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SYNTHETIC PORPHYRINS BEARING A LATERAL PEPTIDE CHAIN—V

ON THE LACK OF EVIDENCE FROM EXAFS FOR A SHORT AXIAL ZN–S BOND IN THE CASE OF *CIS*-ENDO-3-PROPENAMID-ZnTPP DERIVATIVES FEATURING A TERMINAL S-ALKYL CYSTEINE RESIDUE

J. Goulon et Ch. Goulon
Laboratoire de Chimie Théorique (ERA CNRS 22) et Laboratoire pour l'Utilisation du Rayonnement Electromagnétique, Laboratoire propre du CNRS, associé à l'Université de Paris-Sud, Orsay
et
F. Niedercorn, C. Selve et B. Castro*
Laboratoire de Chimie Organique II (ERA CNRS No. 558), Université de Nancy I, Case Officielle 140, 54037 Nancy Cédex, France

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Abstract—"Extended X-Ray Absorption Fine Structure" (EXAFS) spectroscopy has been used for probing the environment of the Zn^{2+} cation in three 3-propenamid-Zn tetraphenyporphyrin complexes. The lack of evidence for a short axial bond of the zinc atom to the sulfur atom of the cysteine terminal residue of the lateral peptidic chain is discussed, together with the few indications which suggest that a Zn–S interaction, if any, can only be weak and probably long range (4.1 Å ?).

INTRODUCTION

An exciting challenge for chemists is presently the synthesis of model compounds which could "mimic" the natural catalytic properties of the monooxygenases of the family of the cytochrome P-450. Our approach to this difficult problem consists in branching on a porphyrin a lateral peptide chain which might stabilize a weak, axial metal–sulfur bond.[1,2] Recently we have been able to prepare various metal complexes of *cis*- and *trans*-3-propenamido-mesotetraphenyl porphyrins, in which a dipeptide chain: Gly-(S-R)Cys-OEt, where R denotes either a methyl or trityl substituent, was coupled to the propenoic acid.[3–6] For the particular *cis*-endo configuration of the peptide chain, there is some reasonable chance to observe such a conformationally stabilized metal–sulphur bond involving the terminal cysteine residue. Indeed, spectroscopic investigations, namely ^{1}H NMR and magnetic circular dichroïsm (MCD) of this *cis*-endo compound might afford such a presomption: However a definitive proof of such a metal–sulphur interaction has still to be given.

The so called "Extended X-Ray Absorption Fine Structure" (EXAFS) spectroscopy which is directly probing the nature of the local environment of the metal, has already been used for detecting such an axial metal–sulphur bond in other model compounds of cytochrome P-450.[7] We wish to report in this paper on our EXAFS investigations of three complexes (Fig. 1): *cis*-endo 3-Prop Zn-TPP-Gly-(-SMe)Cys-OEt (CM), *cis*-endo 3-Prop Zn-TPP-Gly-(-STr)Cys-OEt (CT), *trans*-3-Prop Zn-TPP-Gly-(-STr)Cys-OEt (TT) in the solid state. The lack of evidence for an axial Zn-S bond will be discussed below.

MATERIALS AND METHODS

The synthesis of these three complexes has already been detailed in our previous papers and is not to be further developed here. Pellets of appropriate geometry were prepared from the powdered materials and were protected in the sample holder by air-tight Kapton windows. For the air sensitive CM complex, all the preparations of the sample took place in a small, portable argon filled dry box: no appreciable degradation of the product was detected by chromatography after the X-ray irradiation. All spectra were normally taken at room temperature, except for the more reactive CM complex which was studied at low temperature (25 K).

