Poly(1-vinyl-2-pyrrolidone)- and Dextran-Bound Protoheme Mono[*N*-[3-(imidazol-1-yl)propyl]amide] and Mono[*N*-[5-(2-methylimidazol-1-yl)pentyl]amide] and Their Reversible Oxygen Binding in an Aqueous Medium

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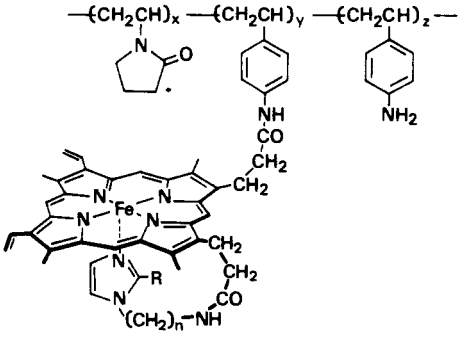
ABSTRACT: Protoheme mono[*N*-[3-(imidazol-1-yl)propyl]amide] and mono[*N*-[5-(2-methylimidazol-1-yl)pentyl]amide] were covalently bound with poly(1-vinyl-2-pyrrolidone) (**1**) and dextran (**2**). The polymer-bound hemes formed oxygen adducts with lifetimes of ca. 1 h for **1** and a few minutes for **2** in an aqueous ethylene glycol (1/1) solution cooled to −30 °C, whereas nonbound heme analogues did not form under the same conditions. It is considered that the water-soluble but hydrophobic poly(vinylpyrrolidone) protects the heme-oxygen adduct from its proton-driven irreversible oxidation. Oxygen binding rate constant of **1** was reduced by the surrounding polymer, and oxygen binding affinity was close to that of hemoglobin. Mössbauer parameters of the iron-57-labeled **1** were also measured.

Introduction

Much effort has been made to mimic the oxygen-transporting function of hemoglobin (Hb) by using an iron–porphyrin complex. Various extensive investigations on sterically modified iron–porphyrins have been reported,[1-3] but most of these were restricted to the oxygen binding reaction in nonaqueous media. In order to study the oxygen binding ability of these modified iron–porphyrin complexes in aqueous media, we have attempted to replace the role of Hb's globin protein in the oxygen binding with that of a synthetic maclomolecule or a macromolecular assembly. Modified and lipophilic or amphiphilic iron–tetraphenylporphyrin complexes were covalently bound to the central hydrophobic block of a poly(ethylene oxide)–polystyrene–poly(ethylene oxide) triblock copolymer[4] or were embedded in the hydrophobic inner region of a phospholipid bilayer.[5] These micelle-forming polymer-bound and lipid-bilayer-embedded iron–porphyrin complexes bind molecular oxygen reversibly under physiological conditions (in pH 7 aqueous media at 37 °C). It was thought that hydrophobic microenvironments around the iron–porphyrin complexes solubilized in the aqueous media protected their oxygen adducts from their irreversible oxidation.

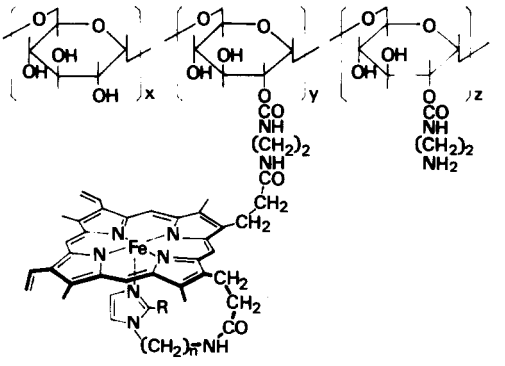
It is also important for a more accurate model of Hb to use an iron(II)–protoporphyrin IX derivative (heme) instead of the iron–tetraphenylporphyrin. This should lead to physicochemical properties of the iron–porphyrin such as visible absorption spectral feature and chemical reactivity of the porphyrin such as porphyrin cleavage reactions in vivo similar to those of the natural iron–protoporphyrin IX of Hb.

