Coordination Behavior of Cyanide Ion to Hemin Uncomplexed and Complexed with Several Synthetic Polymer Ligands

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Synopsis

Hemin (H) (ferri–protoporphyrin IX chloride) was complexed in aqueous media with the following polymer ligands; poly(L-lysine), poly(L-histidine), branched polyethyleneimine, and two copolymers of 1-vinyl-2-pyrrolidone with 1-vinylimidazole and with 1-vinyl-2-methylimidazole. The resulting water-soluble complexes were either the five- or six-coordinate hemichromes, respectively, which coordinated one or two ligand units in polymer to ferri–protoporphyrin IX (FP). The coordination behavior of cyanide ion to the hemichromes and also to H was investigated by means of spectroscopic titration method. It was found that one cyanide ion binds not only to the five-coordinate hemichromes, but also to the six-coordinate ones by the ligand exchange reaction with the cyanide ion, whereas two cyanide ions coordinate to H, which is free from polymer ligand able to occupy the coordination site(s) of FP. Morever, the degree of saturation of the iron(III) in FP with cyanide ion for all the hemichromes was found to be much larger than that for H. These results indicate that the ability of FP to coordinate cyanide ion is closely associated with whether one coordination site of FP is occupied by polymer ligand or not.

INTRODUCTION

Previous publications[1-5] from this laboratory have described the syntheses of several water-insoluble polymers able to function as a cyanide ion exchanger. Such polymers involve ternary polyelectrolyte complex composed of ferri–hemoglobin, potassium poly(vinyl alcohol) sulfate and poly-(diallyldimethylammonium chloride),[1-3] copolymer of hemin (H) (ferri–protoporphyrin IX chloride) and styrene (St),[4] and two terpolymers of H and St with acrylamide and with 1-vinyl-2-pyrrolidone (VP).[5] In a series of previous papers, we assumed that a dominant factor affecting their abilities to adsorb and desorb cyanide ion is coordination structure of polymer-holding ferri–protoporphyrin IX (FP) as the adsorption site. In order to provide an approach for elevating the adsorbing capability of cyanide ion exchanger, therefore, it is necessary to make clear the change in the affinity of polymer-holding FP for cyanide ion depending upon whether the coordination site(s) of the FP is occupied by ligand unit in the polymer or not. However, there is as yet no detailed study dealing with this problem, although some authors[6,7] have investigated the coordination property of cyanide ion to

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polymer-holding ferro– and ferri–protoporphyrinIX complexes with a view to obtaining information about the function of heme-proteins in living system.

In this paper, H was complexed with various synthetic polymer ligands in aqueous media to obtain water-soluble polymer-holding FP samples, i.e., five- and six-coordinate hemichromes, respectively, which coordinated one and two ligand units in the polymer to FP. Poly(L-lysine) (PLL),[6,8-12] poly(L-histidine) (PLH),[7] branched polyethyleneimine (BPEI),[13] and two copolymers[14] of VP with 1-vinyl-imidazole (VI), copoly(VP, VI), and with 1-vinyl-2-methylimidazole (VMI), copoly(VP, VMI) were chosen as the polymer ligands in view of the available information about the preparations of their hemichromes. The coordination properties of cyanide ion to the hemichromes were investigated by spectroscopic titration method. The results obtained were compared with that for H, and discussed in terms of a difference in the affinities of FP for cyanide ions caused by whether one coordination site is occupied by polymer ligand or not.

EXPERIMENTAL

Materials

Chemicals. H and VP were the same samples as used previously.[5] VMI and VI were commercially obtained from BASF Co. and Tokyo Kasei Kogyo Co., respectively. They were twice distilled under reduced pressure (79°C/3 mmHg for VMI and 45°C/9 mmHg for VI) immediately before use.

