On the Function of the Polyion Complex of Methemoglobin and Polyelectrolytes as Cyanide Ion Exchanger

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Synopsis

The binding of cyanide ion to Fe(III) in the hemes of bovine methemoglobin and insoluble polyion complex (KPVS-Hb-PDDA complex) prepared by the complexation of the methemoglobin with potassium poly(vinyl alcohol) sulfate and poly(diallyldimethylammonium chloride) was investigated as functions of pH and potassium cyanide concentration by spectrophotometric method and adsorption experiment. The degree of saturation of the cyanide ligand on the heme showed a maximal value at pH range 8–9, whereas this was reduced to zero in the strongly acidic and basic regions, below pH 3.5 and above pH 13.5. These results were in agreement, in a qualitative way, with the theoretical results represented by four equilibrium reactions between methemoglobin and potassium cyanide at different pH and cyanide concentrations. The separation of cyanide ion in $Na_2B_4O_7$–KH_2PO_4 buffer solution at pH 9.0 was also investigated by using a KPVS-Hb-PDDA complex column. The cyanide ion in the solution was mostly or entirely bound to the complex until the heme was saturated with cyanide ion, and most of the bound cyanide ion was eluted with 0.1*N* NaOH solution. These results indicate that KPVS-Hb-PDDA has a function as cyanide ion exchanger and that the exchange reaction is analogous to that for methemoglobin.

INTRODUCTION

At present there are numerous reports on the method of preparation and the reactions of ion exchangers. Applications of ion exchangers are found in analytical chemistry, hydrometallurgy, antibiotic purification, separation of radioisotopes, water treatment and pollution control, and so forth.[1,2] However, little attention has been paid to selective exchangers of a specific anion. Until recently, no information about cyanide ion exchangers has been available, although selective separation of cyanide ion is of interest in connection with protection against environmental pollution with cyanides.

It is well known that the affinity of cyanide ion for methemoglobin (ferrihemoglobin) in which iron carries an extra positive charge is very high since the cyanide ligand neutralizes the extra iron charge.[3,4] On the other hand, we have recently demonstrated that methemoglobin forms a stoichiometric polyion complex with potassium poly(vinyl alcohol) sulfate (KPVS) at pH 2–2.5 since the basic (amino, imidazolyl, and guanidyl) groups in the hemoglobin are salt-linked with $KOSO_3$ groups in KPVS.[5,6] Moreover, the acidic (carboxyl, phenolic, and cysteinyl) groups in the complex are also salt-linked with quarternary ammonium ions in poly(diallyldimethylammonium chloride) (PDDA) at pH 7–13, and the polyion complex composed of KPVS, methemoglobin, and PDDA

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(KPVS-Hb-PDDA) is precipitated from aqueous solution.[6] These facts give an indication as to the use of KPVS-Hb-PDDA as an exchanger of cyanide ion. The present investigation was undertaken to obtain fundamental information about the functional property of the complex as cyanide ion exchanger and about the exchange reaction.

In this report, the theoretical estimation for the equilibrium reaction of methemoglobin with potassium cyanide at a wide pH range is first described to elucidate the exchange reaction. Next, these results are compared with the experimental results for bovine methemoglobin and KPVS-Hb-PDDA complex to investigate the functional properties of methemoglobin and the complex as cyanide ion exchanger. Finally, the separation of cyanide ion from potassium cyanide solution was carried out using a column packed with KPVS-Hb-PDDA complex.

THEORETICAL ESTIMATION OF THE EXCHANGE REACTION OF METHEMOGLOBIN WITH CYANIDE ION

Methemoglobin consists of two different types: one is acidic methemoglobin (Hb^+OH_2), where the water molecule is attached to the sixth coordination position of the Fe(III) atom in the protoporphyrin IX of methemoglobin, and the other is alkaline methemoglobin (HbOH), where the water molecule of Hb^+OH_2 is substituted by hydroxyl ion. The equilibrium reaction between the acidic and alkaline forms is represented by[7]

$$Hb^+OH_2 + H_2O \rightleftharpoons HbOH + H_3O^+ \qquad \text{(R-1)}$$

The ionization equilibrium of hydrocyanic acid is also represented by

$$HCN + H_2O \rightleftharpoons H_3O^+ + CN^- \qquad \text{(R-2)}$$

Thus, the formation of cyanide methemoglobin (HbCN) from methemoglobin and potassium cyanide in a wide pH region may reasonably be pictured as

