Direct Structural Determination of the Short-lived Intermediate Formed in the Dynamic Incorporation of a Copper(II) Ion into a Mercury(II) Porphyrin by the Stopped-flow EXAFS Method

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Structure parameters around the Cu^{II} ion in a reaction intermediate formed in the course of Cu^{II} ion incorporation into an Hg^{II} porphyrin were directly determined using a new stopped-flow EXAFS apparatus, and elongation of the Cu–N bond in the unstable heterodinuclear porphyrin intermediate was observed

A number of kinetic studies of metalloporphyrin formation have been carried out.[1] Stereochemical and kinetic studies on metalloporphyrins are important in order to understand metal incorporation *in vivo*.[2] We have investigated the kinetics of reactions of 5,10,15,20-tetraphenylporphyrin and *N*-methyl-5,10,15,20-tetraphenylporphyrin with some metal(II) ions in *N,N*-dimethylformamide and pointed out the mechanistic differences in the metallation of these two porphyrins.[3,4] It has been demonstrated that in metalloporphyrin formation, large metal ions such as cadmium(II), lead(II) and mercury(II) ions accelerate the incorporation of medium-sized transition metal(II) ions such as the manganese(II) ion.[5] The large metal(II) ions cannot fit into the porphyrin nucleus and sit above the porphyrin plane. Such a complex forms rapidly and deforms the porphyrin nucleus favourably for attack by another metal ion from the opposite side. These facts indicate that the deformation of the porphyrin is a rapid process prior to the rate-determining step.[5]

Recently, Tabata and Miyata[6] and Robinson and Hambright[7] obtained evidence for an intermediate heterodinuclear metalloporphyrin complex in which two different metal ions are bound simultaneously to a porphyrin on the opposite side. We recently developed a stopped-flow EXAFS apparatus (SF-EXAFS, Type FIT-6) by combining stopped-flow and EXAFS methods.[8] The present study has concentrated on the direct determination of the structure of an unstable intermediate formed in the metal substitution of metalloporphyrins by the newly developed SF-EXAFS method.

The reaction studied was a metal substitution reaction of a mercury(II) porphyrin complex of 5,10,15,20,-tetrakis(4-sulfonatophenyl)porphyrin (H_2tpps^{4-}) with aqueous copper(II) ions. The kinetics of the metal substitution reaction have been thoroughly investigated.[6] The reaction mechanism in eqns. (1) and (2) with [Cu] > [Hg] ≫ [tpps] in weakly acidic media has been proposed. The reaction proceeds with a two-stage kinetic behaviour. The first- and second-step reactions correspond to

$$[Hg_2(tpps)]^{2-} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [Hg(tpps)]^{4-} + Hg^{2+} \qquad (1)$$

$$[Hg(tpps)]^{4-} + Cu^{2+} \xrightarrow[\text{first}]{k_2} [Hg(tpps)Cu]^{2-} \xrightarrow[\text{second}]{k_3} [Cu(tpps)]^{4-} + Hg^{2+} \qquad (2)$$

the formation of the heterodinuclear intermediate $[Hg(tpps)Cu]^{2-}$ and the product $[Cu(tpps)]^{4-}$, respectively. The rate constants at 25 °C are as follows: $k_1 = 1.95 \times 10^8$ $mol^{-1}\ dm^3\ s^{-1}$, $k_2/k_{-1} = 7.14$ and $k_3 = 1.00 \times 10^{-2}\ s^{-1}$.[6]

Compositions of sample solutions and conditions for EXAFS measurements are shown in Table 1. We focused on the reaction intermediate formed by mixing a solution containing the same total concentrations of mercury(II) and H_2tpps^{4-} and an equivalent solution of copper(II) ions in an acetate buffer. Under such conditions, before mixing, the mononuclear $[Hg(tpps)]^{4-}$ will be formed quantitatively and after mixing the formation rate of the reaction intermediate $[Hg(tpps)Cu]^{2-}$ should be very fast because the k_2 path is where copper(II) ions complex. Furthermore, knowing the rate constant values, we expect that almost all the copper(II) ions at pH 5.80 exist in the form of the intermediate during the 10 s measurement (gate time) with a delay of 1 s after mixing the copper(II) acetate solution (solution A) and the mercury(II) porphyrin complex solution (solution C). Thus, the 10 s measurement with a delay of 1 s after mixing was repeated 180 times to accumulate the EXAFS data for solution D.

