A rapid direct analysis of the structure of reaction intermediates by the EXAFS method combined with a stopped-flow technique

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Abstract - A partial structure of the copper(II) ion of a reaction intermediate at the formation of a copper(II) porphyrin complex in the metal substitution reaction of the mercury(II) porphyrin complex with the copper(II) ion in an aqueous acetate buffer solution has been determined by using a newly developed rapid EXAFS apparatus, which is constructed by combining a stopped-flow and a laboratory-scale EXAFS equipments. The porphyrin used is 5,10,15,20-tetrakis(4-sulfonatophenyl)porphine. Two Cu-N bonds in the reaction intermediate have been found to be 204 pm, which are longer than the Cu-N bonds (200 pm) in the final product of the copper(II) porphyrin complex, and it has been estimated that the copper(II) ion in the intermediate is about 40 pm out from the center of the porphine ring, and thus the mercury(II) and copper(II) ions are almost in contact in the heterodinuclear complex. Since we did not clearly see the Hg···Cu nonbonding interaction in the EXAFS spectrum, the position of the atoms may be significantly fluctuating due to the repulsion between the atoms.

INTRODUCTION

The structural determination of reaction intermediates has been attempted by various workers by using different methods. Time resolved EXAFS measurements have been attempted (Refs. 1-3) for determining structural parameters around the central metal ions in biological systems (Refs. 3-5). In these studies the point-to-point method has been adopted. However, the energy dispersive method, which can be used much more conveniently and faster for the collection of EXAFS data, has not been successfully employed. Attempts have been reported for the use of the dispersive SOR-EXAFS method which is combined with a stopped-flow method (Refs. 6-8), but no successful result has been obtained.

We constructed a laboratory scale EXAFS apparatus by employing a rotatory anode X-ray source equipped with a stopped-flow cell along the X-ray path. A pulse X-ray beam has been passed through the cell within a certain time to extract the EXAFS spectrum of the reaction intermediate formed in the cell. By selection of a suitable system, we could determine a partial structure of a reaction intermediate in a solution. This is the first achievement of the direct structural analysis of reaction intermediates by an energy dispersive EXAFS method combined with a stopped-flow technique.

EXPERIMENTAL

A rapid EXAFS apparatus was constructed by using a rotatory anode X-ray generator (RU-300, RIGAKU, Tokyo, Japan) with an Mo target as an X-ray source and a stopped-flow cell (FIT-6, Yunisoku, Hirakata, Japan), which was placed between the X-ray source and an LiF(200) monochromator. The schematic picture of the rapid EXAFS apparatus is shown in Fig. 1. The windows of the stopped-flow cell were made of boron
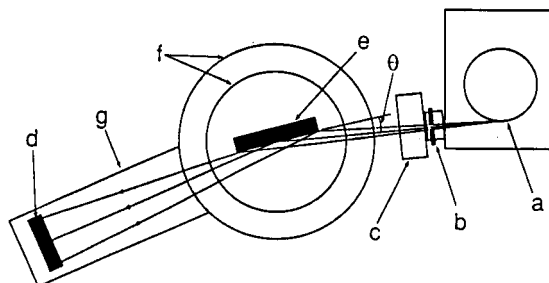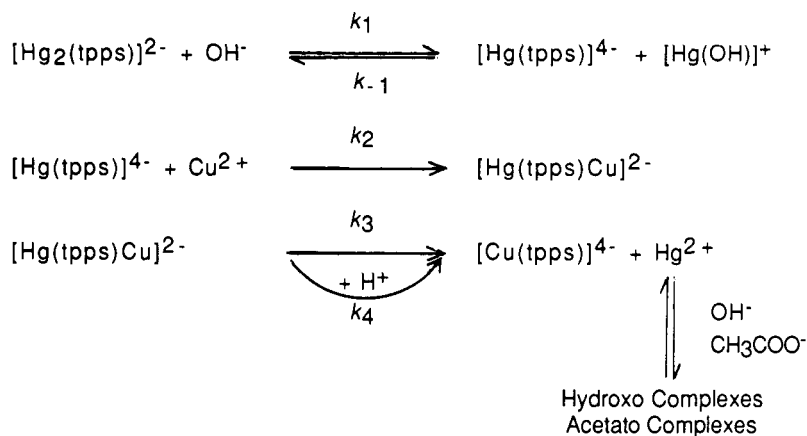