$$Hb^+OH_2 + HCN \rightleftharpoons HbCN + H_3O^+ \qquad \text{(R-3)}$$

$$Hb^+OH_2 + CN^- \rightleftharpoons HbCN + H_2O \qquad \text{(R-4)}$$

$$HbOH + HCN \rightleftharpoons HbCN + H_2O \qquad \text{(R-5)}$$

$$HbOH + CN^- \rightleftharpoons HbCN + OH^- \qquad \text{(R-6)}$$

The equilibrium constants for the six reactions mentioned above may be defined as

$$K_1 = \frac{a_{HbOH}a_{H_3O^+}}{a_{Hb^+OH_2}a_{H_2O}} \qquad (1)$$

$$K_2 = \frac{a_{H_3O^+}a_{CN^-}}{a_{HCN}a_{H_2O}} \qquad (2)$$

$$K_3 = \frac{a_{HbCN}a_{H_3O^+}}{a_{Hb^+OH_2}a_{HCN}} \qquad (3)$$

$$K_4 = \frac{a_{HbCN}a_{H_2O}}{a_{Hb^+OH_2}a_{CN^-}} = \frac{K_3}{K_2} \qquad (4)$$

$$K_5 = \frac{a_{HbCN}a_{H_2O}}{a_{HbOH}a_{HCN}} = \frac{K_3}{K_1} \qquad (5)$$

$$K_6 = \frac{a_{HbCN}a_{OH^-}}{a_{HbOH}a_{CN^-}} = \frac{K_4K_w}{K_1} \tag{6}$$

where a_x signifies the activity of the species represented by the subscript X and K_w is the ionic product of water.

If we now consider a system containing Hb^+OH_2, HbOH, HbCN, HCN, and CN^-, the total concentration (C_1) of methemoglobin and that of cyanide (C_2) in the system will be given by

$$C_1 = [Hb^+OH_2] + [HbOH] + [HbCN] \tag{7}$$

$$C_2 = [HCN] + [CN^-] + [HbCN] \tag{8}$$

where the brackets signify the concentration of the species. Assuming that the activity coefficients of the respective species are unity, $[Hb^+OH_2]$ and [HbOH] in eq. (7) may be given by

$$[Hb^+OH_2] = \frac{[HbOH][H_3O^+]}{K_1} \tag{9}$$

$$= \frac{[HbCN][H_3O^+]}{K_3[HCN]} \tag{9'}$$

$$= \frac{[HbCN]}{K_4[CN^-]} \tag{9''}$$

and

$$[HbOH] = \frac{K_1[Hb^+OH_2]}{[H_3O^+]} \tag{10}$$

$$= \frac{[HbCN]}{K_5[HCN]} \tag{10'}$$

$$= \frac{[HbCN][OH^-]}{K_6[CN^-]} \tag{10''}$$

since a_{H_2O} is taken as unity at the condition of where the concentration of dissolved substances in the water is not too large. Similarly, $[CN^-]$ in eq. (8) is given by

$$[CN^-] = \frac{K_2[HCN]}{[H_3O^+]} \tag{11}$$

$$= \frac{[HbCN]}{K_4[Hb^+OH_2]} \tag{11'}$$

$$= \frac{[HbCN][OH^-]}{K_6[HbOH]} \tag{11''}$$

When eqs. (9') and (10) are combined with eq. (7), we obtain

$$[HCN] = \frac{[HbCN][H_3O^+]}{K_3(C_1 - [HbCN])}\left(1 + \frac{K_1}{[H_3O^+]}\right) \tag{12}$$

Equation (13) is obtained by substituting eq. (11) into eq. (8):

$$[HCN] = \frac{[H_3O^+](C_2 - [HbCN])}{K_2 + [H_3O^+]} \tag{13}$$

Combining eq. (12) with eq. (13) leads to

$$\frac{(C_1 - [\mathrm{HbCN}])(C_2 - [\mathrm{HbCN}])}{[\mathrm{HbCN}]} = \frac{[\mathrm{H_3O^+}]}{K_3}\left(1 + \frac{K_2}{[\mathrm{H_3O^+}]}\right)\left(1 + \frac{K_1}{[\mathrm{H_3O^+}]}\right) \quad (14)$$