A rotating anode X-ray generator (RU-300, RIGAKU Co., Ltd., Tokyo, Japan) was used as an X-ray source and a self-scanning photodiode array (S3904-1024Q, Hamamatsu Photonics K. K.) and a flat LiF(200) crystal were used as the X-ray detector and the monochromator, respectively.

The solutions of copper(II) acetate (pH 5.67, solution A), $[Cu(tpps)]^{4-}$ (pH 5.70, solution B), and copper(II) sulfate (solution E as a standard sample) were measured by the same procedure, *i.e.* each solution was passed through the mixing chamber in order to check any disturbance caused by the mixing process and repetition of the measurements. EXAFS spectra of solutions A, B and C, were also measured under static conditions using synchrotron radiation (SR) at the Photon Factory of the National Laboratory for High Energy

Table 1 Compositions of sample solutions and conditions for SF-EXAFS measurements

Solution	Solutes	Concentration/ $mol\ kg^{-1}$	Gate time[a]/s	No. of accumulations	Total time/s
A	$Cu(MeCO_2)_2$	0.20	25	48	1200
	$MeCO_2Na$	1.58			
	$MeCO_2H$	0.20			
B	$Cu(MeCO_2)_2$	0.10	25	36	900
	H_6tpps	0.10			
	$MeCO_2Na$	0.50			
	$MeCO_2H$	0.20			
	NaOH	0.66			
C	$Hg(MeCO_2)_2$	0.20			
	H_6tpps	0.20			
	$MeCO_2Na$	1.61			
	$MeCO_2H$	0.20			
	NaOH	1.01			
D[b]			10	180	1800
E[c]	$CuSO_4$	0.20	5	750	3800

[a] Measuring time of X-ray intensity for each stopped-flow run. [b] Prepared by mixing solutions A and C. [c] Standard sample for EXAFS analysis.

Physics (Tsukuba, Japan). The EXAFS spectra were analysed as follows. The pre-edge absorptions and the post-edge smooth absorptions were approximated by the Victoreen formula and the six-order polynomial function, respectively. To obtain structural parameters, the model function of EXAFS oscillation[9] was fitted to the Fourier filtered $k^3\chi(k)$ values ($4.5 < 10^2\ k/\text{pm}^{-1} < 8.0$) by least squares, where $k^3\chi(k)$ is the normalised EXAFS oscillation and k is the photoelectron wave vector. For the backscattering amplitude and the total scattering phase shift the reported values were used.[10] For the EXAFS data analyses of solutions A, B and D, the values of the threshold energy E_0 and the mean free path λ, determined by analysis of the EXAFS spectra for the standard sample of the $[Cu(OH_2)_6]^{2+}$ ion (solution E), were fixed during the least-squares calculation. The structural parameters around the copper(II) ion in solutions A and B obtained by the SF-EXAFS apparatus and by the static measurement with SR agreed well with each other (Table 2). Thus, it was found that no significant errors were introduced in the mixing process by comparison with SR data.

Figs. 1 and 2 show the observed $k^3\chi_{\text{obs}}(k)$ curves and the Fourier transforms calculated from the EXAFS data for solutions A, B, D and E obtained at the Cu-K edge, respectively. The structural data of solutions A, B, D and E are summarised in Table 2. The Fourier filtered and theoretical $k^3\chi(k)$ curves are depicted in Fig. 3; the theoretical curve was computed using the obtained parameters in Table 2. For the structural analysis around the copper(II) ion in the intermediate, both a two-shell model (2[Cu–N], 2[Cu–O]) and a three-shell model (2[Cu–N], 2[Cu–O(short)], 2[Cu–O(long)]) were examined. Values of the error squares sum $[\Sigma\{k^3\chi_{\text{filt}}(k) - k^3\chi_{\text{calc}}(k)\}^2/10^{-12}]$ and the R-factor were 0.76 pm^{-6} and 7.6% for the two-shell model, and 0.28 pm^{-6} and 4.6% for the three-shell model, respectively. Thus the three-shell model better reproduced the observed data than the two-shell model. Moreover, the environment around the copper(II) ion in the intermediate on the basis of the three-shell model is quite similar to that of $[Cu(en)(H_2O)_4]^{2+}$.[11]† Therefore, the three-shell model seems to be reasonable.

