

Very first tests on SOLEIL regarding the Zn environment in pathological calcifications made of apatite determined by X-ray absorption spectroscopy.

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Abstract : This very first report of a X-ray absorption spectroscopy experiment on Soleil is part of a more large long term study dedicated to ectopic calcifications. Such biological entities composed of various inorganic and/or organic compounds contain also trace elements. In the case of urinary calculi, different papers already published point out that these oligo elements may promote or inhibit crystal nucleation or growth of mineral or organic species involved. By using such tool specific to synchrotron radiation i.e. determine the local environment of oligoelements and thus their occupation site, we contribute to our understanding of the role of trace elements in ectopic calcifications.

1. Introduction

Pathological calcifications, associated to very different pathologies ranging from to atherosclerosis to gastric cancer [see for examples 1,2] may be composed of various inorganic and/or organic compounds. If we restrict the scope of such biological entities to kidney stones, the principal component founded for the mineral part, which has a hierarchical structure [3,4] are calcium oxalate (70% of the cases), calcium and magnesium phosphates (15%), uric acid (10%) and cystine (1%). By using tools implemented at the hospitals consistent with this chemical complexity such optical microscope and infrared spectrophotometry, it is possible to related their chemical nature and macrostructure to a pathology. For example, in the case of urinary calculi, it has been established a link between cystine and cystinuria while struvite (Magnesium Ammonium Phosphate) kidney stones often called an "infection stone" are usually caused by urinary tract infections with certain types of bacteria. It is also of note that such correlation can be far more subtle, a same chemical phases being related to very different pathologies. In that case, an explanation can be found taking into account the hierarchical structure of the mineral part.

An additional level of complexity is given by the presence of trace elements. Among them, some elements such Zn, due to their special affinity for some crystalline species, may play a role in crystal formation and/or organization of the stones [5]. In previous studies [67], we tried to classify the different elements through synchrotron radiation μ -X-ray fluorescence. Obviously, the presence of major elements which form crystalline phases, i.e. Ca and S (S for cystine) are measured, then oligo elements such as Sr, Se, "metal of life" such Fe, Cu and Zn and finally pathological elements such as Pb. Note that the presence of Sr is linked to the fact that Ca and Sr share the same column in the periodic table (same for S and Se). More precisely, the complete set of data which include the different family of kidney stones (whewellite, weddellite, apatite, cystine, uric acid, struvite) is highly suggestive of a substitution process between calcium and the other metals (and not a catalytic one).

We focus the first report of a XAS (X-ray absorption spectroscopy) experiment on Soleil on one of these oligo-elements i.e. on Zn in order to precise the substitution process. Different studies have already shown that XAS can be employed in order to precise the

environment of one selected element through its absorption edge. More precisely, information regarding the average coordination geometry as well as the average effective charge are available even for low Zn concentration materials [8].

2. Experimental

2.1 Material

The biological samples (Table 1) used in the present study comes from French patients and were first characterized by infrared spectrophotometry using a Fourier transform infrared spectrometer Vector 22 (Bruker Spectrospin, Wissembourg, France) according to the analytical procedure previously described [9, 10].

Table 1. Composition as given by Fourier Transform Infra Red Spectroscopy (F.T. I.R.) and the origin of the ectopic calcifications.

Sample	Origin of the calcification	Composition as given by F.T. I.R.
N 9009	kidney	55% CA, 38% MAP, 3% Prot
N 13066	kidney	79% CA, 10% C2, 7% Prot
N 13086	Prostatic	84% CA, 12% Prot
N 20208	kidney	79% CA, 15% MAP, ?

