Copolymers of haemin and vinyl monomers as cyanide ion exchangers: Modifications of the ion exchange properties by introduction of acrylamide residues

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Ternary copolymers of haemin (H) and styrene (St) with acrylamide (AAm), copoly(H, St, AAm), and with 1-vinyl-2-pyrrolidone (VP), copoly(H, St, VP), were synthesized by radical copolymerization. It was found that the H content of the resulting copolymer generally agrees with that of the corresponding monomer feed. Moreover, the yield and reduced viscosity decrease in proportion to the feed concentration of the H monomer. Each copolymer prepared showed hydrophilic character compared with the copolymer of H and St. The adsorption of cyanide ion onto the copolymer was investigated at different pHs to clarify the functional capability as cyanide ion exchanger. The amount of cyanide ion adsorbed on 1 g dry polymer (A_{cn} in mg/g) was found to be dependent on the H content of the copolymer, thus indicating that the haem-iron is the adsorption site of the cyanide ion. From the curves of A_{cn} against the AAm or VP content of the copolymer and against pH for copoly(H, St, AAm) and copoly(H, St, VP), it was observed that the adsorptive capacity of the cyanide ion is increased by the introduction of the AAm residue. The same result was obtained for column experiments. These results were discussed in connection with the reaction of a cyanide ligand with the haem-iron site in the copolymer.

Keywords Copolymers; haemin monomers; vinyl monomers; cyanideon exchangers; exchange properties; acrylamide residues

INTRODUCTION

The affinity of the cyanide ion for methaemoglobin (ferrihaemoglobin), in which the haem-iron carries an extra positive charge, is very high since the cyanide ligand neutralizes the extra charge[1]. Theoretical estimation[2] of the equilibrium reaction of methaemoglobin with potassium cyanide at different pHs shows that the binding of a cyanide ligand to the haem-iron follows an approximately stoichiometric relationship at pH 8–9 but is inhibited by increasing acidity or basicity because of the coordination of the water molecule or hydroxyl ion to the haem-iron site. These results prompted us to investigate the preparation of a cyanide ion exchanger by immobilization of methaemoglobin. We have studied the synthesis of water-insoluble ternary polyelectrolyte complex of methaemoglobin with acidic and basic polyions[3,4] and reported a functional property of the complex acting as cyanide ion exchanger[2].

Ferri-protoporphyrin IX in the haemoglobin has two vinyl groups in the 2- and 4-positions of the porphyrin ring, which can be allowed to react with vinyl compounds with the aid of radical initiator[5,6]. This also gives an indication as to the preparation of a cyanide ion exchanger by radical copolymerization of the porphyrin with vinyl monomers. Recently it has been demonstrated that haemin (H) (ferri-protoporphyrin IX chloride) reacts with styrene (St) to yield a water-insoluble copolymer able to function as a cyanide ion exchanger[7]. However, the copolymer of H and St, copoly(H, St), is extremely hydrophobic; thus, it is preferable to introduce hydrophilic monomer residues into the copolymer to improve its dispersing properties.

In this study, acrylamide (AAm) or 1-vinyl-2-pyrrolidone (VP) was copolymerized with a mixture of H and St monomers to modify the hydrophobic character of the copoly(H, St). It was found that the introduction of an AAm residue not only gives the hydrophilic character but also improves the cyanide ion exchange property. The present paper describes in detail the preparation data and the cyanide ion exchange properties for both copoly(H, St, AAm) and copoly(H, St, VP). These results were compared with that of copoly(H, St) already reported[7] and discussed in terms of the coordination reaction of cyanide ion with the haem-iron in the copolymer.

EXPERIMENTAL

Materials

H and St monomers were the same samples as used in a previous study[7]. AAm and VP were commercially obtained from Tokyo Kasei Kogyo Co., Ltd. AAm was purified by recrystallization from benzene. VP was distilled under reduced pressure (96°C/14 mmHg) to remove the polymerization inhibitor.

The copolymerization was carried out in a sealed tube at 85°C for 48 h under reduced pressure using purified pyridine as the solvent and α,α'-azobisisobutyronitrile

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(AIBN) as the initiator. A large scale copolymerization was also carried out under a nitrogen atmosphere with stirring at 85°C for 48–50 h. The reaction mixture was poured slowly into methanol to precipitate the copolymer, which was then separated by filtration, washed with methanol, and dried under vacuum. The crude copolymer was reprecipitated three times from chloroform with methanol, dried under vacuum, washed with a large amount of 0.5 N NaOH, rewashed with distilled water, and then dried at 55°C for 2 days under vacuum. In addition, the previously prepared copoly(H, St)[7] (sample code, HS1–HS6) was also used in the present study.

