Calcium L-edge XANES study of some calcium compounds

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The Ca $L_{3,2}$-edge XANES spectra of six calcium salts have been measured in both total electron and fluorescence yields using a high-resolution spherical grating monochromator. The compounds investigated were; CaF_2, $CaCO_3$, $CaCl_2 \cdot 2H_2O$, calcium phosphate, calcium glycerophosphate and calcium gluconate. We find that the fine structure of the Ca $L_{3,2}$-edges for each compound is unique and relates to the local structure of the Ca atom. The implications of these results to the study of the structure of calcium binding sites in systems of biologically interest will be discussed.

Keywords: Ca $L_{3,2}$-edge XANES

1. Introduction

The chemistry of calcium and its compounds is important in a wide variety of technically important fields both old, as in the making of concrete, and new, such as chemical sensors (Fergus, 1997), artificial bone implants (de Groot, 1983), photonics (Kulatov *et al.*, 1997) and medical treatments (Sargent *et al.*, 1999). The perhaps most important area of calcium research today is the biochemistry of calcium.

Calcium is not only an essential component of leaves, bones, teeth and shells it also plays an important role in the biochemistry of living organisms being involved in many functions, including muscle contraction, blood clotting, neurotransmitter release, microtubule formation, hormonal response, protein stabilization, intracellular communication among others. Many of the biological functions of calcium occur via interactions of the calcium ion Ca^{2+} found in solution with proteins such as calmodulin (Spiro, 1983).

The abundance and uses of calcium and its compounds both in biology and in industry have contributed to their extensive study. Even with this intense and long term interest, calcium compounds have not been studied extensively using soft X-ray absorption spectroscopy at the Ca L-edge. Although, high resolution Ca L-edge XANES spectra of CaF_2 have been obtained recently (de Groot *et al*., 1990, Himpsel *et al*., 1986, and Rieger *et al*., 1986), CaF_2 is, to the best of our knowledge, the only calcium compound studied by Ca L-edge XANES even with calcium's obvious importance in biomedical, industrial and environmental areas.

The aim of this and subsequent studies is to generate a catalogue of reference spectra which can then be used to help determine the structural environment surrounding Ca atoms in enzymes and proteins through the use of Ca L-edge XANES.

2. Experimental

Powdered samples of each of the calcium salts were obtained from commercial suppliers. [CaF_2, $CaCO_3$, $CaCl_2 \cdot 2H_2O$, calcium phosphate ($Ca_5(PO_4)_3(OH)$): Aldrich, calcium glycerophosphate: ICN Biomedicals and calcium gluconate: J T Baker Inc.] The fine powders were spread on double-sided carbon tape and mounted onto a stainless steel sample disk before introduction to the experimental chamber.

Ca $L_{3,2}$-edge X-ray absorption spectra were taken on a recently implemented high resolution Spherical Grating Monochromator (SGM) of the Canadian Synchrotron Radiation Facility (CSRF), located at the Synchrotron Radiation Center (SRC), University of Wisconsin-Madison. The spectra were taken in both the Total Electron Yield (TEY) using specimen current and the Fluorescence Yield (FLY) using a channel plate detector. A 1200 l/mm grating provides high resolution light for the range of 270 – 700 eV (Yates et al. 2000). The incoming monochromatic light intensity (I_0) was measured using a gold mesh detector downstream of the sample chamber.

3. Results and Discussion

Fig. 1 shows the XANES spectra of $CaCl_2 \cdot 2H_2O$ and CaF_2 powdered samples. Both spectra have been normalized to I_0 and had a linear pre-edge background removed before they were scaled for clarity. Spectra recorded in FLY exhibit identical spectral patterns albeit suffer some thickness effects (self-absorption). The experimental resolution for the spectra was approximately 0.2 eV.