Recently we have found that the heme complexed with water-soluble poly(1-vinyl-2-methylimidazole) forms a semistable oxygen adduct in aqueous ethylene glycol solution cooled to −30 °C; while the low-molecular-weight analogous heme complex does not form under the same conditions.[6] We suggested that the reason for the oxygen binding in aqueous solution was that the heme complex takes a pentacoordinate structure which leaves the sixth coordination site vacant to bind molecular oxygen and that the oxygen adduct is surrounded with a hydrophobic environment of the polymer. We also have reported in a preliminary publication that poly(1-vinyl-2-pyrrolidone) (PVP)-bound iron(II)–protoporphyrin IX mono[*N*[3-(imidazol-1-yl)propyl]amide] (**1a**) gave its oxygen adduct in a cold aqueous solution:[7] PVP was used because it is a hydrophobic but water-soluble polymer. This paper describes preparation and oxygen binding ability of the PVP-bound heme derivatives (**1**) in an aqueous ethylene glycol solution cooled at 0 to −30 °C. Oxygen-binding



1a; R = H, n = 3
1b; R = CH_3, n = 5

kinetic constants, oxygen-binding affinity, and Mössbauer parameters of the polymer-bound hemes **1** are discussed in comparison with those of the nonbound analogues, iron(II)–protoporphyrin IX mono[*N*-[3-(imidazol-1-yl)-propyl]amide] (**3a**) and mono[*N*-[5-(2-methylimidazol-1-yl)pentyl]amide] (**3b**), and those of Hb. This paper also includes preparation and oxygen binding of the dextran-bound heme derivatives (**2**).



2a; R = H, n = 3
2b; R = CH_3, n = 5

Experimental Section

Iron(III)–protoporphyrin IX mono[*N*-[3-(imidazol-1-yl)-propyl]amide chloride] (**3a′**) was synthesized according to literature methods.[8,9] Protoporphyrin IX mono[*N*-[5-(2-methyl-

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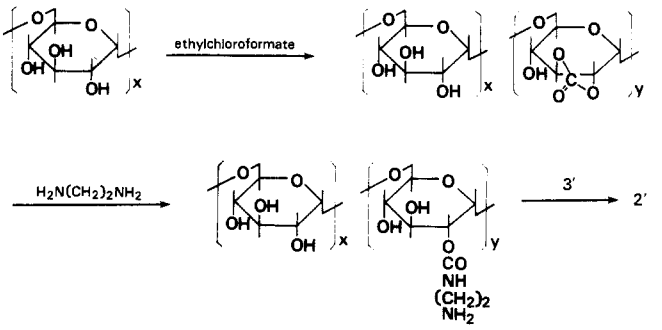
imidazol-1-yl)pentyl]amide] mono(ethyl ester) was synthesized from the reaction of protoporphyrin IX mono(ethyl ester) and 1-(5-aminopentyl)-2-methylimidazole according to the previous paper.[10] Anal. Calcd for $C_{45}H_{53}N_7O_3$: C, 73.1; H, 7.17; N, 13.3. Found: C, 73.1; H, 7.21; N, 13.2. ^1H NMR ($CDCl_3$, Me_4Si standard, δ_H, ppm) 3.55–3.80 (12 H, ring H), 0.30 (3 H, imidazole H), 4.70 (1 H, imidazole H), 4.10 (4 H, propionic α-CH_2), 3.15 (4 H, propionic β-CH_2), 0.90 (2 H, α-CH_2), 0.20 (2 H, β-CH_2), 2.35 (2 H, γ-CH_2), 1.95 (2 H, δ-CH_2), 2.83 (2 H, ϵ-CH_2), 5.80 (1 H, NH), 9.70–9.90 (meso H), 5.00–5.50, 6.90 (3 H, vinyl H), 4.10 (2 H, $-OC_2H_5$), 2.05 (3 H, $-OC_2H_5$), -4.50 (2 H, pyrrole H); IR (KBr pellet, cm^{-1}) 1550, 1640 (amide, $\nu_{C=O}$), 1730 (ester, $\nu_{C=O}$); MS, m/e 740 (M^+). Iron insertion and hydrolysis of the ethyl ester yield iron(III)–protoporphyrin IX mono[*N*-[5-(2-methylimidazol-1-yl)pentyl]amide chloride] (**3b′**). The iron insertion was as follows. The protoporphyrin derivative was dissolved in dimethylformamide. $FeCl_2 \cdot nH_2O$ was added to this solution, and the mixture was heated to 70 °C under nitrogen for 1 h. The solvent was removed by evaporation, and the residue was extracted with a chloroform–methanol mixture. The crude product was chromatographed on a basic alumina column, eluted with chloroform–methanol. The first fraction was collected and dried to give the iron(III)–protoporphyrin derivative.

