CHEMISTRY LETTERS, pp. 1727-1730, 1989.

Oxygenation of Porphinatoiron(II) Complexes with Imidazole-Containing Glycerophosphocholines in Phospholipid Bilayers

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Diacylgylecero-3-phosphocholine derivatives having an imidazole ligand at the terminal of an acyl-chain on the 2nd position of glycerol backbone were synthesized. The amphiphilic lignds formed lipid bilayers with phospholipids and the heme complexes gave oxygen complexes in water (pH 7.4) at 25 ℃.

Hemoglobin and myoglobin contain a heme as a prosthetic group and oxygen is bound to the heme iron located at the center of the porphyrin ring. Various factors control the reactivity of heme complexes. For example, axial ligands of natural hemoproteins mainly determine their functions in vivo. Although many ironporphyrin-based models for oxygen-carrying hemoproteins have been prepared, those were designed with primary attention to the modification of a planar porphyrin ligand without considering the roles of axial ligands.[1] Simple ligands such as 1- or 2-methylimidazole, 1,2-dimethylimidazole etc. are used. However, an interesting approach has appeared by ligand-containing cyclophane[2] and basket-handle hemes,[3] in which an axial ligand is hung and fixed by a bridge on the porphyrin ring. It has been demonstrated that an axial ligand has an important role in the reversible oxygen binding of a specially modified amphiphilic heme, 5,10,15,20-tetra(α,α,α,α-o-(2',2'-dimethyl-20'-(2"-trimethylammonioethylphosphonatoxy)eicosanamido)phenyl)porphinatoiron(II) (1), which can bind oxygen at 37 ℃ in water (pH 7.4) when it is embedded in the hydrophobic region of phospholipid bilayers.[4] A hydrophobic ligand but not low-hydrophobic one can form a complex with the amphiphilic heme (1) in the bilayers to give an oxygen adduct. In this work, amphiphilic ligands, 1-tetradecanoyl-2-(11-(1-imidazolyl)- and 11-(1-(2-methylimidazolyl))-sn-glycero-3-phosphocholine (2a and 2b), were synthesized, and the oxygenation reaction of their complexes with 1 in lipid bilayers was studied thermodynamically and kinetically to elucidate their effects on the reactivity of 1.

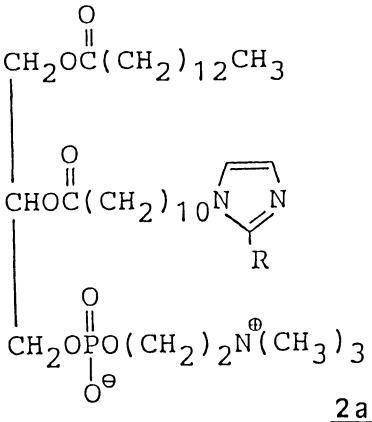
The synthetic procedures for the amphiphilic ligands, 2a and 2b, are as follows. 1,2-Ditetradecanoyl-sn-glycero-3-phosphocholine (3, DMPC) was hydrolyzed by phospholipase A_2 in 25 mmol dm^{-3} Tris buffer (pH 8.0) having 0.08 mol dm^{-3} $CaCl_2$ at 37 ℃ with vigorous stirring for 2 days. 1-Tetradecanoyl-sn-glycero-3-phosphocholine (4) was obtained by chromatographic purification on silica gel

eluted with chloroform/methanol/water (65/25/4) (yield, 52%). On the other hand, the lithium salt of imidazole was allowed to react with t-butyl 11-bromo-undecanoate (5a) in tetrahydrofuran under reflux for 12 h to give t-butyl 11-(1-imidazolyl)undecanoate (5b) in 85% yield. 5b was hydrolyzed in trifluoroacetic acid at room temperature to give 11-(1-imidazolyl)-undecanoic acid (5c) after chromatographic purification on silica gel (yield, 64%; mp, 93.5-95.5 ℃). 11-(1-(2-Methylimidazolyl))undecanoic acid (6c) was also prepared in a same manner as described above (yield, 41%; mp, 96-98 ℃). The carboxylic acids, 5c and 6c, underwent condensation with 4 in N,N-dimethylformamide in the presence of dicyclohexylcarbodiimide and 4-(N,N-dimethylamino)pyridine under argon atmosphere at room temperature for 1 day. After chromatographic purification on silica gel (chloroform/methanol/water), 2a and 2b were obtained in the yields of 40% and 20%, respectively. All the analytical data (FAB MS, IR, ^{1}H or ^{13}C NMR, elemental analyses, and TLC (silica gel)) confirmed the structures of the products.[5)]