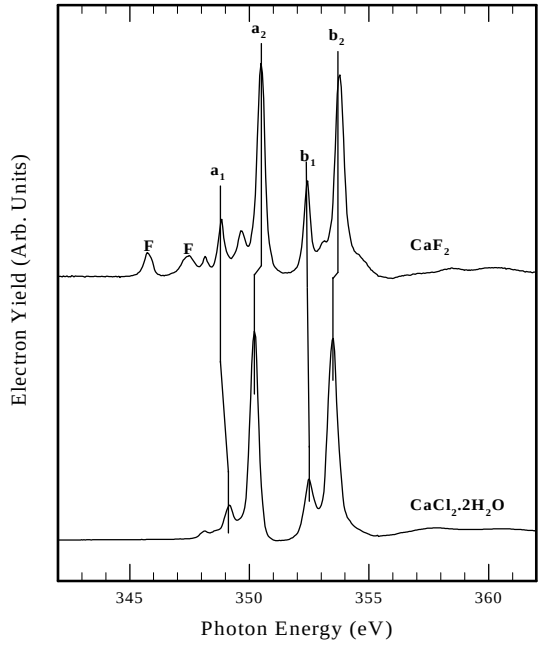

Figure 1.
Ca $L_{3,2}$-edge XANES spectra of CaF_2 and $CaCl_2.2H_2O$. Peaks not related to the Ca spectrum of CaF_2 are labeled "F".

The photon energy scale has not been calibrated against an absolute standard and it should be noted that the positions of the L_3 and L_2-edges that we observe (350.5 eV and 353.8 eV respectively) for CaF_2 will correspond to those reported by Rieger *et al.* (1986) by adding 1.2 eV. Our CaF_2 spectrum reproduces all the features seen in that of Rieger et al. except that our spectrum contains some additional spectral features at both lower and high photon energies. These extra peaks (below 348 eV and above 355 eV) are the F K-edge XAFS from CaF_2 produced by the second order light. The noticeable amount of second order light present in the SGM has since been minimised by the addition of a filter before the sample chamber. Fortunately, the presence of second order light in the beamline only affects the CaF_2 spectrum in this experiment as none of the other samples contain atoms with energy levels in the correct range of the second order light. Since CaF_2 is a scintillator, photoluminescence yield was also used successfully to record the XANES. The results together with theoretical calculations of the CaF_2 spectra will be reported elsewhere in some details (Naftel et al., unpublished).

The Ca $L_{3,2}$-edge XANES of the halides (fluoride and chloride seen in Fig. 1) consist of two main spin-orbit related peaks, L_3 and L_2, along with a number of smaller peaks which appear to precede the L_3 and L_2-edges main peaks. The origin of these multi-peak pattern is known to be the crystal field arising from the symmetry of the atoms surrounding the Ca^{2+} ion in the first co-ordination sphere. It is interesting to note that the relatively small energy separation of the spin-orbit splitting in 2p levels of the alkali, alkali-earth and early 3d row elements, together with the crystal field produces a variety of very complicated $L_{3,2}$ XANES features, a situation not often encountered in the 4d and 5d d counterparts (Hu et al. 2000, Sham, 1982). The effect of the crystal field on the atomic multiplets is to add lines forbidden in a spherically symmetric environment and redistributes the intensity over all the lines in the spectrum (de Groot *et al.*, 1990). The number of lines seen can be related to the symmetry around the Ca^{2+} ion. Following the analysis of de Groot et al. (1990) we have labelled the main peaks a_1 and a_2 (L_3-edge) and b_1 and b_2 (L_2-edge). The splitting of a_1 and a_2 (as well as b_1 and b_2) is related non-linearly to the value of the crystal field parameter (10Dq). One must be careful, however, as the sign of the crystal field parameter within a specific space group also affects the shape of the spectra.

The Ca L-edge spectra for all six compounds studied are shown in Fig. 2. Clearly each spectrum has a rich fine structure. The splitting in the L_3 and L_2-edges along with the co-ordination number of Ca and the symmetry about the Ca site, if known, are given in Table 1