Polymer Ligands. Copoly(VP, VMI) and copoly(VP, VI) were prepared by the radical copolymerizations[14] of an equimolar (175 mmol) mixture of VP (19.44 g) with VMI (18.92 g) and with VI (16.47 g), respectively. The copolymerization was made in a sealed tube at 80°C for 2 h under reduced pressure using purified methanol (60 mL) as the solvent and α,α'-azobisisobutyronitrile (7.3 mmol, 1.2 g) as the initiator. The reaction mixture was poured slowly into diethyl ether (1 L), and the precipitate was collected and dried *in vacuo.* The crude copolymer was then purified by reprecipitation three times from a methanol–diethyl ether system, and dried *in vacuo* at 40°C for 3 days. Yield: 21.5 g for copoly(VP, VMI) and 24.2 g for copoly(VP, VI); IR(KBr), 1640, 1510, 1480, 1450, 1430, and 1410 cm^{-1} for copoly(VP, VMI) and 1640, 1480, 1450, 1430, and 1405 cm^{-1} for copoly(VP, VI); $[\eta]^{30}$ (dL/g), 0.83 (ethanol) for copoly(VP, VMI) and 0.76 (methanol) for copoly(VP, VI). The compositions of the copolymers were VP:VMI = 3.0:2.0 and VP:VI = 2.9:2.1, as determined by the results of elemental analyses: copoly(VP, VMI): C, 65.56%; H, 7.95%; N, 17.84%; copoly(VP, VI): C, 64.46%; H, 7.59%; N, 19.13%.

BPEI ($\overline{M}_w = 1.1 \times 10^5$; ratio of primary, secondary, and tertiary amino groups = 1:2:1) was the same sample as used previously.[15] PLL (HBr salt) and PLH were purchased from Sigma Chemical Co. and purified according to the literature (see Ref. 16 for PLH and Ref. 17 for PLL). The values of $\overline{M}_n$ for both samples were roughly determined by the osmometric method[18]: 2×10^4 for PLH and 1×10^4 for PLL.

Complexation of H with Polymer Ligands

The complexation was carried out at 25 $\pm$ 0.1°C by dissolving H (1 $\times$ 10^{-6} mol) into the solution (50 mL) containing different amounts of polymer ligand, followed by permitting to stand for 2–4 h. Various buffer solutions (ionic strength = 0.15; pHs = 9 and 12) were used as the solvents. In the complexation with copoly(VP, VMI) or copoly(VP, VI), however, ethylene glycol (50 v/v %) was added into the solvent for preventing the aggregation of FP (see Ref. 14). The absence of aggregated FP in this solvent was confirmed by the method of Nishide et al.[19] The prepared water-soluble hemichromes were subjected to spectral measurements to obtain spectroscopic titration curves which are available for examining their coordination structures. The measurement was carried at 25 $\pm$ 0.1°C using a Hitachi 200-20 spectrophotometer and a 1 cm path quartz cell. The optical density was then determined at the wavelength (nm) of a maximum absorption in the Soret region.

Spectroscopic Titrations of Hemichrome and H with Cyanide Ligand

The titration was made at 25 $\pm$ 0.1°C with the same apparatus as used for the hemichromes. The spectral data were determined at 540 nm corresponding to a maximum absorption of the cyanide-coordinated H and hemichromes. The samples were prepared by adding different amounts of KCN into the solutions (50 mL) containing H or hemichromes (1 $\times$ 10^{-6} mol as FP). They were immediately sealed in a measuring cell to avoid the volatilization of hydrocyanic acid, and then permitted to stand at 25 $\pm$ 0.1°C for 3–4 h. The solvents for the hemichromes were the same as those used in the complexations, while NaC1–NaOH buffer solution (ionic strength = 0.15; pHs 9 and 12) containing 2 wt % polyvinylpyrrolidone (PVP) was used for H to avoid the aggregation of the sample (see Ref. 19). The lack of aggregation was also confirmed in the same manner as described in the previous section.

RESULTS AND DISCUSSION

Coordination Structures of Hemichromes

It is necessary for the present purpose to define the coordination structures of the hemichromes prepared by complexing H with polymer ligands. If the formation of the hemichrome (H $(L)_{n_p}$) from H and ligand units (L) in polymer may be pictured as

$$H + n_p\, L \rightleftarrows H\,(L)_{n_p} \tag{1}$$

the axial coordination number (n_p) and stability constant (K_p) of the hemichromes can be defined as

$$K_p = \frac{[H\,(L)_{n_p}]}{[H]\,[L]^{n_p}} \tag{2}$$

where the brackets signify the equilibrium concentration (mol/L or unit mol/L) of the species. According to Miller and Dorough's method,[20] the ratio (R) of [H (L)$_{np}$] to [H] can be determined spectrophotometrically by

