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A STUDY OF CYTOCHROME c AND CYTOCHROME b_2 CORE IN THE OXIDIZED AND REDUCED STATES USING EXAFS AND XANES SPECTROSCOPIES

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RESUME

La spectroscopie EXAFS nous a permis d'atteindre pour la première fois des informations structurales sur le cytochrome b_2 : la distance moyenne Fe...N (1.97±0.02Å) est comparable à celle du cytochrome c et d'autres complexes porphyriniques hexacoordinées de fer bas spin. En ce qui concerne le cytochrome c, aucune variation supérieure à 0.02Å de la distance moyenne Fe...N n'intervient entre les formes oxydée et réduite. En revanche la contribution de la couche Fe...S parait être assez faible et dépendante du mode de préparation des solutions. Un décalage systématique de 2.1 eV du seuil est observé entre les états oxydés et réduits du cytochrome b_2 ou c, alors que le pic de préseuil, bien résolu dans le cas du cytochrome c oxydé, parait se déplacer seulement de 1.0 eV.

ABSTRACT

We report the first structural investigation of cytochrome b_2 core : the average Fe...N distance (1,97±0,02Å) is quite comparable to the Fe...N distance of cytochrome c and of other low spin hexacoordinated porphyrin complexes. As regards cytochrome c no difference of the average Fe...N distance greater than 0.02Å was detected between reduced and oxidized states. However the contribution of the axial Fe...S shell can be weak and obviously depends upon the sample preparation. A systemate shift of 2.1 eV of the edge is observed between oxidized and reduced states but the prepeak which is well resolved for oxidized cytochrome c exhibits a smaller shift of less than 1.0 eV

I INTRODUCTION

Cytochromes are a wide class of hematin compounds which are involved in electron-transfer processes. They are for instance essential components of the respiratory chain in mitochondria. Biodegradation of sugars produces electrons or reducing equivalents which are transferred from one cytochrome to the next until they reach some terminal component having the capability of activating oxygen, while the energy produced all along this chain of reactions is stocked in "highly energetic" molecules of ATP. Haem is the prosthetic group of these proteins while the electron transfer is associated with the iron (III)- iron (II) redox couple. A better knowledge of the coordination geometry of iron and of the actual charge partition in different redox states is a prerequisite to the comprehension of the electron transfer mechanisms.

In order to make some progress in that direction, we have recorded the XANES and EXAFS spectra of two typical members of this class of hemoproteins:
(i) the cytochrome c (from horse heart muscle-SIGMA type VI)
(ii) the cytochrome b_2 core, i.e a tryptic fragment of flavocytochrome b_2 (from *Hansenula anomala* yeast) [1].

In both of them, the haem-iron ions are hexacoordinated but the nature of the axial ligands, with respect to the porphyrin plane is different : in the case of the cytochrome c, the two axial ligands are one imidazole group (from histine 18) and one thioether group (from methionine 80), whereas in the case of cytochrome b_2 core, both axial ligands are expected to be histidines. The three-dimensional structure of cytochrome b_2 core has not yet been solved to a low resolution [2] but it can be anticipated from the amino-acid sequence homology with liver microsomal cytochrome b_5, that the 2.8Å resolution crystal structure [3] of the latter protein, should be a reasonably good model also for cytochrome b_2 core. The crystal structures of tuna heart ferro- and ferri-cytochromes c have been refined to lower resolutions i.e. 1.5Å and 1.8Å respectively [4] but even at such resolutions the iron-ligand bond lengths were not determined with sufficient accuracy: as regards for example the $Fe...N_{\epsilon,\delta}$ distances relative to the haem, the values quoted in reference [4] are 2.06±0.07Å and 2.05±0.11Å for the reduced and oxidized protein while in all relevant hexacoordinated low spin iron (II) or iron(III) porphyrin complexes, the $Fe...N_{\epsilon,\delta}$ distances are always found slightly shorter than 2.00Å [5]. Under proper conditions EXAFS spectroscopy can afford to determine the haem geometry with a much better accuracy and is particularly well suited to detect structural differences as small as 0.02Å or even less in bond lengths. Previous reports on EXAFS measurements on cytochrome c [6-7] have confirmed the sensitivity of the method but the short truncation of the k-space experimental data and the crudeness of the Fourier analyses shown, suggested us that the quality and reliability of the structural informations extracted from EXAFS spectra could still be improved by taking advantages of recent technological [8] or methodological developments [9].

