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SYNTHESIS OF 10-DECYL-3-(2'-HYDROXYMETHYL) BENZO[g] PTERIDINE -2, 4-DIONE PHOSPHATE AND ITS INCORPORATION IN CATIONIC VESICLES

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ABSTRACT
Synthetic flavin molecules are important biomolecules which when incorporated into lipid bilayers by designing them as amphiphilic compounds with both a hydrophilic flavin head and a hydrophobic tail, can act as catalysts for electron transport across the membrane, mimicking processes seen in cellular respiration and photosynthesis. In the present work a new anionic flavin was synthesized and incorporated in cationic (DDAB) vesicles. The electron micrograph and UV-visible spectra show that amphiphilic isoalloxazine is located in more polar inner water bilayer interface of the vesicles.

KEYWORDS: Flavins, cationic vesicles, SEM, UV-Visible spectra.

1. INTRODUCTION

Electron transfer, activation of molecular oxygen and hydrogenation reactions are catalysed by flavoenzymes. These flavoenzymes are also involved in transmembrane electron transfer process in respiratory and photosynthetic system of mitochondria and thylakoid membranes. Amphiphilic flavins (also known as isoalloxazine or benzo[g]pteridine-2,4-dione) have been proven very appropriate compounds for probing membrane micro environments. The knowledge of relative location and orientation of flavoenzymes in model membranes might help to understand the molecular mechanism of different membrane bound flavoenzymes. Light absorption spectra of a flavin family have characteristic dependence upon the medium polarity and have been used in enzymatic and membrane biology. N,N-Dimethyl Didodecyl Ammonium Bromide (DDAB) form organized structure, vesicles in aqueous solution. The physical and functional properties of these synthetic vesicles are similar to vesicles formed by phospholipids and glycolipids.[1,2] Riboflavin, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) are prosthetic groups in flavoenzyme. These are efficient catalysts in the electron transfer enzymatic reactions across bio membrane.[3,4,5]

2. Experimental

2.1. MATERIALS

O-chloro nitro benzene was purchased from Cisco Chem industries, Mumbai, India. Decyl Amine and alloxan monohydrate were obtained from Fluka AG Switzerland. N-N dimethyl Didodecyl Ammonium bromide was obtained from Aldrich. 2-iodo ethanol and phosphorus oxychloride were purchased from sigma .Seralose 4B was obtained from SRL. Double distilled water was used in preparation of vesicles.

2.2. METHODS

The melting points (uncorrected) were determined on a Thomas Hoover Unimelt Capillary melting point apparatus and are expressed in degree centigrade. IR spectra were recorded on a Shimadzu IR-435 or Perkin Elmer FT-1710 spectrophotometer and υmax are expressed in nm. The electronic spectra were recorded on

a Shimadzu UV-260 spectrophotometer and the λ max are expressed in nm. The PMR spectra were recorded on a Perkin Elmer R-32 (90 MHz) spectrometer in $CDCl_3$ and using tetramethyl silane as the internal standard and the chemical shifts are expressed in δ *ppm.* Mass spectra were recorded on a Jeol JMS-D300 spectrometer using electron impact method (70 eV). Electron micrograph was taken on a Phillips EM 300 (at 80 KV) electron microscope.

Synthesis

N-Decyl-2-nitroaniline

A mixture of 1 -chloro-2-nitrobenzene (2.7 g, 0,018 mol), decylamine. (0.013 mol) and anhydrous sodium acetate (1.5 g, 0.019 mol) was stirred at 110 C for 24h, cooled to room temperature, diluted with water (4 ml) and chloroform (15 ml) and the chloroform layer was separated. The aqueous layer was extracted with chloroform (2 × 15 ml) and combined chloroform extracts were dried (sodium sulphate) and evaporated under reduced pressure. Excess of l-chloro-2-nitrobenzene was then removed by distillation under reduced pressure. The physical and spectral data of N-decyl-2-nitroaniline (3) are given below.

