Biochem. J. (1984) **220**, 591–595
Printed in Great Britain

The nickel ion environment in jack bean urease

Lucilla ALAGNA,*§ S. Samar HASNAIN,* Brian PIGGOTT†‖ and David J. WILLIAMS‡
*Science and Engineering Research Council, Daresbury Laboratory, Daresbury, Warrington WA4 4AD, U.K.,
†Inorganic Chemistry Research Laboratory, Division of Chemistry, School of Natural Sciences,
The Hatfield Polytechnic, Hatfield AL10 9AB, U.K.,
and ‡Department of Chemistry, Imperial College of Science and Technology,
London SW7 2AY, U.K.

(*Received 3 April 1984/Accepted 10 April 1984*)

Preliminary results of an extended X-ray absorption fine structure (e.x.a.f.s.) and X-ray absorption near edge structure study of jack bean urease have recently been reported [Hasnain & Piggott (1983) *Biochem. Biophys. Res. Commun.* **112**, 279]. These results indicated that the environment of the nickel ion in the enzyme is similar to that in the model compounds $Ni(L)_2(L')_1(ClO_4)_1$ (where L is 1-*n*-propyl-2-α-hydroxybenzylbenzimidazole and L′ is the deprotonated form) and $Ni(HMB)_3(Br)_2$ (where HMB is 2-hydroxymethylbenzimidazole), the closest similarity being with $Ni(L)_2(L')_1(ClO_4)_1$. A detailed e.x.a.f.s. analysis has now been carried out and the crystal structures of the two model compounds solved. These results are reported here.

Urease in crystalline form was first isolated by Sumner (1926), but 50 years elapsed before it was shown that jack bean urease was in fact a metalloenzyme (Dixon *et al.*, 1975). The M_r of the enzyme is 590000 ± 30000 and it contains 12 nickel atoms/molecule. Since the urease molecule is hexameric, it follows that there are 2 nickel atoms/subunit (Dixon *et al.*, 1980). Urease catalyses the hydrolysis of urea to CO_2 and NH_3 with extreme efficiency (Blakely *et al.*, 1969) and it is also highly specific as to substrate (Dixon *et al.*, 1980). A mechanism for the hydrolysis reaction has been proposed in which one nickel ion binds the substrate and the other nickel ion a hydroxyl ion. This hydroxyl ion acts as a potent nucleophile which attacks the carbonyl carbon of the co-ordinated substrate (Dixon *et al.*, 1980). It was clear from our preliminary study that the environment of the nickel ion in urease was similar to that in the model compounds $Ni(L)_2(L')_1(ClO_4)_1$ and $Ni(HMB)_3(Br)_2$. In both cases the nickel ions co-ordinate to three nitrogen atoms and three oxygen atoms. Qualitative comparison suggested the environment to be more like that in $Ni(L)_2(L')_1(ClO_4)_1$. The structures of both the model compounds have been determined crystallographically. In parallel, detailed e.x.a.f.s. analyses of these two model compounds have been carried out; the results of these are in good agreement with the crystallographic data.

Abbreviations used: e.x.a.f.s., X-ray absorption fine structure; L and L′, 1-*n*-propyl-2-α-hydroxybenzylbenzimidazole and its deprotonated form; HMB, 2-hydroxymethylbenzimidazole.

§ Permanent address: Consiglio Nazionale delle Ricerche, Via Salavia KM29500, Casella Postale 10, I-00016 Monterotondo Stazione, Roma, Italy.

‖ To whom requests for reprints should be addressed.