Taking into account that the degree of saturation (Y) of the sixth coordination position of the Fe(III) atom in the protoporphyrin IX of methemoglobin with cyanide ligand is given by $Y = [\mathrm{HbCN}]/C_1$, the left-hand side of eq. (14) is rewritten as

$$\frac{(C_1 - [\mathrm{HbCN}])(C_2 - [\mathrm{HbCN}])}{[\mathrm{HbCN}]} = \frac{1}{Y}(1 - Y)(C_2 - C_1Y)$$

and eq. (14) becomes

$$\frac{1}{Y}(1 - Y)(C_2 - C_1Y) = \frac{[\mathrm{H_3O^+}]}{K_3}\left(1 + \frac{K_2}{[\mathrm{H_3O^+}]}\right)\left(1 + \frac{K_1}{[\mathrm{H_3O^+}]}\right) \quad (15)$$

This equation can also be derived by using the other equilibrium constants (K_4, K_5, and K_6) because these constants are correlated to each other by eqs. (4), (5), and (6). Therefore, the concentration of HbCN is expressed as a function of C_1, C_2, and pH if the triplet of K values is known.

The different pK ($= -\log K$) values available in the literature[7–12] are listed in Table I. In the present study, bovine methemoglobin was used as the sample, and measurement of Y was always carried out in different buffer solutions of ionic strength (μ) 0.15. Thus, pK values of bovine methemoglobin at $\mu = 0.15$ were selected from Table I: 8.11 for pK_1, 9.18 for pK_2, and 1.26 for pK_3. When these values are substituted into eq. (15), we obtain

$$\frac{1}{Y}(1 - Y)(C_2 - C_1Y) = \left(1 + \frac{10^{-9.18}}{[\mathrm{H_3O^+}]}\right)(10^{1.26}[\mathrm{H_3O^+}] + 10^{-6.85}) \quad (16)$$

TABLE I
pK Values for the Equilibrium Reactions Represented by (R-1) to (R-6)

pK Value	Species	μ	Temp., °C	Reference
pK_1				
8.12	dog	0.10	—	8
$7.88 + 0.59\sqrt{\mu}$ [a]	bovine	—	24 ± 2	9
8.10	human	0.05	24.0	7
8.21	human	1.0	25.4	7
pK_2				
$9.31 - 0.34\sqrt{\mu}$ [b]	—	—	25.0	11
pK_3				
1.26	bovine	independent	24 ± 2	9
1.01	—	—	—	12
pK_4				
−8.31	human	0.05	27.0	10
−7.92[c]	bovine	0.15	24 ± 2	—
pK_5				
−5.5	—	—	—	12
−6.85[c]	bovine	0.15	24 ± 2	—
pK_6				
−2.03[c]	bovine	0.15	24 ± 2	—

[a] pK_1 in Ref. 8 is reported by the equilibrium constant $(6.12 - 0.59\sqrt{\mu})$ for the dissociation of OH^- ion from alkaline methemoglobin.

[b] The correction of ionic strength (μ) was made by using the Debye-Hückel relation.

[c] Calculated by eqs. (4), (5), and (6) from the pK values used here (pK_1 = 8.11, pK_2 = 9.18, and pK_3 = 1.26 for bovine methemoglobin at $\mu = 0.15$).

As will be shown in Figures 2 through 5, the curves calculated from eq. (16) indicate that the reaction of methemoglobin with cyanide ligand approximately follows the stoichiometric relationship at pH 8–9, whereas the reaction is inhibited by increasing acidity or basicity because of the coordination of the water molecule or hydroxyl ion to methemoglobin. Furthermore, no reaction of methemoglobin with cyanide ligand takes place at pH below 3.5 and above 13.5. In theory, therefore, the exchange reactions can be explained by the binding of cyanide ligand to Fe(III) in the heme moiety of methemoglobin and by the replacement of the cyanide ion bound to the heme by water or hydroxyl.

EXPERIMENTAL

Materials

Bovine carboxymethemoglobin was obtained from Wako Pure Chemical Industries Ltd. The sample was purified in the same manner as described in the literature.[13] The purity was confirmed by visible spectrum, iron content, and amino acid composition. Hemin was available commercially from Tokyo Kasei Kogyo Co., Ltd., and its purity was confirmed by visible spectrum and iron content. The polyelectrolytes (KPVS and PDDA) were the same samples as used in the previous studies[5,6,14–16]; the electrochemical and conformational properties[14,16] have already been described.