3′ was allowed to react with poly[(1-vinyl-2-pyrrolidone)-*co*-(4-aminostyrene)]. 4-Aminostyrene was prepared by dehydration of (4-aminophenyl)ethyl alcohol with potassium hydroxide.[11] 4-Aminostyrene and 1-vinyl-2-pyrrolidone were copolymerized with 2,2′-azobisisobutyronitrile in tetrahydrofuran at 60 °C. The copolymer was purified by reprecipitating it twice in ethyl ether. 4-Aminostyrene residue content of the copolymer was determined to be 1.0 mol% by the potential titration with perchloric acid in acetic acid. Molecular weight of the copolymer was measured by vapor pressure osmometry in methanol; 3.65×10^4. The copolymer (130 mg, 4-aminostyrene unit; 0.024 mmol) was allowed to react with **3a′** (0.24 mol) or **3b′** (0.24 mol) in dry dimethylformamide in the presence of ethyl chloroformate (0.24 mmol) and triethylamine (0.24 mmol) at 0 °C for 2 h and at room temperature for 3 days to complete the reaction. After concentration the residue was dissolved in chloroform and the insoluble part was removed by filtration, then the solution was poured into ethyl ether. The crude product was chromatographed on a Sephadex LH-20 column. The first fraction was collected and brought to dryness, which was precipitated in ethyl ether to give poly[(1-vinyl-2-pyrrolidone)-*co*-(4-aminostyrene)]-bound iron(III)–protoporphyrin IX mono[*N*-[3-(imidazol-1-yl)propyl]amide chloride] (**1a′**) and poly[(1-vinyl-2-pyrrolidone)-*co*-(4-aminostyrene)]-bound iron(III)–protoporphyrin IX mono[*N*-[5-(2-methylimidazol-1-yl)pentyl]amide chloride] (**1b′**). The control experiment was carried out with the mixture of PVP (mol wt 4.50×10^4) and **3a′**, which showed that noncovalently bound **3′** was completely separated from the polymer by the purification procedure used. The bound-heme content in the polymer was determined from the absorbance at 412 nm, refered to that of **3a′** and **3b′**; $x = 99$, $y = 0.75$, $z = 0.25$ mol % for **1a′** and $x = 99$, $y = 0.78$, $z = 0.22$ mol % for **1b′**. These PVP-bound hemes were water soluble up to ca. 5 wt %.

The iron-57-labeled **1a** was prepared as follows. Protoporphyrin IX mono[*N*-[3-(imidazol-1-yl)propyl]amide] mono(ethyl ester) and $^{57}Fe(AcO)_2$[12] were dissolved in glacial acetic acid, and the mixture was refluxed under nitrogen for 10 min. The solvent was removed, and the residue was extracted with chloroform. The crude product was purified by chromatography on a basic alumina column with chloroform. The first and second fractions were collected and washed with 5% hydrochloric acid and water and dried. The iron-57-labeled derivative of **3a′** was reacted with the poly[(1-vinyl-2-pyrrolidone)-*co*-(4-aminostyrene)] under the same conditions mentioned above to give the iron-57-labeled derivative of **1a′**.

The dextran-bound hemes were prepared as in Scheme I. First dextran carbonate was prepared from the reaction of dextran (mol wt 4.0×10^4) with ethyl chloroformate, by modifying the literature method.[13] Fifty grams of dextran was dissolved in dry dimethyl sulfoxide (Me_2SO) (500 mL), and to the solution dry dioxane (150 mL) and dry triethylamine (250 mL) were added. After the solution was cooled at ca. 10 °C, dry dioxane solution (25 mL) of ethyl chloroformate (20 mL) was added dropwise to the vigorously stirred dextran solution. Then 150 mL of concentrated hydrochloric acid was slowly added to acidify the solution while keeping the temperature below 17 °C. The reaction mixture was poured into ethyl alcohol/water (10 L/0.4 L). The precipitate was filtrated, washed with ethyl alcohol and then ether, and dried in vacuo at 40 °C. The dextran carbonate was obtained as a white powder, of which yield was quantitative. IR (KBr pellet, cm^{-1}) 1815 (intramolecular cyclic carbonate). Content of the carbonate is 4.6 mol % determined by the titration with barium hydroxide and dilute hydrochloric acid.

