

## Determination of double decker sandwich structured La-substituted chlorophyll a by EXAFS

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As a representative natural porphyrin derivative, it is interesting to know the chemical form of lanthanide in chlorophyll a. We select the natural fern *Dicranopteris dichotoma*, the most lanthanide-concentrated plant known, to isolate the chlorophyll a for our study. It is found by EXAFS that lanthanum substitutes in the magnesium position in chlorophyll and coordinates with the porphyrin ring. The lanthanum is seen to have a similar coordination structure to a sandwich-type lanthanide complex, with the La surrounded by eight nitrogen atoms from two porphyrin rings with average La-N bond length of 2.65 Å.

**Keywords:** EXAFS Chlorophyll Lanthanum Molecular structure

### 1. Introduction

The biological effects of Lanthanide elements impress us in increasing the agriculture yield by its influence on photosynthesis (Li, 1995). To elucidate the role of lanthanide in photosynthesis, it is essential to clarify the chemical form of rare earth elements (REE) in chlorophyll, which is essential to the photosynthesis. Because of the low content of La and other rare-earth elements in natural chlorophyll, EXAFS is a unique method to study the chemical form of these metals in natural systems.

The fern plant, *Dicranopteris dichotoma*, to the best of our knowledge is the most REE-concentrated plant (Li et al, 1992), growing on the erosion layer of ion-adsorption REE deposits, south of China. We chose the lanthanum-riched sample for our EXAFS measurement. We start with a known sandwich structured complex, La[T(tBu)PP]Nc, to determine its structure parameters and, more importantly, to construct a double deck model. We then apply this model in the EXAFS data refinement of La L<sub>3</sub> edge data of Chlorophyll a in plant.

### 2. Experimental section

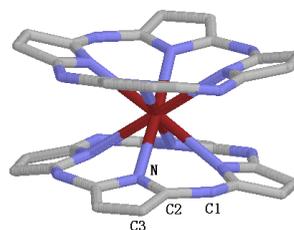
La[T(tBu)PP]Nc, a kind of sandwich-type heteroleptic phthalocyaninato (Pc) and porphyrinato (Por) lanthanide complex, was synthesized as the reported methods (Ng and Jiang, 1997). The chlorophyll was extracted from the leaves of *Dicranopteris dichotoma* with acetone. Chlorophyll a was separated by microcrystalline cellulose column. The content of lanthanide in chlorophyll a was measured by ICP-MS (Total REE 445 µg/g, La

300 µg/g).

X-ray absorption spectroscopic data at La L<sub>III</sub> edges were recorded at the Beijing Synchrotron Radiation Facility (BSRF) on unfocused wiggler beamline 4W1B, using a Si(111) double crystal monochromator. During the experiment, the ring condition was 2.2 GeV and 80–60 mA. Detuning the incident intensity by approximately 30% completed harmonic rejection. The complex was measured in transmission mode while a Lytle-type fluorescence detector with TiO<sub>2</sub> filter was used for chlorophyll sample. The edge position was calibrated by measuring the absorption spectrum of La<sub>2</sub>O<sub>3</sub>. The Fluorescence data represents an average of four scans. The gel-like deep green chlorophyll sample was dispersed on the adhesive tape for measurement. The measurement was performed in room temperature under dark environment. The samples were renewed after every scan. The x-ray spot size is about 20 × 2 mm at sample position. Possible radiation damage to samples was examined by taking optical spectra before and after the X-ray exposure. Little difference was seen, indicating damage to be negligible.

Background-subtracted EXAFS data were obtained using program EXBACK with three region cubic splines. Spherical wave curve fitting, by least-squares refinement of Fourier-filtered EXAFS, was completed using EXCURV92 program (Binsted et al, 1992), implemented in the Cerious<sup>2</sup> module developed by Molecular Simulation Inc., where the phase and amplitude theoretically calculated with the von Barth approximation for ground state potential and Hendin-lunqvist for exchange potentials. With respect to the wavevector range for analysis, K<sub>min</sub> was selected to minimize the low-R (<1 Å) peaks and K<sub>max</sub> selected to about 10 Å<sup>-1</sup> where the La L<sub>II</sub> edge occurs. A Gaussian window function was used in Fourier transform.

### 3. Data analysis and results

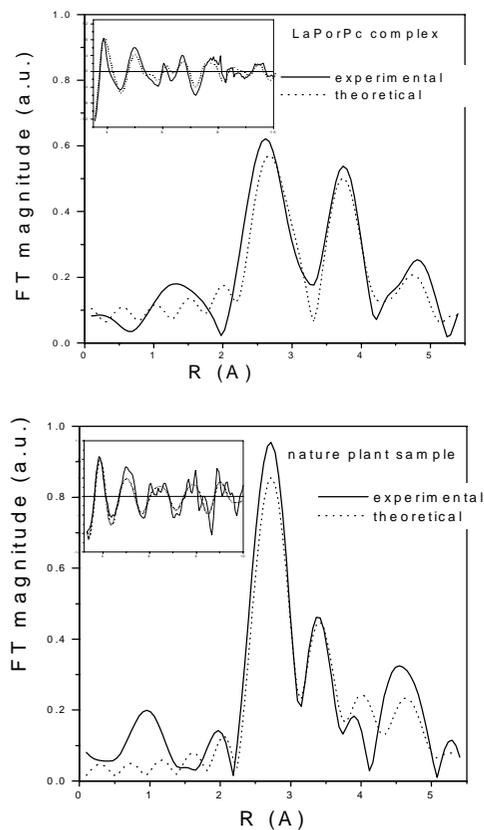


**Figure 1**

Double deck sandwich structured model of La phthalocyaninato and porphyrinato complex. For clarity, only the major part is presented. N and C position also indicated.