$$R = \frac{[H (L)_{n_p}]}{[H]} = \frac{D_m - D_h}{D_c - D_m}$$ (3)

where D_h denotes the optical density of H without a polymer ligand, D_c is that of completely complexed H (i.e., hemichrome), and D_m is that in mixing H and polymer ligand arbitrarily. Now considering that the use of the original ligand concentration ([L]$_0$ in unit mol/L) instead of [L] is permissible under the conditions of [L] $>>$ [H], we obtain the following relation useful in determining n_p and K_p:

$$\log R = n_p \log[L]_0 + \log K_p$$ (4)

The hemichrome samples for the spectral measurements were prepared at the original H concentration ([H]$_0$) = 2×10^{-5} mol/L (constant through all the preparations) and [L]$_0$ = 2×10^{-5} to 6 unit mol/L. Figure 1 depicts the spectroscopic titration curves expressed by plotting $\log R$ against $\log$ [L]$_0$. All of the plots are straight lines with the correlation coefficient of 0.998–0.991. The values of n_p and K_p can be thus determined by means of the slope and intercept of the straight line, respectively. The least-squares method was employed to estimate accurately these values. The results obtained are summarized in Table I. The n_p values for the hemichromes obtained by complexing H with BPEI and with copoly(VP, VMI) are found to
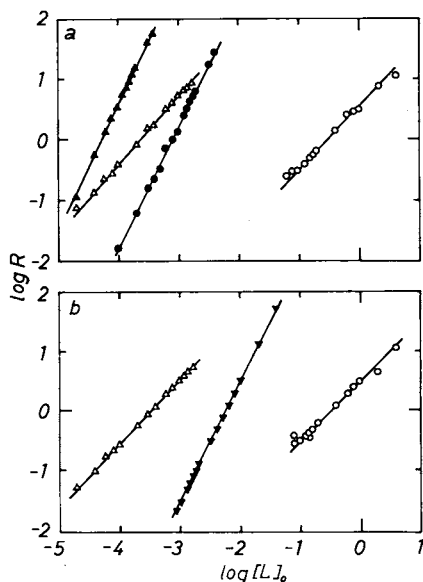

Fig. 1.   Plots of log R vs. log [L]$_0$ at pH's 9 (a) and 12 (b) as a function of the species of polymer ligands: ($\triangle$) copoly(VP, VMI); ($\bullet$) copoly(VP, VI); ($\bigcirc$) BPEI; ($\blacktriangle$) PLH; ($\blacktriangledown$) PLL. The samples were prepared by use of the solvents shown in Table I.

TABLE I
Axial Coordination Number (n_p) and Stability Constant (K_p) for Hemichromes Obtained by
Complexing H with Various Polymer Ligands[a]

Polymer ligand	pH	n_p	K_p[b]	Solvent[c]	λ_{max}[d] (nm)
Copoly(VP, VMI)	9.0	1.12	1.15×10^4	I	404
BPEI	9.0	1.04	3.55×10^0	II	410
PLH	9.0	2.07	7.24×10^8	III	415
Copoly(VP, VI)	9.9	2.01	1.74×10^6	I	413
Copoly(VP, VMI)	12.0	1.06	4.79×10^3	IV	401
BPEI	12.0	1.03	3.16×10^0	V	410
PLL	12.0	2.00	3.16×10^4	III	425

[a] Determined from Figure 1.

[b] Expressed in L/unit mol and L²/unit mol² for the hemichromes with $n_p \cong 1$ and 2, respectively.

[c] Abbreviations used: (I) a 1:1 mixture of ethylene glycol (EG) and $NaHCO_3$–Na_2CO_3 buffer; (II) $NaHCO_3$–Na_2CO_3 buffer; (III) NaCl–NaOH buffer; (IV) a 1:1 mixture of EG and Na_2HPO_4–NaOH buffer; (V) Na_2HPO_4–NaOH buffer.