The physical data[6] (MW 6.45×10^4; total acidic groups, 1.24×10^{-3} mol/g; total basic groups, 1.52×10^{-3} mol/g; iron content, 6.20×10^{-5} mol/g) of bovine methemoglobin estimated by the amino acid sequence[17] and the equivalent weight of KPVS[14] (166) and PDDA[16] (158) obtained previously were taken into consideration for the preparation of the KPVS-Hb-PDDA complex described below.

Preparation of KPVS-Hb-PDDA Complex

Methemoglobin (10 g) was dissolved in aqueous solution (5 L) adjusted to pH 2.2 with 1*N* HCl. The polymer solution (2 L) containing the calculated amount (2.52 g) of KPVS was slowly added to the hemoglobin solution to form and precipitate the polyion complex composed of KPVS and Hb. The complex was separated by decantation and centrifugation, washed with distilled water, and then dried at 50°C for ten days under reduced pressure (yield 91–96%). The KPVS-Hb complex (10 g) was redissolved in aqueous solution (5 L) adjusted to pH 12 with 1*N* NaOH, and then the polymer solution (2 L) containing the calculated amount (1.64 g) of PDDA was slowly added to the complex solution. The KPVS-Hb-PDDA complex precipitated was separated and dried in the same manner as described above (yield 92–94%). The iron content of KPVS-Hb-PDDA is $4.31 \pm 0.12 \times 10^{-5}$ mol/g, as analyzed spectrophotometrically by the color development between *O*-phenanthroline and iron. This is in agreement with that (4.61×10^{-5} mol/g) calculated on the basis of the assumption that the complexation of methemoglobin with both polyelectrolytes is stoichiometric. The infrared spectrum of KPVS-Hb-PDDA in KBr disks shows absorption bands at 1575 and 1380 cm^{-1} (COO^-), 1640 cm^{-1} (amide I), 1520 cm^{-1} (amide II), and 1230 cm^{-1} (S=O). From the scanning electron micrograph shown in Figure 1,

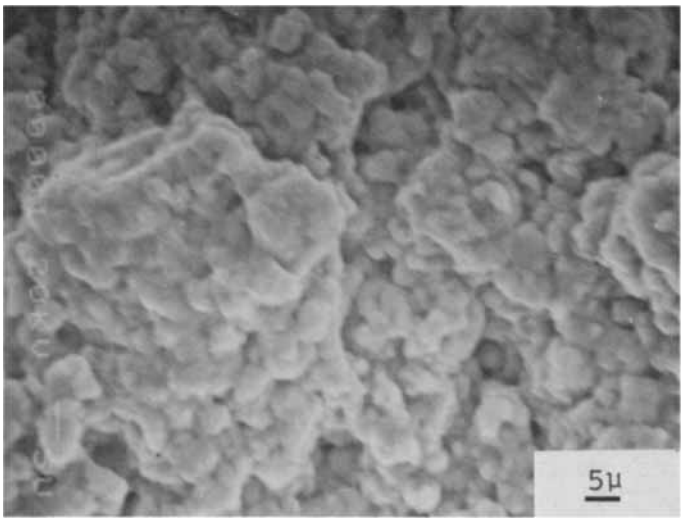

Fig. 1. Electron micrograph of KPVS-Hb-PDDA complex measured with a Hitachi scanning electron microscope (model S-450).

it is observed that the KPVS-Hb-PDDA precipitate is composed of a large number of granular aggregates.

Determination of Degree of Saturation (Y) for Methemoglobin, Hemin, and KPVS-Hb-PDDA Complex