Scheme I



The amino derivative of dextran was prepared as follows. Dry Me_2SO solution (300 mL) of the dextran carbonate (50 g) was added dropwise to 120 mL of ethylenediamine dissolved in Me_2SO/water (100 mL/120 mL). The mixture was stirred overnight at room temperature and poured in ethyl alcohol/water (10 L/0.4 L). The white precipitate was filtrated, washed with ethyl alcohol and then ether, and dried in vacuo. This was purified by reprecipitating twice from Me_2SO to ethyl alcohol, washed with ether, and dried in vacuo. Content of the amino group was 3.2 mol % determined by elemental analysis.

The amino derivative of dextran (124 mg, amino group 0.024 mmol) was allowed to react with **3a′** (0.24 mol) or **3b′** (0.24 mol) in dry Me_2SO in the presence of ethyl chloroformate. The mixture was kept at room temperature for 2 days. The product was purified by precipitating twice from Me_2SO to ethyl alcohol. Then the product was further purified by gel permeation chromatography (Biobeads column, Me_2SO) to give dextran-bound iron(III)–protoporphyrin IX mono[*N*-[3-(imidazol-1-yl)propyl]amide chloride] (**2a′**) and dextran-bound iron(III)–protoporphyrin IX mono[*N*-[5-(2-methylimidazol-1-yl)pentyl]amide chloride] (**2b′**). The control experiment was carried out with the mixture of dextran and **3a′**, which showed that noncovalently-bound **3a′** was completely separated from dextran by the purification procedure used. The contents of **3′** in the dextran were 1.4 and 1.5 mol % for **2a′** and **2b′**, respectively, determined by the absorption at 400 nm (**2a′**, $x = 96.8$, $y = 1.4$, $z = 1.8$ mol %; **2b′**, $x = 96.8$, $y = 1.5$, $z = 1.7$ mol %). The dextran-bound hemes were water soluble up to ca. 10 wt %.

The aqueous medium was an oxygen-free mixture of a buffer solution (pH 7.48, 0.2 M phosphate buffer) with ethylene glycol (volume ratio = 1/1). Ethylene glycol was added as an antifreeze agent. The **1** solution was prepared by adding sodium dithionite to the **1′** solution (molar ratio, $[Na_2S_2O_4]/[Fe(III)] = 5$) under nitrogen atmosphere to reduce iron(III) of **1′** to iron(II) of **1**. The reduction of **1′** to **1** was also carried out with ascorbic acid, glucose, or met-Hb reductase. The last was prepared according to literature procedures.[14]

Flash photolysis and stopped flow measurements were performed by the use of a pulse flash spectrophotometer (Unisoku FP-2000) and a stopped flow spectrophotometer (Unisoku SF-1000) equipped with a kinetic data processer. Rate constants of the oxygen and carbon monoxide binding and dissociation were determined with pseudo-first-order kinetics.[15] The oxygen and carbon monoxide binding and dissociation equilibrium curves were monitored from spectroscopic measurements taken after bubbling the gaseous oxygen and carbon monoxide premixed with nitrogen through solutions containing the polymers.

Mössbauer spectroscopic measurements were carried out as follows. The spectrometer was of the constant-acceleration type. The source was used at room temperature and consisted of about 10 mCi of ^{57}Co diffused in palladium foil. The absorbers with

Table I
UV-Vis Absorption Maxima for the Polymer-Bound Hemes in pH 7.4 Buffer Solution/Ethylene Glycol (1/1) at −30 °C

heme	λ_{max}, nm deoxy	CO adduct	O_2 adduct	ref
PVP–heme **1a**	428, 530, 560	417, 534, 564	410, 540, 572	this work
PVP–heme **1b**	433, 557	418, 537, 566	412, 547, 577	this work
dextran–heme **2a**	427, 530, 560	416, 534, 565	410, 542, 572	this work
dextran–heme **2b**	432, 557	418, 538, 567	411, 547, 577	this work
heme **3a**	427, 530, 558	418, 538, 566	oxidized	10, this work
heme **3b**	432, 557	420, 540, 569	oxidized	17, this work
Hb[a]	430, 556	418, 539, 570	414, 542, 578	16

[a] At room temperature.