II EXAFS MEASUREMENTS

The EXAFS spectra shown below have all be obtained at LURE on the EXAFS-II station equipped with a two crystal (Si-311) monochromator and with an efficient harmonics rejector made of a two parallel, flat mirrors. The spectra were recorded in the fluorescence detection mode using two different prototypes (P-I and P-II) of a new detector consisting of an array of cooled silicon photodiodes [8]. The FT-spectra $Im[\tilde{\chi}_1(R)]$ discussed below were all corrected for the phase shift and scattering amplitude of a standard Fe*...N pair according to: [10]

$$\tilde{\chi}_j(R) = \int_0^\infty dk\ w(k)\ \chi(k)\ \frac{k\ R_j^2}{N_j\ F_j\ A_j}\ \exp[\ 2\sigma_j^2 k^2 - ikR - i\Psi_j(k)\ ]. \qquad (1)$$

where w(k) is a KAISER-BESSEL window function, $\Psi_j(k)$, $F_j(k)$ being tabulated functions. $A_j(k)$ refers also to standard corrections for inelastic processes and multielectron relaxation effects.

We have reproduced in figure 1 the FT-spectra $Im[\tilde{\chi}(R)]$ of an aqueous solution of cytochrome c (0.003M) in the reduced and oxidized forms, while figure 2 is comparing the FT-spectra of two different preparations of oxidized cytochrome c (*A*=0.003M - 3 added scans/P-I ; B=0.007M - single scan/P-II). As for all hexacoordinated porphyrin complexes, these spectra exhibit well resolved typical signatures for the Fe*...N, $Fe^*...C_\alpha$, $Fe^*...C_{meso}$ and $Fe^*...C_\beta$ shells. The average Fe...N bond length (1.97±0.02Å) for the oxidized form is definitively shorter than the crystal structure data [4] but is in good agreement with the EXAFS results of KORSZUN et al. [7]. A small shift of c.a. 0.01$_5$Å was found to be reproducible between the average Fe...N distances of the oxidized and reduced forms. Due to its inversed phase the Fe...S signal is never well resolved and is interfering with the negative feet of the Fe...N signature. Moreover as illustrated by figure 2, the intensity of the Fe...S signal seems to depend upon the sample preparation. It is our interpretation that under specific experimental conditions the Fe...S distance becomes poorly defined and/or that fast axial ligand exchange can occur. More work is now being done in order to rationalize our observations.

We have reproduced in figure 3 a comparison of the FT spectra of an aqueous solution (0.003M) of cytochrome b_2 core in the oxidized state and of a model compound : 1=TPP:Fe(II)(N-Methyl-Imidazole)$_2$. It is also possible to compare in figure 4 the FT-spectra of the solutions of cytochrome b_2 core and cytochrome c both in the oxidized state. Clearly the average Fe*...N bond length (1.97±0.02Å) is not significantly different from the Fe*...N distance found in cytochrome c. Of course we expected to find such similarities between the FT-spectra of 1 and cytochrome b_2 core but we also expected more difference between the two spectra shown in figure 4 : again this result suggests that for this particular preparation of cytochrome c the contribution of the Fe...S shell is very weak.

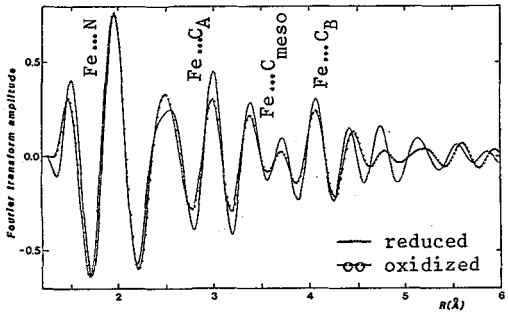

Figure 1 : Comparison of the FT spectra $\overline{Im[\chi_1(R)]}$ of an aqueous solution (0.003 M) of cytochrome c in the oxidized and reduced states.

