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Preparation of Copoly(Styrene-Divinylbenzene) Covalently Bonded Protohemin-mono-N-Histidyl Amide and Its Functional Property as Cyanide Ion Exchanger

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Summary

2,7,12,18-Tetramethyl-3, 8-divinyl-17-(2-carboxyethyl) - ferri-porphyrin-N-histidyl-13-propylamide was bound to a styrene-divinylbenzene copolymer in the form of beads. It was performed by the sequential addition of histidine and hemin to the aminated copolymer according to the method of solid phase peptide synthesis. The obtained polymer showed a functional capability as cyanide ion exchanger.

Introduction

The coordination of cyanide ion to ferri-protoporphyrin IX in methemoglobin follows an approximately stoichiometric relationship at pH 8 - 9 but is inhibited by increasing acidity or basicity because of the binding of water molecule or hydroxyl ion (Kokufuta et al. 1981a). This fact indicates that hemin-containing polymer functions as a cyanide ion exchanger. The previous publications from this laboratory have described that water-insoluble polymers having a functional capability as cyanide ion exchanger can be synthesized by radical co- or terpolymerization of hemin with several vinyl monomers (Kokufuta et al. 1981b and 1982).

Recently some authors (Bayer and Holzbach 1977; Tsuchida et al. 1982) reported the synthesis of water-soluble polymer with covalently bonded hemin by forming amide linkage between carboxyl group in hemin and amino group in polymer support. These papers prompted us to study the preparation of hemin bound to solid polymer having a suitable form for the column separation of cyanide ion. In this communication, we describe the synthesis of cyanide ion exchanger in the form of beads by linking protohemin-mono-N-histidyl amide with the aminated copolymer of styrene with divinylbenzene according to the procedures of solid phase peptide synthesis.

Results and Discussion

The evidence has accrued which indicates that an important factor affecting the ability of hemin-containing polymer to bind cyanide ion is the coordination structure of the hemin; i.e., in order to increase the affinity of the hemin for cyanide ligand, one coordination site of the iron(III) must be occupied by a polymer-bound coordinative group (Kokufuta et al. 1981c and 1982). Thus, histidine was bound to the support together with hemin to obtain the polymer containing the five-coordinate type of hemin. The synthetic outline is schematically shown in Figure 1.

The aminated copolymer (1) [NH_2, 1.19 mmol/g; IR, 3450 and 3385 cm^{-1} (ν_{N-H})] was obtained from commercially available polystyrene beads with chloromethyl groups (1.26 mmol/g) by the method of Weinshenker and Shen (1972). 1,N-Dicarbobenzoxy-L-histidine [Z-His(Z)] was coupled with 1 in dichloromethane (DCM) using dicyclohexylcarbodiimide (DCC) as a condensing reagent. The IR spectrum for 2 [Z-His(Z), 0.80 mmol/g] showed the absorption band at 1660 cm^{-1} [$\nu_{C=O}$(amide)] which was not observed for a mixture of Z-His(Z) and 1. The treatment (25 °C, 30 min) of 2 with acetic acid containing 30% HBr produced 3. The complete cleavage of the α-amino-protecting group (α-Z) was indicated by the fact that the absorption at 1720 cm^{-1} [$\nu_{C=O}$(α-Z)] in the spectrum of 2 was perfectly removed by treating with the acetic acid-HBr system. In addition, it was found that this treatment did not have a pronounced effect on the imidazole-protecting group (*im*-Z), because no remarkable difference in the absorption at 1760 cm^{-1} [$\nu_{C=O}$(*im*-Z)] was observed between 2 and 3. The coupling of hemin to 3 with DCC in a 1:1 mixture of dimethylformamide (DMF) and DCM yielded a black-colored polymer (4) (Fe, 0.578 mmol/g). In this case, unfortunately, the IR analysis was not useful for determining whether hemin is covalently or adsorptively attached to 3, because of the existence of amide groups in 3. Thus, a careful control experiment was made by treating 3 in the absence of DCC under the same conditions as described in the preparation and purification of 4 (see Experimental section), followed by subjecting to the analysis of iron. It was found that the control sample was completely decolorized during the course of the purification and also that no iron remained in the decolorized sample. The final product (5) (Fe, 0.418 mmol/g) was obtained by the treatment (25 °C, 2 hr) of 4 with 30% HBr in acetic acid. The complete removal of the *im*-Z group was confirmed by the disappearance of the absorption band at 1760 cm^{-1}.