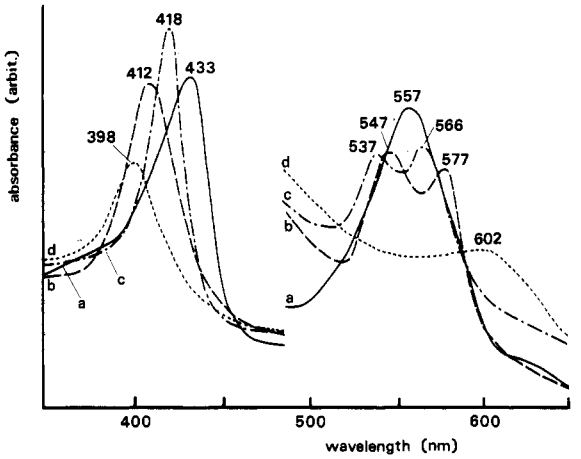


Figure 1. UV–vis absorption spectra of the PVP-bound heme **1b**: (a) deoxy; (b) oxygen adduct; (c) CO adduct; (d) oxidized; [**1b**] = 0.1 mmol/L, in pH 7.4 buffer solution/ethylene glycol (1/1) at −30 °C.

a thickness of about 0.2 mg of iron per cm^2 were kept at 77 K. The Doppler velocity was calibrated with natural iron foil kept at room temperature and zero velocity was taken as the centroid of its Mössbauer spectrum at room temperature. The spectra were fitted to Lorentzian line shapes by using the least-squares fitting program. Statistical uncertainties were 0.01 mm s^{-1} for all Mössbauer parameters.

Results and Discussion

The UV and vis absorption spectra of **1b** are shown in Figure 1, and the absorption maxima of **1**, **2**, and **3** are listed in Table I. For example, the spectrum of the deoxy species **1b** agrees with that of **3b** which has been assigned to a pentacordinate heme complex.[16]

3a and **3b** are known to be immediately oxidized on exposure to oxygen. Irreversible oxidation was also observed for the **3** solution at 30 to −30 °C and in the presence of 2% PVP or dextran. However, when the deoxy and dark red solution of PVP-bound heme **1** was cooled to −30 °C and exposured to oxygen, it became brilliant red and produced a spectrum, which resembles that of oxy-Hb (e.g., as seen in Figure 1 for **1b**). Spectra of the oxygen adducts could be changed to those of the carbon monoxide (CO) adducts by bubbling CO through the polymer solution. Spectra of the deoxy complexes reappeared when nitrogen gas was carefully introduced. This suggests that the oxidation number of the central iron ion (Fe(II)) has not been changed during the exposure to oxygen.

Any contribution of the reducing agent to this reversible oxygenation is contraindicated by the following results. (i) The deoxy heme complex was prepared by reducing the iron(III) derivative with a small excess of sodium dithionite, no trace of which remained before the oxygen exposure. (ii) When dithionite was added again after the oxygenation, the oxy heme was reduced to the deoxy heme in the same manner as oxy-Hb was. (iii) The deoxy heme prepared with organic reductants, such as ascorbic acid, glucose, or the reductase system also formed the oxygen adduct with same absorption spectrum and lifetime.

Table II
Lifetime of the Oxygen Adduct of the Polymer-Bound Hemes[a]

heme	ethylene glycol content, vol %	temp, °C	lifetime τ, min
PVP–heme **1a**	50	−30	80
PVP–heme **1a**	50	−15	10
PVP–heme **1a**	70	−30	44
PVP–heme **1a**	40	−20	18
PVP–heme **1b**	50	−30	60
dextran–heme **2a**	50	−30	5
dextran–heme **2b**	50	−30	4
heme **3a**	50	−30	0
heme **3b**	50	−30	0

[a] In the pH 7.4 buffer solution/ethylene glycol.

