Adsorption and Desorption of Molecular Oxygen in Solid State of Polymeric Hemochrome

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The oxygenation of a pyridine-hemochrome bound to a polymer ligand has been studied in solid state. The oxygenation rate and the amount of oxygen adsorbed were determined by volumetry. The rate of adsorption of oxygen depends upon the structure of polymer matrix. Pyridine hemochrome included in a polyion complex takes up oxygen more rapidly than others. The rate of adsorption depends a great deal upon the surface condition of polymer matrix in case of oxygen binding in solid state. The degree of oxygenation was nearly equal in all cases. The difference in the rate of adsorption of oxygen may be due to the difference in gas permeability of the polymer matrix. This was supported by the fact that the hemochrome bound to a porous polymer matrix adsorbs oxygen more rapidly than the hemochrome bound to a non-porous polymer matrix. The surface condition of the polymer matrix greatly affects the reversibility of oxygenation.

An artificial oxygen carrier which can reversibly take up and release molecular oxygen with a relatively small difference in the partial pressure of oxygen in solution or solid state would meet extensive requirements.

Pfeiffer *et al.*[1] found the reversible binding of molecular oxygen of *N,N'*-ethylene bis(salicylideneiminato)-cobalt(II) complex (salcomin) in crystalline state. Calvin *et al.*[2] developed reversible oxygenation systematically for a series of salcomin derivatives in solid state. Studies on a reversible oxygen complex have actively been made in various fields. However, no metal complex which can reversibly take up oxygen in a neutral aqueous solution at room temperature has been synthesized. The failure in synthesizing a stable oxygen complex is due to the rapid irreversible autoxidation of a central metal ion. Wang *et al.*[3] synthesized the imidazole-hemochrome as a hemoglobin model included in polystyrene film and examined the stability and reversibility of the oxygen complex imbedded in the film in the presence of water.

The present authors[4-9] have found that oxygen complex can exist in a stable form even in aqueous dimethylformamide solution at room temperature by modification of a polymer ligand of heme-iron. Recent studies on oxygen complex have been made mainly in organic solvents.[10-15] In contrast, few studies in solid state have been carried out except for the series of Calvin's studies. In this paper, the oxygenation of the polymer-heme complex in solid state will be discussed. The correlation of the oxygenation reactivity to the surface condition of the polymer matrices has been studied.

Experimental

Materials. Poly(4-vinylpyridine) (PVP; $\overline{P}_n=49$) and partially-quaternized poly(4-vinylpyridine) (QPVP: $\overline{P}_n=49$, 24% of quaternization by benzyl chloride) were prepared and purified according to the conventional method.[4,9] Polystyrene sulfonic acid (PSS; $\overline{P}_n=321$, sulfonation=77%), polyglutamic acid (PGA; $\overline{P}_n=331$) and alginic acid (Alg) were purified according to directions reported in previous papers.[16,17] Isolation of chlorohemin from blood was carried out by Willstatter's method.[18] Sodium dithionite ($Na_2S_2O_4$) of a reagent grade was used without purification and α,α'-azobisisobutyronitrile(AIBN)was recrystallized from methanol.

Preparation of Powdery Polymer Matrix Including Hemochrome. A powdery sample of a polymer-bound hemochrome was prepared as follows. Chlorohemin (150 mg) was dissolved in 150 ml of an aqueous solution (pH=12). An aqueous solution (20 ml) containing 4.0 g of $Na_2S_2O_4$ was added, and then 150 ml of an aqueous solution containing 0.6 g of Q PVP was added immediately in an atmosphere of nitrogen. The complex formation between heme and Q PVP was accompanied by a color change from black brown to reddish brown. Another component of polyion such as PSS, PGA, and Alg was added in order to form a polyion complex; An aqueous solution (80 ml) containing PSS (0.38 g), PGA (0.35 g) or Alg (0.38 g) was slowly added to the above solution. The resulting precipitate was filtered off and dried completely *in vacuo* at 70—80 °C. This type of polymer-heme complex is called "complexed-type hemochrome".

Another type of polymer-heme complex "single-type hemochrome" was also prepared. PVP (1.06 g) and chlorohemin (68 mg) were dissolved in 150 ml of methanol. Methanol (140 ml) containing 0.9 g of L-ascorbic acid was then slowly added with vigorous stirring in an atmosphere of nitrogen. The resulting reddish solution was evaporated and dried at 50 °C *in vacuo*.

