

# AXIAL-LIGAND REPLACEMENT OF [TETRAKIS(4-SULFONATOPHENYL)PORPHINATO]IRON(III) IONS BY CYANIDE IONS IN ALKALINE SOLUTION

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The axial-ligand replacement reaction of [tetrakis(4-sulfonatophenyl)porphyrinato]iron(III) ions (abbreviated to Fe(III)-TPPS) by cyanide ions was studied by means of spectrophotometric methods in aqueous alkaline solutions over the pH range of 11–13 at 25°C.

The spectrophotometric data showed that long periods are necessary to attain equilibrium for the replacement reaction, i.e., 6, 24 and 28 hr at pH 11.0, 12.0 and 12.8, respectively. It was found by spectrophotometric cyanide ion-titration that the diaqua  $\mu$ -oxo-dimer of Fe(III)-TPPS(I) is transformed to monocyano- and dicyano-monomers at equilibrium, the former of which increases with an increasing pH of the solution.

Kinetic measurements by stopped-flow spectrophotometry enabled us to obtain the pseudo first-order rate constant,  $k'_{f(I \rightarrow I')}$ , where  $I'$  is an intermediate species in the reactions, in an early stage of the reaction (within  $\sim 10$  s), which is inversely proportional to the concentration of hydroxyl ions. The intermediate species  $I'$  was proposed to be dicyano monomer of Fe(III)-TPPS based on the properties of Fe(III)-TPPS in solution, such as time-course spectral changes, the number of transferred cyanide ions in the reaction, and aggregate formation in alkaline solution at pH  $\sim 13$ .

## Introduction

A number of heme proteins include an iron(III)protoporphyrinIX (heme) complex as active center in their pockets [1–3]. The heme proteins play a biologically important role in transport and storage of oxygens, transfer of electrons, and transformation of substrates. A group of iron(III)porphyrin complexes are regarded as a model compound of heme proteins for studying their functions mentioned above [3–5]. On the other hand, cyanide ions act as one of desperate inhibitors against the activi-

ty of heme proteins, such as hemoglobin, myoglobin, cytochromes and peroxidases [1, 5]. In a previous paper, we reported cyanide-ion replacement on the axial-ligand of [tetrakis(1-methyl-4-pyridinio)porphine]iron(III) ions, which possesses four positive charges around its porphyrin periphery [6]. As one of the most important results in the study, it was found that the overall reaction rate of its axial-ligand replacement by cyanide ions is very slow in spite of cationic circumstance of the periphery.

In order to investigate the influence of anionic surroundings of the periphery on the reaction,

we have performed spectrophotometric measurements on cyanide ion-replacement reaction on the axial-ligand of [tetraakis(4-sulfonatophenyl)-porphinato]iron(III) ions (abbreviated as Fe(III)-TPPS), which has four negative charges around its porphyrin periphery.

### Experimental

#### Materials and Sample Solutions

Fe(III)-TPPS was prepared by a metal insertion method with 5, 10, 15, 20-tetraphenyl-21H, 23H-porphinetetrasulfonic acid (Dojindo Laboratories, abbreviated as TPPS [7]) and FeSO<sub>4</sub> · 7H<sub>2</sub>O according to Fleischer et al [8]. All other chemicals of guaranteed reagent grade (Wako Pure Chemical Industries) were used without further purification.

The concentration of Fe(III)-TPPS solutions was determined spectrophotometrically by the use of a molar absorption coefficient at 394 nm;  $1.50 \times 10^5 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  at pH 3 [8]. The pH values of sample solutions were adjusted with concentrated sodium hydroxide solutions. The ionic strength of the sample solutions was adjusted to  $0.10 \text{ mol dm}^{-3}$  with a concentrated sodium sulfate solution.

#### Spectrophotometric Measurements

A Hitachi 228A spectrophotometer was used for spectrophotometric measurements. An initial part of the ligand replacement of Fe(III)-TPPS by cyanide ions (within 10 s) was followed by the use of a Union Giken RA401 stopped-flow spectrophotometer. The pH of the sample solutions was measured by means of a TOA HM-20S pH meter. All the measurements were performed at 25°C.

