Direct structural information for the copper site of dopamine β -mono-oxygenase obtained by using extended X-ray-absorption fine structure

S. Samar HASNAIN,* Gregory P. DIAKUN,* Peter F. KNOWLES,† Norman BINSTED,‡
 C. David GARNER‡ and Ninian J. BLACKBURN§

*Science and Engineering Research Council, Daresbury Laboratory, Warrington WA4 4AD, U.K.,

†Department of Biophysics, University of Leeds, Leeds LS2 9JT, U.K., ‡Department of Chemistry,

University of Manchester, Manchester M13 9PL, U.K., and §Department of Chemistry,

University of Manchester Institute of Science and Technology, P.O. Box 88, Manchester M60 1QD, U.K.

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Copper *K*-edge e.x.a.f.s. (extended X-ray-absorption fine structure) was measured for dopamine β -mono-oxygenase in aqueous solution. Comparison with the Cu *K*-edge e.x.a.f.s. of bovine erythrocyte superoxide dismutase shows a close resemblance. Detailed analysis of the e.x.a.f.s. indicates that the copper atom is bound to four imidazole groups at 0.201 nm with one or two oxygen atoms at 0.23 nm.

The copper-containing enzyme dopamine β -mono-oxygenase (EC 1.14.17.1) catalyses the β -hydroxylation of dopamine [2-(3,4-dihydroxyphenyl)ethylamine] to noradrenaline [norepinephrine; 2-(3,4-dihydroxyphenyl)-2-hydroxyethylamine] (Skotland & Ljones, 1979; Villafranca, 1981) according to the equation:

The four functional copper atoms per molecular mass of 290 000 daltons appear to be structurally homogeneous and are classified as Type 2 (Malkin & Malmström, 1970) on the basis of optical and e.p.r. spectra (Skotland & Ljones, 1979; Blackburn *et al.*, 1984b). The copper sites are notable for their extreme lability (Skotland & Flatmark, 1983), and spectroscopic studies indicate available inner-sphere co-ordination sites for cyanide and azide (Blackburn *et al.*, 1984b). These features are suggestive of a solvent-accessible copper site in the native enzyme. A redox role has been established for the copper centres (Skotland & Ljones, 1980), but the extent to which they participate in dioxygen binding and oxygen atom transfer is uncertain. We report here results of an e.x.a.f.s. study of native dopamine β -mono-oxygenase that

for the first time identifies imidazole co-ordination at the copper site.

Materials and methods

Native enzyme was prepared and reconstituted

to full functional copper content as described previously (Blackburn *et al.*, 1984b). The samples used contained 3.95 g-atoms of Cu(II)/mol of enzyme. E.p.r. at 9 and 35 GHz gave no evidence of more than a single species of Cu(II) present.

X-ray-absorption measurements were carried out on concentrated aqueous solutions (1.18 mM in total copper) maintained between 10 and 15°C during exposure to radiation as a precautionary measure to avoid any radiation damage. Experiments were performed on the synchrotron radiation source at the S.E.R.C. Daresbury Laboratory with the use of the E.X.A.F.S. Station 7.1 instrument with fluorescence detection. The synchrotron radiation source was operating at an energy of 2 GeV with an average current of 150 mA. The data presented are an average of ten scans with a total exposure time of 25 h. Each scan was checked individually and the edge structure was used to monitor the sample integrity. Methods

Abbreviation used: e.x.a.f.s., extended X-ray-absorption fine structure.

for data collection and analysis were as previously described (Blackburn *et al.*, 1983).

Results and discussion

Fig. 1 compares the e.x.a.f.s. of dopamine β -mono-oxygenase with that of native superoxide dismutase, and the close similarities between the two spectra suggest close similarities in copper co-ordination. Our interpretation of e.x.a.f.s. of native superoxide dismutase in solution (Blackburn *et al.*, 1984a) has indicated that the four imidazole groups are equidistant from copper at 0.199 nm with an oxygen atom from a water molecule at 0.224 nm. The e.x.a.f.s. of the mono-

oxygenase clearly shows the characteristic beat pattern prominent in the first two e.x.a.f.s. peaks known to originate from beating of first-shell nitrogen atoms and second-shell carbon atoms of imidazole rings (Blackburn *et al.*, 1983, 1984b).