CA : Carbonate hydroxylapatite , MAP = Magnesium Ammonium Phosphate (struvite)

Prot : Protein, C2 (Wd) = weddellite

Then the stones were investigated either on the H10 beamline [11] at LURE (Orsay, France) or on the New DIFFABS beamline [12] at SOLEIL (St Aubin) in order to determine the electronic state as well as the coordination of Zn atoms. DIFFABS offer the possibility of studying the short and medium-range characteristics of materials by the almost simultaneous combination of XAS and wide angle scattering (WAXS). DIFFABS, situated at the D13-1 bending magnet is equipped with a focused, monochromatic beam (“normal mode”, energy range: 3-23 keV) provided by two bent, Rh-coated, mirrors and a double-crystal Si(111) sagittally focusing monochromator situated in the optic hutch. More details are available on the web site. In our case, the experiment was performed without a focusing device ...

2.2 Analysis procedure

X-ray Absorption Near Edge Structure (XANES) spectra were normalized to an edge jump of unity. A prior removal of the background absorption was done by subtraction of a linear function extrapolated from the preedge region.

Following a monoelectronic description of the absorption phenomenon, electronic transitions are allowed between 1s and 4p levels but prohibited toward 3d levels (the dipole selection rule gives $\Delta l = \pm 1$). In fact, hybridizations between 4p and 3d atomic orbitals allow transition between molecular orbitals with 3d and 4p mixed character. At this point, we would like to recall that for an octahedral symmetry, the first unoccupied orbital, t_{1u}^* possesses a 4p character and is associated to the main jump observed at 9665-9668 eV for Zn K-edge spectra. The case of tetrahedral symmetry is more complicated [13]. From an experimental point of view, G. A. Waychunas et al. [14] have discussed extensively the form and the position of the Zn edge. In the case of an octahedral first-neighbour anion shell, a strong initial peak followed by a well-defined second peak is measured. Eventually, a small number of other contributions are also visible in the second peak. At the opposite, tetrahedral Zn

(willemite, zincite, and franklinite), is associated to a more complex XANES. At least three well-defined edge features can be visible, and there is no large drop in absorption after the first peak, as with the octahedral XANES. In one particular case (franklinite), the edge part of the absorption spectrum is highly unusual and additional detailed structure are measured.

Regarding the XAS oscillations, we can use the Zn-O interatomic distance as a fingerprint of the Zn geometry. The characteristic Zn-O distance in tetrahedral coordination has been reported to be between 1.92 and 1.99 Å, while Zn in octahedral coordination with first-shell O has a Zn-O distance between 2.02 and 2.12 Å [15]. For example, for the following compounds namely ZnO, ZnFe₂O₄, Zn(OH)₂, Zn₂SiO₄, and CaZn₂(OH)₆·2H₂O in which Zn is in tetrahedral coordination, the Zn-O distances are respectively equal to 1.98, 1.98, 1.97, 1.96, and 1.94 Å.

3. Results

The X-ray absorption spectra at the Zn K edge are characterized by two main features, labelled A and B in the figure 1 [16]. The peak A is correlated to transitions over the Fermi level and was found to be sensitive to interatomic distances [17] and/or coordination geometry [18]. The almost constant value of the edge position indicates a relatively constant value for the effective charge of the inserted zinc ions for all compounds.