[d] Denotes a maximum absorption in the Soret region which was used for obtaining the spectroscopic titration curve of H with polymer ligand (see Fig. 1).

be close to unity. This means that the FPs in both hemichromes adopt the five-coordinate structure; that is, the polymer ligand coordinates to only the fifth coordination site of the Fe(III). On the other hand, the n_p values for the hemichromes obtained from PLH, copoly(VP, VI), and PLL are close to 2, indicating that they consist of the six-coordinate FP which arises from occupying both fifth and sixth coordination positions of the Fe(III) by the polymer ligand. The coordination structures described above compare favorably with those previously reported by many authors,[6-13] even if they studied the hemichrome formations utilizing procedures which may or may not coincide with those in the present study.

The stabilities of the hemichromes can be evaluated from the values of K_p shown in Table I, though it is impossible to compare directly the results for the five-coordinate hemichromes ($n_p \cong 1$) with those for the six-coordinate ones ($n_p \cong 2$). This is because K_p for the six-coordinate type is defined as the product of K_1 and K_2, where K_1 and K_2 are the stability constants related to the fifth coordination position and to the sixth coordination position, respectively. Thus, the ability of the polymer ligand to stabilize FP was examined only for the hemichromes with the same n_p values: copoly(VP, VMI) >> BPEI for the five-coordinate types, and PLH > copoly(VP, VI) > PLL for the six-coordinate types [it should be noted that the comparison of K_p for PLL at pH 12 with those for PLH and copoly(VP, VI) at pH 9 could be permissible because the K_p values for copoly(VP, VMI) and BPEI were little altered by pH].

From the results obtained here, it became apparent that the hemichromes composed of the five- or six-coordinate type of FP can be obtained by complexing H with the synthetic polymer ligands used. A detailed discussion of hemichrome formation has been made by considering species of polymer-bound ligands, their distribution and density, and conformational property of polymer chain, etc.[6-14] However, this subject lies outside the purpose of

the present paper which is to clarify the coordination behavior of cyanide ion to FPs with different n_p values.

Coordination Behavior of Cyanide Ion to H and Hemichromes

If the coordination of n_{cn} mol cyanide ions to 1 mol FP of the hemichrome may be expressed by

$$H (L)_{n_p} + n_{cn} CN^- \rightleftarrows H (L)_{n_p} \bullet (CN)_{n_{cn}} \tag{5}$$
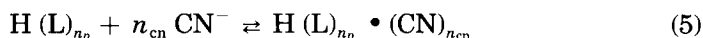
the equilibrium constant (K_{cn}) can be defined as

$$K_{cn} = \frac{[H (L)_{n_p} \bullet (CN)_{n_{cn}}]}{[H (L)_{n_p}] [CN^-]^{n_{cn}}} \tag{6}$$

where the brackets signify the equilibrium concentration (mol/L or unit mol/L) of the species. Taking into account that the degree of saturation (Y) of FP with cyanide ion can be given by

$$Y = \frac{[H (L)_{n_p} \bullet (CN)_{n_{cn}}]}{[H (L)_{n_p}] + [H (L)_{n_p} \bullet (CN)_{n_{cn}}]} \tag{7}$$

we obtain

$$\log \left(\frac{Y}{1 - Y} \right) = n_{cn} \log [CN^-] + \log K_{cn} \tag{8}$$

The value of Y can be determined spectroscopically by using the following equation[3]:

$$Y = \frac{D_s - D_0}{D_\infty - D_0} \tag{9}$$

where D_0 denotes the optical density of the hemichrome free of potassium cyanide, D_∞ is that of the sample containing excess of potassium cyanide, and D_s is that of the sample with potassium cyanide not in excess. These relationships can be applied to uncomplexed hemin if H (L)$_{n_p}$ in eqs.(5)–(8) is replaced by H.

To obtain the hemichrome sample used for the titration with potassium cyanide, the complexation was made at the following ratio of [L]$_0$/[H]$_0$: 18 for PLH; 282 for copoly(VP, VI); 354 (pH 9) and 629 (pH 12) for copoly(VP, VMI); 1.99×10^3 for PLL; 6.30×10^5 for BPEI. As can be seen from Figure 1, under these mixing ratios, all the hemichromes are formed in the yield above 98%. The sample of uncomplexed H was prepared by using 2 wt % PVP-containing aqueous solvent to prevent the aggregation of FP by stacking (PVP serves to interfere the aggregation but not as a polymer ligand[19]).

