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Preparation of Copolymer of Styrene with Hemin and Its Functional Property as Cyanide Ion Exchanger

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Summary

Copolymers of hemin and styrene with different hemin contents were prepared by radical polymerization, using pyridine as the solvent and α,α'-azobisisobutyronitrile as the initiator. It was found that the obtained copolymers are insoluble in aqueous solvents and have function as cyanide ion exchanger.

Introduction

Numerous studies have dealt with the preparation and characterization of various ion exchangers. However, little attention has been paid to an ion exchanger having function of selective separation for specific anion such as cyanide ion. It is well known that the affinity of cyanide ion to ferriheme in methemoglobin (ferri-hemoglobin) is very high because the cyanide ligand neutralizes the extra charge of the heme-iron (Rifkind 1973). Previously we reported that polyelectrolyte complex containing methemoglobin has a function as cyanide ion exchanger (Kokufuta et al. 1981). On the other hand, ferriprotoporphyrin IX in the hemoglobin has two vinyl groups in the 2- and 4-positions of the porphyrin ring. These facts prompted us to investigate the preparation of cyanide ion exchanger by radical polymerization of the porphyrin with vinyl monomer. The present paper describes the copolymerization of hemin (H) (ferri-protoporphyrin IX chloride) with styrene (St) and a functional property of the copolymer as cyanide ion exchanger.

Materials and Methods

H monomer was obtained from Tokyo Kasei Kogyo Co., Ltd. The purification was carried out in the same manner as described in the literature (Tsuchida, Honda 1975). Reagent grade St monomer (Wako Pure Chemical Industries Ltd.) was distilled under reduced pressure (48°C/20mmHg) to remove polymerization inhibitor. The copolymerization was carried out in a sealed tube at 85 °C for 48 hr under reduced pressure by using purified pyridine as the solvent and α,α'-azobisisobutyronitrile (AIBN) as the initiator. The reaction mixture was poured slowly into methanol to precipitate copolymer,

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which was separated by filtration, washed with methanol, and dried under vacuum. The crude copolymer was reprecipitated 3 times from chloroform with methanol, dried under vacuum, washed with a large amount of 0.5N NaOH, rewashed with distilled water, and then dried at 55 °C for 2 days under vacuum.

The amount (A_{cn}) of cyanide ion adsorbed on the copolymer of H and St [copoly(H, St)] was determined at different pH. The copoly(H, St) was dispersed in 50 ml of various buffer solutions (ionic strength 0.15) containing 0.726 mg potassium cyanide. The sample dispersion was always sealed in a vial to avoid the volatilization of hydrocyanic acid, stirred at 25 °C for 12 hr, and then allowed to stand for 12 hr to precipitate the copolymer. The cyanide concentration of the supernatant was measured by pyridine - pyrazolone method (Epstein 1947).

The separation of cyanide ion from potassium cyanide solution was carried out by using a column packed with copoly(H, St). The copolymer (5 g) was swelled in Na_2HPO_4 - KH_2PO_4 buffer solution (pH 8 and ionic strength 0.15) and then packed in a glass column (1m x 10mmϕ). The same buffer solution containing potassium cyanide (13.9 mg/l) was effused through the column at a constant flow rate (0.5 ml/min). After copoly(H, St) was saturated completely with cyanide ion, the cyanide was eluted with 0.5N NaOH solution.

Results and Discussion

H monomer reacts with St monomer to yield the copolymers at different H contents, although the homopolymer of H can not be obtained by radical polymerization. The obtained copolymers are insoluble in aqueous solvents. The infrared spectrum of the copolymer washed with 0.5N HCl shows the absorption bands assigned to phenyl groups (1602 cm^{-1}) in St residues and to carboxyl groups (1720 cm^{-1}) in H residues. The visible and ultraviolet spectra also show typical pattern of heme compounds.

The results of the copolymerization are summarized in Table I. It is found that the content of H monomeric units in copoly(H, St) mostly agrees with that of H monomer in the monomer feed. Thus, it is considered that H monomer becomes incorporated quantitatively into the copolymer under the condition where the feed concentration of H monomer is much smaller than that of St monomer. Another feature in Table I is the decrease in the yield with increasing the feed concentration of H monomer. It has been reported that St monomer shows the low tendency to polymerize in the presence of ferri-protoporphyrin IX dimethyl ester, and the polymer obtained contains the porphyrin residue in the terminal of the polymer chain (Nishide et al. 1979). Taking into account these facts, the decrease in the yield with increasing the feed concentration of H monomer may be ascribable to degradative chain transfer of propagating styrene radicals to H monomer. This could be supported by the fact that the reduced viscosity (η_{sp}/C) decreases with increasing the H content of monomer feed.

TABLE I Copolymerization of Hemin and Styrene[a)]

No	Monomer (m mol)		Yield (%)	H content (mol%)[b)]	$\eta_{sp}/C \times 10^2$ (dl/g)[c)]
	H	St			
1	0.288	58.7	75.6	0.53	9.96
2	0.549	58.4	68.5	0.93	9.67
3	1.13	57.9	60.7	1.94	8.66
4	1.47	57.5	52.4	2.55	7.82
5	1.96	57.0	41.7	3.31	7.65
6	2.82	56.2	37.3	4.49	6.87

[a)] Carried out in 6.14 ml of pyridine containing 1.83 m mol of AIBN.