Characterization

The obtained copolymers were identified by spectroscopic analyses. The measurement of infra-red spectra was made in KBr disc using a Hitachi i.r. spectrophotometer (model 260-50). Visible and ultra-violet spectra were measured in purified chloroform[5] using a Hitachi spectrophotometer (model 220-20).

The copolymer composition was determined by a combination of elemental and iron analyses. For the iron analysis, the copolymer was decomposed completely by alternating treatment with nitric and perchloric acids at 180°C for 24 h, and then evaporated to dryness. The iron concentration was spectrophotometrically measured by the colour development between *o*-phenanthroline and iron.

The reduced viscosity (η_{sp}/C) was estimated by measuring the viscosity in purified dimethylformamide (DMF) at 25 ± 0.005°C with an Ubbelohde viscometer having a flow time of 184.7 s for DMF at 25°C. The polymer concentration was always held at 1.0 g/dl.

The physical state of the powdered copolymer surface was characterized by measuring the surface area and also by studying the morphological properties. The surface area was determined by the BET nitrogen adsorption method. The morphological observation was made by using a Hitachi scanning electron microscope (model S-450).

Adsorption experiment

The amount of cyanide ion adsorbed on the copolymer was determined at different pHs. The copolymer was dispersed in various 50 ml buffer solutions (ionic strength 0.15) containing 0–98 mg potassium cyanide. The buffer solution systems were: NaCl–HCl, pH 0.9–3.2; 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_4OH–NH_4Cl, pH 8–11.0; Na_2HPO_4–NaOH, pH 11.0–12.0; NaOH–NaCl, pH 12.0–13.7. The sample dispersion was always sealed in a vial to avoid volatilization of hydrocyanic acid, stirred at 25 ± 0.1°C for 12 h, and then allowed to stand for 12 h to precipitate the copolymer. The cyanide concentration of the supernatant was measured by the pyridine–pyrazolone method[8].

Column experiment

The separation of the cyanide ions from potassium cyanide solution was carried out using a column packed with the copolymer. The copolymer (5 g) was swelled in $Na_2B_4O_7$–KH_2PO_4 buffer solution (ionic strength 0.15 and pH 9) and then packed in a glass column (1 m × 10 mm diameter). The same buffer solution containing potassium cyanide (CN^- ion, 5.56 mg/l) was effused through the column at a constant flow rate of 0.5 ml min^{-1}. To avoid volatilization of hydrocyanic acid, the column experiment was carried out in a closed system, and the effluent fraction (10 ml) was received in a measuring flask (20 ml) into which 0.1 N NaOH (10 ml) was admitted before receiving the effluent. The copolymer, completely saturated with cyanide ion, was washed with buffer solution at pH 9 (50 ml), and then the cyanide ion bound to the copolymer was eluted with 0.5 N NaOH.

RESULTS AND DISCUSSION

Preparation and characterization of the copolymers

Ternary copolymers of H and St with AAm, copoly(H, St, AAm), and with VP, copoly(H, St, VP), were synthesized by radical copolymerization. The copolymers obtained were insoluble in aqueous solvents if the AAm or VP content of the copolymer is less than about 30–40 mol%. The infra-red spectra of copoly(H, St, AAm) and copoly(H, St, VP) showed the absorption band assigned to the H_2NCO– groups (1665 cm^{-1}) and =NCO– groups (1690 cm^{-1}), respectively. The characteristic absorption bands due to phenyl groups (1495, 755, and 700 cm^{-1}) were observed in the spectra of both copolymers. The existence of the haemin residue was confirmed by the fact that the visible spectra show the adsorption bands characteristic of haem compounds[5].

The results for the preparation and characterization of copoly(H, St, AAm) and copoly(H, St, VP) are summarized in *Table 1*, together with those for copoly(H, St) already reported[7]. The content of H residue in the copolymer was determined by iron analysis, and those of AAm and VP residues were also determined on the basis of the nitrogen content obtained by subtracting the nitrogen due to the porphyrin ring (H residue) from the total nitrogen. It is found that, for each copolymer, the H residue content generally agrees with the molar ratio of H monomer. Moreover, the contents of AAm and VP residues are found to be independent of the H concentration of the monomer feed. From these results, it is thought that St, AAm and VP monomers may not be different from each other with respect to polymerizability with H monomer. However, Nishide *et al.*[6] reported that the dimethyl ester (HDME) of H reacts with St and AAm monomers to yield the corresponding copolymer but not with VP monomer. Taking into account this fact, it seems that H and St residues are contiguous with each other in copoly(H, St, VP). From the compositions of copoly(H, St, AAm) and copoly(H, St, VP) however, it becomes apparent that the AAm monomer is easily incorporated into the copolymer as compared with the VP monomer. This is qualitatively related to the difference in the monomer reactivity ratios of St with AAm and with VP (see refs. 9 and 10).