Materials and methods

The experimental details of the X-ray absorption measurements have been given in our preliminary communication (Hasnain & Piggott, 1983). X-ray absorption spectra were recorded in the standard transmission mode at the Daresbury Synchrotron Radiation Source operating at an energy of 2 GeV and average current of 80 mA. A double crystal Si220 monochromator was used and harmonic rejection achieved by offsetting one crystal with respect to the other. Data analysis utilized the single scattering spherical wave method for calculating e.x.a.f.s. with phase shifts derived from '*ab-initio*' calculations as described previously (Lee & Pendry, 1975; Perutz *et al.*, 1982). The e.x.a.f.s. is plotted in k-space where $k = \sqrt{0.263(E-E_0)}$, E = energy of the beam and E_0 = energy zero of the photoelectron wave. The e.x.a.f.s. amplitude is multiplied by k to enhance the important contribution of the nearest shell at higher k and also where

single scattering theory is most accurate. The quality of the fits was assessed by using criteria described by Perutz *et al.* (1982) and by using a non-linear least squares minimization program. Least squares refinement was carried out with k and k^3 weighting and a minimization was considered reliable only when both converged to similar results. Minimization was carried out for distance parameters only; co-ordination number and Debye–Waller constant were kept fixed during a minimization.

Crystalline jack bean urease was supplied by Sigma Chemical Co. (type C-3; activity 60000–100000 units/g). The nickel content of this sample was determined by atomic absorption spectroscopy and the protein content by the Lowry method. The nickel content of 1150p.p.m. is close to the theoretical value of 1170p.p.m. and the nickel-to-protein ratio of 1:866 in agreement with the value of 1:852 calculated by assuming an enzyme M_r of 600000 and 12 nickel atoms/molecule. The crystalline enzyme was gently crushed and the measurements carried out on the resulting powder. The preparation of the model compounds is described elsewhere (Piggott, 1967).

Crystals of $Ni(L)_2(L')_1(ClO_4)_1$, $C_{51}H_{53}N_6O_3NiClO_4$, are monoclinic with $a = 1.4549(2)$nm, $b = 1.9812(3)$nm, $c = 1.6988(3)$nm, $\beta = 96.05(1)°$, U 4.869nm^3, space group $P2_1/n$, $Z = 4$, $M = 954$, $D_c = 1.31\,g\cdot cm^{-3}$, (Cu-$K\alpha$) = 15cm^{-1}. Data were measured on a Nicolet R3m diffractometer with graphite monochromated Cu-$K\alpha$ radiation and using the ω-scan measuring routine. The data were corrected for Lorentz and polarization factors and an analytical absorption correction was applied. The structure was solved by the heavy atom method and refined anisotropically to $R = 0.056$ for 3915 independent observed reflections ($\theta \leqslant 50°$, $|F_o| > 3\sigma|F_o|$). Crystals of $Ni(HMB)_3(Br)_2$, $C_{24}H_{24}N_6O_3NiBr_2$, are monoclinic with $a = 1.2072(3)$nm, $b = 1.3071(3)$nm, $c = 1.8469(4)$nm, $\beta = 96.07(2)°$, $U = 2.898$nm^3, space group $P2_1/n$, $Z = 4$, $M = 663$, $D_c = 1.53\,g\cdot cm^{-3}$, (Cu-$K\alpha$) = 45cm^{-1}. Data collection and processing were as above, although for this complex an empirical absorption correction (based on 324 azimuthal scans) was applied. Structure solution and refinement were as above to give $R = 0.055$ for the 2606 independent observed reflections.

Results and discussion

Table 1 summarizes the results of the detailed analysis of the e.x.a.f.s. data of urease, $Ni(L)_2(L')_1(ClO_4)_1$ and $Ni(HMB)_3(Br)_2$. Also included in the Table are averaged distances from the crystallographic study of the two model compounds. To facilitate the discussion and presentation of the results a schematic picture of $Ni(L)_2(L')_1(ClO_4)_1$ is shown in Fig. 1, where the bonds to the atoms included in the e.x.a.f.s. analysis are drawn in bold; corresponding atoms, except of course for the phenyl carbon atoms C_C, were used in the e.x.a.f.s. analysis of $Ni(HMB)_3(Br)_2$. Fig. 2(*a*) shows the theoretical e.x.a.f.s. of $Ni(L)_2(L')_1(ClO_4)_1$ which is based on the crystallographic data of this compound, i.e. two Ni–O distances of 0.207nm, one Ni–O distance of 0.223nm and three Ni–N distances of 0.205nm. Also shown in Fig. 2(*a*) is the experimental e.x.a.f.s. spectrum of $Ni(L)_2(L')_1(ClO_4)_1$. The corresponding Fourier