The spectra were collected at LURE, the French synchrotron radiation facility, using the X-rays emitted by the ultra relativistic electron beam of the storage ring

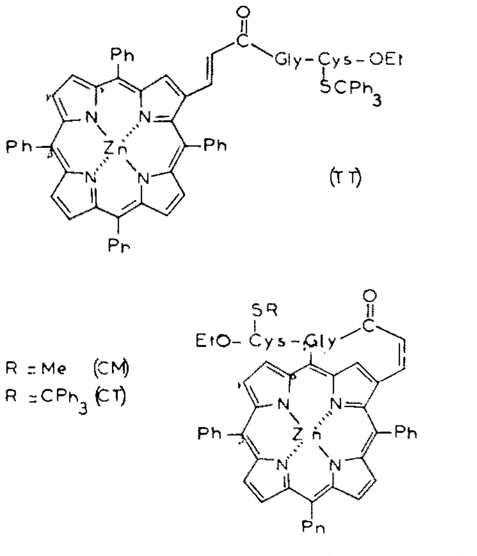

Fig. 1. *Trans*-3-prop-ZnTPP-Gly-(STr)-L-Cys-OEt (TT), *cis*-endo-3-prop-ZnTPP-Gly-(SMe)-L-Cys-OEt (CM), *cis*-endo-3-prop-Zn-TPP-Gly-(STr)-L-Cys-OEt (CT).

DCI running typically at 1.8 GeV. The experimental setup of the EXAFS-I station has been described elsewhere.[8] Zinc complexes were chosen as no glitch is apparent at the Zn-K-edge. Ten successive scans were accumulated for both *cis*-derivatives CM, CT, whereas only 3 scans could be added for the *trans*-compound TT.

The analysis of the spectra includes the usual "preparation" of the data,[9] i.e. (i) removal of the pre- and post-edge background contribution to the whole absorption coefficient $\mu(E)$ by a fast smoothing procedure, (ii) normalization of the spectra with respect to the edge jump (iii) transformation from the photon energy scale to the photoelectron wavevector scale $k = [(2me/h^2)(E-E_0)]^{1/2}$. For a discrete, gaussian distributed lattice, the normalized oscillatory component $\chi(k)$ of the absorption coefficient is given by the approximate but now standard formula:[10–13]

$$\chi(k) = \frac{1}{k}\sum_j \frac{N_j}{R_j^2} \exp(-2\sigma_j^2 k^2)\,|F_j(k,\pi)|\,A(R_j,k)\sin[2kR_j + \psi_j(k)] \qquad (1)$$

where R_j is the distance which separates the absorbing atom from the N_j scattering atoms defining the jth shell. Here also $F_j(k)$ and $\psi_j(k)$ denote in a standard way respectively the backscattering function and the total phase shift relative to this jth shell. The amplitude of the EXAFS oscillations clearly depends upon the function $F_j(k,\pi)$ and upon the effective Debye–Waller factor $\exp\{-2\sigma_j^2 k^2\}$ resulting from the vibrational distorsion of the equilibrium structure. The multiplicative factor $A(k)$ has to be introduced in order to take into account inelastic processes which induce some loss of coherence of the photoelectron.[14]

A nice way of displaying the EXAFS results consists in Fourier Transforming the spectra $\chi(k)$ according to (2):

$$\tilde{\chi}_j(R) = \int_0^\infty dk\, g(k)\chi(k)\frac{R_j^2 k \exp\{+2\sigma^2 k^2\}}{N_j F_{j\kappa}(k) A_j(k)} \exp\{-2jkR - j\psi_j(k)\} \qquad (2)$$

where a Kaiser window function $g(k)$ has been introduced in order to minimize the side lobes of the FT-spectra.[9] Clearly the amplitude and phase shift corrections made in eqn (2) are exact only for a selected shell (e.g. $j = 1$: 4 nitrogen atoms), but as long as only light scatterers (C, N, O) are involved, the differences remain small and a good representation of the radial distributions of the closest neighbours can be then obtained. Fitting techniques which are described elsewhere[9,17] can also be used for refining the analysis. This way of processing the data is very convenient if a semi-empirical parametrization of the functions $F(k,\pi)$ and $\psi(k)$ can be used.[12] It becomes possible also to adjust the energy offset E_0 by following the Lee-Beni criterion of coincidence of the maxima of $|\tilde{\chi}(R)|$ (dotted lines) and $\mathrm{Im}\,\tilde{\chi}(R)$ (full lines).[11]

RESULTS AND DISCUSSION

The corrected pseudo radial distribution $\tilde{\chi}_1(R)$ relative to each one of the derivatives CM, CT and TT are reproduced in Fig. 2, 3 and 4 respectively. Obviously, as a natural consequence of the different number of added scans, a slightly better resolution as well as a somewhat better signal/noise ratio were achieved for the spectra of both *cis* compounds, as compared to the spectrum of the *trans* complex.