Fig. 1. Schematic picture of the laboratory-scale rapid EXAFS apparatus. a: Rotating anode target. b: Slit. c: Stopped-flow cell. d: Detector. e: Monochromator. f: Goniometer. g: Detector arm.

nitride of 150 μm thick pyrolitically prepared. The assembly of the stopped- flow equipment and the cell constructed are depicted in Fig. 2 (Ref. 9). A 1024 channel image-sensor cooled to about -20 °C was used as the detector.

The reaction examined was a metal substitution reaction of a mercury(II) porphyrin complex with a copper(II) ion in aqueous solution. The porphyrin used was 5,10,15,20-tetrakis(4-sulfonatophenyl)porphine (H_6tpps), which is highly soluble in water, and the kinetics of the metal substitution reaction has been thoroughly investigated. The mechanism of the reaction has been proposed as follows:

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

$$[Hg(tpps)]^{4-} + Cu^{2+} \xrightarrow{k_2} [Hg(tpps)Cu]^{2-}$$

$$[Hg(tpps)Cu]^{2-} \underset{+ H^+ \atop k_4}{\overset{k_3}{\rightleftharpoons}} [Cu(tpps)]^{4-} + Hg^{2+}$$

$$\Big\downarrow \begin{array}{l} OH^- \\ CH_3COO^- \end{array}$$

Hydroxo Complexes
Acetato Complexes

and the formation of a heterodinuclear reaction intermediate [Hg(tpps)Cu]$^{2-}$ has been suggested. The rate constants at 25 °C are: k_1 = 1.95 × 10^8 mol^{-1} dm^3 s^{-1}, k_2/k_{-1} = 7.14, k_3 = 1.00 × 10^{-2} s^{-1}, and k_4 = 2.70 × 10^3 mol^{-1} dm^3 s^{-1} (Ref. 10).

According to the rate constants, the half-life time of the reaction intermediate should be 75.3 s at pH 5.4, and the most favorable 10 s period was used for opening the gate of the X-ray beam at one EXAFS measurement (the time is called "Gate Time" in Table 1). After 1 s of mixing of the solutions of the mercury(II) porphyrin complex and a copper(II) acetate solution in a stopped-flow cell, the intermediate should be formed almost quantitatively and the concentration of the final product in the period should be only 0.54 % of the total concentration of the complexes. Thus, the 10 second measurement was repeated 180 times to accumulate
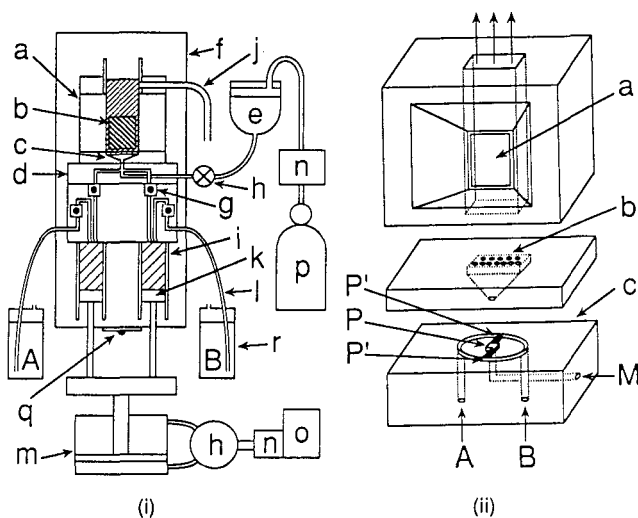

