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A Model of Membrane-bound Heme Proteins: Incorporation of a Hemin–Lipophilic Imidazole Complex into Liposomes

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Heme proteins such as cytochrome P-450 and cytochrome b_5 function in the membrane-bound state and the native environment of these heme proteins is lipid bilayers of the endoplasmic reticulum [1]. The incorporation of a simple heme complex into an artificial lipid bilayer membrane has not so far been reported. In the present work, we incorporated a heminlipophilic imidazole complex into egg yolk phosphatidylcholine liposomes and studied its optical properties on comparison with those of cytochrome b_5 [2, 3], cytochrome P-450 [4, 5] and myoglobin to obtain information on the cordination entity of the heme moiety.

Materials and Methods

Egg yolk phosphatidylcholine was prepared by the method of Pangborn [6]. Its fatty acid composition, determined by gas chromatography after methnolysis in acid methanol [7], was as follows: palmitic acid 38.7%, oleic acid 33.9%, linoleic acid 12.3%, stearic acid 12.0%, arachidonic acid 2.2% and linolenic acid 1.0%. 2-Undecylimidazole($C_{11}H_{23}$-Im), 2-undecyl-4-methyl-imidazole($C_{11}H_{23}$-CH_3-Im) and 2-heptadecyl-imidazole($C_{17}H_{35}$-Im) were generous gifts from Shikoku Fine Chemicals, Co. Hemin chloride (bovine, type I) and myoglobin (type II) were obtained from Sigma Chemical Co. and were used without further purification. Concentrations of phospholipids were determined by the method of Chalvardjian *et al.* [8].

A mixture of the desired amount of egg yolk phosphatidylcholine and lipophilic imidazole in chloroform was evaporated under a stream of nitrogen gas and residual solvent was further removed *in vacuo* overnight. The dried film of lipid was dispersed in 0.1 *M* phosphate buffer (pH 7.4) to give a concentration of 800 nmol of phosphatidylcholine per mol. Then hemin chloride solution [9] (usually 40 nmol) was added to 4.0 ml phosphatidylcholine dispersion, and the resultant suspension was clarified by brief sonication.

The sample was applied on a Sephadex G-25 column (2.0 35 cm) previously equilibrated with 0.1 *M* phosphate buffer (pH 7.4), and material was eluted with the same buffer. The turbidity due to liposomes and the absorption due to hemin were measured at 660 nm and 390 nm, respectively. The optical spectrum of the complex was measured with a Union Giken SM-302 spectrometer at room temperature (22 °C). Sodium dithionite was used to reduce ferric heme complex.

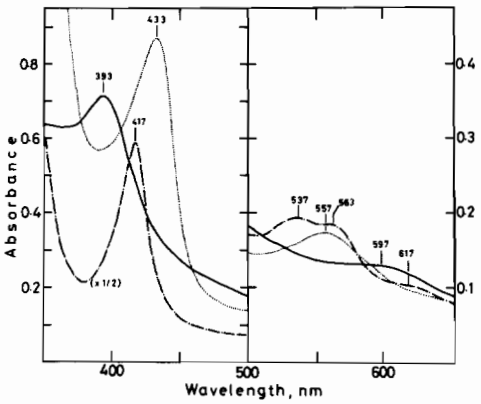

Fig. 1. Optical spectrum of the hemin-2-undecylimidazole complex incorporated into liposomes. The mixture (1 ml) consisted of 0.1 *M* phosphate buffer (pH 7.4) and liposomes containing 800 nmol of egg yolk phosphatidylcholine, 100 nmol of 2-undecylimidazole and 10 nmol of hemin chloride. —— oxidized form, ... reduced form, –.– CO-binding form.

Results and Discussion

Studies by Sephadex gel filtration showed that hemin was completely incorporated into liposomes containing lipophilic imidazole, but was not incorporated into liposomes without lipophilic imidazole and was separated from vesicles.

The visible absorption spectra of the oxidized, reduced and CO-binding forms of hemin incorporated into liposomes containing lipophilic imidazole (Hm-Im-Lip) are shown in Fig. 1. The optical spectra observed with different types of lipophilic imidazole were similar (Table I). These spectra, especially in the reduced form, were similar to those of myoglobin. The optical spectrum in the absence of lipophilic

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TABLE I. Absorption Maxima of Various Hemin–Lipophilic Imidazole Complexes Incorporated into Egg Yolk Phosphatidylcholine Liposomes.[a]

System	λ_{max} (nm)								
	Oxidized form				Reduced form		CO-Binding form		
EPC + $C_{11}Z$ + Hemin	393	597			433	557	417	537	563
EPC + $C_{11}MZ$ + Hemin	394	594			432	556	416	536	566
EPC + $C_{17}Z$ + Hemin	394	596			430	554	415	532	563
EPC + $C_{18}NH_2$ + Hemin	395	564		604sh	404	530	416	531	566
EPC + Hemin	396				406		413	532	566
Myoglobin	408	502	581	533	433	558	422	540	577

[a]Mixtures (1 ml) consisted of 0.1 *M* phosphate buffer (pH 7.4) and liposomes containing 800 nmol of egg yolk phosphatidylcholine, 100 nmol of lipophilic imidazole and 10 nmol of hemin chloride. The abbreviations used are: EPC, egg yolk phosphayidylcholine; $C_{11}Z$, 2-undecylimidazole; $C_{11}MZ$, 2-undecyl-4-methyl-imidazole; $C_{17}Z$, 2-heptadecylimidazole; $C_{18}NH_2$, stearylamine.