Another important feature of *Table 1* is that the yield and viscosity decrease proportionally with the feed concentration of H monomer, whereas the effects of the AAm and VP monomers are not as marked as that of the H monomer. It is known that the homopolymers of the H monomer[7,11] and other metalloporphyrin monomers[11] are not synthesized by radical polymerization. Futhermore, the St monomer shows a low tendency to polymerize in the presence of haemin or HDME, and the

Table 1 Preparation and characterization of copoly(H, St), copoly(H, St, AAm) and copoly(H, St, VP)[a]

Sample code	Feed (mmol)				Yield (%)	Composition of copolymer (mol %)			$\eta_{sp}/C \times 10^2$ (dl/g)	S (m^2/g)
	H	St	AAm	VP		H	AAm	VP		
HS1[b]	0.228	58.7			75.6	0.53			9.96	4.20
HS2[b]	0.549	58.4			68.5	0.93			9.67	4.21
HS3[b]	1.13	57.9			60.7	1.94			8.66	4.22
HS4[b]	1.47	57.5			52.4	2.55			7.82	4.22
HS5[b]	1.96	57.0			41.7	3.31			7.65	4.22
HS6[b]	2.82	56.2			37.3	4.49			6.87	4.20
HAS1	0.387	38.5	38.5		57.1	0.57	36.8		6.82	4.32
HAS2	0.917	38.2	38.2		42.8	1.26	35.4		6.50	4.45
HAS3	1.51	37.9	37.9		26.3	2.14	37.4		5.58	4.50
HAS4	1.89	37.7	37.7		14.6	2.67	35.7		4.93	4.62
ASH1	1.97	69.7	7.1		54.9	2.32	12.9		7.43	5.22
ASH2	1.97	64.0	12.8		50.9	2.34	16.0		6.87	5.24
ASH3	1.97	59.1	17.8		54.1	2.57	19.9		6.82	5.25
ASH4	1.97	51.3	25.5		52.1	2.45	27.7		6.50	5.26
ASH5	1.97	44.6	32.2		41.2	2.48	29.1		5.52	5.25
ASH6	1.97	43.0	33.8		41.9	2.40	33.1		5.17	5.25
ASH7	1.97	39.5	37.4		32.2	2.58	38.2		4.90	5.25
HVS1	0.390	38.5		38.5	47.4	0.65		25.2	6.77	4.25
HVS2	0.773	38.3		38.3	35.7	0.99		27.3	5.85	4.50
HVS3	1.52	37.9		37.9	21.9	2.01		24.2	4.86	4.75
HVS4	1.88	37.7		37.7	12.3	2.69		26.4	4.39	4.92
VSH1	1.97	69.1		7.9	52.6	2.53		1.3	7.54	5.72
VSH2	1.97	59.2		17.8	57.9	2.74		4.1	7.00	5.84
VSH3	1.97	50.9		26.1	44.9	2.53		11.5	6.66	5.95
VSH4	1.97	42.7		34.3	31.4	2.81		23.1	5.67	6.00
VSH5	1.97	40.5		36.5	22.7	2.68		24.3	3.53	7.42
VSH6	1.97	38.5		38.5	21.9	2.90		25.2	4.39	6.70
HS4L10[c]	14.3	560			56.3	2.52			7.86	4.37
HAS4L15[c]	28.8	560	560		29.2	2.69	37.3		5.21	4.69
HVS4L15[c]	28.8	560		560	24.3	2.71		25.2	4.67	5.20

[a] Copolymerization was carried out in 6.14 ml of pyridine containing 1.83 mmol of AIBN

[b] Cited from ref. 7

[c] Denotes a large scale copolymerization which was carried out in 62 ml of pyridine containing 18.3 mmol of AIBN for copoly(H, St) and in 92 ml pyridine containing 27.5 mmol of AIBN for the other copolymers

polymers obtained contain the haem residue in the end of the polymer chain[6,7]. Taking the above into account, the changes in the yield and viscosity with H monomer may be ascribed to degradative chain transfer of propagating chain radicals to the H monomer. Thus, it is difficult to synthesize the copolymers having high molecular weight, by radical copolymerization of the H monomer with various vinyl monomers. The value of η_{sp}/C for the prepared copolymers is, in fact, found to be relatively small. However, the copolymers obtained in this study are adequate for use as cyanide ion exchangers, since no dissolution of the haem was observed even when the extraction with 1 N NaOH was allowed to continue for a total time of 2 months at 30°–40°C.