### Results and Discussion

#### Axial-Ligand Substituted Species of Fe(III)-TPPS in Alkaline Solutions

In previous spectrophotometric investigations, it has been established that diaqua  $\mu$ -oxo-dimer is a predominant species in aqueous solutions of Fe(III)-TPPS at pH above 6 [8-10]. Addition of potassium cyanide to the Fe(III)-

TPPS solutions over the pH range of 11-13 brought about a marked spectral change. Figure 1 shows a typical example at pH 11.1, where the maximum wavelength of Soret band shifted from 410 to 425 nm with an isosbestic point at  $\sim 415 \text{ nm}$ . On the other hand, characteristic two peaks of Q band at 570 and 610 nm changed into three peaks at 540, 560 and 600 nm overlapped in part. These results suggests that the symmetric diaqua  $\mu$ -oxo-dimer was transformed into other species with poor symmetry, i. e., presumably monocyano monomer or monocyano  $\mu$ -oxo-dimer. It took 6 hr to reach until the equilibrium of the substitution reaction.

Similar spectral changes owing to the CN-substitution of Fe(III)-TPPS were also observed on alkaline solutions of Fe(III)-TPPS at pH 12.0 and 12.8, although periods for equilibration were elongated to 24 and 28 hr at respective pHs. The molar absorption coefficients of CN-substituted Fe(III)-TPPS species at 425 nm

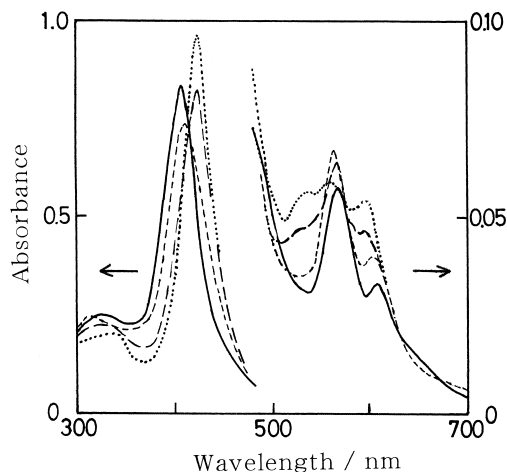


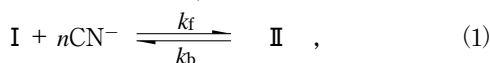
Fig. 1. Time-course variation of the absorption spectrum of Fe(III)-TPPS at pH 11.1 after the addition of cyanide ions.

— : no addition, - - - - : 4 min,  
- · - · : 60 min, ····· : 300 min

[Fe(III)-TPPS] :  $1.00 \times 10^{-5} \text{ mol dm}^{-3}$ ,  
[KCN] :  $2.00 \times 10^{-2} \text{ mol dm}^{-3}$ .

after equilibration were equal to one another within experimental errors regardless of different pH values, i. e.,  $9.76 \times 10^4$ ,  $9.73 \times 10^4$  and  $9.78 \times 10^4$   $\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$  at pH 11.0, 12.0 and 12.8, respectively. This fact indicates that the CN-substituted species show the same spectral characteristics at 425 nm, while those do considerably dissimilar behaviors on the Q band, at least, after 1 hr.

In order to elucidate the CN-substituted species, we carried out the spectrophotometric cyanide ion-titration experiments with the Fe(III)-TPPS solutions of pH 11.0, 12.0 and 12.8 at 410 nm, results of which are shown in Fig. 2. The reaction scheme concerned was postulated as follows:



where I and II represent the initial diaqua  $\mu$ -oxo-dimer of Fe(III)-TPPS, and final species formed, respectively, and  $k_f$  and  $k_b$  the forward

and backward reaction rate constants, respectively. Accordingly,  $(k_f/k_b)$  corresponds to the equilibrium constant  $K$  for the reaction (1), as defined by the following equation.

