>. On a coarse scale of interactions, the properties of such binding sites are governed by the nature of the ion-coordinating ligands and the structure of their binding pockets. These pockets are often constructed by a combination of carbonyl and carboxylate functional groups that interact with the ion<sup>5</sup>, e.g., in many chelating agents, and in proteins such as calmodulin and other EF hand proteins<sup>6</sup>. On a more subtle level, the behavior of an ion binding site also depends on the chemical environment of the pocket. Apart from protein environments, water molecules play a particularly important role in this context, since they can interact with both the metal ion and the constituents of the binding pocket, and thus have the potential to alter the interplay between the host molecule and the guest ion. In addition, the competition between ion binding and ion hydration plays an important role in binding and selectivity, and this issue has been at the forefront of host-guest chemistry in recent years<sup>7</sup>.

Infrared (IR) spectroscopy has been shown to offer powerful probes for the structure and dynamics 15 of ion binding sites<sup>8,9</sup>. Aminopolycarboxylic acids offer molecular models for binding pockets, with ethylenediaminetetraacetic acid (EDTA) being the most prototypical member of this group, and such models afford simplified access to the IR signatures of ion binding structures compared to the study of full proteins. However, the condensed phase environment complicates the direct 20 connection of IR spectroscopic information even to such structural models due to the complex chemical environment of solutions.

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Fig. 1. Geometry of EDTA complexes with alkali earth dications. (A)  $[M(II) \cdot EDTA]^{2-}$  line drawing. (B) Ball-and-bond type structure of  $[M(II) \cdot EDTA]^{2-}$  highlighting the binding pocket. Axial and equatorial carboxylate ligands are labeled "ax" and "eq", respectively. (C) Axial slice of  $[M(II) \cdot EDTA]^{2-}$  with the definition of the axial opening angle,  $\theta_{ax}$ . (D) Equatorial slice of  $[M(II) \cdot EDTA]^{2-}$  with the definition of the equatorial opening angle,  $\theta_{eq}$ , and the ion position, *d*. (E) Lowest energy structure of  $[M(II) \cdot EDTA]^{2-} \cdot H_2O$ . The water molecule is in front of both axial and equatorial carboxylate ligands. In parts C and D, the  $C_2$  axis is shown as a dashed line, and light grey arrows indicate the direction of the transition dipole in each carboxylate group. Colors for the highlighted atoms: red for O, dark grey for C, blue for N, and yellow for the metal ion.

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Cryogenic ion spectroscopy methods<sup>10-19</sup> provide a complementary approach to uncover structural details of complex molecular systems through mass selection of spectroscopic targets in vacuo, affording a high level of control of the chemical environment, including an ability to probe the effects of a small number of water molecules on the molecular structure of solute ions. EDTA is an archetypal and widely used chelator, with four carboxylate groups and two nitrogen atoms forming a binding pocket for cationic guests (Fig. 1), and it has been used as a model system for  $Ca^{2+}$  binding pockets in EF hand proteins<sup>8</sup>. It binds metal ions in a hexadentate fashion, forming complexes with  $C_2$  symmetry and approximately octahedral coordination in the case of alkali earth dications as shown in Fig. 1. These complexes are accessible to cryogenic ion spectroscopy

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methods through electrospray ionization, and their electronic structure has been studied by photoelectron spectroscopy in this fashion<sup>20-22</sup>. Here we employ this method<sup>23</sup> to characterize the IR signatures encoding the structure of EDTA binding alkali earth metal ions  $M^{2+}$  (M = Ca, Mg, Sr, and Ba).

# Bare M<sup>2+</sup>-EDTA complexes

Fig. 2 shows the vibrational spectra of all four complexes in the region of the antisymmetric OCO stretching vibrational bands of the carboxylate groups (full IR fingerprint region shown in Fig. S1). The vibrational modes assigned to each spectral feature are identical for all four complexes, and the measured transition energies as well as a detailed assignment are given in supplementary text and Table S1.

