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Solvation of the photodynamic therapy agent in the model lipid–protein system: The evidence of porphyrin preferential solvation by apolar environment

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This paper is dedicated in memoriam of professor Boris D. Berezin.

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ABSTRACT

This paper presents the results of the first accurate study of the energetics of solvation of the well-established agent for photodynamic therapy of cancer–hematoporphyrin dimethylester in the 1-octanol–N,N-dimethylformamide mixed solvent modeling the smooth transition from porphyrin solvation in an apolar lipid-like phase to a polar protein-like environment. Our results do indicate that porphyrin molecule is preferentially solvated by the amide in the physiological temperature range mainly due to the influence of polar functional groups which interact stronger with N,N-dimethylformamide molecules. In contrast, macrocycle itself interacts stronger with 1-octanol which results in some excess of alcohol molecules in its nearest vicinity. Thus, polar side-chains of hematoporphyrin are better solvated in a protein-like environment, whereas macrocycle shows a tendency to be located in a lipid-like phase.

1. Introduction

Porphyrins, chlorins and phthalocyanines are most popular ingredients in the photodynamic therapy (PDT) drugs which are currently in use [1–4]. The photosensitizers induce a generation of reactive oxygen species that cause a selective destruction of cancer cells, the relative amount of the reactive oxygen forms depends strongly on the macroheterocycle environment [3]. It is obvious that the most effective way to destroy such cells is to affect a cellular nucleus inducing apoptosis and necrosis mechanisms [4]. Although photosensitizers contain polar functional groups increasing solubility in biological liquids, their behavior *in vivo* is found to be rather hydrophobic and they often locate in apolar sites of cells such as lipid membranes [2,4]. To explain such solute behavior the detailed information on the porphyrin–environment interaction should be available. However, even for simple model systems the driving forces of macrocycle solvation are not fully understood [4,5].

In this paper we provide much deeper insight into the problem of the interaction of such drugs with liquid environment and study for the first time the thermochemical behavior of slightly soluble hematoporphyrin dimethylester (HPDME, see Fig. 1) and some non-electrolytes modeling its side chains in the 1-octanol–N,N-dimethylformamide mixed solvent at 318 K and compare the results obtained with those reported earlier [6,7]. 1-Octanol (OctOH) has been chosen as a model for a cell membrane [8] and dipolar N,N-dimethylformamide (DMF) is used as a model of internal surface of global proteins [9]. The OctOH–DMF mixture allows to simulate the smooth transition from porphyrin solvation in an apolar lipid-like phase to a polar protein-like environment.

2. Experimental

2.1. Materials

DMF (Fluka, >98 mass%) was dried with 4 Å molecular sieves for several days and then distilled under reduced pressure at 303 K, the middle fraction being selected. 1-octanol (Reachem, chemical purity) was dried with 4 Å molecular sieves for several days and then distilled under reduced pressure at 360 K. Karl Fisher titration showed that water content in both solvents did not exceed 0.02 mass%. EtOAc (Reachem, chemical purity) was shaken with Na_2CO_3, dried with 4 Å molecular sieves for several days and then

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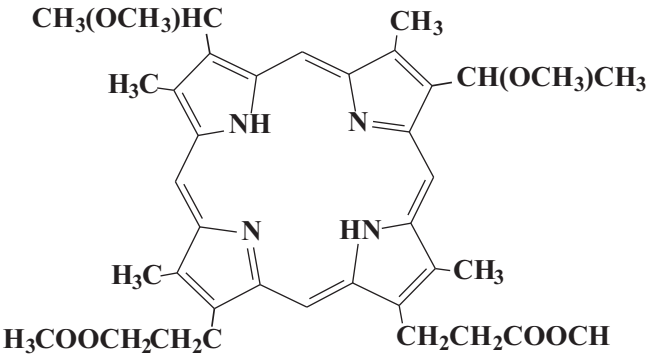

Fig. 1. Structure of HPDME molecule.

distilled twice at 350 K. Decane (Reachem, for chromatography) was used as supplied.

Hematoporphyrin dimethylester was obtained from ferriheme chloride according to the procedure described in detail elsewhere [10,11]. Cold methanol was used to precipitate HPDME crystals from fresh chloroform. Then the solute was dried under reduced pressure at 353 K for 10 h. Structure and purity of HPDME were proved with the elemental analysis, absorption and NMR-spectra. NMR ^{1}H studies were performed with a Bruker-500 (500 MHz) spectrometer in $CDCl_3$ at 298 K – (δ, ppm): δ, ppm): 10.60, 10.56, 10.17, 10.13 (4s, 1H each, H (5,10,15,20), 6.09 (br. q, 2H, J = 6.2 Hz, H (3-1, 8-1)), 4.47 (t, 4H, J = 7.2 Hz, H(13-2), H(17-2)), 3.75 (s, 6H, $COOCH_3$), 3.69–3.71 (br s, 12H, CH_3 (2, 7, 12, 18)), 3.66 (s, 6H, OCH_3 (3-1, 8-1)), 3.34 (t, 4H, J = 7.2 Hz, H(13-1), H(17-1)), 2.31 (br. m., 6H, CH_3 (3-2, 8-2), −3.65 (br. s., 2H, NH).The impurity content in the final product did not exceed 1%.