Yield : 6.3 g (76%).
IR(film) : 1030, 1050, 1150, 1230, 1260, 1375, 1410, 1480, 1510, 1530, 1560, 1620, 2850, 2900 and 3400cm^{1},
^{1}H NMR ($CDCl_3$) : 0.73-1.05 (3H, t, -CH_3), 1.05 - 1.90 (16H, m, - $(CH_2)_{8}$-), 3.27 (2H, q, $NHCH_2$), 6.58 (1H, dd, J_{4H-3H} =J_{4H-5H}=8.0 Hz, J_{4H-6H}=2.0 BHz, 4-H), 6.82 (1H, dd, J_{6H-5H}=8.0 Hz, J_{6H-4H}=2.0 Hz, 6-H), 7.41 (1H, dd, J_{5H-4H}=J_{5H-6H}=8.0 Hz, J_{5H-3H}=2.0 Hz, 5-H), 7.86-8.23 (1H, br s, NH) and 8.13 (1H, dd, J_{3H-4H} = 8.0 Hz, J_{3H-5H} = 2.0 Hz, 3-H).

10-Decyl benzo[g] pteridine-2, 4-dione (1).[9]

The platinum oxide (0.075 g) was added to a solution of N-decyl-2-nitrobenzene (0.025 mol) in absolute ethanol (40 ml) and the reaction mixture was hydrogenated with vigorous stirring at ambient temperature and at 60 psi pressure in dark for 20 h. When the solution became colourless, IN HC1 (20 ml) was added to the reaction mixture and the catalyst was filtered off. The alloxan (0.028 mole) was added to the filtrate and the mixture refluxed for 30 minutes. The reaction mixture was cooled to 0°C, the resulting brown precipitate was collected by filtration and washed with ethanol (10 ml) to give yellowish green solid. The filtrate on concentration gave second crop of the product.
Yield : 3.4g (40%)
M. P. : 245-247°C (lit. [9,10] m.p. 245-247°C).
IR (Nujol) : 820, 1100, 1170, 1220, 1270, 1400, 1450, 1500, 1540, 1580, 1610, 1670, 1720 and 3500 cm^{-1}.
UV (DMSO) : $\lambda_{max}\left(\varepsilon_{max} mM\right)$: 268.8 (33.581), 329.2 (2.624) and 434.8 (6.232)nm.
Uv ($CHcl_3$) 268.2, 334.0, 416.0 440.6 and 464nm.
^{1}H NMR (DMSO-d_6) : 0.90 (3H, t, -CH_3), 1.04-1.08 (16H, m, - $(CH_2)_{8}$-), 4.24 (2H, t, N-CH_2), 7.6-8.6 (4H, m, 6, 7, 8 and 9 aromatic protons).
Ms (m/z) (rel. int.) : 354 (22.4 M^{+}), 215 (100), 171 (22.9) and 143 (68.5).

10-Decyl-3-(2'-hydroxy ethyl) benzo[g]pteridine-2,4-dione(2)

To a solution of 10-decyl benzo[g]pteridine-2,4-dione (1) (0.1 g, 0.0002 mol) in acetone was added iodoethanol (0.048 g, 0.0002 mol) and potassium carbonate. The reaction mixture was refluxed and monitored by TLC. The potassium carbonate was filtered off and the solvent was removed under reduced pressure to get 2.
Yield: 0.09 g (80%).
IR(KBr): 1060, 1170, 1270, 1540, 1580, 1610, 1650, 1670, 1720 and 3300-3400 cm^{-1}.
UV(MeOH) : $\lambda_{max}\left(\varepsilon_{max} mM\right)$: 225.0(9.95), 266.0(13.03), 332.2 (4.48) and 433.0(4.68)nm.

10-Decyl-3-(2'-hydroxy ethyl) benzo[g]pteridine-2,4-dione monophosphate(3)

To a mixture of $POCl_3$ (1.2 g, 0.008 mol) and water (0.29 g, 0.016 mol) was added 10-decy1-3-(2'-hydroxy ethyl) benzo[g] pteridine-2,4-dione (2) (0.119 g, 0.0003 mol). The reaction mixture was kept aside for 24 h at room temperature, HC1 was removed by flushing dry nitrogen through the reaction mixture for 1 h. The flavin phosphate was precipitated by pouring the mixture into dry cold ether (400 ml). After decanting ether, the hygroscopic residue dissolved in a mixture of water (1 ml) and dioxane (10 ml). After 2 h at room temperature, 10-decyl-3-(2-hydroxy ethyl)benzo[g]pteridine-2,4-dione monophosphate was precipitated by addition of ether (100 ml).
Yield: 0.10 g (80%).
IR(KBr) : 710, 770, 1090, 1170, 1280, 1400, 1500, 1520, 1670, 1720 and 3400 cm^{-1}.
UV(MeOH): $\lambda_{max}\left(\varepsilon_{max} mM\right)$: 265,4(4.16), 332.6(1,02) and 433.6 (1.38) nm.
MS(m/s)(rel. int.): 382(5.29), 354(44.7), 256(2.0), 242 (3.5), 228(54.1), 214(81.4), 185(16.3), 170(13.0), 143(50.0), 130(5.6) and 103(8.1).