The spectroscopic titration curves at pHs 9 and 12 are shown in Figure 2. The results were obtained by use of the initial concentration ([KCN]$_0$ in
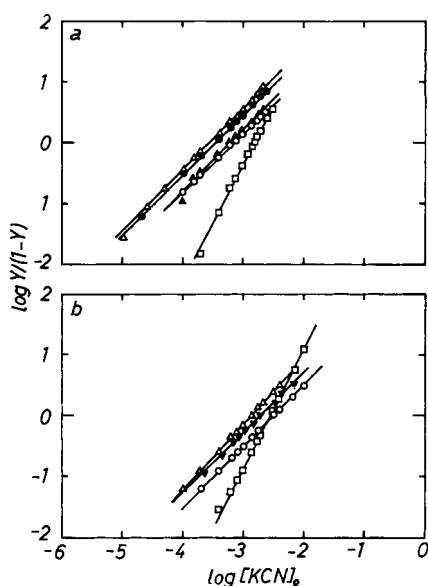
Fig. 2.   Plots of log $Y/(1 - Y)$ vs. log $[KCN]_0$ at pH's 9 (a) and 12 (b) obtained by the spectroscopic titrations with potassium cyanide of H ($\square$) and also of hemichromes composed of copoly(VP, VMI) ($\triangle$), copoly(VP, VI) ($\bullet$), BPEI ($\bigcirc$), PLH ($\blacktriangle$), and PLL ($\blacktriangledown$). The methods for the titration and sample preparation are described in the text.

mol/L) of potassium cyanide instead of [CN⁻] because the titration was made with a excess or large excess of potassium cyanide, and also because both cyanide ion and hydrocyanic acid react with H or hemichromes to yield the cyanide-coordinated FP[3]. It is observed that all the titration curves are straight lines (correlation coefficient 0.998–0.993). The values of n_{cn} and K_{cn} were thus estimated in the same way as used for determining the n_p and K_p values. The results obtained are listed in Table II. The n_{cn} values for all the hemichromes are found to be close to unity regardless of their n_p values (see Table I). This reveals that one cyanide ion binds not only to

TABLE II
Coordination Properties of Cyanide Ion to H and Hemichromes[a]

Sample	pH	n_{cn}	$K_{cn} \times 10^{-3}$ [b]
Hemichromes composed of			
Copoly(VP, VMI)	9.0	1.02	3.55
BPEI	9.0	0.98	1.25
PLH	9.0	1.01	1.58
Copoly(VP, VI)	9.0	0.98	2.82
Copoly(VP, VMI)	12.0	1.06	1.19
BPEI	12.0	1.02	0.35
PLL	12.0	0.98	0.50
Uncomplexed H	9.0	1.91	223.8
	12.0	1.98	112.1

[a] The moles (n_{cn}) of cyanide ion bound to 1 mol FP in the H or hemichrome and the equilibrium constant (K_{cn}) were determined from Figure 2.
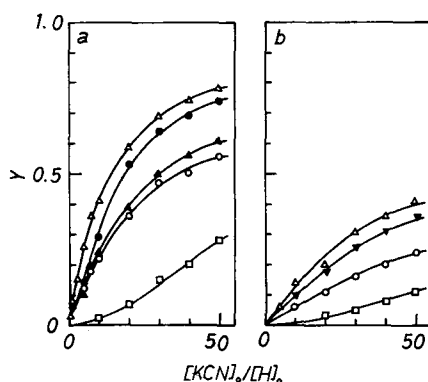[b] Expressed in L/mol for the hemichromes and in L²/mol² for H.

Fig. 3.   Curves of Y vs. $[KCN]_0/[H]_0$ at pH's 9 (a) and 12 (b) for H and hemichromes. Symbols for each of the plots correspond those in Figure 2.

the five-coordinate hemichromes ($n_p \cong 1$), but also to the six-coordinate ones ($n_p \cong 2$) owing to the ligand exchange reaction with the cyanide ion. In contrast to the hemichromes, the n_{cn} values for H are about 2, indicating that two cyanide ions coordinate to FP when both fifth and sixth coordination positions of the Fe(III) are free from polymer ligand.

