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AN EXAFS STUDY OF JACK BEAN UREASE, A NICKEL METALLOENZYME

S.S. Hasnain[+] and B. Piggott[*]

[+]Science & 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, Herts. AL10 9AB, U.K.

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SUMMARY: EXAFS and XANES spectra have been recorded above the nickel K edge of urease and three model compounds. Preliminary results indicate that the local environment of the nickel ions in urease resemble most closely that of the nickel ions in the model compound $[Ni(L)_2(L^*)_1]$ $(ClO_4)_1$, where L is 1-n-propyl-2-α-hydroxybenzyl benzimidazole and L* is the deprotonated form.

Urease in crystalline form was first isolated in 1926 by Sumner (1) thus demonstrating that the enzyme is a protein. Some fifty years elapsed before Dixon et al (2,3) showed that jack bean urease (urea amidohydrolase) was in fact a metalloenzyme (which has a molecular weight of 590,000 ± 30,000 and contains 12 nickel atoms per molecule). Prior to this discovery nickel had been the only metal in the first transition series beyond titanium which had not been shown to have a biological role.

Urease catalyses the hydrolysis of urea to carbon dioxide and ammonia with extreme efficiency (4)

$$H_2N\text{-}CO\text{-}NH_2 \xrightarrow[H_2O]{\text{urease}} CO_2 + 2NH_3$$

It is also highly specific; N-hydroxyurea, N,N'-dihydroxyurea, N-aminourea, N-methylurea, formamide and acetamide (3) being the only other known substrates. Little is known about the mechanism of this reaction although there has been some speculation as to the involvement of the nickel ions

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(3). However, since the identity of the nickel ion coordination sphere is unknown suggestions as to their role in the hydrolysis reaction remains speculative. It was with the aim of identifying the metal ion environment that this X-ray absorption study was undertaken.

A plot of X-ray absorption against photon energy shows damped oscillations on the high energy side of the edge. These oscillations are the EXAFS spectrum which arises from interference of emitted photo electrons with those scattered back by neighbouring atoms; the spectrum is thus very sensitive to the immediate chemical environment of the absorbing atom (5). We have now succeeded in recording spectra, using the newly commissioned Synchrotron Radiation Source at Daresbury Laboratory which provide information on the metal ion site in jack bean urease.

MATERIALS AND METHODS

The enzyme urease from jack bean (canavalia ensiformis) was generously supplied by SIGMA LONDON CHEMICAL COMPANY LTD. About 200 mg of the finely ground enzyme was packed into an aluminium holder and held in place with Sellotape. X-ray absorption spectra in the (EXAFS) and (XANES) regions were obtained at the Ni K-edge in the standard transmission mode at the Daresbury Synchrotron Radiation Source (6). The storage ring was operating at 2 GeV and an average beam current of 80 mA. Harmonic contamination ($\lambda/2$, $\lambda/3$ .....) was minimised by using a double crystal Si 220 monochromator and by offsetting the first crystal to give I/I_{max} = 70%, where I_{max} is the intensity when the two crystals are set parallel. For the enzyme, six scans were used to produce the average spectrum shown in Figs.1 and 2 while for the model systems single scan data is presented.

RESULTS

EXAFS spectra of the enzyme and three model compounds are shown in Fig.1. Each of these spectra show strong beat regions typical of a histidine type environment (7). However, the spectra differ in detail, the profile of individual beat regions in each of these spectra is different. From past experience, we suggest that the differences arise from small structural differences in the first 2 shells of atoms.

Fig.1(d) shows the spectrum of $[Ni(IMIDAZOLE)_6](ClO_4)_2$ in which the nickel ion is in an octahedral co-ordination sphere of six nitrogen atoms. In