The dextran-bound hemes **2** show the same UV–vis absorption spectra for the deoxy, oxy, and CO adducts of **1** (Table I), although the oxygen adducts of **2** were only observed by cooling the solution to −30 °C.

Recently Allcock et al.[18] reported that the water-soluble poly(phosphazene)-bound iron(II)–protoporphyrin IX mono[*N*-[3-(imidazol-1-yl)propyl]amide] gives a stable oxygen adduct at −30 °C in dimethylformamide but is irreversibly oxidized in aqueous ethylene glycol (1/1) solution at −30 °C. Bayer and Holtzbach[19] report reversible oxygenation of histidylheme bound to poly(ethylene oxide) in an aqueous medium at room temperature, but the poly(ethylene oxide)-bound heme synthesized by us shows only a simple irreversible oxidation in the aqueous ethylene glycol solution at 30 to −30 °C. These results indicate that only PVP-bound heme can form a semistable oxygen adduct in cooled aqueous solution probably due to the hydrophobic property of PVP.

The oxygen adducts are not stable and slowly degrade to the iron(III) derivative through isosbestic points as seen in the change of the visible absorption spectrum. Its degradation obeys first-order kinetics from which the lifetime (half-life period) of the oxygen adducts can be calculated (Table II). The lifetime of ca. 1 h for **1** at −30 °C is longer than that of poly(1-vinyl-2-methylimidazole) heme (in pH 10 aqueous ethylene glycol solution; 24 min at −30 °C) previously reported by us[6] and much longer than those of the dextran-bound hemes **2**. Table II also shows that the oxygen adduct is observed regardless of the ethylene glycol content. The oxygen adduct is not observed for polymer-nonbound protoheme analogues alone or with 2% PVP.

The lifetime of the oxygen adduct of **1a** depends on the pH of the aqueous buffer solution mixed with ethylene glycol: 6 h at pH 10 (0.2 mol/L carbonate buffer), 80 min at pH 7.4, and 12 min at pH 6 (0.067 mol/L phosphate buffer). Its lifetime is independent of the heme concentration, ranging from 0.15 to 0.01 mmol/L. These data

Table III
Oxygen and Carbon Monoxide Binding Rate Constants and Affinities for the PVP-Hemes[a]

heme	O_2			CO			ref
	k_{on}, M^{-1} s^{-1}	k_{off}, s^{-1}	p_{50}, mmHg	k_{on}, M^{-1} s^{-1}	k_{off}, s^{-1}	p_{50}, mmHg	
PVP-heme **1a**	7.9×10^5	5.0	3.8	1.0×10^5	0.0021	0.027	this work
PVP-heme **1b**	$.5 \times 10^5$	4.2	17	4.6×10^4	0.0063	0.75	this work
heme **3a**[b]				2.4×10^6	0.020	0.0060	this work
heme **3b**[b]				7.7×10^5	0.63	0.60	this work
heme **3a**	2.6×10^7[c]	47[c]	1.0[c]	3.6×10^6	0.0089	0.0018	20
Hb (R)	3.3×10^7	12–13	0.22–0.36	4.6×10^6	0.0090	0.0014	21–23
Hb (T)	$(0.29–1.2) \times 10^7$	180–2500	40–140	2.2×10^5	0.090	0.30	21–23
Mb	$(1.0–2.0) \times 10^7$	10–30	0.46–0.72	$(3.0–5.0) \times 10^5$	0.0017–0.040	0.018–0.034	16

[a] In the pH 7.4 buffer solution/ethylene glycol at room temperature (20 °C). [b] The UV–vis absorption maxima of the deoxy **3** are consistent with those of the deoxy **1** in Table I, which indicates that **3** is solubilized without aggregation in the aqueous solvent. [c] Estimated by the CO protection method.

suggest that the irreversible oxidation to Fe(III) of the oxygenated heme proceeds mainly via a unimolecular process caused by the attack of a proton on the heme-coordinated oxygen (proton-driven oxidation) rather than via a binuclear μ-dioxo dimer.