$$K = \frac{[\text{II}]}{[\text{I}][\text{CN}^-]^n} \quad (2)$$

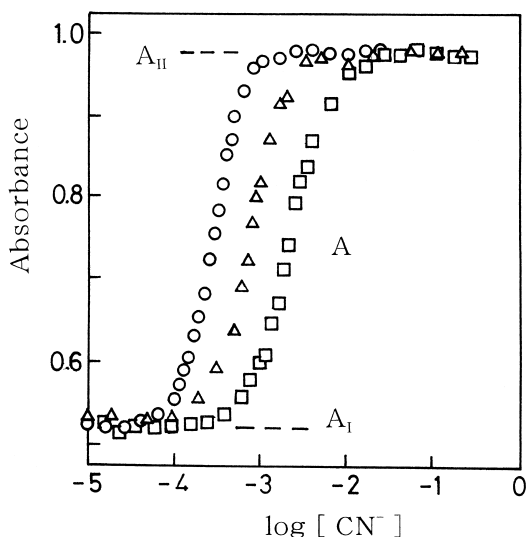
This can be rewritten in logarithmic form as follows:

$$\log \frac{[\text{II}]}{[\text{I}]} = \log K + n \log [\text{CN}^-] \quad (3)$$

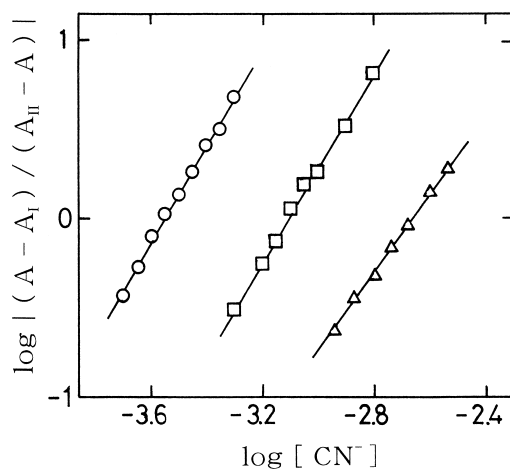
By introducing absorbances of a plateau at low concentrations of cyanide ions,  $A_I$ , at high concentrations,  $A_{II}$ , and at an arbitrary cyanide ion concentration between the two plateaus,  $A$ , into eq. 3, we obtain the following relationship [6].

$$\log \frac{|A - A_I|}{|A_{II} - A|} = \log K + n \log [\text{CN}^-] \quad (4)$$

Figure 3 shows plots of the left hand side of eq. 4 against  $\log [\text{CN}^-]$ , which gave linear correlations at respective pHs. Therefore, we



**Fig. 2.** Spectrophotometric cyanide ion-titration curves of Fe(III)-TPPS at 425 nm.  
[Fe(III)-TPPS] :  $1.00 \times 10^{-5}$   $\text{mol dm}^{-3}$ ,  
○ : pH 11.0, △ : pH 12.0 and □ : pH 12.8.



**Fig. 3.** Plots of eq. 4 for evaluating the number of transferred cyanide ions,  $n$ , in the axial-ligand replacement reaction.  
[Fe(III)-TPPS] :  $1.00 \times 10^{-5}$   $\text{mol dm}^{-3}$ ,  
○ : pH 11.0, △ : pH 12.0 and □ : pH 12.8.

can obtain the number of transferred cyanide ions,  $n$ , from the slope and  $K$  from the intercept on the axis of abscissas, provided that eq. 1 holds for the CN-substitution reaction proceeded. The  $n$  and  $K$  values obtained are listed in Table 1. It should be noted that the  $n$  value at pH 12.8 is nearly equal to 2, from which species **II** is assumed to be the dicyano  $\mu$ -oxo-dimer or the cyanohydroxo monomer of Fe(III)-TPPS. On the other hands, those values at pH 11.0 and 12.0 are 2.6 and 2.7, respectively. This fact rejects the dicyano  $\mu$ -oxo-dimer as species **II**, because it may not possess any substitution sites more than two. Thus, it is most likely that species **II** is the cyanohydroxo monomer and that possible other species co-existing with species **II** is the dicyano monomer, as shown later in Fig. 8. It could also be referred on the basis of deviations of  $n$  from 2 that about 34, 31 and 6% of Fe(III)-TPPS exist as dicyano monomer at pH 11.0, 12.0 and 12.8, respectively.