Under the present conditions, di-, tri- and tetra-acetatocopper(II) complexes would be formed in solution A in a ratio of 3 : 10 : 6 which was estimated from the available formation constants.[12] Four equatorial positions of the copper(II) ion in solution A seem to be occupied by acetate ions and/or water molecules, and two water molecules are interacting in the axial position. For solution B, the Cu–N bond distance is in good agreement with the value determined by static EXAFS measurements at the SR beam line (Table 2) and the distance in the crystalline phase.[13] Thus there should be no interaction in the axial positions of the copper(II) ion.

Although a Cu···Hg nonbonding interaction is not observed in Fig. 2 due to the fluctuation in the relative positions of both metal ions and/or a long Cu···Hg distance in the intermediate, the intermediate contains the mercury(II) ion on the basis of the kinetic study.[6] The Cu–N bond length in the intermediate $[Hg(tpps)Cu]^{2-}$ was determined to be 204 pm, longer than the Cu–N bonds (200 pm) in the final product, $[Cu(tpps)]^{4-}$.

Table 2 Structural parameters around the copper(II) ion in solutions A, B, C, D and E[a]

Solution	Interaction[b]	r^c/pm	σ^d/pm	n^e
A	Cu–O$_{\text{eq}}$	196(1)	5.5(0.3)	4[k]
	Cu–O$_{\text{ax}}$	225(3)	8.9(0.7)	2[k]
A[f,g]	Cu–O$_{\text{eq}}$	197(1)	7.4(0.1)	4.4(0.1)
	Cu–O$_{\text{ax}}$	225(1)	10.1(0.5)	2.1(0.2)
A[f,h]	Cu–O$_{\text{eq}}$	197(1)	5.8(0.4)	4[k]
	Cu–O$_{\text{ax}}$	226(2)	11.9(0.9)	2[k]
B	Cu–N	200(1)	8.0(0.6)	3.8(0.2)
B[f,i]	Cu–N	200(1)	4.8(0.1)	3.5(0.1)
C[f]	Hg–N	223(1)	8.9(0.5)	2.2(0.3)
	Hg–O	260(3)	11.0(3.0)	0.7(0.5)
D[j]	Cu–N	204(1)	8.0[k]	2[k]
	Cu–O$_{\text{eq}}$	195(1)	8.1[k]	2[k]
	Cu–O$_{\text{ax}}$	238(2)	16.4(0.7)	2[k]
E[a]	Cu–O$_{\text{eq}}$	196(1)	8.1(0.2)	4[k]
	Cu–O$_{\text{ax}}$	229(3)	11.8(0.4)	2[k]

[a] The values of threshold energy E_0 and mean free path λ used for the EXAFS data analyses of solutions A, B and D are 9.004(0.002) keV and 540(30) pm, respectively, which were obtained for soltuion E. [b] The subscripts eq and ax represent the equatorial and axial postions, respectively. [c] The interatomic distance between the central metal(II) ion and the scatterer. [d] The Debye-Waller factor. [e] The number of scatters. [f] The structural parameters are obtained by the analysis of EXAFS spectra measured by using the synchrotron radiation beam line. [g] $[Cu(MeCO_2)_2]$ = 0.10 mol dm^{-3}, $[MeCO_2Na]$ = 0.50 mol dm^{-3} and $[MeCO_2H]$ = 0.20 mol dm^{-3} in water. [h] $[Cu(NO_3)_2]$ = 1.0 mol dm^{-3} in water. Copper(II) ion exists as $Cu(H_2O)_6^{2+}$. [i] $[Cu(tpps)^{4-}]$ = 0.10 mol dm^{-3}, $[MeCO_2Na]$ = 0.50 mol dm^{-3} and $[MeCO_2H]$ = 0.20 mol dm^{-3} in water. [j] Three-shell model (see text). [k] Fixed during a least-squares calculation.

