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REACTION OF POLYMER-HEME COMPLEXES WITH CARBON MONOXIDE OR MOLECULAR OXYGEN

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Summary Interactions of some polymer-heme complexes with carbon monoxide or molecular oxygen were studied spectrophotometrically and manometrically. Effects of additives on the reactions were also studied. It was found that the carbon monoxide equilibrium curve of the complex with poly-L-lysine was sigmoid and its cooperative parameter for the reaction was determined to be 2.1 by the Hill's plots. In the reaction with molecular oxygen, it was also sigmoid. However,other polymer-ligands except poly-L-lysine showed no cooperativity. From the results of the additive effects, it was suggested that a helical conformation of the polymer-ligand may play an important part in raising cooperativity.

Introduction The present authors recently reported formations of complexes of heme with polymer-ligands and their characteristics in the complex formation and in the reaction with molecular oxygen (1,2,3) and found a reversible oxygenation of the polymer-heme complexes in organic solvents at room temperature (1). In this communication, we report interactions of these polymer-heme complexes with carbon monoxide or molecular oxygen and discuss on the roles of the conformation of polymer-ligand in a cooperativity found.

Method and Materials Polymer-ligands, that is, poly-L-lysine, poly-4-vinylpyridine, and branched-type polyethyleneimine (degree of branching = 0.42) were prepared according to the previous papers (1,3). Hemin (ferri-protoporphyrine IX chloride) was prepared by the method of Willstaetter (4). 0.05M Phosphate buffer (pH12.0) and N,N-dimethylformamide were used as solvents. Structures and formation constants of heme complexes were determined spectrophotometrically using the method of Miller and Dorough (5). Absorption of carbon monoxide or molecular oxygen was determined by the use of a Warburg's manometer and by a spectrophotometer.

Results and Discussion Visible absorption spectra were measured on the reaction between a poly-L-lysine-heme complex and carbon monoxide in a 0.05M phosphate buffer (pH12.0). Spectral changes observed in a Q-band region of heme occured with isosbestic points and the absorption bands were still optically active even after the reaction with carbon monoxide, while heme in the absence of poly-L-lysine was not active. These results indicated that the complex formed is one with ε-amino group of poly-L-lysine and carbon monoxide as axial ligands of a central iron of heme. Figure 1 shows an absorption behavior of carbon monoxide by a poly-L-lysine-heme complex. Only the absorption curve by the complex with poly-L-lysine was found to be sigmoid, but others were hyperbolic. The Hill's coefficient (a cooperative parameter for a reaction,n) of the poly-L-lysine system was determined to be 2.1 from the slope of the straight line in Figure 1(B) by Hill's plots (6). This large value indicated

$$\log(Y/(1-Y)) = \log K + n \log P_{CO}$$

a cooperative interaction between heme complexes situated on an α-helical poly-L-lysine. As shown in Figure 2, such a cooperative reaction was also observed in the absorption of molecular oxygen by the poly-L-lysine-heme complex,

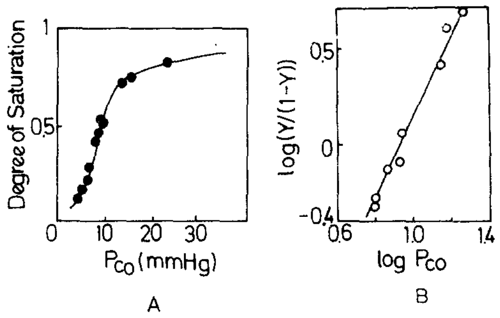

Figure 1 Carbon monoxide equilibrium curve of poly-L-lysine-heme complex (A) and Hill's plots (B) in phosphate buffer (pH12.0) at 25°C. $[\text{polylysine}]_0$= 45.5 unit-mmole/l, $[\text{heme}]_0$= 3.0 mmole/l, Total pressure of carbon monoxide and molecular nitrogen was kept constant (760 mmHg).

since n was equal to 2.0. This revealed that a cooperativity was raised not only in an equilibrium reaction but also in an absorption kinetics.

Subsequently, effects of types of polymer-ligands and their conformations were studied. When random-coiled polymer-ligands, that is, poly-4-vinylpyridine and branched polyethyleneimine, were used, no cooperative reaction was observed since a cooperative parameter was small (Table 1). Such a different behavior dependent on polymer-ligands may be caused by the differences in original structure of each complexes and conformation of polymer-ligands in solution. As previously reported, only a poly-L-lysine-heme complex has two axial ligands from the same polymer chain (3), which were summarized in Table 1. Therefore, P_{50} was higher in a poly-L-lysine system than in others because a ligand-exchange reaction by molecular oxygen was retarded in the former. The results in Table 1 suggest that it may be important to cause cooperative interaction : (a) a regular conformation of a polymer-ligand and (b) a structure of a heme complex with coordination number 2 before reaction. Actually, the fact that an α-helical content of poly-L-lysine in its heme complex increased after the reaction with carbon monoxide, although it had been partially destroyed by the complex formation with a heme due to steric hinderance