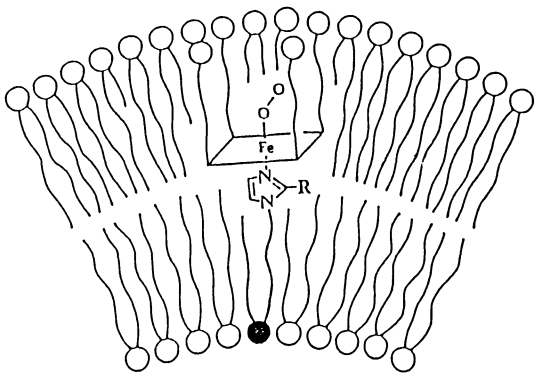
A lipid-suspension was prepared in water by a thin film method with a Vortex mixer. Transmission electron microphotographic measurements by staining with uranyl acetate showed the formation of multilamellar vesicles (diameter:0.2-0.4μm), indicating that 2a and 2b can form bilayers by themselves.

The heme complexes were embedded in liposome by the method described in literatures.[4)] For example, 1 (0.5 μmol), ligand 2a (1 μmol) or 2b (2.5 μmol,) and phosphatidylcholine 3 (25 or 100 μmol) were dissolved in chloroform(1 ml)/methanol(1 ml), and the solvents were removed by evaporation under reduced pressure to form thin films in a round-bottomed flask. To this was added 10 ml of N_2-saturated 1/30 mmol dm^{-3} phosphate buffer (pH 7.4) and then it was ultrasonicated and homogenized in an ice-water bath under N_2 atmosphere (40W, 20 min). The iron(II) complexes were prepared by the reduction with sodium dithionite (30-fold mol relative to the content of the iron(III) complexes) under N_2 atmosphere for a half hour. The average diameter of the liposomes was 30 nm determined by a submicron particle analyzer (Coulter Electronics, N4SD).

The visible absorption spectra under N_2 atmosphere of 2a and 2b complexes with 1 indicated formation of the deoxy hexacoordinated heme (λ_{max}: 435, 536 nm) and the deoxy pentacoordinated heme (λ_{max}: 439, 565 nm), respectively. The ligand titration measurements showed that the amount of 2b for preparing the deoxypenta-



2a ; R= H
2b ; R= CH3



Liposome/1/2a or 2b

coordinated 1 complex is one fifth of that of 1-dodecyl-2-methylimidazole (abbreviated as L2MIm). The heme complexes formed oxygen complexes at 25 ℃ by bubbling oxygen gas through the suspensions. The visible absorption maxima under O_2 atmosphere were 421 and 548 nm for the 2a complex and 421 and 546 nm for the 2b complex, which correspond to those of the oxygen complexes.[4,6] The spectra under O_2 atmosphere reversibly returned to those of the deoxy complexes by bubbling N_2 gas through the suspensions. By bubbling carbon monoxide gas through the oxygenated solutions, those of the carbon monoxide complexes (λ_{max}: 425, 544 nm for 2a, 424, 543 nm for 2b) were observed. They showed stability against autoxidation and the half-life times at 25 ℃ were 6 hours and 3 hours for the 2a and 2b complexes, respectively.

Values of the affinity and kinetic constants for O_2 binding are summarized in the Table. The oxygen binding affinity (P_{50}) was determined by measuring absorption change at 439 nm due to the heme at partial oxygen pressure. Well defined isosbestic points (λ: 425, 456 nm) which indicate a simple equilibrium between deoxy and oxy complexes of 1/2b were observed. The oxygen binding and dissociation equilibrium curve for the 1/2b complex is hyperbolic, indicating no interactions between hemes (i.e. Hill's coefficient(n)=1.0) and P_{50} is 9 mmHg at 25 ℃. This indicates there is rather higher affinity in comparison with those of the complexes of L2MIm with a picket-fence heme[6] (7) or 1 embedded in 3 lipsomes. The thermodynamic parameters (ΔH, ΔS) indicate the high affinity is driven by an entropy effect. The small kinetic constants for O_2-binding and dissociation show that both the on- and off-reaction are retarded considerably in the 1/2b complex embedded in liposomes, but the ratios (the equilibrium constants, $K(O_2)$) are in the same order of magnitude. This indicates that in both reactions the preformed complexes are much more stable against structural changes during reactions. The activation parameters for the oxygen binding reaction of the 1/2b complex indicate the presence of a high reaction barrier and the formation of a weak complex in the transition state. These results are well understood by considering a difference in nature of axial ligand; that is, the hydrophobic ligand (L2MIm) and the