The content of heme of the polymer complex was determined by spectrometry to be 5.0×10^{-5} mole per unit gram of the polymer matrix. The powdery sample used for oxygenation analysis was ground to particles under 48 mesh.

Measurement of Magnetic Susceptibility. Metal complexes used were the monomeric and polymeric pyridine hemichromes. The measurement of magnetic susceptibility of powdery samples was carried out by the Faraday method. A magnetic torsion balance was operated at a field strength of 10000 G over the temperature range from liquid nitrogen temperature to room temperature. Gd_2O_3 was used as a standard sample. The magnetic susceptibility was recorded automatically and continuously as a function of temperature on an X-Y recorder. The apparatus used for the measurement is the same as that reported by Kohn.[19] The errors inherent in this method of analysis are of the order of ten per cent.

Manometric Measurement of Oxygen Uptake in Solid State. The time-conversion curves of oxygenation in solid state of polymer-bound hemochrome were obtained by use of a Warburg apparatus. Heme (1.0×10^{-5} mole) imbedded in 0.2 g of the matrix was put into a cuvette in an atmospheric oxygen pressure, the amount of oxygen adsorbed by the powdery sample being checked by use of a manometer. The amount of oxygen adsorbed by the hemochrome was corrected with a small quantity of oxygen adsorbed by the

powdery polymer and an excess of the reductant.

Results and Discussion

Coordination Structure of the Polymer-Heme Complex. The coordination numbers ($\bar{n}$) of the axial ligand and the equilibrium constants (K) of the hemichrome and hemochrome were determined by the method of Miller and Dorough.[20] The parameters $\bar{n}$ and K were calculated on the basis of the analysis of the spectrophotometrical titration for the complexation between hemin or heme and various bases. Application of this method to polymer ligands was reported previously[4,9]

The results given in Table 1 show that the axial 5th site of heme-iron is occupied by the pyridine molecule of PVP or QPVP. Another axial site of heme-iron might be either occupied by water or vacant. However, all the coordination numbers ($\bar{n}$) of the axial ligands were intermediate between 1.0 and 2.0 in both cases of the hemichrome and hemochrome. It is reasonable to consider that the six-coordinate pyridine hemichrome or hemochrome, whose axial sites are both occupied by two pyridine molecules, is mixed with the five-coordinate complex. The result of magnetic susceptibility also suggests that the high spin complex and the low spin complex are in equilibrium, because the molar magnetic susceptibility (X_M) of the pyridine hemichrome was intermediate between the calculated values of the high spin and low spin states (Table 1). From the temperature dependence of the magnetic susceptibility (Fig. 1), it was found that the high spin complex and the low spin complex are in spin equilibrium at the iron(III) state.[21] On the other hand, the equilibrium constant of spin exchange between the high spin state and the low spin state of the heme-ion(II) does not depend upon temperature. The observed value of the magnetic susceptibility was nearly equal to the calculated one of the high spin complex. Thus, in the case of the hemochrome, the six-coordinate complex can hardly be mixed with the five-coordinate complex.

Table 1. Axial coordination numbers ($\bar{n}$) and magnetic susceptibility of hemichrome and hemochrome bonded to polymer ligand

Axial base	$\bar{n}$		λ_{max} (nm)		$\chi_M \times 10^6$ (e.m.u.)
	Fe^{3+}	Fe^{2+}	Fe^{3+}	Fe^{2+}	Fe^{3+} at 0 °C
Py	1.02	1.11	424	418	13900
PVP	0.95	1.32	424	418	7900
QPVP	1.30	1.25	397	419	14200
QPVP-PSS	—	—	397	422	9900

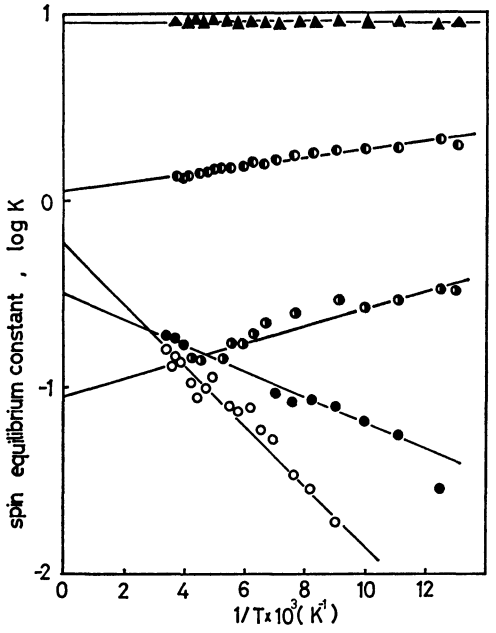

Fig. 1. Correlation of the spin equilibrium constant to inverse temperature for various types of polymer-hemin complexes.
◐ QPVP, ◑ QPVP-PSS, ● Py, ○ PVP, ▲ QPVP-PSS-Heme.