The Y values of bovine methemoglobin and hemin at different pH were determined by the measurement of optical density at 540 nm using a Hitachi spectrophotometer (model 200-20). The Y value was calculated by the following equation[10]:

$$Y = \frac{D - D_0}{D_\infty - D_0} \tag{17}$$

where D_0 represents the optical density of the sample free of potassium cyanide, D_∞ is the optical density of the sample containing excess of potassium cyanide, and D is the optical density of the sample with potassium cyanide not in excess. The three samples contain equimolecular amounts of the heme, and the pH of the sample for the measurement of D_0 was adjusted to that for the D value. The sample for D_∞ measurement was adjusted to pH 9.0. All samples (50 ml each) were prepared by using various buffer solutions at ionic strength 0.15 and were allowed to stand at 25 ± 0.1°C for 4 h. The buffer solution systems are: CH_3COOH–CH_3COONa, pH 3.2–5.5; $Na_2B_4O_7$–KH_2PO_4, pH 5.5–9.2; $Na_2B_4O_7$–Na_2CO_3, pH 9.2–11.0; NH_4Cl–NH_4OH, pH 8.0–11.0; Na_2HPO_4–NaOH, pH 11.0–12.0; NaOH–NaCl, pH 12.0–13.7. In order to avoid the volatilization of hydrocyanic acid, the sample containing potassium cyanide was always sealed in a vial when allowed to stand.

The Y value for KPVS-Hb-PDDA was determined by adsorption of cyanide ion on the complex. The complex was dispersed in various buffer solutions (50 ml) containing potassium cyanide and was sealed in a vial to avoid the volatil-

ization of hydrocyanic acid. The dispersion was stirred at 25 ± 0.1°C for 6 h and was then allowed to stand for 2 h to precipitate the complex. The adsorbed amount of cyanide was determined by the spectrophotometric measurement of the residual cyanide in the supernatant according to the pyridine–pyrazolone method.[18]

Separation of Cyanide Ion by KPVS-Hb-PDDA Column

KPVS-Hb-PDDA complex (12 g) was swelled in $Na_2B_4O_7$–KH_2PO_4 buffer solution at pH 9.0 and then packed in a glass column (1 m × 10 mm d.). The buffer solution containing 20 ppm cyanide ion was effused through the column at constant flow rate of 0.5 ml/min. In order to avoid the volatilization of hydrocyanic acid, the column experiment was carried out in a closed system, and the effluent fraction (5 ml) was received in a measuring flask (10 ml) into which 5 ml 0.1*N* NaOH was admitted before receiving the effluent. The complex saturated completely with cyanide ion was washed with buffer solution at pH 9.0 until the cyanide concentration of the washing was no more than 1 ppm, and then the cyanide ion bound to the complex was eluted with 0.1*N* NaOH.

RESULTS AND DISCUSSION

Equilibrium Reaction of Methemoglobin with Potassium Cyanide at Different pH

The reaction of cyanide ligand with methemoglobin was investigated to confirm the theoretical results mentioned previously and to compare with the results of KPVS-Hb-PDDA complex described below. The plots of Y vs. C_2/C_1 at different pH and those of Y vs. pH at $C_2/C_1 = 1$ are shown in Figures 2 and 3, together with the theoretical results. The experimental results show that the Y value approaches unity in the pH 8–9 region if the concentration (C_2) of cyanide is equal to or more than that (C_1) of the heme of methemoglobin, while this value is reduced to zero in the pH regions below 3.5 and above 13.5. These are in agreement, at least in part, with the curves calculated by eq. (16). Thus, it can be seen that the cyanide ligand is stoichiometrically bound to Fe(III) in the heme of methemoglobin in the pH 8–9 region, whereas the coordination of the water molecule or hydroxyl ion to the heme becomes the dominant factor in the strongly acidic or basic regions, respectively.

When the experimental points are compared in detail with the calculation curves, a considerable difference is found between both results in the pH range 4–6. It is known that in the acidic region two protons associate with (or dissociate from) the pyrrole nitrogens of protoporphyrin IX of acidic methemoglobin, and thus two different types are present in acidic methemoglobin.[19] These equilibrium reactions are characterized by pK values[19,20] of 5.35 and 6.65. In the theoretical estimation, however, the reactions of cyanide ligand with the two types of acidic methemoglobin were not taken into account, so that the difference between the theoretical and the experimental results could arise from this neglect.

