# METAL COORDINATION IN ZINC ENZYMES

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# METAL COORDINATION IN ZINC ENZYMES

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Résumé

Nous avons étudié des exo-enzymes de Bacillus Cereus, phospholipase-C et β-lactamase II, par zinc et cobalt K-edge EXAFS. Nous rapportons que les zincs dans les centre structural et catalytique de phospholipase-C ont cinq ligants, tandis que le cobalt, substitué dans le centre catalytique, a six ligants. Le zinc dans le centre actif de β-lactamase II a un ligant soufre, à bas nombre de coordination ou haut facteur Debye-Waller, outre des ligants imidazoles.

Abstract

We have studied the Bacillus Cereus exo-enzymes, phospholipase-C and β-lactamase II, by Zn and Co K-edge EXAFS. We report that the zincs in the structural and catalytic sites of the former enzyme are 5-coordinate, whereas cobalt, substituted in the catalytic site, is 6 coordinate. The β-lactamase II active site zinc has sulphur coordination, with either low occupancy or high Debye-Waller factor, in addition to imidazole coordination.

As zinc is normally spectroscopically inaccessible, zinc enzymes have often been studied by substitution of the zinc by cobalt, a process which usually retains enzyme activity to some extent [1]. Apart from crystallography, which is not always applicable, EXAFS is the only technique by which zinc and cobalt-substituted enzymes can be compared and the validity of extrapolation of the spectroscopic studies of cobalt enzymes to zinc enzymes checked. For example, crystallographic studies suggest 4-coordination for zinc in carbonic anhydrase, whereas UV/vis spectroscopic studies suggest that cobalt in this enzyme is 4- or 5-coordinated, depending on pH and presence of inhibitors. X-ray absorption studies give definite evidence that differences between zinc and cobalt-substituted carbonic anhydrase exist [2].

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For Bacillus cereus phospholipase C, chemical modification studies have indicated the absence of sulphur ligation, and involvement of four histidine ligands, possibly one bridging, for the two zinc atoms [3], which are 5.7 Å apart [4]. Selective substitution by cobalt is feasible, and spectroscopic studies suggest that the cobalt-substituted sites are similar, interact with each other, and have distorted octahedral ligand geometry [5]. We have interpreted the Zn-EXAFS of the structural and catalytic sites, and the Co-EXAFS of the latter, and compared the results. These suggest that zinc is 5-coordinated in both sites, with 3 N(His) ligands at 2.01-2.02 Å and 2 N or O, possibly water oxygens, at 2.13-2.15 Å (Fig. 1), whereas cobalt, in agreement with the spectroscopic studies, has 6-coordination in the catalytic site (Fig. 2).

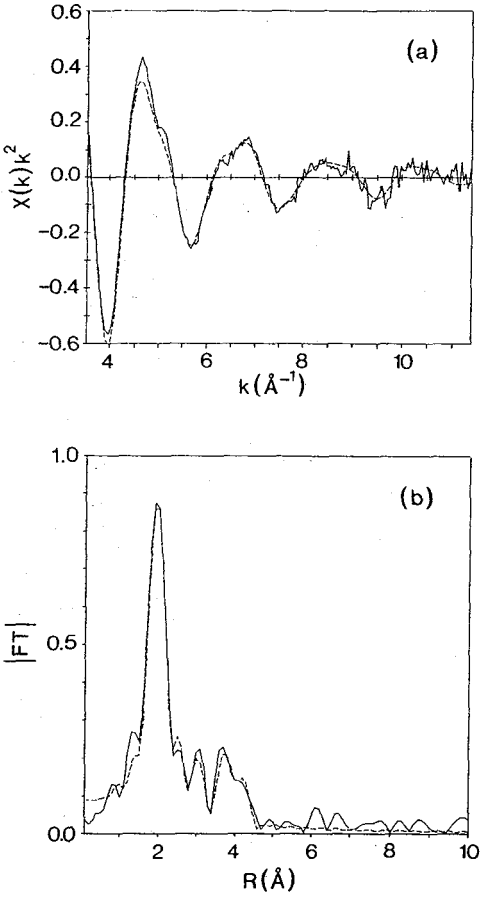


Fig. 1. EXAFS (upper panel) and phaseshift-corrected modulus of the Fourier transform (lower panel) experimental (solid line) and simulation (dashed line) of Zn in phospholipase-C, structural site.

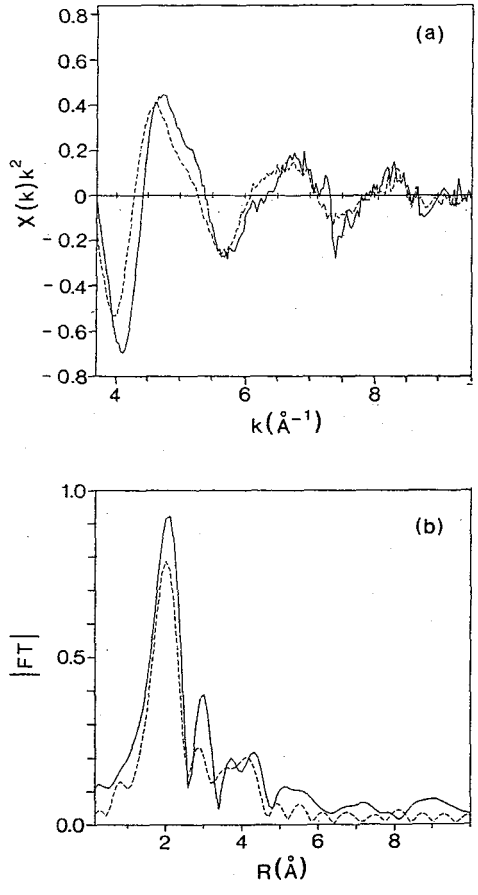


Fig. 2. As Fig. 1, but the solid and dashed lines represent Co and Zn, respectively, in the phospholipase-C catalytic site.