Next, if we try to superpose (Fig. 5a, b) the spectra of both *cis* compounds CM and CT, it appears that the two spectra $\mathrm{Im}\,\tilde{\chi}(R)$ do fit perfectly in phase but also in amplitude, excepted in the range 4-4.5 Å. One is therefore led to conclude first that there can hardly be any major difference in the local environment of the zinc atom in CM and CT for $R \leq 4$ Å although significant differences do exist in their MCD spectra. Now, if one also argues that there cannot be any direct interaction between the metal and the cysteine residue in the *trans* conformer, then one is led to the second major conclusion, that there is no evidence from their EXAFS spectra for a short metal-sulphur bond in anyone of the *cis* conformer: although the quality of the data are not quite as good for the *trans* and *cis* conformers, it is clear

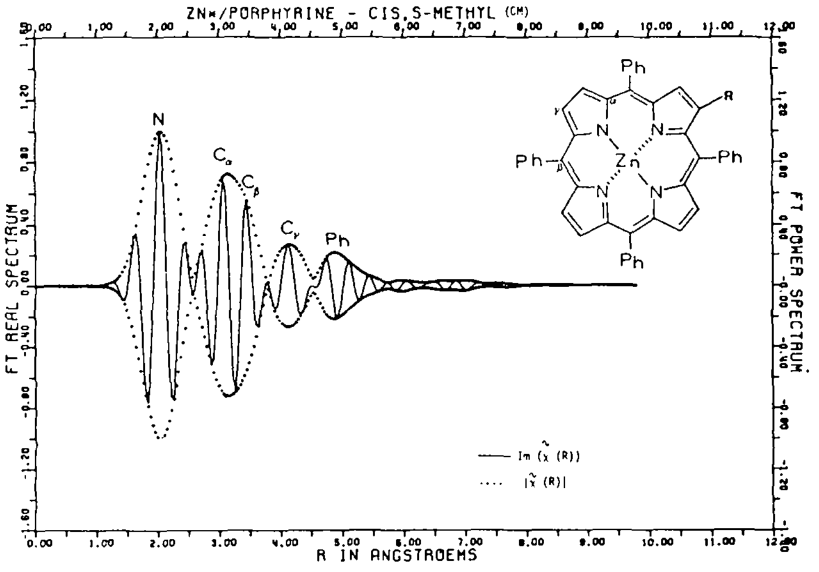

Fig. 2. EXAFS corrected pseudo radial distribution $\tilde{\chi}(R)$ (10 scans) for the *cis*-endo-3-prop-ZnTPP-Gly-SMe-Cys-OEt (CM) complex.

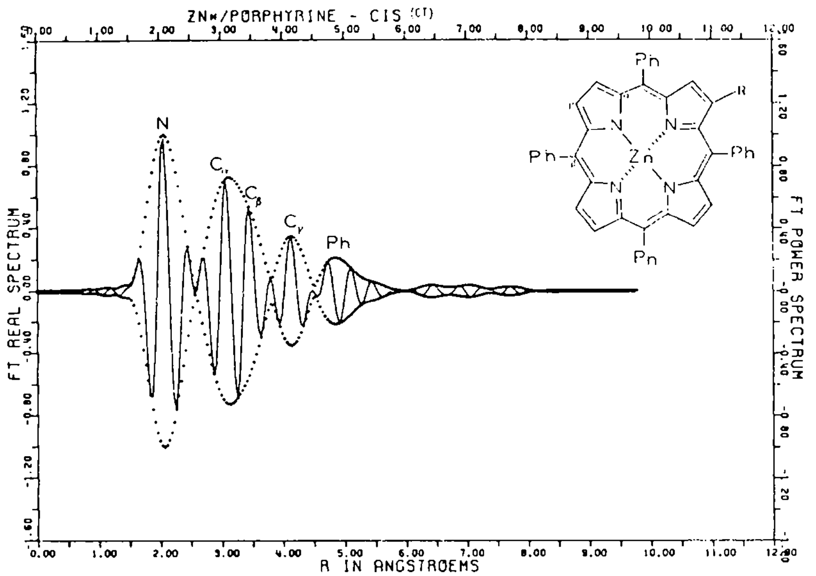

Fig. 3. EXAFS corrected pseudo radial distribution $\tilde{\chi}$ (*R*) (10 scans) for the *cis*-endo-3-prop-ZnTPP-Gly-STr-Cys-OEt (CT) complex.