The surface state of the powdered copolymers is a significant factor in the investigation of the adsoprtion behaviour of the cyanide ion; thus, the specific surface area (S) was measured by the BET method (see *Table 1*). A well-defined correlation between the surface area and the copolymer composition is not observed in the results for copoly(H, St) and copoly(H, St, AAm), except that the S value for copoly(H, St, VP) increases with increasing content of VP residue in the copolymer. As shown in *Figure 1*, the results of the morphological observation indicate that copoly(H, St, VP) is more porous than the other copolymers. The value of S is therefore related to the porosity of the copolymers, but it is doubtful whether the porosity arises simply from the copolymerization of the VP monomer.

Adsorption of cyanide ion onto the copolymers

It was found that, in contrast with copoly(H, St), copoly(H, St, AAm) and copoly(H, St, VP) are easily dispersed into aqueous media. Furthermore, the adsorptive amount of water vapour on both copolymers is larger than that for copoly(H, St) and increases with increasing content of hydrophilic monomer residues. From these results, it becomes apparent that the hydrophobic character of copoly(H, St) can be improved by the introduction of AAm and VP residues.

Figure 2 shows the change in the adsorptive amount (A_{cn}) of cyanide ion with the H content of the copolymer. The value of A_{cn} was expressed as the weight of cyanide ion adsorbed on 1 g dry polymer. On the whole, the increase in the H content tends to increase the A_{cn} value; therefore, it is evident that the haem-iron is the adsorption site for the cyanide ion. Another remarkable feature of *Figure 2* is that the value of A_{cn} for copoly(H, St, AAm) is much larger than that for the other copolymers. A similar result is also obtained when the AAm content of the copolymer was varied under conditions where the content of the H residue was kept constant (*Figure 3*). This characteristic of copoly(H, St, AAm) is unaltered even if the A_{cn} value is corrected by the S value shown in *Table 1*.

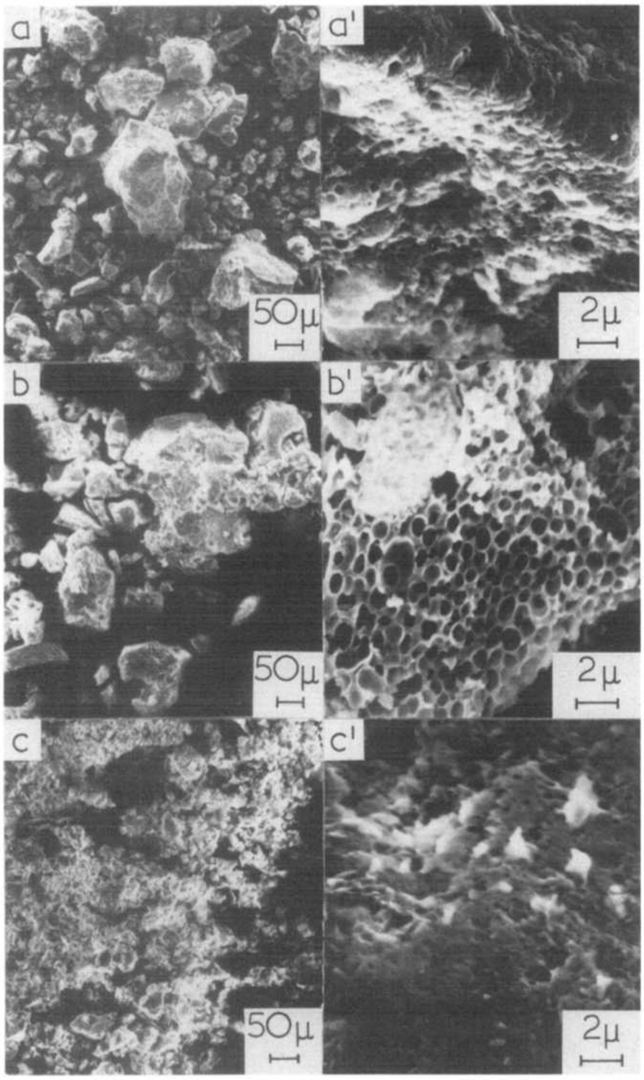

Figure 1 Scanning electron micrographs of copoly(H, St, AAm) (a, a'), copoly(H, St, VP) (b, b'), and copoly(H, St) (c, c'). Sample code (*Table 1*) of the copolymers used for the measurements: HAS4L15 for copoly(H, St, AAm); HVS4L15 for copoly(H, St, VP); HS4L10 for copoly(H, St)

Thus, the copolymerization of AAm monomer appears to be an attractive procedure in increasing the adsorptive capacity of the copolymer for the cyanide ion.