2.2. Apparatus

The calorimetric measurements were carried out with an isoperibol ampoule calorimeter described in detail previously [12,13]. The detection limit of the apparatus was 10 μK. The temperature instability in the "Thermostat A 3" system (BIC, Minsk) was less than 1 mK in the temperature range of 275–350 K. The enthalpies of solution were measured by a comparative method with the digital Standard Temperature Measuring Instrument (BIC, Minsk). An electrical calibration was carried out before each experiment. The enthalpies of solution were corrected to the side effects associated with an ampoule crushing on the vessel bottom and solvent vaporization into free space of the calorimetric vessel. For this purpose thin empty ampoules ($V \approx 0.7\,cm^3$) were crushed in liquid DMF and 1-octanol. This side effect was found to be equal to -0.03 ± 0.01 and -0.04 ± 0.01 J, respectively. It was assumed that it varied linearly with the mixed solvent composition. Duration of porphyrin dissolution in our cell equipped effective stirring was found to be equal to 5 min in DMF and about 10–15 min in pure 1-octanol. The standard enthalpies of solution in pure solvents listed in Tables 1 and 2 represent the mean value from eight independent measurements in the range of solute molalities of 0.0002–0.001 mol kg^{-1} for hematoporphyrin and the mean value from four measurements in the range of molalities of 0.01–0.04 mol kg^{-1} for other non-electrolytes, where experimental enthalpies of solution do not depend on the solute concentration. The ΔH^m (sol) $\cong \Delta H^0$ (sol) values in the mixed solvent listed in Tables 1 and 2 reflect the result of one experiment in this molality range. According to our previous studies, the overall uncertainty of the ΔH^0 (sol) is estimated to be within 1%.

Table 1
Standard enthalpies of solution $\Delta_{sol}H^0$ in kJ·mol^{-1} of non-electrolytes in 1-octanol–DMF mixtures at 298.15 K.

DMF [6]		OctOH [6]		EtOAc [6]	
X_{DMF}	$\Delta_{sol}H^0$	X_{DMF}	$\Delta_{sol}H^0$	X_{DMF}	$\Delta_{sol}H^0$
0	5.63	0	0	0	7.54
0.1013	4.50	0.1019	0.02	0.1039	5.97
0.2381	3.23	0.2428	0.36	0.2422	4.27
0.3517	2.31	0.3521	0.61	0.3058	3.64
0.4625	1.60	0.4656	1.22	0.4642	2.27
0.6210	0.95	0.6219	1.96	0.6236	1.32
0.7626	0.41	0.7648	3.22	0.7635	1.01
1	0	1	6.50	1	0.83

HPDME [7]		Decane	
X_{DMF}	$\Delta_{sol}H^0$	X_{DMF}	$\Delta_{sol}H^0$
0	27.26	0	1.63
0.1046	23.28	0.1276	2.03
0.3137	16.58	0.2422	2.52
0.5039	12.60	0.3058	3.18
0.6207	11.44	0.4642	4.30
0.7212	10.49	0.6236	5.57
0.7999	10.10	0.7635	8.45
0.8392	9.87	1	11.24
0.9206	9.48		
1	9.12		

3. Results and discussion

The standard enthalpies of solution for HPDME, OctOH, DMF, ethyl acetate (EtOAc) and decane are given in Tables 1 and 2. As can be seen, the dissolution process is endothermic at both temperatures, polar DMF, EtOAc and HPDME being more exothermically solvated in polar DMF, whereas apolar decane and OctOH being better solvated in slightly polar 1-octanol. To recover more detailed information about the porphyrin–solvent and solvent–solvent interactions from the experimental data, we have applied the well-established theoretical approach [14,15] which connects standard enthalpies of solute transfer with thermodynamic properties of a binary mixture. Briefly, it takes into account all possible effects accompanying the solute molecule transfer from a gas phase to

Table 2
Standard enthalpies of solution $\Delta_{sol}H^0$ in kJ mol^{-1} of non-electrolytes in 1-octanol–DMF mixtures at 318.15 K.

DMF		OctOH		EtOAc	
X_{DMF}	$\Delta_{sol}H^0$	X_{DMF}	$\Delta_{sol}H^0$	X_{DMF}	$\Delta_{sol}H^0$
0	5.10	0	0	0	7.75[12]
0.04984	4.65	0.1132	0.05	0.1011	6.10
0.1115	4.20	0.2941	0.45	0.1480	5.34
0.2980	2.62	0.4942	1.26	0.2755	3.44
0.4940	1.37	0.6821	2.36	0.4601	2.32
0.6392	0.76	0.7901	3.28	0.6896	1.26
0.7897	0.28	0.9904	6.18	0.7897	0.99
1	0	1	6.38	1	0.82

HPDME		Decane	
X_{DMF}	$\Delta_{sol}H^0$	X_{DMF}	$\Delta_{sol}H^0$
0	37.95	0	2.13
0.1084	32.96	0.1198	2.29
0.2934	25.24	0.2392	2.69
0.4689	20.51	0.4062	3.35
0.5503	18.54	0.5442	4.45
0.6623	16.88	0.5673	4.58
0.7011	15.97	0.7486	6.29
0.7722	15.08	0.8751	8.99
0.8600	13.95	1	11.94
1	13.09		