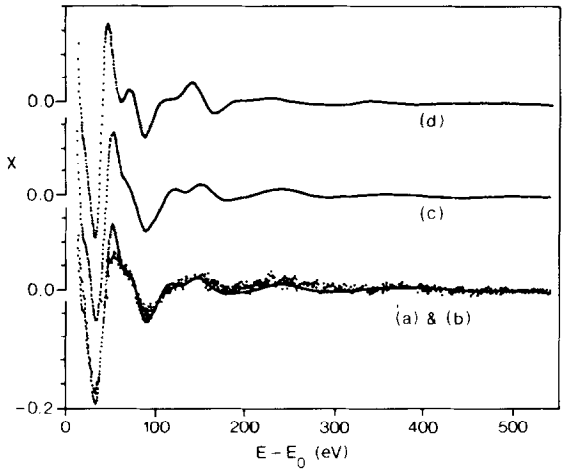
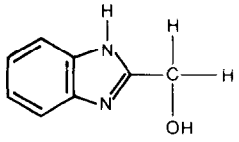
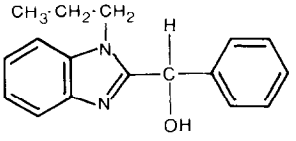


Fig.1 EXAFS spectra of (a) jack bean urase (b) $[Ni(L)_2(L^*)_1](ClO_4)_1$ (c) $[Ni(HOMB)_3](Br)_2$ where HOMB is 2-hydroxymethylbenzimidazole and (d) $[Ni(IMIDAZOLE)_6](ClO_4)_2$.

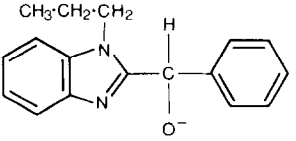
Fig.1(c) the spectrum of $[Ni(HOMB)_3](Br)_2$ is shown. In this compound the nickel is octahedrally co-ordinated to three nitrogen and three oxygen atoms (8). HOMB is



The best match to the enzyme spectrum is obtained with $[Ni(L)_2(L^*)_1](ClO_4)_1$, whose spectrum, fig.1(b), is superimposed on that of the enzyme, fig.1(a).The nickel ion in this model compound is in a quasi octahedral environment of three nitrogen atoms and three oxygen atoms (one of which is negatively charged (8)). L is



and L^* is



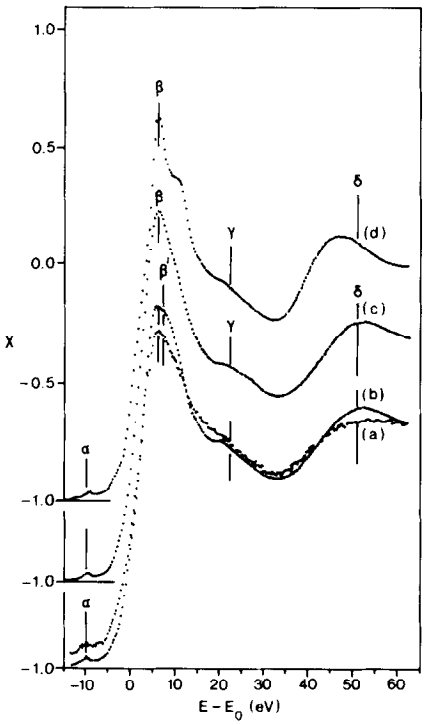


Fig.2 XANES spectra of (a), (b), (c) and (d) as defined in Fig.1.

Fig.2 shows a similar comparison to that in Fig.1, of the near edge spectrum (XANES). In this region, the contribution from multiple scattering dominates and thus geometrical information of the ligands surrounding the metal atom is preserved (9). Also distant shells (up to 3rd) make strong contributions. The overall profile of these spectra is similar with differences in details, e.g. in the case of $[Ni(IMIDAZOLE)_6](ClO_4)_2$, a sharp white line, β, is observed with a well defined shoulder between β and γ; the sharpness of β reduces in going from (d) to (b) and (c) where a broad white line, with unresolved features β and β', is observed. The close similarity of the XANES spectra (a) and (b) is in accord with the EXAFS spectrum; thus we suggest that structurally the environment of the Ni atom in urease is very similar to that in $[Ni(L)_2(L^*)_1(ClO_4)_1$.

DISCUSSION

Of the three model compounds studied the EXAFS and XANES spectra of $[Ni(L)_2(L^*)_1](ClO_4)$, are most similar to those of urease. This suggests

that the local environment (up to 4 Å) of the nickel ions in the two systems are similar and that nickel ions in urease are bound in part to histidine or histidyl residues.

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