The coordination sphere around the central iron of the hemin in 5 was characterized by electron spin resonance (ESR) spectroscopy. The measurements were made at liquid nitrogen temperature for two samples prepared by dispersing 5 into

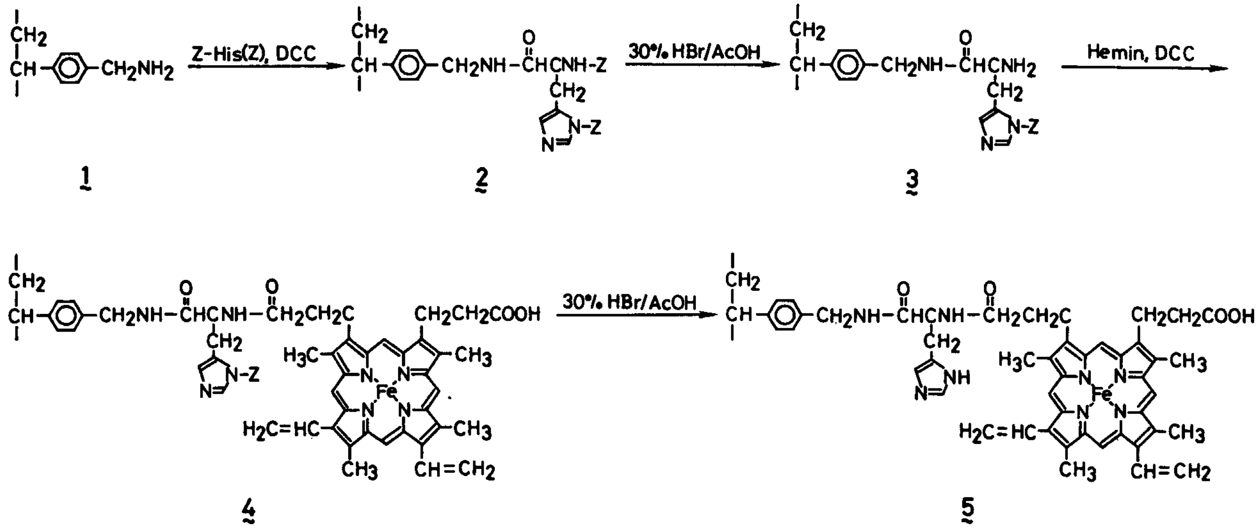

Fig. 1 The scheme for the preparation of hemin-containing polymer

0.001N HCl (pH 3) and into phosphate buffer (pH 9) containing 0.1M KCN. The spectrum in the HCl solution indicates the five-coordinated high spin type of iron(III) porphyrins characterized by the ESR signal with g=6, while that in the KCN solution is typical for low spin iron(III) in octahedral ligand field (see Figure 2). On the other hand, the axial coordination number of cyanide ion for 5 was close to one, as estimated in the same manner as used previously (Kokufuta et al. 1981a and 1982). These results could mean that 5 consists of the hemin in which the fifth coordination site of the iron(III) is occupied by a coordinative group (probably, imidazolyl group), and cyanide ion binds to the sixth coordination position.

The functional capability of 5 as cyanide ion exchanger was examined by column experiment (Figure 3). When the phosphate buffer solution (pH 9) containing 15.0 mg/l cyanide ion was effused through a column of the polymer (5 g), the cyanide concentration of the fractions was observed to be kept below 0.01 mg/l until the total fraction volume exceeded 180 ml. The adsorbed cyanide ion (0.581 mg/g, corresponding to 5.4 mol% of total hemin bound to polymer) was then almost entirely eluted with 0.1N NaOH solution (recovery, ca. 98%). The adsorption and desorption were reproduced reversibly over at least 5 runs of the column experiments.