[b)] Determined by the analysis of iron (Kokufuta et al. 1981) and elemental analysis.

[c)] Measured for 1 % N,N'-dimethylformamide solution of copoly(H, St) at 25 ± 0.02 °C by using an Ubbelohde viscometer.

Figure 1 shows the change in A_{cn} with the H content of copoly(H, St). It is found that the A_{cn} value increases with increasing the H content. This indicates that the heme-iron is the adsorption site of cyanide ion. Figure 2 shows the curve of A_{cn} against pH. A maximal value of A_{cn} is observed in the neighborhood of pH 8, while the A_{cn} value is reduced to zero in the pH regions below 2 and above 12. These results mean that the adsorption and desorption of cyanide ion are controllable by the adjustment of pH. Thus, the copoly(H, St) can be expected to have the function as cyanide ion exchanger.

In order to investigate further cyanide ion exchange property for copoly(H, St), the separation of cyanide ion from potassium cyanide solution was carried out by using a column packed with the copolymer. The results are shown in Figure 3. When the potassium cyanide solution, adjusted to pH 8 with phosphate buffer, was passed through the column of copoly(H,St), the cyanide concentration of the fractions is found to be kept below 0.7 mg/l until the heme-iron in the copolymer is saturated with cyanide ion. The ion-exchange capacity for the copolymer used here is 0.14 mg/g (corresponding to 2.5 mol % of total H residues), as estimated from the column experiment. The adsorbed cyanide ion is mostly or entirely eluted with 0.5N NaOH solution (recovery: above 95 %). These adsorption and desorption were reproduced reversibly at different runs of the column experiment.

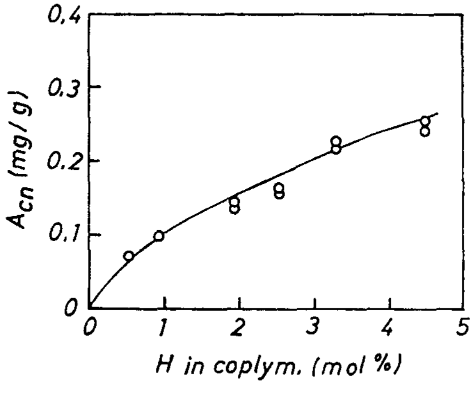

Fig. 1 Change in the adsorbed amount (A_{cn}) of CN^- ion with the H content of copoly(H, St): pH 8 (phosphate buffer); Ionic strength, 0.15; Initial KCN concn., 14.52 mg/l.

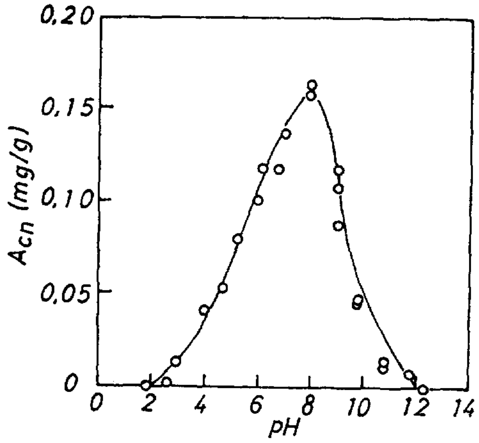

Fig. 2 Dependence of A_{cn} on pH for copoly(H, St) containing 2.55 mol % of H residues: Ionic strength, 0.15; Initial KCN concn., 14.52 mg/l.

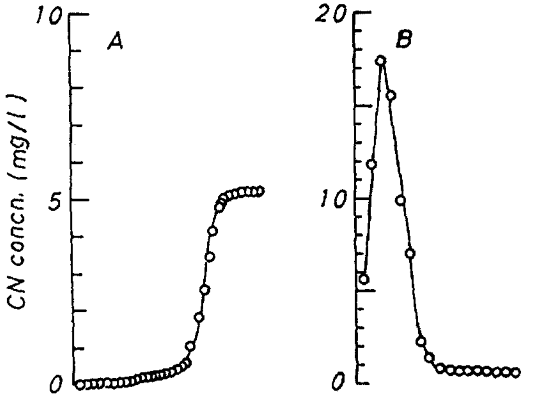

Fig. 3 Column separation of cyanide ion with copoly(H, St) containing 2.55 mol % of H residues. A: Separation of CN^- ion from KCN solution [KCN concn., 13.9 mg/l; pH 8 (phosphate buffer); Ionic strength, 0.15]. B: Elution of the adsorbed CN^- ion with 0.5N NaOH.

From the results described above, it may be concluded that H monomer reacts with St monomer with the aid of radical initiator to yield water-insoluble copolymer having the function as cyanide ion exchanger.

References

EPSTEIN, J.: Anal. Chem. 19, 272 (1947)

KOKUFUTA, E., WATANABE, N. and NAKAMURA, I.: J. Appl. Polym. Sci. (1981) in press

NISHIDE, H., SHINOHARA, K. and TSUCHIDA, E.: Abstr. II, p. 411, 40th National Meeting of the Chemical Society of Japan, Fukuoka, Oct. 1979

TSUCHIDA, E. and HONDA, K.: Polymer J. 7, 498 (1975)

RIFKIND, J. M.: Inorg. Biochem. 2, 832 (1973)

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