A difference in the affinities of the hemichrome-holding FPs for cyanide ion can be investigated by comparing the K_{cn} values of the hemichromes given in Table II. By comparing the data of hemichrome and H, however, it is impossible to state whether or not polymer ligand bound to one coordination site of FP produces a marked effect on the coordination of cyanide ion to the other site. This is because the K_{cn} values for the samples with $n_{cn} \cong 1$ and 2 are different from each other with respect to the dimension (see Table II). Thus, the difference between H and hemichrome was qualitatively studied by examining the dependence of Y on $[KCN]_0/[H]_0$ shown in Figure 3. From the results in Table II and Figure 3, the following three characteristics can be noted: (a) an increase in the affinity of FP for cyanide ion caused by occupying the fifth coordination site of the Fe(III) by polymer ligands, which is clearly indicated by the fact that the initial slope of the Y vs. $[KCN]_0/$ $[H]_0$ curve for all the hemichromes is much larger than that for H; (b) variation in the affinity depending on the species of coordinated polymer ligands but not on whether they form the five- or six-coordinate type, which is drawn on the basis of a distinguishable difference in the values of K_{cn} (and also Y at a constant $[KCN]_0/[H]_0$) between the hemichrome composed of copoly(VP, VI) or copoly(VP, VMI) and the others; (c) a decrease in the affinity with increasing the hydroxyl ion concentration, because the values of K_{cn} (and also Y) at pH 12 for both H and hemichrome are smaller than those at pH 9.

In conclusion, the present study reveals that polymer ligand bound to the fifth coordination site of the iron(III) in FP could play an important role in the coordination of cyanide ion to the other coordination site. This may provide useful information regarding the preparation of cyanide ion exchanger.

References

1. E. Kokufuta, H. Shimizu, and I. Nakamura, *Polym. Bull.*, **2**, 157 (1980).
2. E. Kokufuta, H. Shimizu, and I. Nakamura, *Macromolecules*, **14**, 1178 (1981).
3. E. Kokufuta, N. Watanabe, and I. Nakamura, *J. Appl. Polym. Sci.*, **26**, 2601 (1981).
4. E. Kokufuta, H. Watanabe, and I. Nakamura, *Polym. Bull.*, **4**, 603 (1981).
5. E. Kokufuta, H. Watanabe, and I. Nakamura, *Polymer*, **23**, 1815 (1982).
6. E. Tsuchida, E. Hasegawa, and K. Honda, *Biochim. Biophys. Acta*, **427**, 520 (1976).
7. M. Tohjo and K. Shibata, *Arch. Biochem. Biophys.*, **103**, 401 (1963).
8. G. Blauer, *Nature*, **189**, 396 (1961).
9. G. Blauer, *Biochim. Biophys. Acta*, **79**, 574 (1964).
10. G. Blauer, *Arch. Biochem. Biophys.*, **121**, 587 (1967).
11. T. E. King, F. C. Yong, and S. Takemori, *Biochem. Biophys. Res. Commun.*, **22**, 658 (1966).
12. S. Yamamoto, T. Nozawa, and M. Hatano, *Polymer*, **15**, 330 (1974).
13. E. Tsuchida, in *Kagaku Sosetsu, No. 20*, S. Nakahara et al., Eds., Gakukai Shuppan, 1978, pp. 41–53.
14. H. Nishide, H. Ohno, and E. Tsuchida, *Kobunshi Ronbunshu (Jpn. Ed.)*, **37**, 614 (1980).
15. E. Kokufuta, *Macromolecules*, **12**, 350 (1979).
16. K. S. Norland, G. D. Fasman, E. Katchalski, and E. R. Blout, *Biopolymers*, **1**, 277 (1963).
17. A. Yaron and A. Berger, *Biochim. Biophys. Acta*, **69**, 397 (1963).
18. E. Kokufuta, T. Terada, M. Tamura, S. Suzuki, and K. Harada, *Arch. Biochem. Biophys.*, **196**, 23 (1979).
19. H. Nishide, K. Mihayashi, and E. Tsuchida, *Biochim. Biophys. Acta*, **498**, 208 (1977).
20. J. R. Miller and G. D. Dorough, *J. Am. Chem. Soc.*, **74**, 3977 (1952).