Fig. 2. (i) The stopped-flow assembly. a: Observation cell. b: Window. c: Nozzle. d: Mixing chamber. e: Medium solution vessel. f: Thermostated block. g: Ruby ball. h: Electromagnetic 2-way valve. i: Piston cylinder. j: Drain. k: Piston head. l: Teflon tube. m: Gas cylinder. n: Pressure regulator. o: Air compressor. p: Nitrogen gas cylinder. q: Micro-switch. r: Reservoir.
(ii) Cell. a: Window. b: Nozzle. c: Mixing chamber. A, B: Test solution inlets. P, P': Mixing points. M: Inlet of medium solution for backgound measurement.

the EXAFS data for solution C (denoted as "Accumulation" in Table 1), which consists of two solutions, one containing the mercury(II)-tpps complex and the other copper(II) acetate. Thus, the total length of time for the measurement was 1,800 s ("Total Time" in Table 1), which was enough to obtain a reasonable EXAFS spectrum in this experiment.

Table 1 Composition of Solutions.

Solution	Solute	Concentration mol kg^{-1}	pH	Gate Time s	Accumulation Times	Total Time s
A	Cu(CH$_3$COO)$_2$	0.20	5.67	25	48	1,200
	CH$_3$COONa	1.58				
	CH$_3$COOH	0.20				
B	Cu(CH$_3$COO)$_2$	0.10	5.7	25	36	900
	H$_6$tpps	0.10				
	CH$_3$COONa	0.50				
	CH$_3$COOH	0.20				
	NaOH	0.66				
C	Hg(CH$_3$COO)$_2$	0.20	5.8 (pH=5.43 after mixing)	10	180	1,800
	H$_6$tpps	0.20				
	CH$_3$COONa	1.61				
	CH$_3$COOH	0.20				
	NaOH	1.01				
	Cu(CH$_3$COO)$_2$	0.20	5.67			
	CH$_3$COONa	1.58				
	CH$_3$COOH	0.20				

As references, solutions of copper(II) acetate (pH = 5.67) (solution A) and copper(II)-tpps (pH = 5.7) (solution B) were measured by the same procedure, i. e., the individual solutions were passed through the mixing chamber in order to check disturbance caused by the process and the EXAFS data were collected from repeated measurements. It was found that no significant errors were introduced at the mixing process. The EXAFS spectra of the solutions were also measured under a static condition by using a synchrotron orbital radiation (SOR) at the Photon Factory of the National Laboratory for High Energy Physics. The structural data of copper(II) ions in an acetate buffer solution and the [Cu(tpps)]$^{4-}$ complex in a copper(II)-tpps solution obtained by measurement with the mixing procedure in the present rapid EXAFS apparatus and by the static measurement with SOR agreed well each other. The compositions of the solutions examined are summarized in Table 1.

The Fourier transforms of solutions A, B, and C calculated from the EXAFS data obtained from absorptions at the Cu K-edge are shown in Fig. 3. It is obvious that the peak position of the solution C shifts toward the side of the larger r value compared with the peak position in solution B, and the result indicates that the bonds between the Cu(II) ion and the ligand atoms in the reaction intermediate are longer than those in the product [Cu(tpps)]$^{4-}$. The $k^3\chi(k)$ curves derived from the Fourier transform of the main peak around 100 - 200 pm in Fig. 3 cut with a suitable window function are shown in Fig. 4.

Fig. 3. Fourier transform of the EXAFS data obtained from solutions containing copper(II) acetate, [Cu(tpps)]$^{4-}$ complex, and the reaction intermediate, [Hg(tpps)Cu]$^{2-}$.

RESULTS AND DISCUSSION

In the course of the structural analysis around the copper(II) ion in the intermediate, both of a two-shell (2[Cu-N], 2[Cu-O]) and a three-shell (2[Cu-N], 2[Cu-O(short)], 2[Cu-O(long)]) models have been examined. No significant difference in the Cu-N and Cu-O(short) bond lengths has been found. The structural data of solutions A, B, and C are summarized in Table 2. The $k^3\chi(k)$ curves in Fig. 4 are well reproduced with these parameters.