These facts are interpreted as follows. Although the coordination tendency of hydroxyl ions is considerably weaker than that of cyanide ions[10], it is very likely that a hydroxyl ion substitutes competitively with one of the axial-ligands of the dicyano monomer in a high concentration region of hydroxyl ions. In fact, the concentration ratios of  $[\text{OH}^-]/[\text{CN}^-]$  at pH 11.0, 12.0 and 12.8 are 0.05, 1 and 3, respectively. This agrees very well with reduction in the amount of the dicyano monomer with increasing  $[\text{OH}^-]$ . The dicyano monomer seems to be preferably formed as CN-substituted species of Fe(III)-TPPS from the diaqua  $\mu$ -

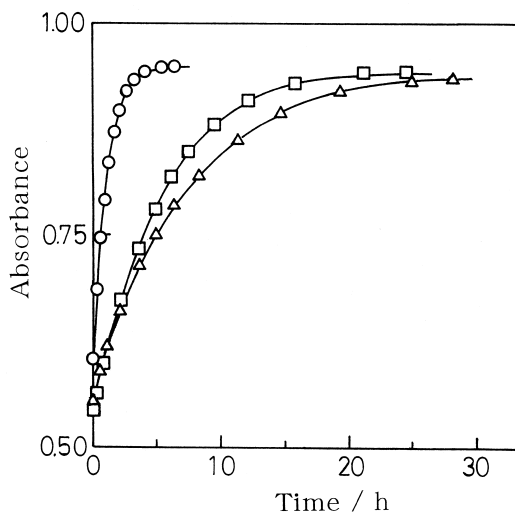
oxo-dimer, but it is susceptible to be substituted by hydroxyl ions in high pH solutions, though the rate of the OH-substitution is very slow, as discussed in the following section. Thus, the  $K$  values obtained from eq. 1 should be regarded as apparent equilibrium constant (Table 1).

*Kinetics of the Axial-Ligand Replacement of Fe(III)-TPPS by Cyanide Ions in Alkaline Solutions*

In the preceding section, it has been revealed that the CN-substitution reaction of Fe(III)-TPPS is very slow and that the cyanohydroxo and dicyano monomers are the final products, as species **II** in eq. 1. Then, we made a kinetic experiment on the overall reaction by tracing time-course variations in absorbance at 425 nm, at which absorbance increased slowly depending on the formation of species **II**. Even though species **II** consists of mono- and di-substituted monomers with cyanide ions, they may exhibit similar characteristics in spectral variation. The results obtained at pH 11.0, 12.0 and 12.8 are shown in Fig. 4.

**Table 1** Numbers of transferred cyanide ions,  $n$ , and equilibrium constants,  $K$ , for the overall reaction represented by eq. 1.

pH	$n$	$K/(\text{mol dm}^{-3})^n$
11.0	2.68	$3.4 \times 10^9$
12.0	2.62	$1.4 \times 10^8$
12.8	2.13	$4.6 \times 10^5$



**Fig. 4.** Time-course variation of the absorbance of Fe(III)-TPPS at 425 nm after the addition of cyanide ions.  $[\text{Fe(III)-TPPS}] : 1.00 \times 10^{-5} \text{ mol dm}^{-3}$ ,  $[\text{KCN}] : 2.00 \times 10^{-2} \text{ mol dm}^{-3}$ ,  $\circ$  : pH 11.0,  $\triangle$  : pH 12.0 and  $\square$  : pH 12.8.