To clarify the function of each copolymer as a cyanide ion exchanger, the adsorption experiments were carried out at different pHs. The sample copolymers used were prepared by a large scale polymerization (see *Table 1*) and unless otherwise noted, the experiments described below were carried out with the same samples. The curves of A_{cn} *versus* pH are shown in *Figure 4*. It is found that the adsorption of cyanide ion has a maximum value at pH 8–9, while cyanide ion is little adsorbed at pH ranges below 2 and above 13 where the binding of water molecules and hydroxyl ions to the haem-iron becomes the dominant factor[2]. This finding means that the adsorption and desorption of cyanide ion are controllable by the adjustment of pH; that is, the copolymers prepared here behave as a cyanide ion exchangers. In *Figure 4*, however, it should also be noted that there is a remarkable difference in the A_{cn} values between copoly(H, St, AAm) and the others.

The dependence of A_{cn} on the initial concentration ($[CN^-]_0$) of cyanide ion was studied at pH 9 to estimate the maximum adsorption capacity (A_{cn}^{max}) of cyanide ion (*Figure 5*). Although the A_{cn} *versus* pH curve for copoly(H, St) showed a maximum value at pH 8, the estimation of A_{cn}^{max} was made at pH 9 because no distinguishable difference between the A_{cn} values at pH 8 and 9 was observed in the $[CN^-]_0$ range above 500 mg/l. The value of A_{cn}^{max} can thus be determined from the A_{cn} value at the plateau region of the curve of A_{cn} *versus* $[CN^-]_0$: 3.5 mg/g for copoly(H, St, AAm); 2.1 mg/g for copoly(H, St); 1.0 mg/g for copoly(H, St, VP). These values correspond to 54%–18% for the H content of the copolymer.

From the results mentioned above, it may be concluded that AAm and VP monomers are copolymerized with a mixture of H and St monomers to yield copolymers having the ability to function as cyanide ion exchangers. In addition, the adsorptive capacity for the cyanide ion is improved by the introduction of the AAm residue.

Column separation of cyanide ion with the copolymers

To test the separation of cyanide ion with the copolymers, the $Na_2B_4O_7$–KH_2PO_4 buffer solution (pH 9) containing 5.56 mg/l cyanide ion was effused through a column containing the copolymer. As shown in *Figure 6*, the cyanide concentration in the fractions is kept below 1 mg/l until the total fraction volume goes up to over 440–450 ml for copoly(H, St, AAm) and 100–120 ml for the