Experimental

Chloromethylated styrene-divinylbenzene copolymer (Bio-Beads, SX-1, 200-400 mesh) was of commercial origin. The amination of the copolymer was made in the same manner as described in the literature (Weinshenker and Shen 1972). Z-His(Z) with one molecule of methanol, Z-His(Z)·MeOH, was prepared in the usual way (e.g., Patchornik et al. 1957): mp. 105-107 °C (dec.); UV (methanol) 238 nm; IR (KBr) 3365, 3125, 1760, 1720, 1685 cm^{-1}. Anal. Calcd. for $C_{22}H_{21}N_3O_6 \cdot CH_3OH$: C, 60.7; H, 5.5; N, 9.2. Found: C, 60.6; H, 5.5; N, 9.3. Z-His(Z)·MeOH (40 mmol) was allowed to react with sufficiently swelled 1 (11.9 mmol of NH_2) containing 40 mmol of DCC at 20 °C with shaking for 12 hr. The obtained product was transferred into a glass column, reciprocally washed with DCM and DMF until the washing has no UV absorption bands (238 - 300 nm), further washed with ethanol, and dried at 50 °C under reduced pressure (this purification method was also used for 4). To remove the α-Z group, 2 (6 g) was shaken for 30 min at 25 °C in 100 ml of acetic acid containing 30% HBr. The resulting 3 was then purified by the same manner as described by Merrifield (1963), and subjected to the coupling reaction without drying. The coupling of hemin (20 mmol) to 3 (6 g as

Fig. 2 ESR spectra of 5 at liquid nitrogen temperature: a, in 0.001N HCl; b, in KCN-containing phosphate buffer (pH 9, 0.1M KCN).

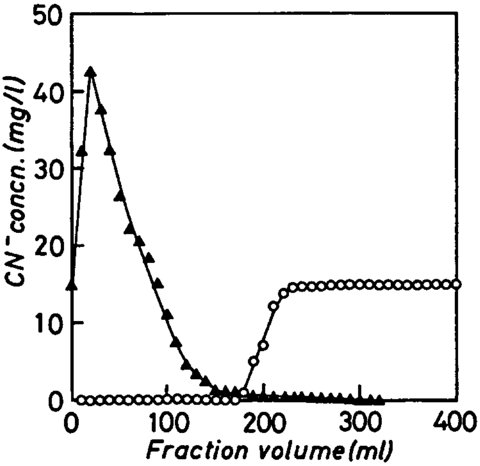

Fig. 3 Column separation of cyanide ion with 5: ○, separation of cyanide ions from KCN-containing buffer solution (pH 9, CN = 15 mg/l); ▲, elution of the adsorbed cyanide ions with 0.5N NaOH solution.

wet polymer) was made in 100 ml of a 1:1 mixture of DCM and DMF with shaking for 50 hr at 10 °C. The purification method has been described above. The im-Z groups of 4 (5 g) were finally cleaved in the same manner as used for 2, except the shaking was made for 2 hr.

The sample obtained in each step of the preparation was characterized by elemental analysis and IR spectroscopy with a Hitachi IR 260-50 spectrophotometer equipped with a Hitachi IRR-8 diffused reflective apparatus. ESR spectroscopy with a JEOL JES-FELX spectrometer was also applied to characterize 5. The capability of 5 to bind cyanide ion was examined by passing $Na_2B_4O_7$-KH_2PO_4 buffer (pH 9, ionic strength 0.1) containing 15 mg/l cyanide ion through a column (1m x 10mm diameter) packed with 5 g of the polymer. After the polymer was completely saturated with cyanide ion, the cyanide was eluted with 0.5N NaOH solution. The cyanide concentration was measured by the pyridine-pyrazolone method (Kokufuta et al. 1981a).

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