Correlation of the Reactivity of Oxygenation to the Surface Condition of the Polymer Matrix. The oxygen adsorption curves of the complexed-type and the single-type polymeric hemochromes are shown in Fig. 2. Table 2 gives the initial rate, the half-saturation time and the degree of oxygen-saturation for the various types of polymer matrices. We see that the initial rate of the complexed-type system was appreciably larger than that of the single-type system. In general, the rate of adsorption of oxygen in solid state is affected by the diffusion of oxygen in solid matrix.[2b] The difference in the rate of adsorption between the complexed-type and single-type hemochromes may be due to the difference in the rate of diffusion of oxygen in the polymer matrices.

In order to clarify the correlation of the reactivity of oxygenation to gas permeability in the polymer matrix, the rate of adsorption of oxygen was checked for a porous polymer-bound hemochrome prepared by treatment of the matrix with a foaming agent such as

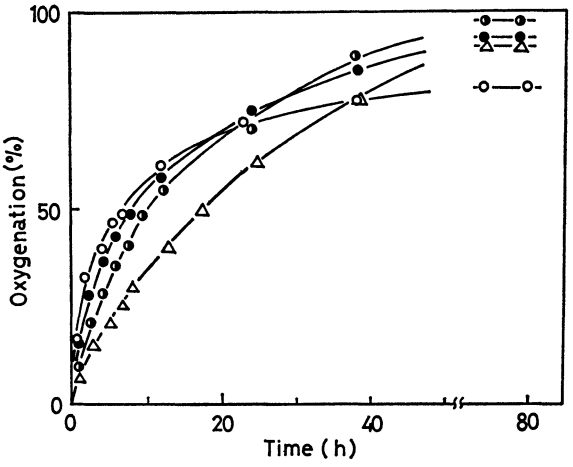

Fig. 2. Time conversion curve of oxygenation of polymer-bound hemochromes in solid state.
Matrix: ○ QPVPP-GA, ◑ QPVP-Alg, ● QPVP-PSS, △ PVP.
Heme: 1.0×10^{-5} mol/0.2 g polymer, at 30° C.

Table 2. Oxygenation of polymeric hemochrome in polymer matrix

System	Matrix	V_i (μl/mol)[a]		T_{50} (h)[b]		D_s (%)[c]	
		solid	dispersion[d]	solid	dispersion[d]	solid	dispersion[d]
Complexed	QPVP-PSS	3.00	65.8	7.5	0.58	93	92
	QPVP-PGA	3.80	96.5	6.6	0.50	80	90
	QPVP-Alg	1.40	60.1	9.7	0.60	100	95
Single	PVP	0.78	53.2	17.1	0.67	93	92

PVP-Heme dissolved in DMF/MeOH=1/1; V_i=280, D_s=92.
a) V_i: initial rate of oxygenation, b) T_{50}: time for half-saturation of oxygen, c) D: degree of saturation of oxygenation, d) dispersed in H_2O (pH=6.0), Heme=1×10^{-5} mol/0.2 g polymer matrix, at 30 °C.

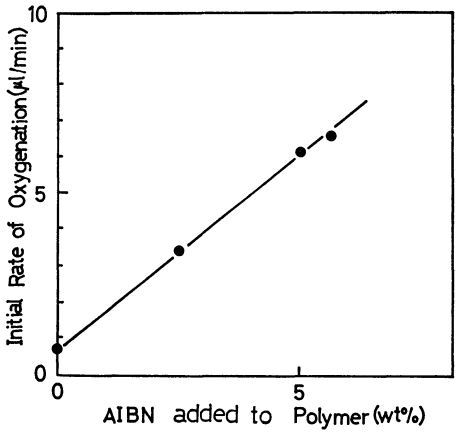

Fig. 3. Effect of porosity of polymer matrix on the oxygenation rate.
Heme: 1.0×10^{-5} mol/0.2 g polymer, at 30 °C.