Preparation of vesicles from didodecyl dimethyl ammonium bromide[9]

A thin film was obtained by dissolution of didodecyl dimethyl ammonium bromide (0.023 g, 4 x 10^{-5} mol) in chloroform (10 ml) and evaporation of chloroform under reduced pressure. The thin film was dried under reduced pressure for 12 h. Deionized water (5 ml) was added to above dried film and suspended film in aqueous solution was sonicated in bath type sonicator at 190 W for 4-5 h at 45-50° C, The solution was cooled to room temperature and was applied to a Seralose 4B column (1 cm × 6 cm). The column eluted with deionized water and different fractions of unilamellar vesicles were obtained.

Incorporation of anionic Flavin in cationic vesicles from didodecyl dimethyl ammonium bromide[9]

A thin film was obtained by the evaporation of the chloroform solution (5 ml) of didodecyl dimethyl ammonium bromide (0.023 g, 4×10^{-5} mol) and desired flavin (0.001 g, x 10^{-6} mol) under vaccum. The resulted thin film was dried and sonicated after addition of deionized water (5 ml) as above at 45-50°C. The sonicated solution was cooled to room temperature and was subjected to gel filtration through Seralose 4B column, eluted by deionized water and different fractions (1 to 1.5 ml) were collected. The incorporation of benzo [g] pteridine in vesicles were followed by UV-Visible spectroscopy.

Electron microscopy

Few drops of cationic vesicles were applied to a carbon coated copper electron microscopic grid (mesh size 150) and dried. Few drops of uranyl acetate were applied to the same grid and after appropriate drying the sample was examined in Philips EM 300 electron microscope operated at 80 KV.

3. RESULTS AND DISCUSSION

10-decyl-3-(2'-hydroxyethyl) benzo [g] pteridine-2,4-dione monophosphate (3) was synthesized from 10-deyl benzo [g] pteridine-2,4 dione.[6,7] and incorporated into synthetic vesicles to understand the molecular mechanism of electron transfer process in bio membranes through flavoenzymes.

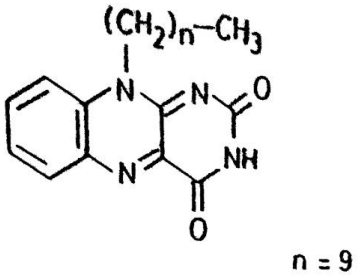
Anionic benzo [g] pteridine -2, 4-dione /flavin was prepared by reaction of 10- decyl benzo [g] pteridine 2, 4-dione (1) with iodoethanol in basic conditions followed by phosphorylation with $POCl_3$ (Scheme-1). Product thus formed was confirmed by usual spectroscopic techniques. Detailed data is given in the experimental section.

A solution of didodecyl dimethyl ammonium bromide (DDAB) (10 mM) was sonicated in bath type sonicator at 40-45°c for 4 hours, which resulted in the formation of vesicles. The electron microscopic studies confirm the existence of unilamellar vesicles (Fig. 1).[8]

Sonication method was used to prepare flavin incorporated vesicles also.[9] Surfactant vesicles solution was subjected to gel filtration for uniformity and to separate the free flavin. Studies have been done with synthetic Flavin incorporated in synthetic peptide by other group.[5,8]

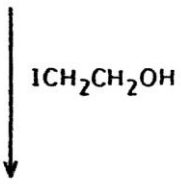
The entrapment of 10-decyl-3-(2'-hydroxymethyl) benzo[g] pteridine -2, 4-dione phosphate (anionic Flavin) in DDAB vesicles show a fine structure in S_1 band 420, 444 and 472 nm in all (I-V) the fractions collected from gel filtration. The S_2 peak appears at 344 nm indicating its presence in more polar bilayer water interface of vesicles (Table-1).

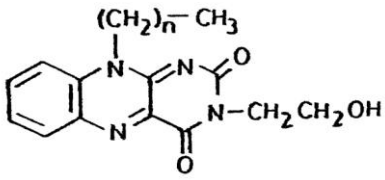
N, N-dimethyl dodecyl ammonium bromide (DDAB) form unilamellar vesicles in aqueous solution by sonication. Amphiphilic flavins get entrapped in inner water bilayer interphase of vesicles, schematic presentation (Fig. 2).