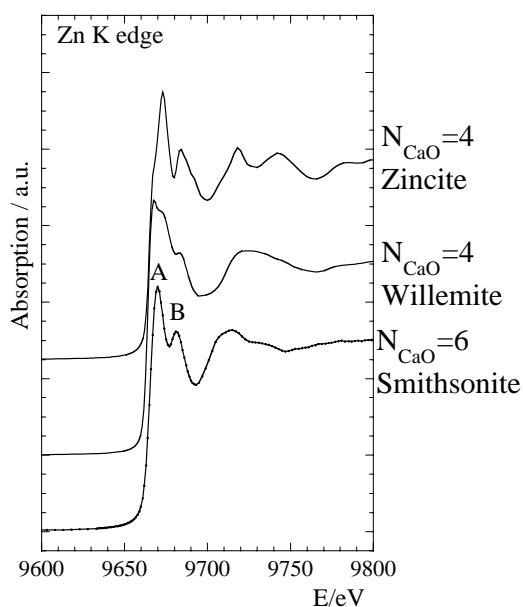


Fig 1

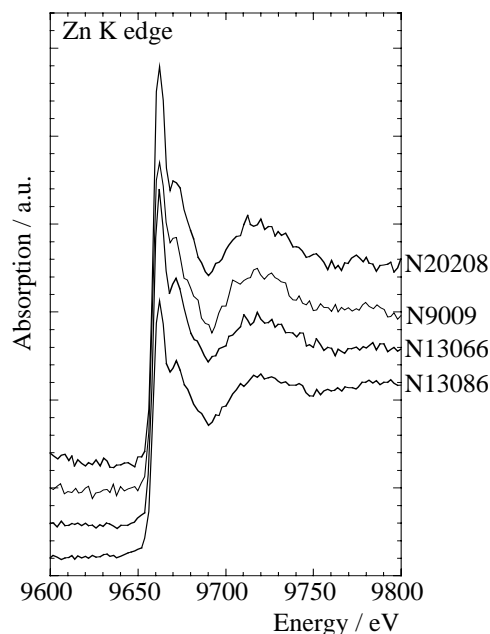


Fig 2

Fig 1 Xanes spectra recorded at the Zn K edge (9659. eV) for a set of references (Zincite, Willemite, Smithsonite). The Zinc environ,ent is indicated in the figure.

Fig 2 Xanes spectra recorded at the Zn K edge (9659. eV) for selected samples

4. Discussion

In the case of our biological samples, powder neutron diffraction experiments have been performed. Although the background level is high due to the hydrogen content of this biological sample, the high quality of the diagram allows a complete Rietveld-type refinement using the structural model where hydroxylapatite, Ca₁₀(PO₄)₆(OH)₂, has a structure which is

hexagonal with space group $P6_3/m$ [19;20]. Four Ca ions, at Ca(I) sites, lie along columns parallel to the *c* axis while six Ca atoms, positioned at Ca(II) sites, are laying on the mirror planes. Thus, there are two distinct calcium environments [21 ;22], Ca(I) which is best described for the different calcium oxygen bonds as $N_{CaO}=6$ ($R_{CaO}=2.43 \text{ \AA}$)+ $N_{CaO}=3$ ($R_{CaO}=2.79 \text{ \AA}$) coordination environment while Ca(II) is best described as either $N_{CaO}=4$ ($R_{CaO}=2.36 \text{ \AA}$) + $N_{CaO}=2$ ($R_{CaO}=2.51 \text{ \AA}$) + $N_{CaO}=1$ ($R_{CaO}=2.71 \text{ \AA}$) or $N_{CaO}=5$ ($R_{CaO}=2.35 \text{ \AA}$) + $N_{CaO}=2$ ($R_{CaO}=2.51 \text{ \AA}$) + $N_{CaO}=1$ ($R_{CaO}=2.71 \text{ \AA}$). Note that Ca(I) and Ca(II) are present in a 2/3 ratio. Note that in the case of urinary calculi, hydroxylapatite may contain a large amount of carbonate group and thus are denominated as Carbonate apatite (CA).

Here, for all the four selected samples we considered, a careful examination of the Xanes part of the absorption spectra indicates an octahedral complex, Thus, the X-ray absorption spectroscopy indicates that Zinc atoms are probably positionned at Ca(I) site. At this point, additional developpement of the synchrotron radiation source as well as of the beam line are required to better describe the site occupation of Zn. It is quite clear that a critical evaluation will be made through a careful analysis of the XAS modulations.

5. Conclusion

This very first set of experimental tests contributes to our understanding of the role of trace elements in ectopic calcifications. By using tools that are consistent with the chemical complexity of the sample i.e. Powder Neutron Diffraction for the matrix here a biological apatite and XAS for the determination of the coordination number and the electronic state of oligoelements here Zn, the medical community should be better positionned to understand the role of trace element in the formation of the ectopic calcification. Motivated by these observations, a second set of experiment has been scheduled.

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