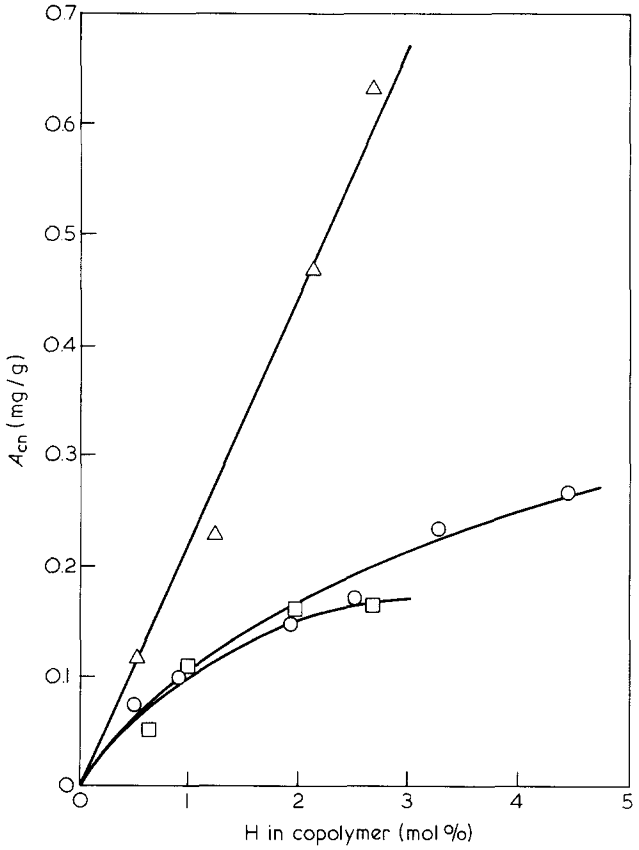

Figure 2 Variation in the adsorptive amount (A_{cn}) of cyanide ion with the H residue content of the copolymers: △, copoly(H, St, AAm) (sample code, HAS1–HAS4); □, copoly(H, St, VP) (sample code, HVS1–HVS4); ○, copoly(H, St)[7] (sample code, HS1–HS6). Adsorption conditions: initial cyanide concentration ($[CN^-]_0$), 5.81 mg/l; pH, 8.0 (KH_2PO_4–Na_2HPO_4 buffer for copoly(H, St) and $Na_2B_4O_7$–KH_2PO_4 for the others); ionic strength, 0.15; adsorbent weight, 10–12 mg. A_{cn} was expressed in milligrams of cyanide ion adsorbed on 1 g dry copolymer

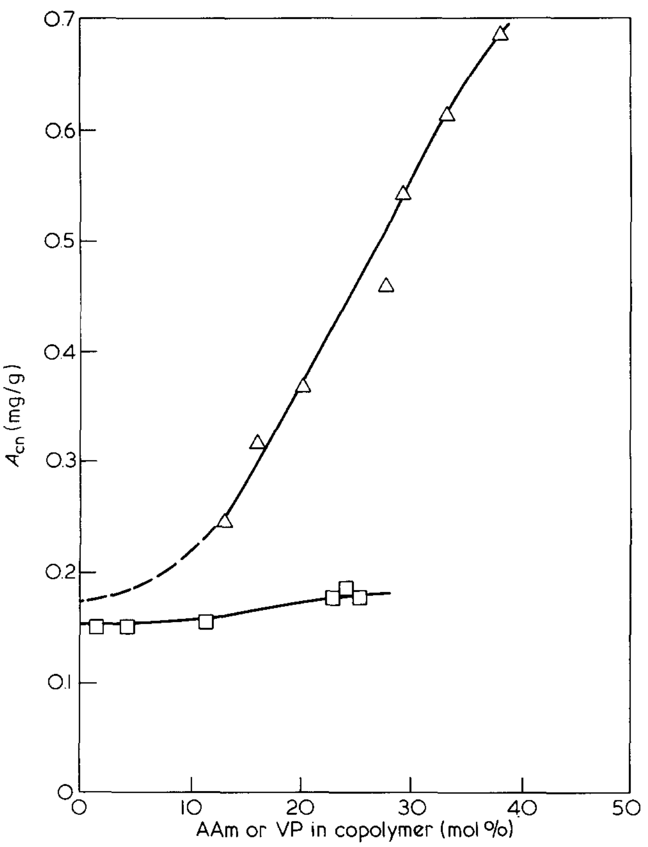

Figure 3 Effect of the hydrophilic monomer residue in copoly(H, St, AAm) (△) and copoly(H, St, VP) (□) on the adsorption of cyanide ion. Sample code of the copolymers used for the measurements: ASH1–ASH7 (H residue content, 2.32–2.58 mol%) for copoly(H, St, AAm); VSH1–VSH6 (H residue content, 2.53–2.90 mol%) for copoly(H, St, VP). Adsorption experiments were carried out under the same conditions as described in *Figure 2*