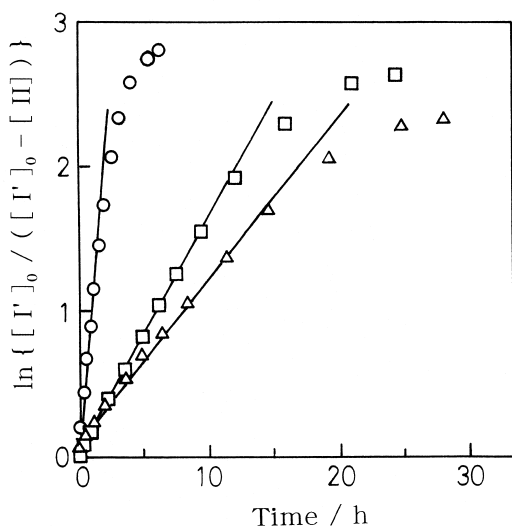
Since the backward reaction in eq. 1 is negligible in an early stage of the reaction under the condition in excess of  $\text{CN}^-$  concentrations compared with those of  $\text{Fe(III)-TPPS}$ , the reaction rate is expressed as follows;

$$\begin{aligned} d[\text{II}]/dt &= k_f [\text{I}][\text{CN}^-] \\ &= k_f [\text{I}]_0 - [\text{II}] ([\text{CN}^-]_0 - [\text{II}]) \\ &\approx k_f' ([\text{I}]_0 - [\text{II}]), \end{aligned} \quad (5)$$

where  $[\text{I}]_0$  and  $[\text{CN}^-]_0$  represent the initial concentrations of species I and cyanide ions, and  $k_f'$  the pseudo first-order rate constant,  $k_f ([\text{CN}^-]_0 - [\text{II}])$ . By integrating eq. 5, the following equation is obtained.

$$\ln \{ [\text{I}]_0 / ([\text{I}]_0 - [\text{II}]) \} = k_f' t, \quad (6)$$

in which  $[\text{I}]_0 = \varepsilon_1 A_0$ ;  $\varepsilon_1$  and  $A_0$  are the molar absorption coefficient of species I and the absorbance at the initial point of the reaction at 425 nm, respectively, and  $[\text{II}] = (A - \varepsilon_1 A_0) / (\varepsilon_{\text{II}} - \varepsilon_1)$ ;  $\varepsilon_{\text{II}}$  and  $A$  are the molar absorption coefficient of species II and an absorbance at an arbitrary reaction time  $t$  at 425 nm, respectively. Figure 5 shows plots of the left hand side of eq. 6 against  $t$ , which

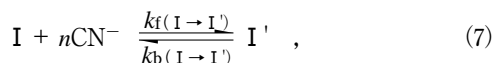


**Fig. 5.** Plots of eq. 6 for evaluating the pseudo first-order rate constants,  $k_f'$ , of the overall replacement reaction.  
 $\circ$  : pH 11.0,  $\triangle$  : pH 12.0 and  $\square$  : pH 12.8.

gave linear relationships in an early stage of the reaction at individual pHs. The  $k_f'$  values obtained from the slope of linear regressions are listed in Table 2, where the  $k_f'$  values decrease with increasing pH. It should be noted that the axial-ligand of  $\text{Fe(III)-TPPS}$  is substituted by cyanide ions at slower rates by a factor of ca. 1/5~1/10 than that of  $\text{Fe(III)-TMPyP}$  is in highly alkaline solutions [6]. The reason of the slow substitution with the former is presumably attributable to either electrostatic repulsion between cyanide ions and negative charges of the periphery or enthalpic disadvantage owing to disruption of the dimer-linkage.

On the other hand, the time-course variations in absorbance at 410 nm is very rapid, which corresponds to the reduction of species I, i. e., the diaqua  $\mu$ -oxo-dimer. Therefore, we did not estimate the number of transferred cyanide ions,  $n$ , in the following eq. 7.

Next, we studied the fast reaction of species I by stopped-flow spectrophotometry (Fig. 6), assuming the reaction scheme as follows;



in which  $\text{I}'$  denotes an intermediate species between species I and II.