The result for hemin obtained at the condition of $C_2/C_1 = 100$ is also included in Figure 3 to compare with that for methemoglobin. A remarkable difference

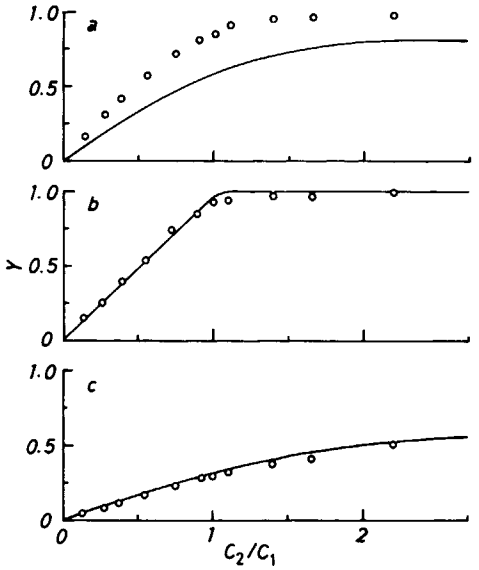

Fig. 2. Curves of Y vs. C_2/C_1 for bovine methemoglobin at pH 6.0 (a), 9.0 (b), and 12.0 (c): plots, experimental points; full lines, calculation curves by eq. (16); C_1 (methemoglobin concentration expressed by molarity of the heme), 6.52×10^{-5} mol/L; C_2 (concentration of potassium cyanide), $0–1.40 \times 10^{-4}$ mol/L.

between the two results is observed: the maximal value of Y for hemin is about 0.7, even at $C_2/C_1 = 100$. Thus, the presence of protein (globin) subunits in methemoglobin plays an important role in the reaction of cyanide ligand with heme.

From the results, it is clear that methemoglobin has the function of cyanide ion exchanger and the exchange reaction is interpreted, at least in a qualitative way, by the four reactions represented by (R-3) through (R-6) in the previous section.

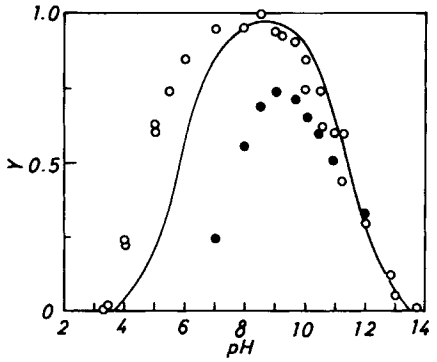

Fig. 3. Curves of Y vs. pH for bovine methemoglobin (○) at $C_2/C_1 = 1$ ($C_1 = C_2 = 6.52 \times 10^{-5}$ mol/L) and hemin (●) at $C_2/C_1 = 100$ ($C_1 = 8.40 \times 10^{-5}$ mol/L and $C_2 = 8.40 \times 10^{-3}$ mol/L): plots, experimental points; full line, curve for methemoglobin calculated by eq. (16).

Adsorption of Cyanide Ion on KPVS-Hb-PDDA Complex

For the purpose of the use of methemoglobin as cyanide ion exchanger, it is necessary to prepare an insoluble material containing methemoglobin. Previously, we have found that methemoglobin forms an insoluble polyion complex with KPVS and PDDA. Thus, the KPVS-Hb-PDDA complex was prepared and subjected to adsorption experiment of cyanide ion in order to obtain information about the equilibrium reaction of potassium cyanide with methemoglobin in the complex.

Figures 4 and 5 show the plots of Y vs. C_2/C_1 at different pH and those of Y vs. pH at $C_2/C_1 = 1$ for KPVS-Hb-PDDA complex, together with the calculated curves of methemoglobin. The adsorption experiment for the complex was not carried out in the acidic region because volatilization of hydrocyanic acid is not negligible during stirring over a long time. From the results in Figures 4 and

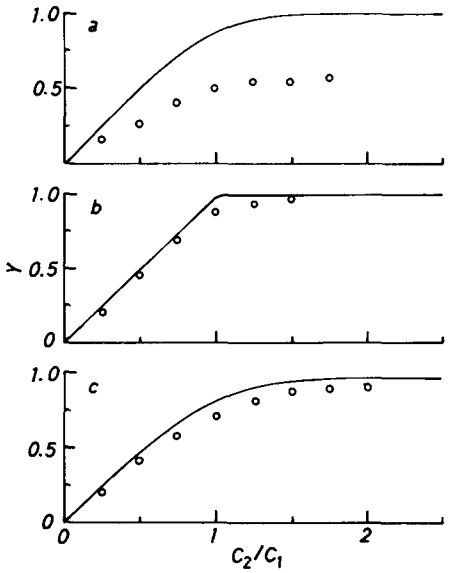

Fig. 4. Curves of Y vs. C_2/C_1 for KPVS-Hb-PDDA complex at pH 7.0 (a), 9.0 (b), and 11.0 (c): plots, experimental points; full lines, calculation curves by eq. (16); C_1 (determined by the iron content of the complex), 2.14×10^{-4} mol/L; C_2, 0–4.28×10^{-4} mol/L.