The two peaks observed in each spectrum (**a** and **b**) contain four partially resolved OCO antisymmetric stretching modes<sup>8</sup> with patterns of motion representing different in-phase vs. outof-phase combinations of these vibrations in all four of the EDTA carboxylate groups (see supplementary text and Fig. S2). The highest frequency feature (**a**) corresponds to the all in-phase combination of these motions, while the most intense feature (**b**) encompasses the remaining three unresolved linear combinations of the local antisymmetric stretching motions.

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Fig. 2. Comparison of experimental (upright traces) and simulated (inverted traces) spectra of  $[M(II) \cdot EDTA]^{2-}$  complexes. The spectra show the region of the antisymmetric OCO stretching vibrations, with the metal indicated below each set of traces (top to bottom, Mg, Ca, Sr, Ba). Simulated IR spectra are based on scaled harmonic calculations with each mode represented by a Lorentzian line shape with 8 cm<sup>-1</sup> full width at half-maximum. For a discussion of features **a** and **b** see text.

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As the size of the chelated ion increases<sup>24</sup>, the opening angles of the binding pocket (defined in Fig. 1) and the distance of the ion from the central C-C bond (ion position, d) increase. Fig. 3 shows the calculated geometry dependence on the ion size. The spectroscopic features of the IR

spectra encode information on the structure of each complex, allowing experimental verification in addition to the computational data. The configuration of the carboxylate groups is reflected in the relative intensity  $I_a$  of peak **a**, defining it as  $I_a = A_a / (A_a + A_b)$ , where  $A_a$  and  $A_b$  are the areas under features **a** and **b**, respectively. We choose the equatorial opening angle of the binding pocket,  $\theta_{eq}$ , and the ion position in the pocket, *d*, both defined in Fig. 1, to illustrate the geometry dependence of  $I_a$ , since they present particularly clear correlations of  $I_a$  with significant geometry changes (Fig. 3).

While the nature of the ion modifies the calculated binding pocket geometry, the relative intensities of the antisymmetric OCO stretching modes in vacuo depend predominantly on the conformation of the carboxylate groups themselves (supplementary text and Fig. S6). This geometry dependence can be traced to the orientations of the local transition dipole moments in each carboxylate group. Choosing the  $C_2$  symmetry axis of the complexes as the *z*-axis, the *x* and *y* components of the local OCO antisymmetric stretching transition dipoles of each equatorial and axial pair of carboxylate groups cancel for the mode giving rise to feature **a** (see Fig. 1 and supplementary text), leaving only the z components for generating IR activity in this mode. As larger ions displace the carboxylate groups outward, and the opening angles of the binding pocket increase, the z projection of the transition dipole increases as well (Fig. 1), resulting in the observed growth of  $I_a$  along the sequence from  $Mg^{2+}$  to  $Ba^{2+}$ . This direct correlation allows us to quantitatively model the binding pocket geometry by predicting the equatorial opening angle,  $\theta_{eq}$ , and the ion position, d, from fits of the calculated geometry parameters as a function of the calculated  $I_a$  (Fig. 3). The ion position scales linearly with  $I_a$ , while the equatorial opening angle is described by a second order polynomial (note that both relationships are empirical). We use the fit functions for  $\theta_{eq}$  and d to determine the geometry parameters in the EDTA complexes of the ions under study from the

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experimental values of  $I_a$ , which we obtain from each spectrum by fitting both features with Lorentzian profiles.

Both the calculated and experimental  $I_a$  values increase along the sequence from Mg<sup>2+</sup> to Ba<sup>2+</sup>, indicating that larger ions are bound closer to the rim of the binding pocket, and that the opening angle of the pocket increases as well. Since the ionic radius also increases along this sequence, the trend in the geometry change is consistent with chemical intuition (Mg<sup>2+</sup>, Ca<sup>2+</sup>, Sr<sup>2+</sup>, and Ba<sup>2+</sup> have ionic radii<sup>24</sup> of 0.72 Å, 1.00 Å, 1.18 Å, and 1.35 Å, respectively).