Because of the similarities to native superoxide dismutase, detailed simulations (Fig. 2 and Table 1) commenced assuming four equidistant imidazole groups with Debye-Waller factors as determined for superoxide dismutase. Least-squares refinement gave a fit index {defined as $\sum k[\chi_{\text{exp.}}(k) - \chi_{\text{theor.}}(k)]^2$ } of 0.0613 with imidazole nitrogen atoms refining at 0.201 nm (Fig. 2a). Inclusion of an oxygen atom refining at 0.232 nm improved the fit index to 0.0376 and produced a better fit most noticeable in the first and third beat regions (Fig. 2b). The fit index further improved to 0.0253 if two oxygen atoms (refining at 0.234 nm) were used instead of one. In this case the e.x.a.f.s. was noticeably improved over the whole range and reproduced the beat patterns well. Simulations assuming three imidazole ligands were also attempted. An adequate fit (fit index 0.040) could be obtained with additional oxygen atoms at 0.201 and 0.232 nm, although, unlike the four-imidazole fits, the phases of experimental and theoretical spectra were not well matched. We note that the oxygen distance of 0.232–0.234 nm obtained in the above simulations is significantly longer than the 0.224 nm found in the case of superoxide dismutase.

Therefore it is not possible at this time to be definite about the imidazole/oxygen ratio of the inner co-ordination sphere of copper(II) in dopamine β -mono-oxygenase, owing to the restricted fitting range, which is a consequence of the poor signal-to-noise ratio of the e.x.a.f.s. data. However, a copper site composed of four equatorially co-ordinated imidazole groups with one or two axial oxygen atoms (Musinu *et al.*, 1983) provides the most satisfactory interpretation of the e.x.a.f.s. data and is consistent with the conclusions of an e.p.r. study that the g -tensor of the copper(II) site is axially symmetric with little or no rhombic

Fig. 1. Cu K-edge e.x.a.f.s. of (a) dopamine β -mono-oxygenase and (b) superoxide dismutase

E.x.a.f.s. [$\chi(k)$] is presented as $\chi(k) \times k$ plotted versus k , where k is in $\text{\AA}^{-1}$ [equivalent to $(10 \text{ nm})^{-1}$]. These experimental spectra are shifted by $\Delta E_0 = 15 \text{ eV}$, which was required for the theoretical simulations of dopamine β -mono-oxygenase.

Table 1. Parameters used to simulate e.x.a.f.s. associated with the Cu K-edge of dopamine β -mono-oxygenase in aqueous solution

R denotes the distance of atoms from the Cu atom.
 σ^2 is a Debye-Waller factor and is equivalent to ΔR^2_{rms} . $\Delta E_0 = 15 \text{ eV}$.

Atom	$\sigma^2 \text{ (nm}^2\text{)}$	$R \text{ (nm)}$
4N _(z)	0.00010	0.202
2O	0.00010	0.234
4C _(\beta)	0.00010	0.277
4C _(B)	0.00010	0.306
4C _(\gamma)	0.00010	0.370
4N _(\gamma)	0.00010	0.387

Fig. 2. Cu K-edge e.x.a.f.s. and Fourier transforms of dopamine β -mono-oxygenase. Continuous lines represent the experimental spectra, broken lines the simulated spectra. (a) Best simulation based on four imidazole groups, fit index = 0.0613, (b) four imidazole groups and one oxygen atom, fit index = 0.0376, and (c) four imidazole groups and two oxygen atoms, fit index = 0.0253.

distortion (Blackburn *et al.*, 1984b). The five-coordinate structure resembles the copper(II) site of native superoxide dismutase; however, the latter is rhombically distorted (Tainer *et al.*, 1982) and has the oxygen atom of its water molecule at 0.224 nm compared with 0.232 nm in dopamine β -mono-oxygenase. The alternative of a six-coordinate copper(II) site in dopamine β -mono-oxygenase, with two axial water molecules, would be unusual in copper proteins and would seem to allow solvent access on both sides of the $\text{Cu}(\text{imidazole})_4$ equatorial plane, although it is possible that a protein-bound carboxylate or phenolate group could provide one of the axial oxygen atoms. However, a six-coordinate $\text{Cu}(\text{imidazole})_4(\text{H}_2\text{O})_2$ site might account for the highly labile nature of the copper atoms of dopamine β -mono-oxygenase, as compared with other Type-2 copper proteins (Skotland & Flatmark, 1983).

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