The Cu-N bonds in the reaction intermediate [Hg(tpps)Cu]$^{2-}$ has been determined to be 204 pm (three-shell model), which is longer than the Cu-N bonds (200 pm) in the final product [Cu(tpps)]$^{4-}$, and thus, the Cu(II) ion in the intermediate is located at about 40 pm out from the center of the porphine ring (Ref. 11). Taking into account the ionic radius of copper(II) ion (ca 90 pm), the copper ion may be almost in contact of the mercury (II) ion (ionic radius, ca 110 pm) in the heterodinuclear complex. Nevertheless we could not see the Cu···Hg nonbonding distance in the EXAFS
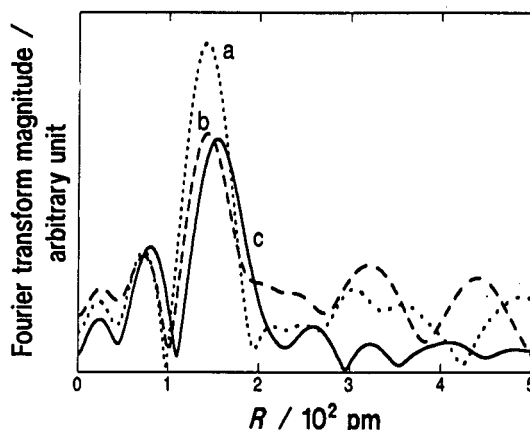
spectrum in Figs. 3 and 4, suggesting that the position of the atoms may be largely fluctuating due to the repulsive force between them. The result also suggests that the prophine ring in the inter-mediate should be deformed. The existence of the Cu-O(long) bond is not conclusive. No structural information around the mercury(II) ion in the reaction intermediate was obtainable, because we measured X-ray absorption at the Cu K-edge for the EXAFS measurements and the absorption at the Hg L-edge was too weak to measure the EXAFS spectra with the present apparatus.
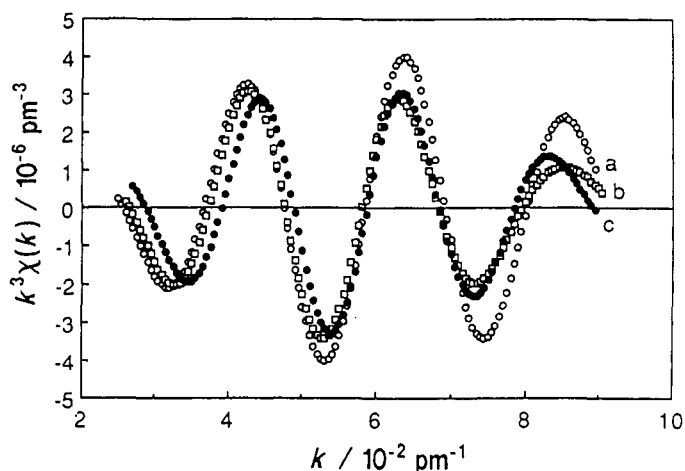
Fig. 4 $k^3\chi(k)$ Curves vs. k of solutions containing copper(II) acetate, $[Cu(tpps)]^{4-}$ complex, and the reaction intermediate, $[Hg(tpps)Cu]^{2-}$.

Table 2. Structural data around the copper(II) ion of the complexes in solutions A, B and C[a].

Solution	Atom-pair	r/pm	σ/pm	n
A	Cu-O$_{eq}$	196(1)	5.5(0.3)	4[d]
	Cu-O$_{ax}$	225(2)	8.9(0.7)	2[d]
B	Cu-N	200(1)	8.0(0.6)	2[d]
C[b]	Cu-N	204(1)	8.0[d]	2[d]
	Cu-O$_{eq}$	195(1)	8.1[d]	2[d]
	Cu-O$_{ax}$	238(2)	16.4(0.7)	2[d]

[a]r: Bond length. σ: Debye-Waller factor. n: Coordination number. [b]Three-shell model.
[c]Fixed during the least-squares refinement.

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