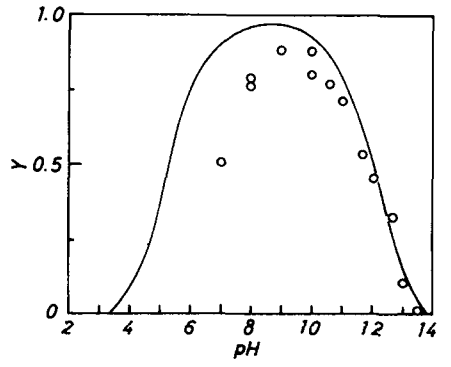

Fig. 5. Curves of Y vs. pH for KPVS-Hb-PDDA complex at $C_2/C_1 = 1$ ($C_1 = C_2 = 2.14 \times 10^{-4}$ mol/L): plots, experimental points; full lines, calculation curve by eq. (16).

5, it is apparent that in the pH region around 9, Y approaches unity as the cyanide concentration (C_2) exceeds that (C_1) of the heme, while this value is reduced to zero in the strongly basic region above pH 13.5. These approximately agree with the theoretical and experimental results of methemoglobin, indicating that little of the function of methemoglobin as cyanide ion exchanger is lost in the process of the complexation with KPVS and PDDA.

In the neutral region, however, some difference between methemoglobin and KPVS-Hb-PDDA complex is observed from the results shown in Figures 3 and 5. In the present stage of the studies on the physicochemical properties of hemoglobin, it is generally known that the change in the globin conformation with any interaction affects the binding of ligands to hemoglobin since some of the amino acid residues in close proximity to the heme are affected by the conformational change.[4] Thus, the difference between methemoglobin and the complex could be explained on the basis of the assumption that the binding of cyanide ligand to methemoglobin is more or less affected by the conformational change caused by the complexation with polyelectrolytes. However, the binding of cyanide ligand to hemin which has no globin subunits is obviously distinguishable from that for methemoglobin and the complex (see Fig. 3). It could be that the condition of globin in the vicinity of the heme for KPVS-Hb-PDDA complex is approximately analogous to that for methemoglobin, although a conformational change of the globin chain is, in fact, produced by the complexation.

In order to obtain further information about the condition of the heme in KPVS-Hb-PDDA complex, Hill plots were investigated for methemoglobin, the complex, and hemin. If the binding of cyanide ion to the hemes in the three samples may be described by

$$\text{heme} + n\text{CN}^- \rightleftharpoons \text{heme(CN)}_n \tag{R-7}$$

the equilibrium constant K' can be defined as

$$K' = \frac{[\text{heme(CN)}_n]}{[\text{heme}][\text{CN}^-]^n} \tag{17}$$

where n represents the moles cyanide ion bound to the heme and the brackets signify the concentration of the species. Taking into account that the degree of saturation (Y) of cyanide ion can be given by $Y = [\text{heme(CN)}_n]/([\text{heme}] + [\text{heme(CN)}_n])$, we obtain

$$\log \frac{Y}{(1 - Y)} = n \log [\text{CN}^-] + \log K' \tag{18}$$

Figure 6 shows the Hill plots at pH 12 where hydrocyanic acid dissociates completely, and the n values obtained are listed in Table II. It is generally believed that the n value for the coordination reaction of cyanide ion with methemoglobin is unity because the cyanide ion is only bound to the sixth coordination position of Fe(III) in the heme, while this value for oxygen equilibrium of hemoglobin is greater than unity because of the cooperative action due to the interaction between the hemes. In the case of methemoglobin, the n value obtained here is also close to unity. In contrast to methemoglobin, the n value of hemin is close to 2, indicating that two cyanide ions are bound to the fifth and sixth coordination positions of Fe(III) in protoporphyrin IX. On the other hand, the result of the KPVS-Hb-PDDA complex is in fair agreement with that of methemoglobin.