Table 1. *Parameters used to simulate the e.x.a.f.s. associated with the Ni K-edge of the crystalline (powdered) samples of jack bean urease, $Ni(L)_2(L')_1(ClO_4)_1$ and $Ni(HMB)_3(Br)_2$*

R denotes the e.x.a.f.s. distance of atoms from the central nickel ion; R' are average values of non crystallographically equivalent distances; σ^2 is a Debye–Waller type factor and is equivalent to $R^2_{r.m.s.}$.

Shell no.	Atom type	Jack bean urease			$Ni(L)_2(L')_1(ClO_4)_1$				$Ni(HMB)_3(Br)_2$			
		Number	σ^2 (nm^2)	R (nm)	Number	σ^2 (nm^2)	R (nm)	R' (nm)	Number	σ^2 (nm^2)	R (nm)	R' (nm)
1	N_α	3	0.00012	0.204	3	0.00006	0.206	0.205	3	0.00002	0.204	0.204
2	O	2	0.00012	0.206	2	0.00006	0.207	0.207	3	0.00008	0.214	0.213
2	O_L	1	0.00010	0.225	1	0.00004	0.228	0.223	–	–	–	–
3	$C_{\beta 1}$	3	0.00015	0.294	3	0.00022	0.294	0.294	3	0.00012	0.289	0.285
3	C_A	–	–	–	3	0.00022	0.294	0.294	3	0.00012	0.299	0.302
3	$C_{\beta 2}$	3	0.00015	0.312	3	0.00015	0.320	0.320	3	0.00012	0.315	0.324
4	C_B	–	–	–	3	0.00015	0.377	0.384	3	0.00010	0.390	0.387
4	N_γ	3	0.00015	0.392	3	0.00015	0.390	0.411	3	0.00010	0.381	0.409
4	C_γ	3	0.00015	0.394	3	0.00022	0.393	0.418	3	0.00010	0.398	0.430
4	C_C	–	–	–	6	0.00022	0.393	0.418	–	–	–	–
					Ni–Ni distance 0.470				Ni–Ni distance 0.900			