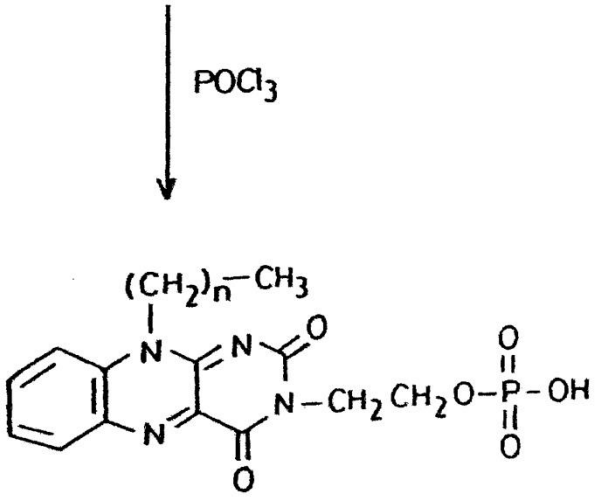
n = 9

(1)





(2)



(3)

Scheme - 1

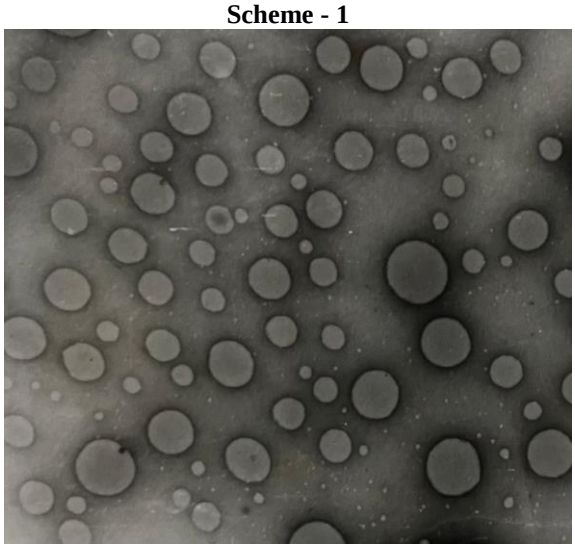


Figure - 1
Didodecyl dimethyl Ammonium Bromide Vesicles × 20,000 (1 mm = 50 nm)

Table 1: UV Spectra of anionic amphiphilic benzo[g] petridine2, 4-dione (3) in organic solvents and vesicles.

Flavin	Solvent/ Vesicle Fraction	$\lambda_{max}\,(nm)$	
		S_2	S_1
3	Chloroform	332	420 444 468
	CTAB	330	436
	Methanol	333	434
	Water	350	449 480
	I-V Fraction	344	420 444 472

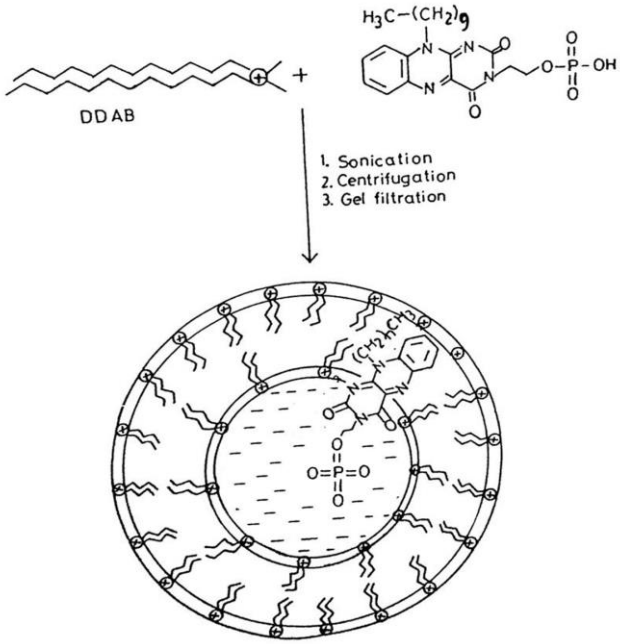


Figure – 2.

4. CONCLUSIONS

The new anionic flavin, 10-decyl-3-(2'-hydroxyethyl) benzo [g] pteridine-2,4-dione monophosphate (3) was synthesized and entrapped in cationic vesicle bilayer interphase. Which can be used as powerful tools for studying membrane-associated redox chemistry, electron transfer reactions as biomimetic artificial enzymes, in a minimal, controllable model to understand molecular mechanism involved in such reactions.

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