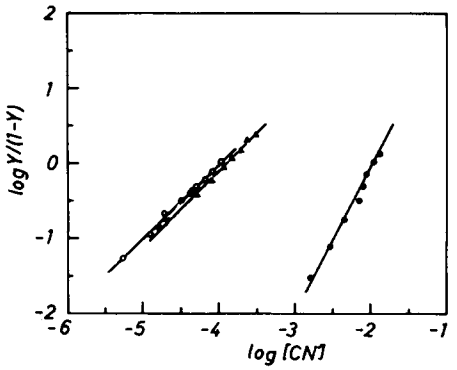

Fig. 6. Hill plots for bovine methemoglobin (○), KPVS-Hb-PDDA complex (△), and hemin (●) at pH 11.97–12.06. Concentration of heme: 6.52×10^{-5} mol/L for methemoglobin; 2.14×10^{-4} for KPVS-Hb-PDDA complex; 8.40×10^{-5} mol/L for hemin. Concentration of potassium cyanide: 0–1.40×10^{-4} mol/L for methemoglobin; 0–4.28×10^{-4} mol/L for KPVS-Hb-PDDA complex; 0–8.40×10^{-3} mol/L.

Therefore, this reveals that the coordinate bond between the heme and imidazolyl nitrogen of hystydyl residue in the globin subunit is not severed by the complexation, and thereby the cyanide ion is not bound to the fifth coordination position of the heme in the complex.

On the basis of the results obtained here, it can be concluded that the methemoglobin in the KPVS-Hb-PDDA complex not only retains the function as cyanide ion exchanger, but the exchange reaction is also analogous to that of methemoglobin.

Separation of Cyanide Ion with KPVS-Hb-PDDA Complex

In order to test the separation of cyanide ion with KPVS-Hb-PDDA complex, the $Na_2B_4O_7$–KH_2PO_4 buffer solution (pH 9.0) containing 20 ppm cyanide ion was effused through a column of the complex. As shown in Figure 7, the cyanide concentration in the fractions is kept below 0.1–0.2 ppm until the total fraction volume goes up to over 600 ml. The adsorbed amount of cyanide ion is 4.58×10^{-5} mol/g-complex, as estimated from the results in Figure 7. This is in accord with that (4.31×10^{-5} mol/g-complex) calculated by the iron content of the complex. Thus, the exchange capacity of KPVS-Hb-PDDA complex agrees with the iron content of the complex. The elution pattern with 0.1*N* NaOH for the complex saturated with cyanide ion is also represented in Figure 7. The cyanide concentration reaches a maximal value (96 ppm) at a fraction volume of 40 ml, and about 80% of the cyanide ion bound to the heme in the complex is recovered when the elution is continued until 500 ml.

TABLE II
n Values in Eq. (18) Obtained by Hill Plots

Sample	*n*	pH
Methemoglobin	1.01	12.02
KPVS-Hb-PDDA complex	1.12	11.97
Hemin	1.96	12.06

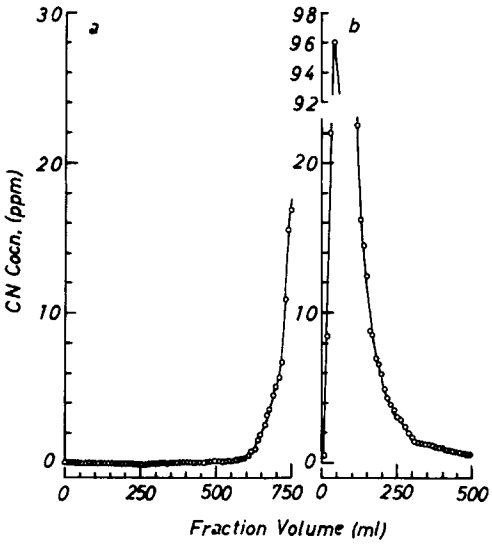

Fig. 7. Separation of cyanide ion with KPVS-Hb-PDDA complex: (a) obtained by passing $Na_2B_4O_7$–KH_2PO_4 buffer solution (pH 9.0) containing 20 ppm cyanide ion through a column of the complex (12 g); (b) obtained by passing 0.1*N* NaOH solution through a column of the complex saturated with cyanide ion.

As mentioned above, the function of KPVS-Hb-PDDA complex as cyanide ion exchanger can also be supported by the results of column separation.

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