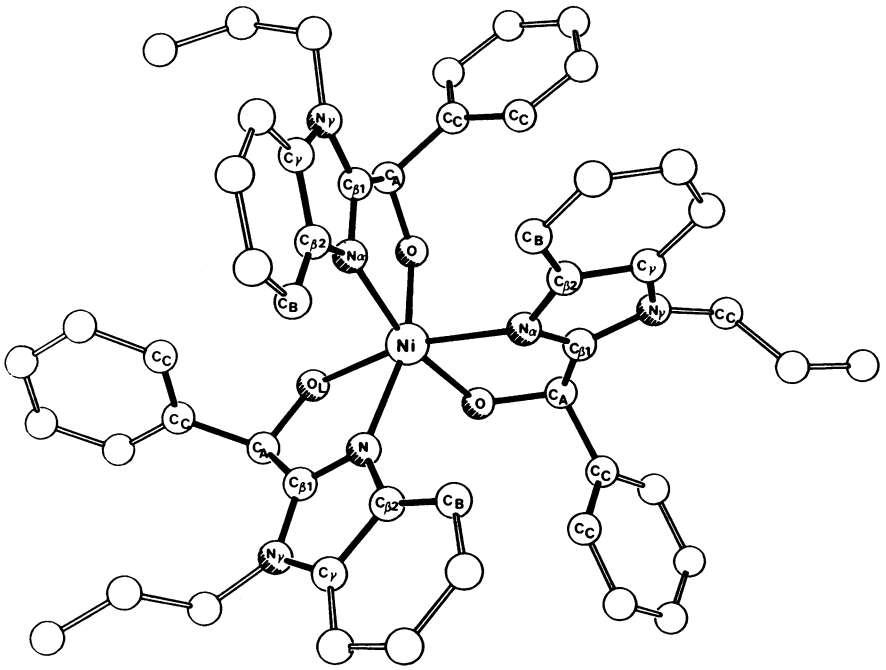

Fig. 1. *Schematic picture of* $Ni(L)_2(L')_1(ClO_4)_1$
Atoms included in the e.x.a.f.s. analysis are labelled and the bonds are shown in bold.

transforms, Fig. 2(*b*), show contributions at 0.2nm (shells 1 and 2), 0.27–0.3nm (shell 3) and 0.4nm (shell 4). In order to assess the confidence of the fit in Fig. 2(*a*), for which the fit index is 0.05, attempts were made to simulate the experimental spectrum by assuming (i) three Ni–O bond lengths of 0.207nm and three Ni–N bond lengths of 0.205nm, or (ii) three Ni–O bond lengths of 0.207nm, two Ni–N bond lengths of 0.206nm and one Ni–N bond length of 0.228nm.

In case (i), reasonable simulations were obtained with deterioration in the first and third beat regions. The fit index increased from 0.05 to 0.16 and the Ni–N, Ni–O and Ni–C bond lengths refined to 0.201nm, 0.210nm and 0.308nm respectively. Other bond lengths altered only slightly. With case (ii), significant deterioration of the simulation occurred but this time in the second and third beat regions; deterioration in the first beat region was less pronounced (Fig. 2*c*). The fit index increased from 0.05 to 0.064.

The experimental e.x.a.f.s. spectrum of $Ni(HMB)_3(Br)_2$ is given in Fig. 2(*d*), and the parameters used to generate the theoretial spectrum are given in Table 1. The fit index is 0.068. Comparison of Figs. 2(*a*) and 2(*d*) shows the difference for the two model compounds in the initial three beat regions. These differences can be attributed mainly to the different Ni–O bond lengths in the two model compounds. Fig. 3 shows the theoretical and experimental e.x.a.f.s. spectra for the nickel *K*-edge of urease. Theoretical simulation was obtained using essentially the same parameters as for $Ni(L)_2(L')_1(ClO_4)_1$ except for a slightly shorter Ni–N bond length of 0.204nm and omission of atoms C_A, C_B and C_C from the analysis. Elimination of C_A, C_B and C_C was done to reduce the number of parameters, given the limited quality of the urease e.x.a.f.s. data. To test the goodness of this fit, simulation of the urease spectrum was attempted using the same parameters as for $Ni(HMB)_3(Br)_2$. This resulted in a much poorer fit, as judged by a dramatic increase in the fit index from 0.11 to 0.28. To refine this fit and make the analysis consistent with that used for $Ni(L)_2(L')_1(ClO_4)_1$, atoms C_A and C_B were omitted. The fit index improved to 0.16, but was still inferior to the value of 0.11; also, the Ni–N and Ni–O bond lengths changed to 0.201nm and 0.210nm respectively.

It is known from spectral studies that the nickel ion in urease is six-co-ordinate (Dixon *et al.*, 1975). Our results are consistent with this and indicate that the nickel ion first co-ordination sphere is some combination of nitrogen and oxygen donors.