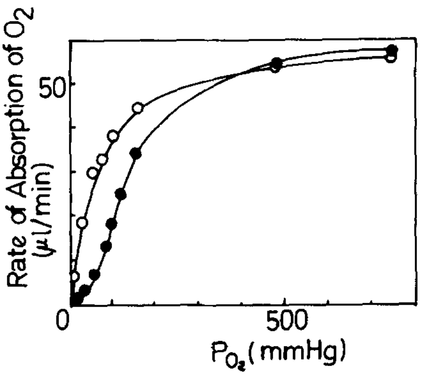

Figure 2 Absorption of molecular oxygen by poly-L-lysine-heme complex (●) and the effect of additive polyethyleneglycole (○). $[\text{polylysine}]_0$= 45.5 unit-mmole/l, $[\text{heme}]_0$= 6.0 mmole/l, $[\text{polyethyleneglycole}]_0$= 60 unit-mmole/l, Total pressure of oxygen and nitrogen was kept constant (760 mmHg) .

between heme and cylindrical poly-L-lysine (3),indicated that a disappearence or reduction of the strain of a helical structure by the reaction with carbon monoxide or molecular oxygen, which resulted in the formation of the complex with coordination number 1,may initiate a cooperative-binding through the polymer chain with regular conformation. However, in a poly-4-vinylpyridine or branched polyethyleneimine system, the complex with axial coordination number 1 are formed, so that such a dynamical change of the structure as expected in the poly-L-lysine-heme complex may not occur and in fact, it was confirmed by viscosity measurement of solution that no conformational changes of polymer-ligand was caused after the reaction. Therefore, no cooperativity may be caused in the systems concerning polymer-ligands of random structure or conformation.

Nextly, we studied the effects of additives on the absorption behavior of molecular oxygen by a poly-L-lysine-heme complex. When various polymers, which do not act as ligand but are able to interact with poly-L-lysine to form polymer aggregates, were added to the system, a cooperative parameter decreased as shown in Table 2. In these cases, it was found that an α-helical conformation was made to be instabilized by the formation of polymer aggregates (7). And no spectral changes was observed in visible region before and after the addition

Table 1 Effects of polymer-ligands on the absorption of molecular oxygen by polymer-heme complexes at 30°C

Polymer-ligand	λ_{max}(nm)	$\bar{n}$	K(20°C)	Solvent	n	P_{50}(mmHg)
poly-4-vinylpyridine	418	1.0	$1.1\times10^3 M^{-1}$	DMF/MeOH	1.1	58
poly-L-lysine	431	2.0	$2.5\times10^8 M^{-2}$	pH12 aq.	2.1	130
branched polyethylene-imine	424	1.0	$7.5\times10^4 M^{-1}$	pH12 aq.	1.0	100

n : Cooperative parameter ; $\bar{n}$: Axial coordination number of heme complex ; K : Formation constant of heme complex ; DMF :N,N-dimethylformamide ; MeOH : methylalcohol ; DMF/MeOH=7/3(v/v) ; λ_{max} Absorption maximum of deoxy-heme complex

Table 2 Effects of additives on the absorption behavior of molecular oxygen by poly-L-lysine-heme complex at 30°C in PH 12 buffer soln.

Additives	Cooperative parameter (n)	P_{50}(mmHg)	$\bar{n}$	K(20°C)(M^{-2})
None	2.0	130	2.0	2.5×10^8
polyethyleneglycole	1.3	62		
polyglutamic acid	1.7	80		
sodium chloride(0.1M)	1.7	140		
sodium chloride(0.4M)	1.3	270	2.0	4.6×10^8

of additive polymers, which shows that the overall structure of deoxy-heme complex was not affected at all by additive polymers. Therefore, the decrease in a cooperative parameter may be caused by the instabilization of an α-helical structure of poly-L-lysine, which may mean a destruction of an information transfer.

On the other hand,when potassium cyanide was used in place of carbon monoxide or molecular oxygen, no cooperative-binding was observed. This difference may not be due to the difference in size of molecule absorbed but due to the difference between a neutral and an ionic species. That is, the essential cause may be the ionic strength because the concentration of potassium cyanide enough to cause a ligand-exchange reaction with a poly-L-lysine-heme complex was much higher than those of molecular oxygen and carbon monoxide. Then, we studied the effect of neutral salt on the absorption behavior of molecular oxygen by a poly-L-lysine-heme complex. As shown in Table 2, with the increase of the concentration of sodium chloride, a cooperative parameter decreased, while the axial coordination number was kept constant and the formation constant increased. Therefore, the increase of P_{50} by the addition of sodium chloride can be explained by the increase in the stability of a heme complex. Generally, it is well known that a helical structure of polypeptides in an aqueous solution becomes instable by adding sodium chloride. So the decrease in a cooperative parameter observed may be explained by the instabilization of an α-helical structure of the polymer-ligand by salt.

This system consisting of poly-L-lysine and heme is extremely interest from the standpoint of a synthetic model of cooperative systems. We are now studying factors by both experimental and theoretical methods of approach. It wiil be reported in the near future.

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