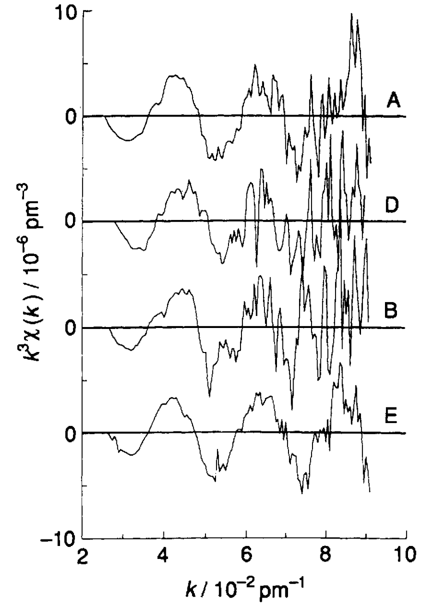

Fig. 1 Observed $k^3\chi(k)$ curves for solutions containing copper(II) acetate (solution A), $[Cu(tpps)]^{4-}$ complex (solution B), intermediate $[Hg(tpps)Cu]^{2-}$ (solution D) and $[Cu(OH_2)_6]^{2+}$ ion (solution E)

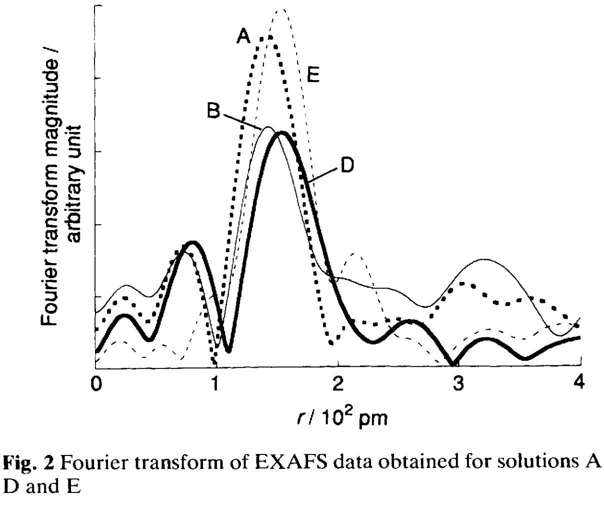

Fig. 2 Fourier transform of EXAFS data obtained for solutions A, B, D and E

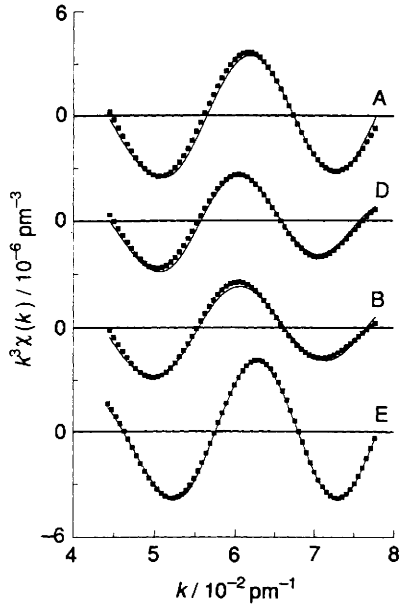

Fig. 3 Fourier filtered (dots) and calculated (solid line) $k^3\chi(k)$ curves for solutions A, B, D and E

Thus, elongation of the Cu–N bond in the unstable intermediate was directly observed. Cu–O interactions were also observed in the intermediate, and acetate ions appear to be bound to the copper(II) ion. Therefore, the results suggest that the porphyrin ring in the intermediate is deformed to allow coordination in the equatorial position of the copper(II) ion. Unfortunately, at present, the absorption at the Hg L-edge is too weak for the EXAFS spectra to be measured with the present apparatus.

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Footnote

† Structure parameters for $[Cu(en)(H_2O)_4]^{2+}$ are as follows: interatomic distance $r(2[CuN-N_{eq}])$ = 200 pm, Debye–Waller factor $\sigma(2[Cu-N_{eq}])$ = 5.8 pm, $r(2[Cu-O_{eq}])$ = 198 pm, $\sigma(2[Cu-O_{eq}])$ = 5.8 pm, $r(2[Cu-O_{ax}])$ = 231 pm and $\sigma(2[Cu-O_{ax}])$ = 12.8 pm.

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