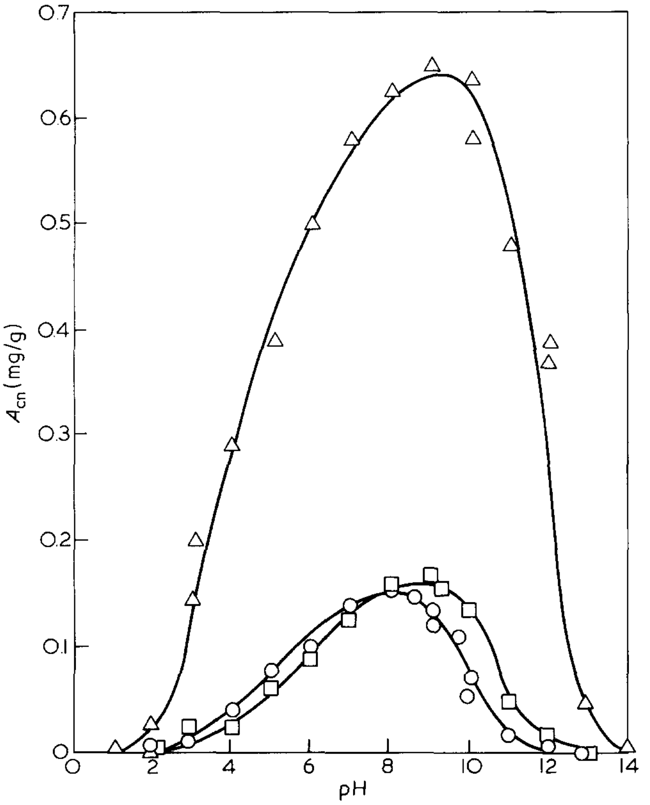

Figure 4 Curves of A_{cn} vs. pH for copoly(H, St, AAm) (△), copoly(H, St, VP) (□), and copoly(H, St) (○). The measurements were made at $[CN^-]_0$ = 5.81 mg/l and ionic strength = 0.15 for each copolymer (10–12 mg) used in *Figure 1*

other copolymers. The adsorbed cyanide ion is 0.69 mg/g for copoly(H, St, AAm) and 0.13–0.18 mg/g for copoly(H, St, VP) and copoly(H, St), as estimated from the column experiments. This amount for copoly(H, St, AAm) corresponds to 20% for A_{cn}^{max}, and those for the others are about 10%. These results also indicate that copoly(H, St, AAm) has a large adsorption capacity for cyanide ion as compared with copoly(H, St, VP) and copoly(H, St). The elution pattern with 0.5 N NaOH, for the copolymer saturated with cyanide ion, is further shown in *Figure 6*. The adsorbed cyanide ion is found to be almost entirely eluted with the NaOH solution, and the recovery is always above 96%. The adsorption and desorption were reproduced reversibly over different runs of the column experiments. The results obtained here thus support the conclusion mentioned previously that the adsorptive capacity of the copolymer is improved by the introduction of the AAm residue.

Coordination of cyanide ion to the haem-iron in the copolymers

The present authors reported in a previous paper[2] that the binding of a cyanide ligand to methaemoglobin is

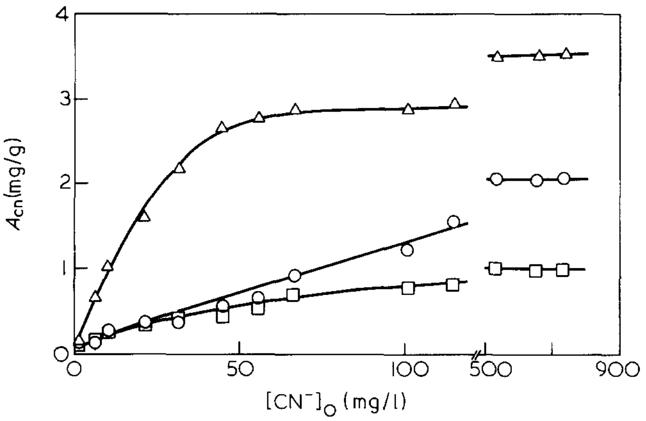

Figure 5 Curves of A_{cn} vs. $[CN^-]_0$ for copoly(H, St, AAm) (△), copoly(H, St, VP) (□), and copoly(H, St) (○). The measurements were made at pH 9.0 ($Na_2B_4O_7$–KH_2PO_4 buffer at ionic strength 0.15) for each copolymer (10–12 mg) used in *Figure 1*

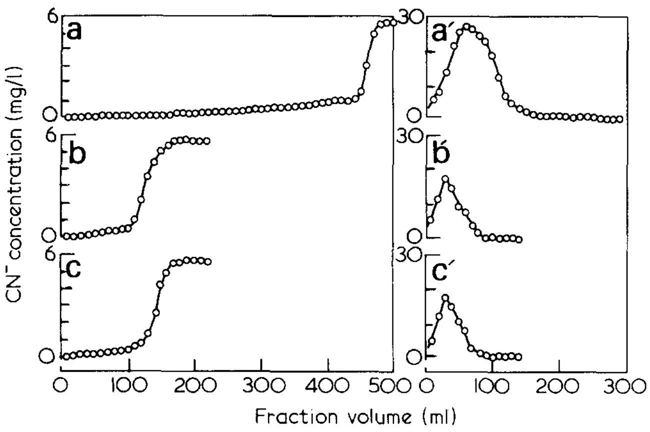

Figure 6 Column separations of cyanide ion with copoly(H, St, AAm) (a, a′), copoly(H, St, VP) (b, b′), and copoly(H, St) (c, c′): a–c, obtained by passing $Na_2B_4O_7$–KH_2PO_4 buffer (pH 9 and ionic strength 0.15) containing 5.56 mg/l cyanide ion through a column of each copolymer (5 g) used in *Figure 1*; a′–c′, obtained by passing 0.5 N NaOH solution through a column of the copolymer saturated with cyanide ion