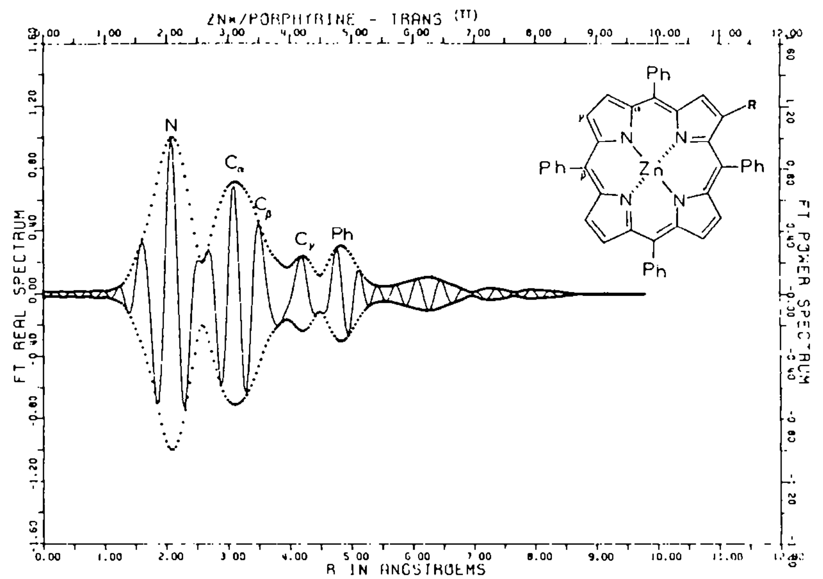

Fig. 4. EXAFS corrected pseudo radial distribution $\tilde{\chi}(R)$ (3 scans) for *trans*-3-prop-ZnTPP-Gly-STr-Cys-OEt (TT) complex.

that there is no appreciable change in the range 2.0–2.5 Å which might suggest the presence in the *cis* derivatives of a short *Zn–S* (~ 2.35 Å) bond as observed by Hodgson *et al.* for other P-450 model compound. Even the phase of the first peak remains unaltered, whereas it is our experience on other model compounds that the phase of the signal is quite sensitive to the presence of both N and S atoms in the coordination sphere of the absorbing Zn^{2+} ion. Finally, coming back again to a direct comparison of the well resolved spectra of the *cis* compounds, there is still a detail which appears to be perhaps noteworthy: the spectrum of the S-methyl derivative CM only differs of that of the S-trityl CT by a small reduction (~ 25%) of the signal peaking at 4.2 Å whereas all other signatures remain unchanged in amplitude by more than 5%. As the noise level is rather identical for both spectra, one may guess that this effect is probably real. However there is no way to interpret such a decrease of intensity by a temperature dependent vibrational effect because the spectrum of CM was recorded at 25 K. Therefore, if this effect is real the only explanation that can be found is the possible interference of two EXAFS oscillations of similar periodicity but nearly opposite phases. If one remembers that a carbon or a sulfur scattering atom precisely differ by a constant phase shift of 3.6 rd, it becomes attracting to consider as a possible explanation the hypothetical interference of the EXAFS signal of the C_γ shell (R = 4.22 Å) with the signal of a sulfur atom distant from the zinc central atom by about 4.1 Å. It is however necessary to emphasize that such an interpretation remains still tentative and cannot be regarded as definitive.