Dixon *et al.* (1980) have postulated that urease in

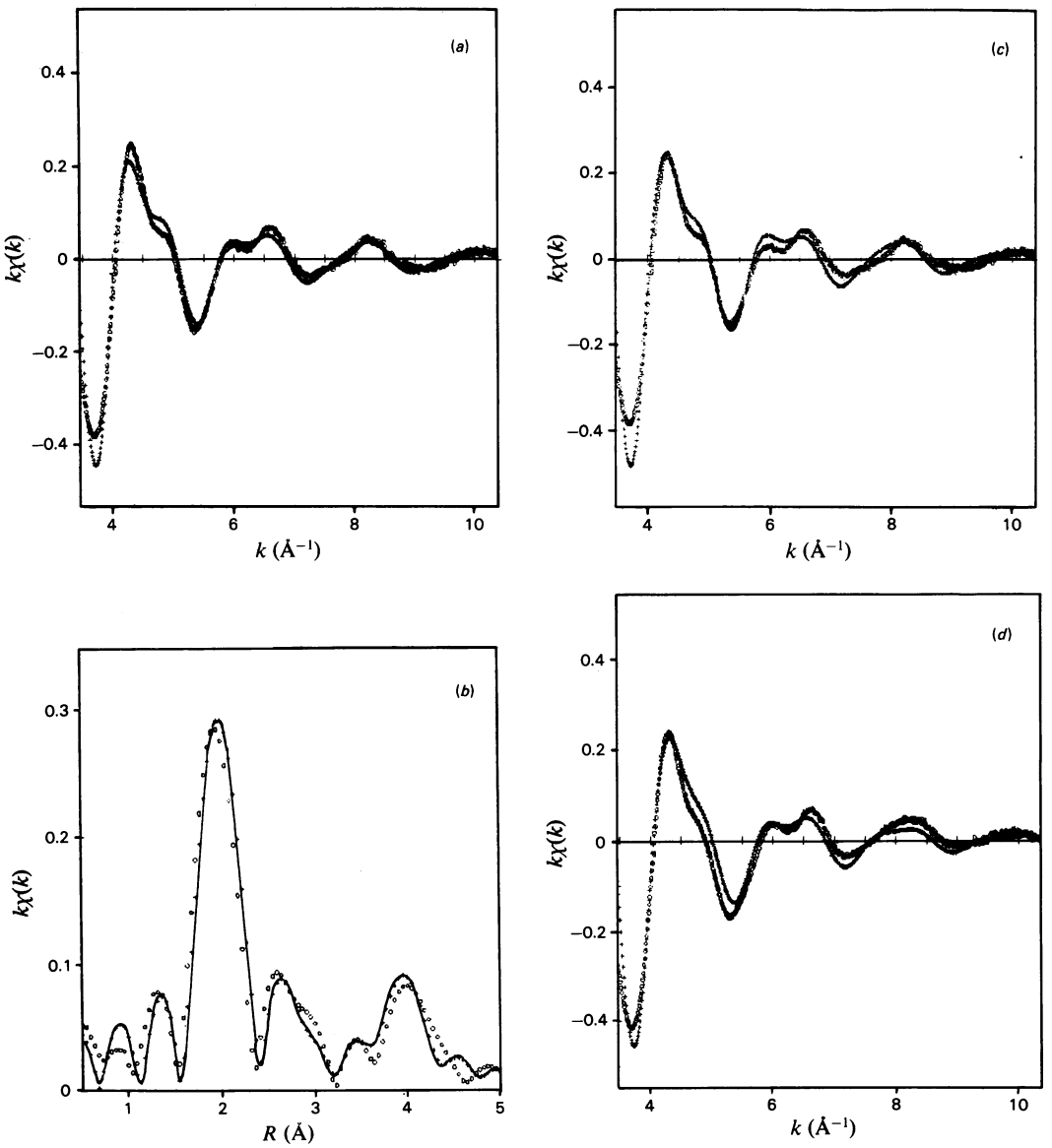

Fig. 2. *E.x.a.f.s. of model compounds*

E.x.a.f.s. is presented as $k\chi(k)$ plotted versus k, where k is in $Å^{-1}$ (equivalent to $10 nm^{-1}$). Experimental data (○) are shown without smoothing. (*a*) Ni *K*-edge e.x.a.f.s. of $Ni(L)_2(L')_1(ClO_4)_1$. The theoretical curve (+) corresponds to the structural parameters given in Table 1. (*b*) Fourier transform of experimental (○) and theoretical (—) e.x.a.f.s. data. (*c*) The theoretical e.x.a.f.s. curve (+) corresponds to the best simulation based on an interchange of oxygen by nitrogen atoms (see the text). (*d*) Ni *K*-edge e.x.a.f.s. of $Ni(HMB)_3(Br)_2$. The theoretical curve (+) corresponds to the structural parameters given in Table 1.

the resting state contains a loosely bound water molecule, and hence a long Ni–O bond, which is displaced by the substrate during turnover. Our results are consistent with the postulate of a long Ni–O bond. Those authors have also suggested that the nickel ions in urease subunits are within 0.6 nm of each other and that this is of fundamental importance in the hydrolysis mechanism. It was

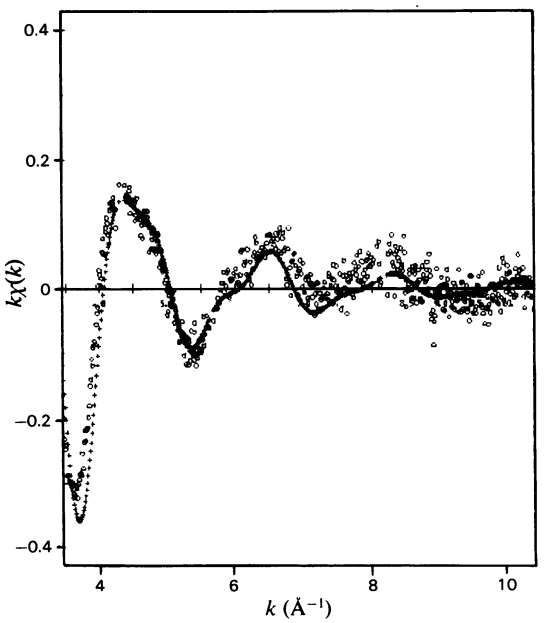

Fig. 3. *Ni K-edge e.x.a.f.s. of jack bean urease*