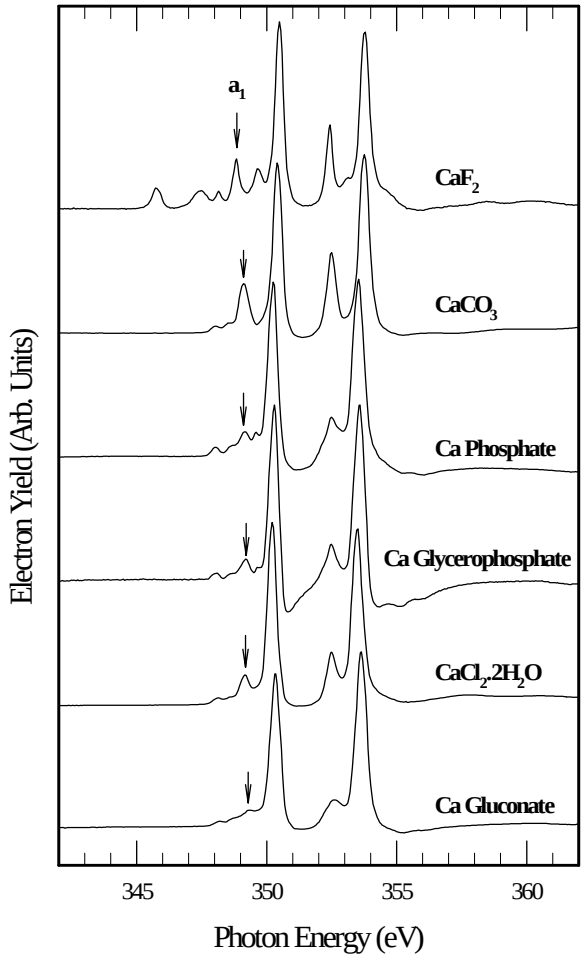

Figure 2. Ca $L_{3,2}$-edge XANES spectra of six Ca compounds.

Table 1. Relevant parameters from the Ca L-edge spectra.

Compound	ΔL_3 (a_2-a_1)	ΔL_2 (b_2-b_1)	Co-ordinationNumber	Site Symmetry
Ca Gluconate	1.0	1.0		
$CaCl_2 \cdot 2H_2O$	1.0	1.0	6	[b]Octahedral O_h
Ca Glycerophosphate	1.1	1.0		
$Ca_5(PO_4)_3(OH)$	1.1	1.0	8 and 9	
$CaCO_3$	1.3	1.2	6	
CaF_2	1.6	1.4	8	[c]Cubic O_h

[b]Le Claire and Borel, 1977, [c]Villars and Calvert, 1991.

Each of the spectra in Fig. 2 is unique in terms of the intensity and number of visible peaks as well as the splittings noted in Table 1. The uniqueness of each spectrum makes the use of Ca L-edge XANES as a probe of the local Ca structure attractive and feasible. Although no structural information about the Ca sites can be quantitatively determine determined from the Ca $L_{3,2}$-edge XANES alone, it possible from the spectra and information that we have to make some interesting observations.

First, from the results of de Groot *et al*. (1990), it appears that for an octahedral crystal field (O_h symmetry, CN = 6, and a positive crystal field parameter) there are usually two small initial peaks, of about the same intensity, with the third peak being more intense (a_1). For a cubic crystal field (O_h symmetry, CN = 8, and a negative crystal field parameter) there is usually only one leading weak peak followed by a more intense peak (a_1). This observation is repeated in Fig. 1 in comparing the spectrum of CaF_2 (Cubic) and $CaCl_2$ (octahedral). From Fig. 2 this observation suggests that at least $CaCO_3$ with a CN= 6 could have its Ca in an octahedral environment.

Second, the intensity of (a_1) relative to (a_2) in general indicates the magnitude of the crystal field. This suggests that Ca Gluconate has the smallest crystal field parameter while CaF_2 has the largest. This trend is in good accord with electronegativity considerations.

Third, the spectra of calcium glycerophosphate and calcium phosphate are very similar suggesting that both have a similar Ca site structure.

4. Summary

The present work represents the first steps in developing a database of the Ca $L_{3,2}$-edge spectra of model calcium compounds. The clear distinction among the spectra of all the compounds studied, we believe, makes XANES spectroscopy a feasible method to elucidate the local electronic structure systematic of Ca binding sites in proteins. This, together with Ca K-edge and other techniques will likely make some future impact on the study of the functionality of Ca in biological systems. Clearly more detailed analysis of a number of biologically important Ca compounds is needed.

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