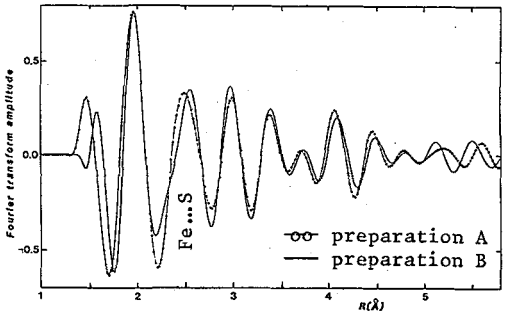

Figure 2 : Comparison of the FT spectra of two different preparations of oxidized cytochrome c : the phase inversed contribution of the Fe...S shell is different between solutions A and B but is never resolved.

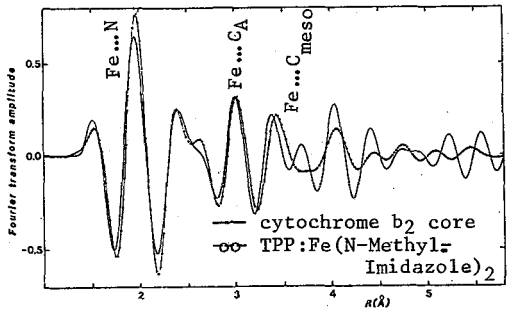

Figure 3 : Comparison of the FT spectra of an aqueous solution of oxidized cytochrome b_2 core and of model compound TPP:Fe(N-Methyl-Imidazole)$_2$.

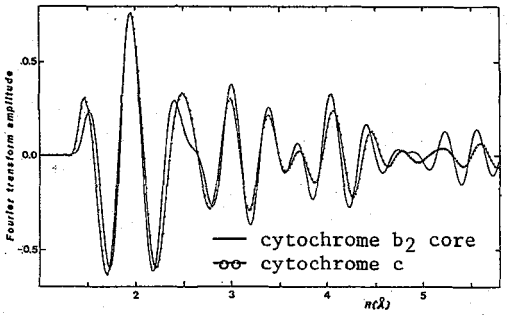

Figure 4 : Comparison of the FT spectra of aqueous solutions of oxidized cytochrome b_2 core and cytochrome c.

III XANES SPECTRA

The XANES first derivative spectra of the oxidized versus reduced forms of cytochrome c and cytochrome b_2 core are compared in figures 5 and 6 respectively. On going from the oxidized to the reduced state, the edge is apparently shifted by $\Delta E = -2.1 \pm 0.2$ eV for both systems. As regards cytochrome c, this value is quite consistent with the earlier reports [6-7]. It has however to be compared with the -1.2 eV shift reported by BIANCONI et al. for Fe(II)/Fe(III)$(CN)_6$ complexes [11]. As in the latter example, part of the observed shift is perhaps to be accounted for by small structural changes of the haem or of the axial ligands during reduction. If real, the small shift of the average Fe*...N peak detected in the EXAFS spectra of the cytochrome c would substantiate this hypothesis. Another indication supporting the same interpretation might be found also in the tiny shift (less than 1.0 eV) of the prepeak which is nicely resolved in the case of the oxidized solution of cytochrome c. However the latter argument is not completely convincing because the location of the prepeak is perhaps less accurate for the reduced species and because the exact nature of the excited states is not known yet unambiguously. Indeed if the relevant excited states are built predominantly with the orbitals of the porphyrin macrocycle, this prepeak should not exhibit any direct sensitivity to variations of the metal charge.