The theoretical curve (+) corresponds to the structural parameters given in Table 1.

found that in the crystal structure of $Ni(L)_2$-$(L')_1(ClO_4)_1$ nickel ions of adjacent molecules are within 0.47nm of each other, whereas the shortest Ni–Ni distance in $Ni(HMB)_3(Br)_2$ is 0.9nm. However, this particular structural feature was not one of the parameters used in the simulation of the e.x.a.f.s. spectrum of urease in the present study, and we therefore do not speculate as to the validity of the suggestion of Dixon *et al.* (1980).

References

Blakeley, R. L., Hinds, J. A., Kunze, H. E., Webb, E. C. & Zerner, B. (1969) *Biochemistry* **8**, 1991–2000

Dixon, N. E., Gazzola, C., Blakeley, R. L. & Zerner, B. (1975) *J. Am. Chem. Soc.* **97**, 4131–4133

Dixon, N. E., Riddles, P. W., Gazzola, C., Blakeley, R. L. & Zerner, B. (1980) *Can. J. Biochem.* **58**, 1335–1344

Hasnain, S. S. & Piggott, B. (1983) *Biochem. Biophys. Res. Commun.* **112**, 279

Lee, P. A. & Pendry, J. B. (1975) *Phys. Rev.* **11B**, 2795–2811

Perutz, M. F., Hasnain, S. S., Duke, P. J., Sessler, J. L. & Hahn, J. L. (1982) *Nature (London)* **295**, 535–538

Piggott, B. (1967) Ph.D. Thesis, University of London

Sumner, J. B. (1926) *J. Biol. Chem.* **69**, 435–441