Initial structure parameters used in theoretical calculation of EXAFS are calculated from the LaPorPc structure model (Fig. 1), constructed by 3D builder of Cerious<sup>2</sup> module after consulting the molecular structure studies of sandwich lanthanoid porphyrins complex (Buchler et al, 1986 and Buchler, 1988). The shell parameters are obtained within 5 Å range from center La ion. This range is enough for EXAFS analysis because most EXAFS contribution comes from pyrrole rings within 5 Å scope. The model was minimized in energy for a chemically reasonable structure. For La in chlorophyll, even if there are some modification reactions during the isolation, separation, demetalization and insertion, their effects on the macrocycle structure should be negligible.

Due to the rigid porphyrin ligand contributing strong multiple scattering (MS) terms to the total EXAFS signal, the MS effects must be considered in the fitting. We assign eight MS unit of pyrroles from the model.



**Figure 2**

Phaseshift-corrected radial distribution function of La in Chlorophyll a, the inset is refinement fit for La L3 edge EXAFS oscillation with  $k^3$  weighting  $\Delta K=2.5-10.3 \text{ \AA}^{-1}$  with  $K^3$  weighting. Experiment curve (solid) and fitting one (dash). upper: Sandwich Lanthanide complex; lower: natural Chlorophyll a La from fern *dicranopteris dichotoma*

**Table 1**

La L3-edge EXAFS-derived Structural Parameters for sandwich structured La complex and La in natural Chlorophyll a

Shell	number of shell atom	R(Å)		$\sigma^2 (\text{Å}^2)$	
		Complex./natural	complex/natural	complex/natural	complex/natural
N	8	2.63(2)/2.65(2)		0.009/0.005	
C2	16	3.65(3)/3.43(3)		0.005/0.007	
C1	8	3.90 (5)/3.85(5)		0.014/0.010	
C3	16	4.76(5)/4.65(5)		0.012/0.008	

$E_f = -15 \text{ eV}$ ; coordination number fixed in fitting;

In the fits, the first peak in FT of complex sample ( $\sim 2.6 \text{ \AA}$ , Fig.2) is well accounted for by strong contribution from eight single-scattering signals, La-N ligands (Fig.1). The determined La-N bond length is impressively the same between the complex and chlorophyll. Sixteen MS La-N-C2 contributions and eight SS La-C1 from the pyrrole rings account for the second peak at  $\sim 3.5 \text{ \AA}$  in the complex, while the peak is split in the natural sample. The peak intensity of the complex is comparatively stronger than that of natural one (for LaPorPc, there are four carbon and four nitrogen in La-C1 shell. Due to little difference in backscattering amplitude and

phaseshift for carbon and nitrogen, we assign them as eight carbons here in fitting for convenience). Considering their distance from the central absorption ion, the magnitude of the fourth peak at about  $\sim 4.5 \text{ \AA}$  in FT is comparatively stronger, even if there are sixteen C3 atoms. In fact, this intensity also comes from the La-N1-C3 MS contributions with shadowing effects to some extent. For the chlorophyll sample, we obtain the same structure configuration (Table 1), although the peak intensity and position in RDF is somewhat different from the complex, especially the peak splitting compared with the second peak of complex. This may reflect the influence of the long chain phytol and the isocyclic ring of chlorophyll on the porphyrin rings structure.

#### 4. Conclusion

In nature, magnesium is the coordination center of chlorophyll. Other metal-substituted chlorophyll has been investigated extensively (Hartwich et al, 1998). These complexes, however, feature the monoporphyry structure. Sandwich-like Lanthanoid porphyrin complexes have been synthesized and characterized systematically (Buchler et al, 1985 and Buchler et al, 1988). As we know, the bis(porphyrinato) metal complexes have been put forward as structural and spectroscopic models for the special pairs found in the reaction center protein of photosynthetic bacteria (Buchler & Heinz, 1996). So, it is worthwhile to know if lanthanide substituted chlorophyll also prefer this kind of coordination structure to monoporphyry.

From our EXAFS results, lanthanum is shown to substitute at the magnesium position in chlorophyll and coordinate with the porphyrin rings. Compared with the model complex, we obtain the same double-decker sandwich structure configuration for La in chlorophyll a in natural fern. As to our best knowledge, it is the first report of lanthanide sandwich structure of chlorophyll in natural sample. Sandwich porphyrin complexes feature in strong  $\pi\pi$  interaction, and we know photosynthesis is a process of electron transfer, where Chlorophyll plays an important role. Our results may help in the elucidation of REE biological effects in photosynthesis.

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