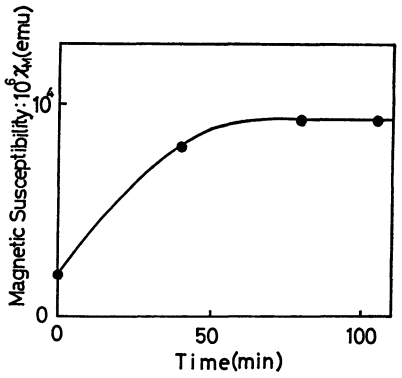

Fig. 4. Time conversion curve of magnetic susceptibility due to deoxygenation of a poly(vinylpyridine)-hemochrome.
X_M: Measured at 25 °C, deoxygenation: atmospheric, at 90 °C. Heme: 1.0×10^{-5} mol/0.2 g polymer.

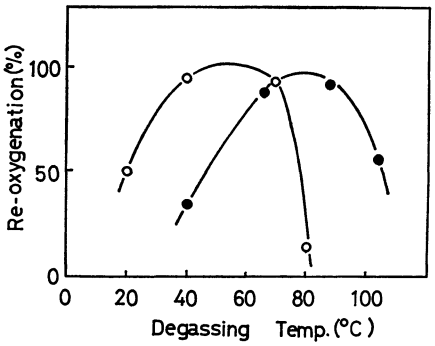

Fig. 5. Temperature effect on deoxygenation of a polymer-bound hemochrome.
Deoxygenation: 3 mmHg, for 1 h.

azobisisobutyronitrile (AIBN). Figure 3 shows the relationship between the initial rate of oxygen uptake and the amount of AIBN added. The adsorption rate is linearly proportional to the amount of AIBN. This might be due to the increase in porosity of the polymer matrix. The porosity of matrix is of importance for oxygen uptake in solid state.

Table 2 also gives the corresponding parameters of oxygenation of the dispersion system in water. The gas permeability calculated by the Fick's Law becomes very large in the presence of water owing to the formation of water-bridge.[2b] We found that the oxygenation rate of the dispersion system is much larger than that of the solid system. This may also be due to the difference in gas permeability between the solid system and the dispersion system. However, the rate of the dispersion system in water was still much smaller than that of the homogeneous solution system.

Reversible Desorption of the Oxygen Complex Bound to Polymer Matrix. The oxygen complex can reversibly desorb molecular oxygen under reduced pressure, by heating or in a nitrogen stream. When the PVP-heme complex was heated at 90 °C, molecular oxygen bonded to heme-iron(II) was reversibly dissociated from heme-iron(II) with increasing magnetic susceptibility (Fig. 4). The spin state of heme-iron(II) of the pyridine hemochrome changed from the low spin state to the high spin state with deoxygenation.

The reversibility of oxygen binding was affected by the temperature of degassing. Figure 5 shows a plot of the degree of re-oxygenation against the temperature. Deoxygenation is complete in heating above 40 °C for one hour. However, at much higher temperatures, the degree of deoxygenation decreases owing to the irreversible autoxidation. Figure 6 shows the reversibility of oxygenation for the solid system and the dispersion system of the PVP-heme and QPVP/PSS-heme complexes. It is due to the irreversible autoxidation that the degree of oxygenation gradually decreases with the number of cycles of deoxygenation. The autoxidation of the complexed-type hemochrome proceeded more rapidly than that of the single-type hemochrome and the autoxidation of the dispersion system in water proceeded much more rapidly.

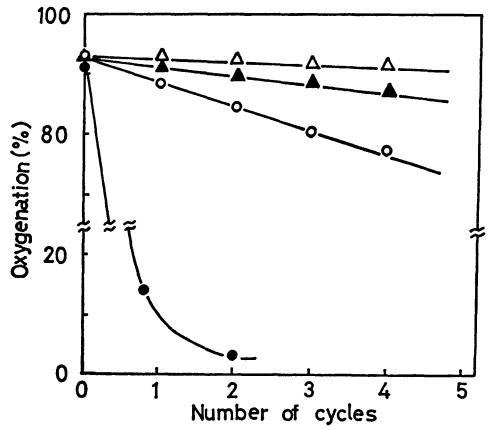

Fig. 6. Reversibility of oxygenation of some types of polymer-bound hemochrome.
PVP: △ solid, ▲ dispersion in H_2O.
QPVP-PSS: ○ solid, ● dispersion in H_2O.

In conclusion, oxygenation in solid state differs from that in solution in the following points: (1) The rate of oxygenation in solid state is smaller than that in solution. (2) Irreversible autoxidation scarcely occurs at room temperature in solid state. (3) The oxygen complex in solid state is much more stable than in solution, especially in comparison with the oxygen complex in an aqueous solution.

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