TABLE II. Ligand-binding Spectra of the Hemin–Lipophilic Imidazole Complex Incorporated into Egg Yolk Phosphatidylcholine Liposomes and of Myoglobin.[a]

Ligand	System	λ_{max} (nm)										
		Oxidized form				Reduced form			CO-Binding form			
	Model	393			597	433		557	417	537	563	617
	Mb	408	502	581	633	433		558	422	540	577	
Im	Model	411	530	566		423	525	556	419	536	566	618
	Mb	413	534	568		432		557	422	540	576	
1-MIm	Model	409	525			424	528	557	418	536	566	618
	Mb	410	531	574	626	434		557	422	541	577	
2-MIm	Model	413	533	562		426	528	557	420	536	565	618
	Mb	415	534	568		432		556	423	541	577	
4-MIm	Model	393			598	433		556	418	536	566	616
	Mb	409	497	581		432		556	422	539	576	
n-$BuNH_2$	Model	396		566	598	420	523	554	416	535	565	622
	Mb	411	541	573		423		556	420	540	574	
Py	Model	398	520	564	598	417	521	554	417	535	565	622
	Mb	408	525		636	421	525	562	420	540	574	
γ-Pic	Model	402	522	552		414	522	554	415	534	558	
	Mb	418	526	568		421	525	555	419	538	558	618
β-Pic	Model	400	522	550		414	521	553	413	532	556	
	Mb	409	535	568		422	526	560	420	540	571	
α-Pic	Model	394		582		416	521	553	414	535	564	618
	Mb	407	502	582	631	428	532	562	421	541	575	
cytochrome	b_5*	413	530	560		423	526	555				
cyrochrome P-448**		393	512		646	411	545		448		550	
cytochrome P-450**		418	534	570		414	545		451		554	

[a]The model (1 ml) consisted of 0.1 *M* phosphate buffer (pH 7.4), various amounts (described below) of ligand and liposomes containing 800 nmol of egg yolk phosphatidylcholine, 100 nmol of 2-undecylimidazole and 10 nmol of hemin chloride. The ligands added in 1 ml of solution were as follows: imidazole derivatives, 67 μmol; n-$BuNH_2$, 0.33 μmol; Py, 0.41 μmol; picoline derivatives, 0.34 μmol. The absorption maxima of the hemin–bisimidazole complex without liposomes were at 405, 532 and 562 nm (oxidized), 415, 523 and 553 nm (reduced), and 413, 537, 566 and 622 nm (CO-binding form). Abbreviations used: Mb, myoglobin; Im, imidazole; 1-, 2-, and 4-Im, 1-, 2-, and 4-methylimidazole; n-$BuNH_2$, n-butylamine; Py, pyridine; α-, β-, and γ-Pic, α-, β-, and γ-picoline.
*Ref. 2, 3. **Ref. 4.

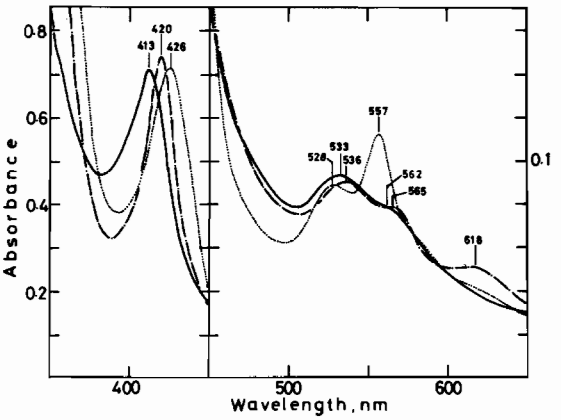

Fig. 2. Ligand-binding spectrum of the hemin-2-undecylimidazole complex incorporated into liposomes. The amount of 2-methylimidazole was 67 μmol/ml, and the amounts of other components were as for Fig. 1. —— oxidized form, ····· reduced form, –·– CO-binding form.

imidazole was very different from that of Hm-Im-Lip and of myoglobin.

The ligand-binding spectra of Hm-Im-Lip (Fig. 2) were compared with those of myoglobin, cytochrome P-450 (Table II). In the presence of a nitrogenous ligand the Soret bands of oxidized Hm-Im-Lip complexes generally showed bathochromic shifts, unlike those in the absence of ligands. The degrees of bathochromic shift of the Soret peaks of the oxidized ligand (imidazole derivative)-bound Hm-Im-Lip complexes were in the order, 2-methylimidazole > imidazole > 1-methylimidazole > 4-methylimidazole, corresponding well with the order of those of ligand-bound myoglobin spectra. Furthermore, the spectra of oxidized and reduced Hm-Im-Lip complexes bound within imidazole derivatives were very similar to those of cytochrome b_5, which has an octahedral geometry with axial Im(N)–heme-(Fe)–(N)Im coordination.

From these results, the hemin–lipophilic imidazole complex incorporated into liposomes is concluded to have a five-coordinating geometry with axial lipophilic Im(N)–heme(Fe) bonding and on addition of a nitrogenous ligand it yields the six-coordinating octahedral geometry of lipophilic Im(N)–heme(Fe)–(N)Im bonding.

In conclusion, we propose that the hemin–lipophilic imidazole complex incorporated into liposomes is useful as a model of heme protein in the membrane-bound state or studies on the relation of heme coordination to functions.

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