In addition to the changes in relative intensity, features **a** and **b** shift to lower frequencies upon changing the ion from Mg<sup>2+</sup> to Ba<sup>2+</sup>, by a total of 23 cm<sup>-1</sup> and 19 cm<sup>-1</sup>, respectively. Different from the intensity ratio discussed above, which depends mainly on the geometry of the binding pocket, the observed frequency shifts are caused by (i) a combination of changes in complex geometry, (ii) the local asymmetry of the C-O bond lengths between the "inner" O atom coordinating the metal and the "outer", free O atom in each carboxylate group (Figs. 1.C and 1.D), and (iii) the charge distribution within the carboxylate groups. As a result of the subtle interplay of multiple factors, the frequency positions of the peaks are less straightforward to use as a tool for determining the geometry of the binding pocket quantitatively, but we can qualitatively associate a lower frequency with a more open binding pocket and an ion position closer to the rim of the pocket.

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Fig. 3. Correlation of the ion size and the relative intensity  $I_a$  with geometry parameters of the binding pocket. (A) Equatorial opening angle and (B) ion position as defined in Fig. 1 determined from the experimental relative intensity of peak **a** for each metal. (C) Equatorial opening angle and (D) ion position as a function of ionic radius for each metal. Circles represent experimental data from  $[M(II) \cdot EDTA]^{2-}$  complexes in vacuo. Diamonds show experimental data taken in aqueous solution (D<sub>2</sub>O). In each of the two data sets,  $I_a$  increases from Mg<sup>2+</sup> to Ba<sup>2+</sup> (data points are color coded for each metal as shown in panel A). The full lines are fits to the calculated data for  $[M(II) \cdot EDTA]^{2-}$  complexes in vacuo, the dashed lines are analogous fits from a polarizable continuum model. The grey shaded areas allow estimates of the geometry parameters of the EDTA binding pocket in aqueous solution. Computational data points and their comparison to the experimental values of  $I_a$  are shown in Fig. S4. Error bars are  $\pm 1 \sigma$  uncertainties arising from a nonlinear parameter fit of the peak areas for peaks **a** and **b**.

#### **Solvation effects**

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The presence of solvent molecules can be expected to perturb the geometry of the binding pocket, but it is challenging to obtain computational predictions for this case. On the one hand, the simulation of a sufficient number of explicit solvent molecules can be computationally expensive to the point of being unfeasible, both regarding the total size of the system and the large number of possible solvent conformations around a solute complex as large as [M(II)·EDTA]<sup>2-</sup>. On the other hand, the use of polarizable continuum models (PCM) for solvation completely neglects the molecular structure of the solvent, which makes the predictions less reliable, particularly in the case of water, which forms hydrogen bonded solvation networks.

To probe solvation effects, we compare the data for bare EDTA complexes in vacuo to IR spectra 10 of EDTA complexes with one water adduct and in aqueous solution. Fig. 4 shows the antisymmetric OCO stretching signatures of [Ca(II)·EDTA]<sup>2-</sup> for the bare ion, the singly hydrated complex, and the fully hydrated complex in D<sub>2</sub>O solution, along with computational predictions (see Fig. S9 for solution phase spectra for all ions). Addition of a single water molecule leads to a weak blue shift of features **a** and **b** by 1 cm<sup>-1</sup> and 5 cm<sup>-1</sup>, respectively. Exploratory measurements 15 on dihydrated  $[Ca(II) \cdot EDTA]^{2-}$  show that the frequency of peak **b** is the same as for the monohydrate (Fig. S8). In contrast, both features **a** and **b** show a red shift of 45 cm<sup>-1</sup> upon full hydration. Interestingly, PCM calculations recover the observed red shift of feature b (Fig. 4) for aqueous solutions rather well. This implies that they capture some of the changes in the charge 20 distribution within the carboxylate groups, which affects the force constants of the CO bonds and thereby the frequency positions of the OCO stretching vibrations. For [Ca(II)·EDTA]<sup>2-</sup>, the relative intensity of peak **a**,  $I_a$ , remains around 0.05 for the bare and singly hydrated complex, but increases to 0.13 in aqueous solution. We use the PCM calculations to construct model curves for the equatorial opening angle and ion position in analogy to the curves derived from calculations for the bare ions. We note that while the calculations for bare complexes recover the splitting between peaks **a** and **b** quite well, the PCM calculations predict a narrowing of the splitting, while the experimental splitting increases for all metals under study, leading to a deviation of the calculations by a factor of two. In addition, the agreement of the PCM values for  $I_a$  with aqueous solution data is lower than for the data in vacuo. In view of these discrepancies, we judge that the accuracy of the PCM based curve is lower than that for the bare complexes, and we therefore treat the model curves from the two scenarios (bare ions vs. PCM) as limiting cases for the modeled pocket geometry in aqueous solution to estimate the geometric parameters for the EDTA binding pocket from the experimental values of  $I_a$  in aqueous solution (grey shaded areas in Fig. 3).