The afore developed analysis tends to suggest that

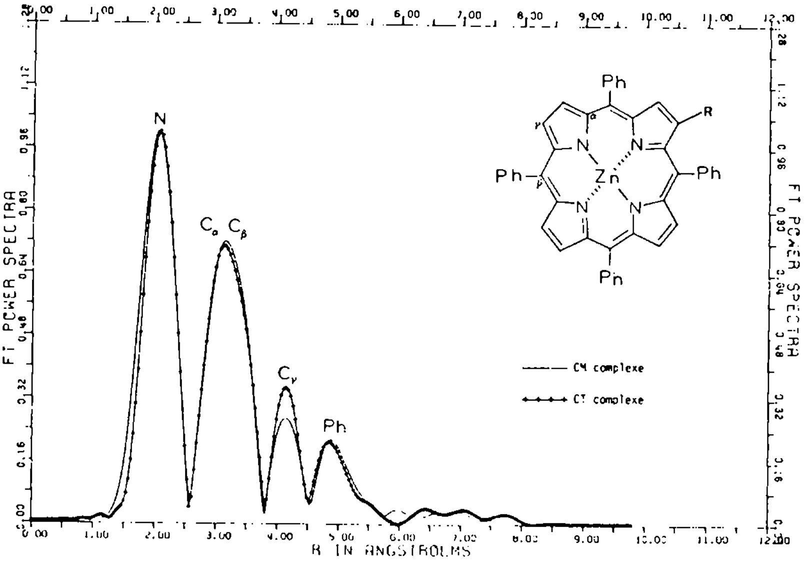

Fig. 5(a). Superposition of the power spectra $|\tilde{\chi}(R)|$ relative to CM and CT complexes.

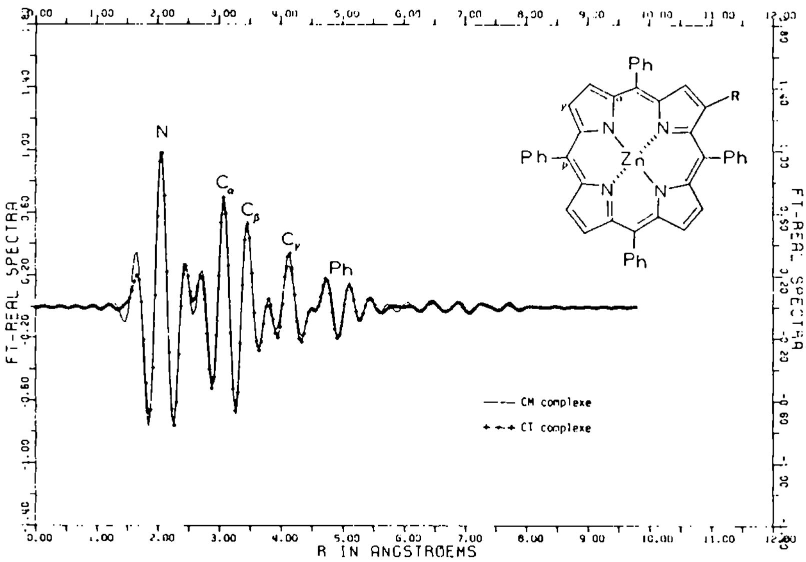

Fig. 5(b). Superposition of the imaginary part of $\tilde{\chi}(R)$ relative to CM and CT complexes.

even in the *cis*-endo conformation of the peptide, the interaction of the cysteine residue with the metal of the porphyrin should remain weak. Let us notice that our previously reported ^{1}H NMR investigations are also supporting this analysis: the increased shielding of the S-methyl protons ($\delta \sim 2.5$ *ppm*) is quite significative but is not large enough to give a clear evidence of a short Zn-S bonds. Besides various steric or torsional hindrances associated with the peptidic chain itself, one may imagine also that an axially bound solvent molecule[15,16] could prevent the cysteine residue to approach the metal atom closer and perturb the symmetry of the electronic distribution around the metal, thus affecting the very sensitive MCD spectra. Even a very weak, direct or not, interaction with the cysteine group might also be enough to alter the MCD spectra.