Table 1. Oxygen binding parameters for various deoxy pentacoordinated iron(II) porphyrine at 25 ℃

	Solvent	P_{50}	$k(O_2)on$	$k(O_2)off$	$K(O_2)$	$\Delta H^{g)}$	$\Delta S^{f,g)}$	$\Delta H^{\ddagger}on^{g)}$	$\Delta S^{\ddagger}on^{f,g)}$
		mmHg	$dm^3mol^{-1}s^{-1}$	s^{-1}	dm^3mol^{-1}	kcal mol^{-1}		kcal mol^{-1}	
1/2b/DMPC	H_2O[a)]	9	8.3×10^2	1.2×10^{-2}	6.8×10^4	-10	-22	13	24
1/L2MIm/DMPC	H_2O[a)]	53	9.8×10^7	8.2×10^3	1.2×10^4	-15	-40	10	13
7/L2MIm/DMPC[b,c)]	H_2O[a)]	49	7.9×10^3	3.2×10^{-1}	2.5×10^4	-14	-42	10	-7.3
7/L2MIm[c)]	toluene	——	1.1×10^8	——	——	——	——	10	11
Chelated heme[d)]	H_2O	——	2.6×10^7	4.7×10^1	5.5×10^5	-14	-35	10	11
Hemoglobin in red blood cell[e)]	H_2O[a)]	27	1.1×10^4	1.6×10^{-1}	6.8×10^4	-14	-41	——	——
Myoglobin[e)]	H_2O[a)]	0.5	1.5×10^7	10,30	0.8×10^4	-13,-21	-35	——	——

a) Phosphate Buffer(pH 7.4). b) E.Tsuchida et al., J.Chem.Soc., Dalton Trans., 1985, 65. c) From Ref.4. d) T.G.Traylor and A.P.Berizinic, Proc.Natl.Acad.Sci.,U.S.A., 77, 3175(1980), Phosphate Buffer(pH 7.3). e) E.Antonini and M.Burunori,"Hemoglobin and Myoglobin in Their Reactions with Ligands," North-Holland, Amsterdam (1971). f) in cal K^{-1} mol^{-1} unit. g) 1 cal = 4.184 J.

amphiphilic ligand 2b. Thus 2b is more compatible with lipid bilayers and well arranged in the bilayer of phospholipid molecules 3. Therefore, not only heme 1 but also ligand 2b are well fixed within the bilayers. Then, in the oxygen binding reaction the structural change from the pyramidal deoxy iron-porphyrin to the planar oxy one[7,8] should be retarded, while in the former complex the axial ligand (1-dodecyl-2-methylimidazole) can move easily in the bilayers during the on-reaction. The same consideration is also applicable to the off-reaction, where the preformed complex is the planar oxygen complex.

Thus the restrain model from the kinetic viewpoint, which is originally proposed for the cooperative binding of O_2 by hemoglobin (the so-called "T-state"),[7] is constructed by arranging both amphiphilic heme and amphiphilic imidazole ligand in phospholipid bilayers.

This work is partially supported by Grant-in-Aids for Scientific Research on Priority Area of Macromolecular Complexes from the Ministry of Education.

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5) 2a: ^{1}H NMR ($CDCl_3$, TMS) δ 3.28(9H, S, choline methyl), 6.90, 7.10, 7.50(3H, s, imidazole). ^{13}C NMR ($CDCl_3$, TMS) δ 173.1, 173.5(ester carbonyl), 118.8, 129.2, 137.0(imidazole). IR(KBr) 1740($\nu_{C=O}$), 1250($\nu_{P=O}$), 1100 cm^{-1}(δ_{P-O-C}). Anal. Calcd for $C_{38}H_{68}O_8N_3P$: N, 5.75. Found: 5.98. FAB MS 702 (M^++1).
2b: ^{1}H NMR ($CDCl_3$, TMS) δ 2.38(3H, s, imidazole-CH_3), 6.82, 6.96(2H, s, imidazole). IR(KBr) 1740($\nu_{C=O}$), 1240($\nu_{P=O}$), 1100(δ_{P-O-C}). Anal. Calcd for $C_{39}H_{70}O_8N_3P$: N, 5.39. Found: 5.34. FAB MS 716(M^++1). Both compounds showed the single spot on TLC (silicagel, chloroform/methanol/water(65/25/4)).

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(Received June 19, 1989)