Both sets of model curves, which relate  $I_a$  to the geometry of the binding pocket, predict that full hydration leads to a more open, and therefore shallower, binding pocket and a binding position of the ion closer to the rim of the pocket than in the bare complex. The opening angle increases by ca.  $6^{\circ}$  for both Mg<sup>2+</sup> and Ba<sup>2</sup>, and full hydration brings Mg<sup>2+</sup> by ca. 0.14 Å further out of the pocket, while Ba<sup>2+</sup> changes its position in the pocket by ca. 0.33 Å. We qualitatively explain the dependence of the binding pocket geometry on the level of hydration with the structural evolution of the solvent environment. A search for the minimum energy conformation of the monohydrated [Ca(II)·EDTA]<sup>2-</sup> complex (Fig. 1.E) reveals that the first water molecule forms hydrogen bonds to the "inner" oxygen atom on two carboxylate ligands, and the oxygen atom of the water molecule interacts with the dication in the pocket. Other low-lying isomers show similar interactions. Judging from the small blue shift of features **a** and **b**, the presence of the water molecule slightly tightens the pocket, but does not deform it significantly, consistent with the negligible change in  $I_a$ . As the hydration shell grows, solvent molecules will also form hydrogen bonds to the "outer" oxygen atoms of the carboxylate groups. This interaction increases the opening angle of the

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binding pocket and pulls the chelated ion closer to its rim, reflected in the increase of  $I_a$  for each complex in aqueous solution compared to the bare complex in vacuo.



Fig. 4. IR spectra of  $[Ca(II) \cdot EDTA]^{2-}$  at different levels of hydration. The spectra of the bare  $[Ca(II) \cdot EDTA]^{2-}$  ion (top trace) and of  $[Ca(II) \cdot EDTA]^{2-} \cdot H_2O$  in vacuo (center) are compared to  $[Ca(II) \cdot EDTA]^{2-}$  in D<sub>2</sub>O solution (pD  $\approx$  8, bottom trace).

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The IR spectra of EDTA complexes clearly illustrate the impact of the solvation environment on the structural and binding characteristics of the binding pocket. In particular, the molecular nature of the hydrogen bonding interactions with the binding pocket needs to be taken into account to

model the binding pocket geometry in the presence of solvent. This is important not only for fully solvated chelators like EDTA, but also for chemical environments that restrict solvent access, such as ion binding pockets in proteins, where only a small number of water molecules interact with the ion and its binding site, but where the presence or absence of water molecules can impact the function of the protein.

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# Author contributions:

M.M.F contributed data acquisition, visualization, and writing. J.M.W. contributed conceptualization, methodology, visualization, funding acquisition, project administration, supervision, and writing.

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Competing Interest Declaration: Authors declare that they have no competing interests.

#### **Additional Information**

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# Supplementary Materials

Materials and Methods

Supplementary Text

Figs. S1 to S9

5 Tables S1-S5

References (25-35)