Equations 5 and 6 are also adaptable to the present case, and then eqs. 8 and 9 hold,

$$-d[\text{I}]/dt \approx k_f'(\text{I} \rightarrow \text{I}') ([\text{I}]_0 - [\text{I}']), \quad (8)$$

$$\ln \{ [\text{I}]_0 / ([\text{I}]_0 - [\text{I}']) \} = k_f'(\text{I} \rightarrow \text{I}') t, \quad (9)$$

where  $[\text{I}]_0 = \varepsilon_1 A_0$ ;  $\varepsilon_1$  and  $A_0$  are the molar absorption coefficient of species I and the

**Table 2** Pseudo first-order rate constants for the overall reaction represented by eq. 1,  $k_f'$ , and those for the fast reaction in its early stage by eq. 7,  $k_f'(\text{I} \rightarrow \text{I}')$ , at 25°C.

pH	$k_f'/\text{s}$	$K_f(\text{I} \rightarrow \text{I}')/\text{s}$
11.0	$2.37 \times 10^{-4}$	5.76
12.0	$4.55 \times 10^{-5}$	$5.88 \times 10^{-1}$
12.8	$3.11 \times 10^{-5}$	$7.50 \times 10^{-2}$

absorbance at the initial point of the reaction at 410 nm, respectively, and  $[I'] = (A - \epsilon_I A_0) / (\epsilon_{I'} - \epsilon_I)$ ;  $\epsilon_I$  and  $A$  are the molar absorption coefficient of species I and absorbance at an arbitrary reaction time  $t$  at 410 nm, respectively. The pseudo first-order rate constants,  $k'_{f(I \rightarrow I')}$ , at pH 11.0, 12.0 and 12.8 were evaluated as in the case of the reaction process  $I \rightarrow II$  (Fig. 6), and then their logarithmic values are plotted against pH in Fig. 7. An excellent linear relationship with a slope of ca. -1 gave the true first-order rate constant,  $k_{f(I \rightarrow I')}$ , shown in eq. 10.

$$k_{f(I \rightarrow I')} = k'_{f(I \rightarrow I')} [\text{OH}^-] / K_w, \quad (10)$$

where  $K_w$  is the ionic product of water at 25 °C. The  $k_{f(I \rightarrow I')}$  value, evaluated from data listed in Table 2, is  $5.5 \times 10^{11} \text{ s}^{-1}$ .

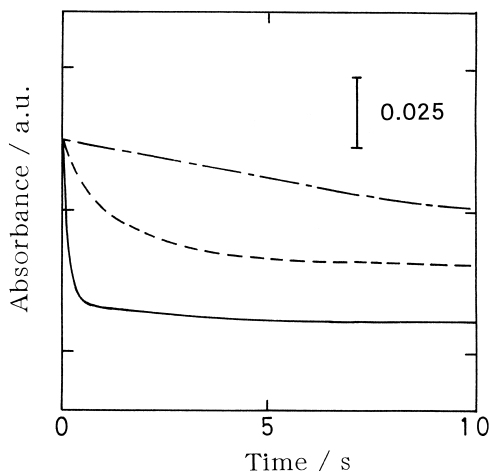
The  $k_{f(I \rightarrow II')}$  and  $k_{f(I' \rightarrow II')}$  values for the aquahydroxo and dihydroxo monomers, respectively, of Fe(III)-TMPyP, not evaluated in a previous study [6], may be several times larger

than the  $5.5 \times 10^{11} \text{ s}^{-1}$  for the diaqua  $\mu$ -oxo-dimer of Fe(III)-TPPS by the reason aforementioned.

*Reaction Scheme for the Axial-Ligand Replacement of Fe(III)-TPPS by Cyanide Ions in Alkaline Solutions*

Figure 8 summarizes plausible schemes for the axial-ligand replacement of Fe(III)-TPPS by cyanide ions in alkaline solutions over the pH range of 11-13.