In comparing the results of these different spectroscopies, one should however keep in mind that the ^{1}H NMR and MCD investigations were carried out on dilute solutions in chloroform or toluene, whereas the EXAFS measurements were obtained from polycrystalline, powder samples. For instance, one may postulate that in solution one is observing an equilibrium between two conformations of the peptidic chain, one of which gives rise to a short range Zn-S interaction: the crystalline field could shift this equilibrium and make only the other one observable in the solid state. EXAFS measurements on dilute solutions are possible, especially using the more sensitive X-ray fluorescence mode of detection,[17,18] but there is still very little chance for such a labile conformer to be detected, as the anharmonic distorsions of the Zn–S bond might then be large enough to let the corresponding signal to vanish at room temperature .[19] Obviously a most serious limitation of EXAFS spectroscopy appears there.

We can now proceed still further in the analysis of the

Table 1.

DISTANCES	EXAFS			CRYSTAL STRUCTURE	
R (Å)	CM	CT	TT	H_2O-ZnTPP[15]	$(Tol)_2$ZnTPP[16]
Zn---N	2.0_6	2.0_6	2.0_7	2.05_0	2.03_5
Zn---C_α	3.0_8	3.0_8	3.1_0	3.08_6	3.06_1
Zn---C_β	3.4_4	3.4_4	3.4_9	3.45_8	3.44_6
Zn---C_γ	4.2_2	4.2_2	4.2_0	4.30_0	4.28_6
Zn---C_Φ*	4.9_3	4.9_3	4.9_3	4.95_2	4.94_6

* Corrected value for multiple scattering effects

EXAFS spectra of our compounds by considering that all the features of the EXAFS spectra Im$\tilde{\chi}$ (R) should be purely representative of the porphyrin macrocycle itself, if as discussed above, the interaction of the peptidic chain with the metal ion is weak and only long range. The assignment of the successive signatures of the spectra becomes quite straighforward if one notices that the various scatterers of the porphyrin ring are only low Z atoms (N, C) featuring fairly similar phase shifts corrections.[12] Clearly the signal of the coordination shell (N) and of the carbon shells $C_\alpha, C_\beta, C_\gamma$ are easily identified on figure 2. The distances R_i associated with each shell were refined using a conventional filtering/fitting procedure[9] and are reported in Table 1 together with known crystallographic data referring to an aquozinc TPP[15] and a toluene solvated Zn-TPP[16]. Indeed the agreement between EXAFS and crystallographic distances appears most encouraging and supports very well our interpretations.

The data seem to indicate also that the zinc ion is probably not lying exactly in the plane of the porphyrin ring but close to it, even for the TT complex. However one needs to be very careful as a firm conclusion is requiring very accurate phase shifts and E_0 values. We may also object that the zinc ion, the carbon C_β and the first bonded carbon C_Φ of the phenyl substituent should be nearly colinear as suggested by the characteristic enhancement, shift and phase distorsion of the peak that we have assigned at ~ 4.8 Å to the EXAFS contributions of the C_Φ shell (Fig. 2). One can find in the literature many reported examples of linear systems where multiple scattering effects give rise to such a typical enhancement of the signal of the remotest shell.[20,21] It is not clear yet if this effect is still observable when the three atoms are not perfectly colinear, as in systems like hemoglobin where the metal is pulled out of the plane of the porphyrin by a large amount.[17]

Finally let us mention here that on fitting the data relative to the first peak of the EXAFS spectrum of CM, we obtained for the number of closest neighbours the value $N \sim 3.8$. We do not believe however that such a determination is accurate enough to exclude, for instance, the fixation of a water molecule in an axial position: the transferability of the amplitude factors is still matter of discussion[13] and it is our experience that the normalization procedure of the EXAFS spectra can be subject to experimental artefacts if the samples used are not perfectly homogeneous and well positioned.

CONCLUSION

The present EXAFS study failed to bring any evidence for a short, axial Zn-S bond, in the case of *cis*-endo 3-propenamid ZnTPP derivatives. However there are in the reported EXAFS spectra a few indications for a weak long range ($R \sim 4.1$ Å?) interaction of the cysteine residue with the metal cation. The well resolved spectra of both *cis* compounds CM and CT reproduce perfectly all the expected features associated with the porphyrin ring and thus give a good illustration of the performances of the EXAFS spectroscopy as a structural tool.

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