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FIRST MEMBER OF ARTIFICIAL FLAVOLIPID FAMILY, ITS SYNTHESIS AND INCORPORATION INTO ARTIFICIAL LIPOSOMES.

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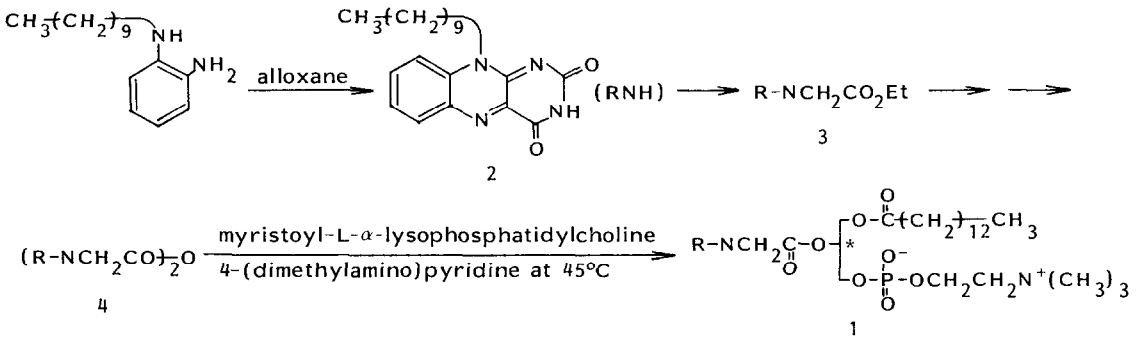
The first member of flavolipid family of artificial flavolipid 1 was synthesized and coincorporated into the bilayer lecithin artificial liposomes. Very fast electron transport from the exterior dithionite to the interior ferricyanide across the flavomembrane was observed.

Membrane-bound flavoproteins are considered to play important roles in many oxidation-reduction reactions in biological systems[1]. Especially important is the electron transport across the membrane aided by flavoproteins[2]. In order to understand the complicated and sophisticated function of the membrane bound flavin, an appropriate simplified model system is necessary and important. In this article authors wish to report the successful synthesis and electron-transporting capacity of the first member of the artificial flavolipid family.

Preparation of the flavolipid 1 was carried out as summarized in Scheme.

Scheme



2-Amino-N-decylaniline was treated with alloxan monohydrate according to the literature[3] to afford 10-decylisoalloxazin 2 in 40% yield: m.p. = 245 - 247°C; NMR (DMSO-d_6), δ: 0.73 - 1.03 (t-like, 3H, CH_3), 1.33 - 1.96 (m, 16H), 4.40 - 4.76 (m, 2H, $-CH_2N$), 7.46 - 8.26 (m, 4H, isoalloxazin ring). IR (KBr): ν_{NH} 3580, 3540 cm^{-1}, $\nu_{C=O}$ 1720, 1670 cm^{-1}. Anal. Cald. for $C_{20}H_{26}N_4O_2$: C, 67.77; H, 7.39; N, 15.81; Found: C, 67.47; H, 7.21; N, 15.65. Compound 3[4], m.p. = 160 - 163°C; NMR($CDCl_3$), δ: 0.73 - 1.03 (t-like, 3H, CH_3), 1.27 (t, 3H, $-OCH_2CH_3$, J = 7Hz), 1.03 - 2.08 (m, 16H, $-CH_2-$), 4.24 (q, 2H, $-OCH_2CH_3$, J = 7Hz), 4.50 - 4.90 (m, 2H, $N-CH_2-$), 4.83 (s, 2H, $-N-CH_2-COO$), 7.43 - 8.42 (m, 4H, isoalloxazin ring). Mass: 440 (M^+). IR (KBr): $\nu_{C=O}$ 1735, 1715, 1660 cm^{-1}. Alal. Cald. for $C_{24}H_{32}N_4O_4$: C, 65.43; H, 7.32; N, 12.72; Found: C, 65.62; H, 7.32; N, 12.52. Phosphatidylcholine moiety was introduced to the alkylated flavin skeleton[5] 3 to yield artificial flavolipid 1 in 62%. 1 is the first

example of the artificial flavolipids. 1 was purified by ion exchange column chromatography (AG-501-x·8(D)) followed by silica gel column chromatography. NMR ($CDCl_3$) δ: 0.87 - 0.89 (m, 6H, $-CH_3$ ×2), 1.23 - 1.86 (m, 38H, $-CH_2-$), 2.30 (t, 2H, $-CH_2COO$, J = 8Hz), 3.39 (broad s, 9H, $-N^+(CH_3)_2$), 3.84 - 5.28 (m, 13H, $-CH_2O$ ×3, -HC-O, $-CH_2N^+$, $-CH_2N$ and $N-CH_2-COO$), 7.64 - 8.32 (m, 4H, isoalloxazin ring). FAB-Mass: 862 ($(M + H)^+$). IR (KBr): $\nu_{C=O}$ 1730, 1705, 1660 cm^{-1}. Electronic spectrum ($CHCl_3$): 290, 334 and 444 nm with a shoulder at 472 nm.