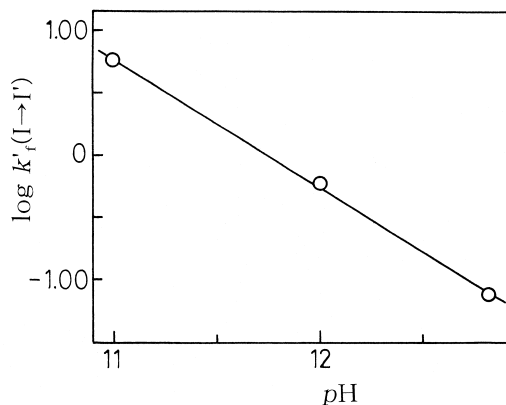
Species I', an intermediate between I and II, has not yet been clarified, because the number of transferred cyanide ions in the fast reaction of species I to I' was not determined. It should, however, be worth pointing out that the shape of Q band relevant to a good symmetrical structure does not change in an early stage of the reaction, at least within 4 minutes (see Fig. 1). Hence, the dicyano  $\mu$ -oxo-dimer (in Scheme A) or the dicyano monomer (in Scheme B) of Fe(III)-TPPS are plausible as species I', as shown in Fig. 8. We presume the latter as species I', because the mono- and di-cyano monomers are very stable compared with the diaqua  $\mu$ -oxo-dimer



**Fig. 6.** Time-course variation of the absorbance of Fe(III)-TPPS at 410 nm in an early stage of the replacement reaction after the addition of cyanide ions.

$[\text{Fe(III)-TPPS}] : 1.00 \times 10^{-5} \text{ mol dm}^{-3}$ ,  
 $[\text{KCN}] : 2.00 \times 10^{-2} \text{ mol dm}^{-3}$ .

———— : pH 11.0,    - - - - - : pH 12.0,  
 - · - · - : pH 12.8,

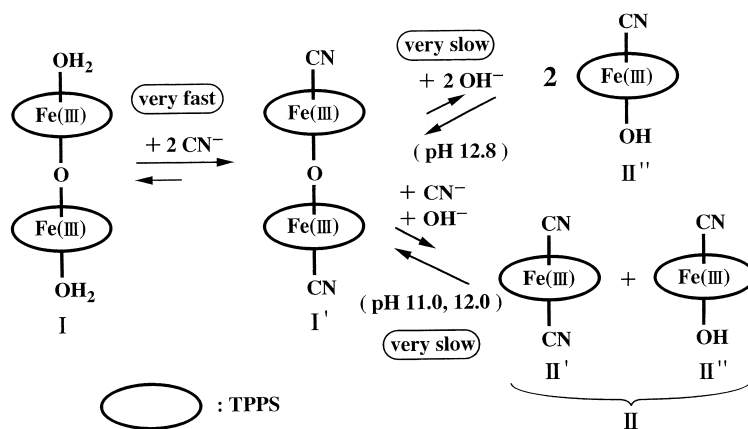


**Fig. 7.** Relationship between the pseudo first-order rate constant,  $\log k'_{f(I \rightarrow I')}$  and pH.

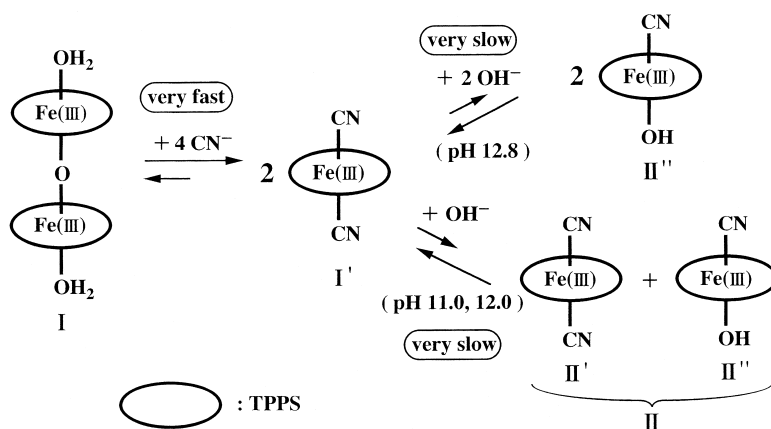
of Fe(III)-TPPS in highly alkaline solutions at pH above 12.5, which gradually transforms into some aggregate [12]. In addition, the amount of the dicyano monomer at pH 11.0 is larger than that at pH 12.8, indicating that the dicyano monomer is more stable than the cyanohydroxo monomer.

### Acknowledgements

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Scheme A



Scheme B

Fig. 8. Plausible reaction schemes for the axial-ligand replacement of Fe(III)-TPPS by cyanide ions in aqueous alkaline solution.

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