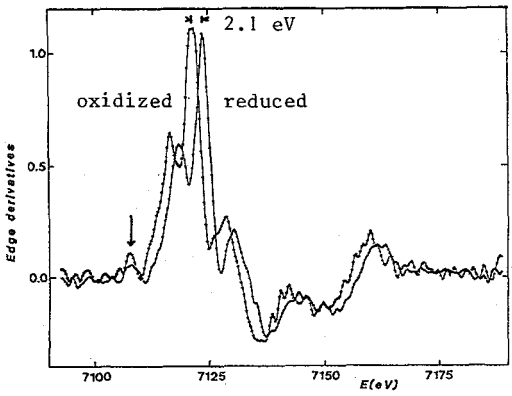

Figure 5 : Comparison of the XANES first derivative spectra of aqueous solutions of cytochrome c in the oxidized and reduced states. The arrow is for the prepeak signatures.

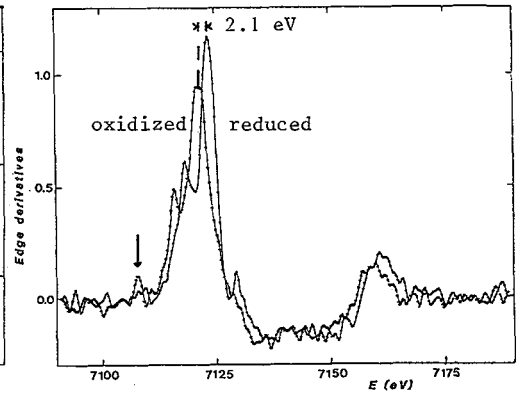

Figure 6 : Comparison of the XANES first derivative spectra of aqueous solutions of cytochrome b_2 core in the oxidized and reduced states.

IV CONCLUSION

This paper reports the first structural investigation of oxidized cytochrome b_2 core : the average Fe...N distance is 1.97±0.02Å as in cytochrome c and in other hexacoordinated low spin complexes, while the metal is sitting unambiguously in the porphyrin ring plane. As regards cytochrome c, our results confirm the basic conclusions of the previous EXAFS/XANES investigations reported by KORSZUN et al. [7]. No differences greater than 0.02Å were detected for the average distance Fe...N between the reduced and oxidized states. However our experiments show that the contribution of the axial Fe...S shell is surprisingly weak and obviously depends upon the mode of preparation of the solutions. Although the quality of the experimental data has already been improved by using new fluorescence detectors [8], still more work and better data are required before perturbed difference Fourier analyses can be carried out successfully on these systems [9] in order to refine selectively the determination of the axial bond lengths which might be more sensitive to the redox process.

REFERENCES

[1] R. GUIARD, O. GROUDINSKI and F. LEDERER, Proc. Nat. Acad. Sci. (USA) 71, 2539 (1974)
[2] P. BETHGE and F.S. MATHEWS, Acta Cryst. A35, 412 (1979)
[3] F.S. MATHEWS, M. LEVINE, and P. ARGOS, J. Mol. Biol. 64, 449 (1972)
[4] T. TAKANO and R.E. DICKERSON, J. Mol. Biol. 153, 79 (1981)
[5] T. MASHIKO, C.A. REED, K.J. HALLER, M.E. KASTNER and W.R. SCHEIDT, J. Am. Chem. Soc. 103, 5738 (1981)
[6] A. LABHARDT and C. YVEN, Nature 277, 150 (1979)
[7] Z.R. KORSZUN, K. MOFFAT, K. FRANK and M.A. CUSANOVICH, Biochemistry 21, 2253 (1982)
[8] A. RETOURNARD, M. LOOS, I. ASCONE, J. GOULON, M. LEMONNIER and R. CORTES, Proc. of the EXAFS-IV Conf. at the Abbaye de Fontevraud (1986), to be published in J. de Physique
[9] J. GOULON, M. LOOS, I. ASCONE, C. GOULON-GINET, P. BATTIONI, J.P. BATTIONI, J.P. MAHY, D. MANSUY and B. MEUNIER, Proc. of the Int. Conf. "Biophysics and Synchrotron Radiation" held at Frascati (1986) - to be published by Springer-Verlag
[10] J. GOULON and C. GOULON-GINET, Pure and Appl. Chemistry, 54, 2307 (1982)
[11] A. BIANCONI, M. DELL'ARICCIA, P.J. DURHAM and J.B. PENDRY, Phys. Rev. B26, 6502 (1982)