From these results, it is connsidered that the proton-driven irreversible oxidation has to be suppressed for oxygenation in aqueous media and that by combining the five-coordinate heme complex with a water-soluble but hydrophobic polymer the oxygen adduct can be observed in the cooled aqueous medium due to the hydrophobic environment around the oxygen adduct.

Oxygen binding kinetic parameters, $k_{on}(O_2)$ and $k_{off}(O_2)$, and carbon monoxide binding kinetic parameters, $k_{on}(CO)$ and $k_{off}(CO)$, for the polymer-bound hemes **1** have been estimated by a stopped flow method, as follows. Because

$$PFe^{II} + O_2 \text{ (or CO)} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} PFe\text{–}O_2 \text{ (or –CO)} \qquad (1)$$

the oxygen adducts of **1** have short lifetimes even at room temperature, the oxygen binding and dissociation rate constants could be estimated from the spectral change within ca. 1 s. By changing the monitoring wavelength from 390 to 450 nm, a difference spectrum before and after the rapid mixing of the deoxy solution with oxygen-saturated solution was obtained. Negative and positive extremes in the difference spectrum, 412 and 433 nm, were selected as the monitoring wavelengths, e.g., for **1b** (Figure 2b). These absorption wavelengths were in accord with the absorption maxima of the oxygen adduct and the deoxy heme, respectively. The validity of this was supported further by the following two results. (i) The absorbance at 417 nm remains constant before and after the rapid mixing (Figure 2a); this wavelength is the same as that of the isosbestic point between the oxygen adduct and the deoxy heme. (ii) The time courses at 412 and 433 nm are symmetric (Figure 2a). For the CO binding, the reactions were followed at 418 and 433 nm, e.g., for **1b**. The reproducibility of the measurements was confirmed by using several batches of the sample.

The time curves for the oxygen and CO binding were that of a monophase one, which was approximated by first-order kinetics, $f(x) = \ln [1 - (x/a)]$, where a is a constant, within the experimental error (e.g., Figure 2b). From the slope of the plots, the k_{obsd} for the oxygen (or CO) binding was obtained. The rate constants k_{on} and k_{off} were calculated from the relation of k_{obsd} with the oxygen or CO concentration:

$$k_{obsd} = k_{on}[O_2 \text{ (or CO)}] + k_{off} \qquad (2)$$

Flash photolysis measurement was also applied to determine the rate constants for the CO binding and dissociation. The $k_{on}(CO)$ and $k_{off}(CO)$ values measured by flash photolysis approximately agreed with those by the stopped flow method.

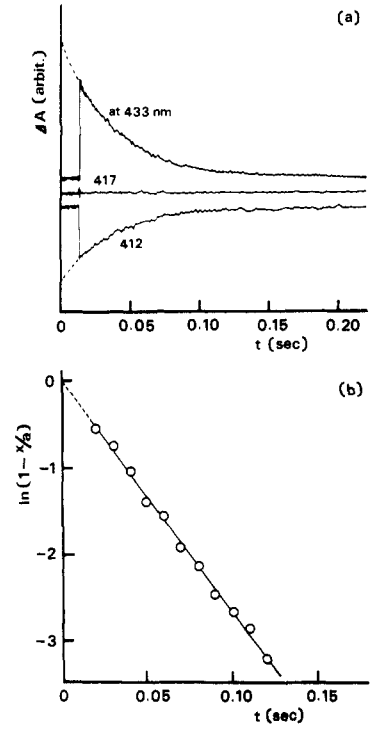


Figure 2. Oxygen binding of the PVP-bound heme **1b** monitored at 412, 417, and 433 nm (a), and the approximation to first-order kinetics (b). ΔA = Differential absorbance; a and x = differential absorbance at 433 nm at time 0 and t, respectively.

The kinetic values are listed in Table III together with reference data. The $k_{on}(CO)$ value for **3a** measured in the present work was approximately consistent with that previously reported by Traylor et al.[20] This also supports the validity of the measurement in the present experiment. $k_{on}(O_2)$ and $k_{on}(CO)$ of the polymer-bound hemes are smaller than those of the nonbound analogues. The heme-bonded polymer chain is considered to sterically retard the coordination rate of gaseous molecules to the heme, while it constructs a relatively hydrophobic environment around the heme and protects the oxygen adduct in the aqueous medium.