For the B.cereus β-lactamase II active site, there is evidence for three histidine ligands and a sulphur ligand [6]. However, the intensity of the Co-S charge transfer band is rather low compared to that of other cobalt-substituted proteins with sulphur ligation [7]. This seems to be reflected in the Zn-EXAFS, which can be simulated with histidine ligands alone. A sulphur ligand, when included in the simulation, comes at a normal Zn-S distance, but with either a high Debye-Waller factor or a low occupancy (Fig. 3).

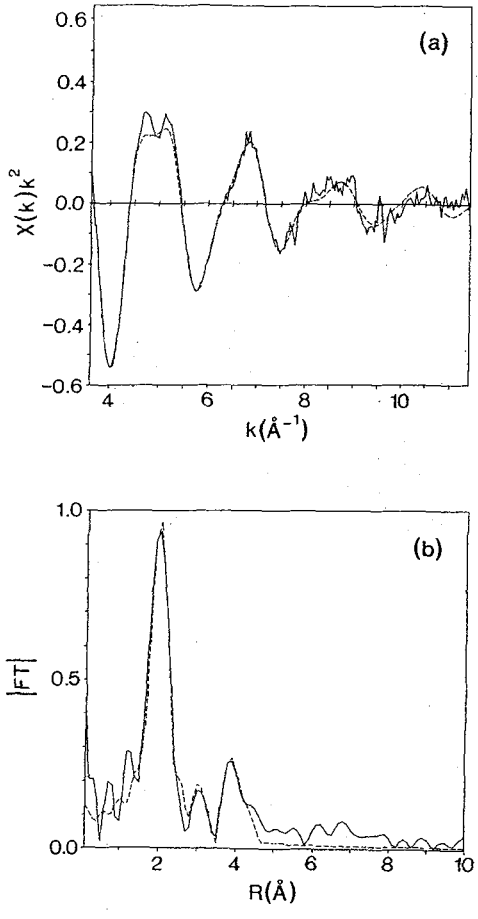


Fig. 3. As Fig. 1, but for Zn in the β-lactamase II active site, the dashed line representing simulation 1 of Table 1.

Table 1. Parameters used for the simulations

Distances in Å, Debye-Waller factors in brackets as a = $2\sigma^2$ in $Å^2$, Fi, fit index; N, nitrogen; O, oxygen; S, sulphur; C, carbon.

| | Phospholipase-C | | β-lactamase II | |
|---|---|---|---|---|
| | Structural | Catalytic | Simulation 1 | Simulation 2 |
| N | 3 at 2.01 (0.004) | 3 at 2.01 (0.003) | 4 at 2.02 (0.010) | 5 at 2.02 (0.015) |
| O | 2 at 2.14 (0.007) | 2 at 2.13 (0.005) | - | - |
| S | - | - | 1 at 2.34 (0.029) | 0.3 at 2.32 (0.008) |
| O | 1 at 2.70 (0.008) | - | - | - |
| C | 2 at 2.98 (0.008) | 3 at 2.91 (0.031) | 2 at 3.00 (0.008) | 2 at 3.00 (0.014) |
| C | 4 at 3.74 (0.017) | 4 at 3.74 (0.017) | 3 at 3.80 (0.005) | 3 at 3.80 (0.007) |
| N | 2 at 4.30 (0.011) | 2 at 4.31 (0.013) | 6 at 4.26 (0.036) | 6 at 4.26 (0.032) |
| FI | 0.08667 | 0.09351 | 0.08856 | 0.07878 |

Acknowledgement

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References

[1] References (1983) in Metal Ions in Biology (T.G. Spiro, editor) Vol.IV: Zinc Enzymes (Wiley: New York).
[2] Yachandra, V., Powers, L. and Spiro, T.G. (1983) J. Am. Chem. Soc. 105, 6596-6604.
[3] Little, C. (1977) Biochem. J. 167, 399-404.
[4] Aalmo, K.H., Hansen, L., Hough, E., Jynge, K., Krane, J., Little, C. and Storm, C.B. (1984) Biochem. Int. 8, 27-33.
[5] Bicknell, R., Hanson, G.R., Holmquist, B. and Little, C. (1986) Biochemistry, submitted for publication.
[6] Hill, H.A.O., Sammes, P.G. and Waley, S.G. (1980) Phil. Trans. R. Soc. Lond. B289, 333-344.
[7] Baldwin, G.S., Galdes, A., Hill, H.A.O., Waley, S.G. and Abraham, E.P. (1980) J. Inorg. Biochem. 13, 189-204.