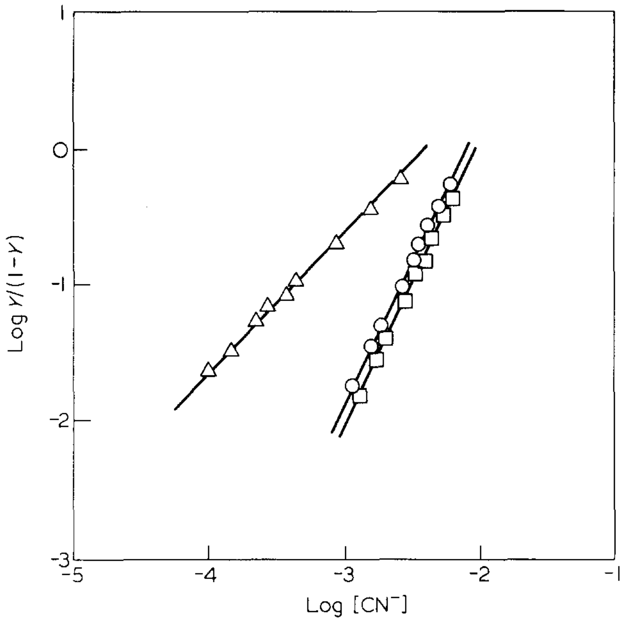

Figure 7 Logarithmic plots of $Y/(1-Y)$ vs. $[\mathrm{CN}^-]$ for copoly(H, St, AAm) (△), copoly(H, St, VP) (□), and copoly(H, St) (○). Adsorption experiments were made at pH 11.5–11.7 (Na_2HPO_4–NaOH buffer at ionic strength 0.15) and at $[\mathrm{CN}^-]_0 = 10^{-4}$–$10^{-2}$ mol l^{-1} for each copolymer (20–24 mg for copoly(H, St, AAm) and 50–68 mg for the others) used in *Figure 1*. Y and $[\mathrm{CN}^-]$ denote the degree of saturation of cyanide ion and the equilibrium cyanide concentration in mol l^{-1}, respectively

clearly distinguishable from that for haemin which has no globin subunits. This result can be related to the fact that the imidazolyl nitrogen of the hystidyl residue in the globin subunit binds to the fifth coordination position of the haem-iron. Thus, a large adsorptive capacity for cyanide ion of copoly(H, St, AAm) may be interpreted by assuming the binding of the AAm residue to the haem-iron in the H residue. In order to confirm this assumption, the axial coordination number (n) of cyanide ion to the haem-iron in the copoly(H, St, AAm) was evaluated and compared with the results found for the other copolymers. The value of n was estimated by the following equation:

$$\log Y/(1-Y) = n \log[\mathrm{CN}^-] + \log K \tag{1}$$

where $Y(=A_{\mathrm{cn}}/A_{\mathrm{cn}}^{\mathrm{max}})$ denotes the degree of saturation of cyanide ion, $[\mathrm{CN}^-]$ is the equilibrium cyanide concentration in mol l^{-1}, and K is the formation constant of the cyanide–haem complex. The plot of $\log Y/(1-Y)$ *versus* $\log[\mathrm{CN}^-]$ was investigated at pH 11.5–11.7 where hydrocyanic acid dissociates completely (*Figure 7*). The n values determined by means of the slopes of the straight lines are 1.1 for copoly(H, St, AAm) and 2.0–2.1 for copoly(H, St, VP) and copoly(H, St). Therefore, it can be indicated that, for copoly(H, St, AAm), one cyanide ion binds to the haem-iron in the copolymer, whereas the fifth and sixth coordination positions of the haem-iron in the copoly(H, St, VP) and copoly(H, St) are saturated with cyanide ions. A primary nitrogen is situated in the amide group of the AAm residue; however, the VP residue has a tertiary nitrogen. Taking into account these facts, the results obtained here could be understood from the explanation that the amide groups in the AAm residue coordinate to the fifth position of the haem-iron in the H residue, while the VP residue does not bind to the haem-iron because of the tertiary nitrogen. This may be due to the difference in the adsorption capacities between copoly(H, St, AAm) and copoly(H, St, VP).

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