The oxygen-binding affinity, $p_{50}(O_2)$, i.e., the oxygen pressure at 50% oxygen binding to heme, was calculated from K ($=k_{on}/k_{off}$) for the polymer-bound hemes and is also given in Table III. It has been suggested that an environment of amide residues around heme stabilizes the heme-oxygen bond and increases the oxygen-binding affinity in the same manner as the heme complexes of Hb and myoglobin (Mb) surrounded with a peptide environment shows higher oxygen-binding affinity. In addition,

Table IV
Mössbauer Parameters[a]

species	δ, mm s^{-1}	ΔE_Q, mm s^{-1}	ref
PVP–heme 1a deoxy	0.95	2.06	this work
PVP–heme 1a carboxy	0.23	0.38	this work
PVP–heme 1a oxy	0.25	2.11	this work
Hb deoxy	0.91	2.26	25, 26
Hb carboxy	0.26[b]	0.26[b]	25, 26
Hb oxy	0.26	2.19	25, 26

[a] Values at 77 K. [b] Values at 4.2 K.

the oxygen-binding affinity of the heme analogous compounds in amide solvents such as dimethylformamide is enhanced in comparison with those in nonpolar solvents.[9,23] But in Table III one notices that the oxygen-binding affinity of the PVP-bound heme is smaller ($p_{50}(O_2)$ is larger) than that of the PVP-nonbound heme analogue. The smaller $k_{on}(O_2)$ value of the polymer-bound heme brings about the lower oxygen binding affinity (larger $p_{50}(O_2)$ value) in comparison with those of the nonbound analogue, and the resultant oxygen-binding affinity of the polymer-bound hemes resembles that of Hb in T state.

Mössbauer parameters, the isomer shift (δ) and quadrupole splitting (ΔE_Q), of the deoxy, carboxy, and oxy PVP–heme are summarized in Table IV with the reference data. For the deoxy PVP–heme, the δ value is larger than that for deoxy Hb, which suggests that the fixation of the imidazole ligand with spacer group and/or the surrounding polymer matrix causes the iron–imidazole bond to weaken. The Mössbauer parameters of the oxygen adduct also were measured for the PVP–heme. The parameters are comparable with those of oxy-Hb; this means that the iron ion in the oxygen adduct is in the ferric low-spin state also for the synthetic polymer-bound heme.

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Registry No. **2**, 9004-54-0; **3a′**, 96964-15-7; **3a′** ^{57}Fe derivative, 108919-00-2; **3b′**, 108918-99-6; CO, 630-08-0; O_2, 7782-44-7; protoporphyrin IX mono[*N*-(5-methylimidazol-1-yl)pentyl]amide mono(ethyl ester), 78897-43-5; 1-(5-aminopentyl)-2-methylimidazole, 78881-19-3; PVP-*co*-(4-aminostyrene), 79031-51-9; 4-aminostyrene, 1520-21-4; (4-aminophenyl)ethyl alcohol, 104-10-9; ethyl chloroformate, 541-41-3; dextran carbonate, 37359-17-4; ethylenediamine, 107-15-3; dextran amino derivative, 75301-70-1.

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Chain Dimensions in Deformed Networks: Theory and Comparison with Experiment

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ABSTRACT: Expressions for the transformation of the mean-squared chain vector and of the mean-squared radius of gyration in a deformed network are obtained according to the constrained junction model of rubber elasticity. Differences between the behavior of chain vectors and radii of gyration are indicated. Dependence of the state of microscopic deformation on the type of macroscopic strain, network chain length, junction functionality, and the extent of dilation during cross-linking is investigated. The microscopic state of strain is described completely in terms of the material parameters κ and ζ of the constrained junction model. Predictions of the theory are compared with results of small-angle neutron-scattering experiments reported by various investigators. Experimentally observed deviations of chain dimensions from those expected from the phantom and affine network models are explained with the present theory.

Introduction

The state of strain at the molecular level in a deformed network in equilibrium differs from the externally applied macroscopic state of strain. Recent results of neutron-scattering experiments on various polymeric networks show that the transformation of chain dimensions is significantly

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