Artificial liposomes incorporating 1 in its bilayer membrane and containing $K_3Fe(CN)_6$ in its interior aqueous phase (Fl-Lip $|^{(i)}K_3Fe(CN)_6$) were prepared as reported by us for cyt *c*-lecithin-liposomes[6,7] (Cyt-Lip$|^{(i)}K_3Fe(CN)_6$). In 10 mL of $CHCl_3$ were dissolved 3 mg of the flavolipid 1 and 63 mg of carefully purified[8] egg lecithin and the mixture was evaporated, then the remaining film was suspended in water and sonicated. To remove large lecithin aggregates, the solution was centrifugated at 20000rpm for 15 min at 4°C. Then the supernatant was treated on Sepharose 4B column (1.2 cm × 40 cm) at 4°C, eluted with 5 mM aqueous Tris-HCl (pH 7.0) in order to remove free $K_3Fe(CN)_6$, surfactant and multilayer liposomes. The isolated liposomes were reasonably stable for at least 96 hours at 4°C, pH 7. Electron micrograghy of the flavolipid/lecithin liposomes stained with 2% aq $(NH_4)_2Mo_7O_{24}$ was studied by use of JEOL JEM 200 CS[9]. The observed electron micrograph demonstrated that the present liposomes have a closed, single-compartment structure with diameter of 600 - 1000 Å[10]. The present flavolipid/lecithin liposomes showed the electronic absorptions at 304, 325 and 444 nm with a shoulder of 472 nm, characteristic of the flavin chromophore. The 444 nm absorption characteristic of flavin was somewhat superimposed by the characteristic absorptions of $K_3Fe(CN)_6$ at 425 nm. However, quantitative determination of both species was successfully achieved by the help of other characteristic absorptions. A 2 mL of 4×10^{-7} M (M; number of liposome particles/Avogadro's number per L) aqueous liposomes solution (5 mM Tris-HCl pH 7.0) was deaerated through careful substitution of air by Ar via repeated evacuation and Ar introduction at room temperature. Then the solution was mixed with 0.2 mL of a freshly prepared aqueous $Na_2S_2O_4$ solution (5 mM Tris-HCl pH 7.0, 10 - 30 fold excess) kept at 25°C by use of a specially designed stopped-flow apparatus as reported in the previous paper[6]. The change of the absorbance was followed at 25°C. Immediately after the mixing, rapid reduction of the flavin moiety was observed as followed by the characteristic absorption at 472 nm without affecting the interior ferricyanide concentration appreciably. This rapid process was followed by the slower decrease of ferricyanide monitored by the 425 nm absorption. The observed decrease of the ferricyanide concentration followed pseudo first order rate equation over the range of the dithionite concentration 3.0 - 13.4 mM;

$$-\frac{d[Fe(CN)_6^{3-}]}{dt} = k_{obs}[Fe(CN)_6^{3-}]$$

The pseudo first order rate constants obtained were listed in Table I. Interestingly, the observed first order rate constants were practically independent of the exterior dithionite concentration. The present electron transport

Table I Reduction rate constant of Interior $K_3Fe(CN)_6$ [a]

Flavolipid[b] (mM)	interior[c] $K_3Fe(CN)_6$ (mM)	bulk aq $Na_2S_2O_4$ (mM)	k_{obs} (sec^{-1})
0.07	0.41	3.0	0.44 ± 0.02
0.07	0.41	7.2	0.45 ± 0.02
0.07	0.41	11.2	0.49 ± 0.02
0.07	0.41	13.4	0.52 ± 0.03

a) at 25°C, pH 7. b) Flavolipid/egg lecithin = 4/96

c) analytical concentration. interior concentration of $K_3Fe(CN)_6$ = 0.75 M.

Table II

electron donor	electron acceptor	mediator [a]	k_{obs} (sec^{-1})	k_{obs}/[mediator]	ref.
ascorbate	$FeCl_3$	chloroplast-pigment	0.007	——— [b]	11
$Na_2S_2O_4$	$K_3Fe(CN)_6$	UQ_{10},PQ	≥0.11	≥16.0 × 10^2	12
		UQ_1	0.011	1.6 × 10^2	12
ascorbate	$K_3Fe(CN)_6$	Ferrocence/Fccp.	0.013	6.5 × 10^2	13
		benzoquinone	0.013	6.5 × 10^2	13
$Na_2S_2O_4$	$K_3Fe(CN)_6$	C_4V^{2+}	0.0023	5.8 × 10^2	6,7
		cyt-c	0.0075	8.6 × 10^2	6,7
		cyt-a_3	0.230	260 × 10^2	6,7
$Na_2S_2O_4$	$K_3Fe(CN)_6$	flavolipid	0.45	64 × 10^2	

a) abbreviations; UQ_{10}, ubiquinone with ten of isoprene unit, UQ_1, ubiquinone with one of isoprene unit, PQ, plastoquinone, FCCP, carbonylcyanide p-trifluoromethoxyphenyl hydrazone, C_4V^{2+}, N,N'-di-n-butylviologen.

b) There is no description of the mediator concentration.

c) reported as mininum value

rate constants were compared with those reported in literatures (Table II). As shown in Table II, apparent electron transport rates for the flavoliposomes are much faster than any of the reported artificial liposomes functionalized with ferrocene, benzoquinone, viologen or cytochrome-c. Only comparable magnitude of the rates was reported for the cytochrome a_3 modified liposomes[6,7] and the ubiquinone or plastoquinone modified liposomes[12]. For the former, statistical aggregation of two cytochrome a_3 molecules to form a

temporary electron channel was concluded mostly responsible to the rapid electron transport rates. Although the details of the latter were not described, aggregation of the quinones was also suggested[14].

For the present flavolipids, statistical aggregation seems plausible based on our preliminary studies on the membrane dynamics, which will appear in a full-length article. As a conclusion, the present flavo-liposomes exhibited one of the fastest electron transport rates across the bilayer membrane from the exterior electron source to the interior electron sink, probably by making a temporary electron channel, without seriously affecting sizes or properties of the bilayer membrane. A variety of versatile applications of the flavo-membrane to preparation of sophisticated artificial cells seem feasible, a few of which are now under ways in our laboratory.

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