Evaluation of meta-tetra(hydroxyphenil)chlorin incorporation into lipid bilayer

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We studied the kinetics of photosensitizer meta-tetra(hydroxyphenil)chlorin (mTHPC) distribution in model lipid membrane. For this purpose the parameters of mTHPC quenching were examined as a function of time after pigment introduction into suspension of small unilamellar vesicles (SULV). The results obtained show that photosensitizer molecules penetrate very slowly into membrane. At equilibrium mTHPC binding sites are localized deeply in hydrophobic region of lipid bilayer.

1 Introduction

The mechanisms of interaction between porphyrins and artificial lipid membranes are of great importance for design of new sensor materials and assembling of biomolecular devices into cellular architectures [1, 2]. This problem attracts a special attention in respect to a new photoactivative drugs development for the purposes of photodynamic therapy (PDT).

The second generation photosensitizer mTHPC (commercial product Foscan®) was proposed for medical treatment of different cancer diseases in 1989 [3]. Its effectiveness exceeds many other widespread sensitizers (e.g. Photofrin®). mTHPC photophysic characteristics are similar to many other photosensitizers, so it is suggested that its effectiveness is a result of the peculiarities of interactions with biological structures, especially membranes.

Our work deals with the study of photosensitizer meta-tetra(hydroxyphenil)chlorin localization in model lipid membranes. Using spectroscopic technique we evaluated the rate of mTHPC penetration into lipid bilayer.

2 Experimental Measurements

We used small unilamellar lipid vesicles (SULV) as a model of biological membrane prepared by sonication of a dispersion of phospholipids in phosphate saline buffer (pH = 7.2) similarly to the procedure described in [4].

We used two technical approaches of mTHPC introduction into lipid vesicles. In the first mTHPC was introduced into phospholipids dispersion at the stage of SULV preparation. In the second, mTHPC from the stock solution (5×10^{-3} M) was injected into prepared SULV suspension. mTHPC/lipid molar ratio was 1:1000 in both cases. The fluorescence spectra were recorded on spectrofluorimeter LSF 1211A (Solar, Belarus). The instrument was equipped with a thermostatted cell holder and magnetic stirrer. Excitation was set at 420 nm.

To study mTHPC localization in lipid bilayer we used the quenching of its fluorescence by KI and Antraquinone-2,6-disulphonate (AQDS) as membrane impermeable quenchers. Fluorescence quenching data were obtained at 30° C and analyzed by modified Stern-Volmer equation (1):

$$F_0/(F_0 - F) = 1/(f_\alpha K_q [Q]) + (1/f_\alpha) \qquad (1)$$

where F_0 and F are mTHPC fluorescence intensities without and with quencher, respectively; [Q] is the quencher concentration; f_α is the fraction of quencher-accessible fluorescence and K_q is the Stern-Volmer quenching constant associated to this fraction. All F values were corrected for the dilution effects due to the added quencher. Evaluation of mTHPC aggregation state in sample was performed by verifying the relative fluorescence intensity change before and after Triton X-100 addition.

3 Results and Discussion

mTHPC is poorly soluble in aqueous media. Pigment introduction into buffer system accompanies by mTHPC aggregation which leads to drastic decrease of fluorescence quantum yield. When SULV suspension is introduced into mTHPC aqueous solution, the disaggregation of photosensitizer is observed as a result of its interaction with SULV. mTHPC fluorescence quantum yield increases and spectrum becomes similar to those for pigment in organic solvent (see Fig. 1). Prolonged incubation does not lead to any change of pigment fluorescence. This fact demonstrates the mTHPC presence in SULV in monomeric state.

The study of the mTHPC fluorescence polarization of in suspension of SULV showed that polarization degree was very high (about 0.38) and independ-

ent of a method of vesicles loading by pigment. This result suggests that mTHPC molecules are strongly fixed in the lipid bilayer.

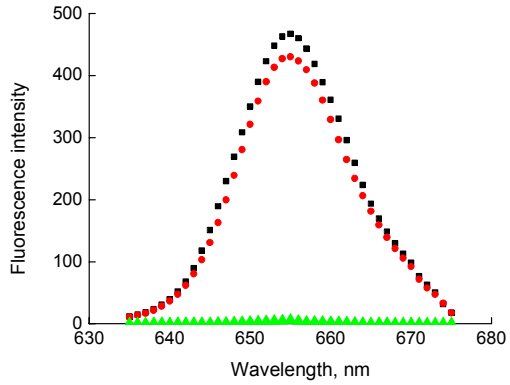

***Fig. 1** mTHPC fluorescence spectra in organic solvent (■), into lipid vesicles (●), aqueous media (▲).*

To evaluate mTHPC localization in lipid bilayer we used fluorescence quenching technique with two membrane impermeable quenchers. The dependence of the fluorescence intensity on the quencher concentration established from the Stern-Volmer equation was found to be nonlinear. This fact is apparently a consequence of the inaccessibility of some mTHPC molecules bound in SULV to water-soluble quenchers. For this reason all fluorescence quenching data were analyzed by modified Stern-Volmer equation. As can be seen from Fig. 2 there is a good linear relationship between fluorescence quenching degree and inverse quencher concentration value.

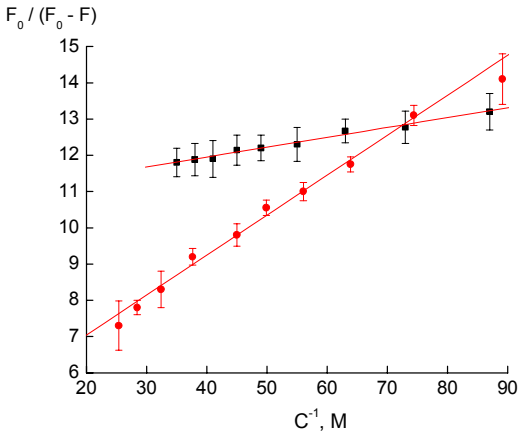

***Fig. 2** Dependence of fluorescence quenching degree of mTHPC in SULV on the KI (■) and ADQS (●) inverse concentration.*

According to the data obtained the fraction of pigment molecules available to the quencher is 0.09 for KI and 0.19 for AQDS, correspondingly. Measurements of quenching characteristics showed that mTHPC binding sites are localized in hydrophobic region of lipid bilayer so the majority of pigment molecules are hidden in lipid bilayer and cannot interact with quencher.

To evaluate the kinetics of mTHPC incorporation into lipid bilayer we studied fluorescence quenching characteristics as a function of time after pigment introduction into SULV suspension. f_α values for 0.5, 6 and 24 hours intervals of incubation and for preloaded SULV are presented in Tab. 1.

t, hours	SULV loaded	0.5	6	24
f_α, (KI as a quencher)	0.09	0.27	0.16	0.12
f_α, (ADQS as a quencher)	0.19	0.5	0.41	0.27

***Tab. 1** Comparison of values f_α obtained after different time intervals.*

Immediately after mTHPC introduction into SULV suspension the overwhelming majority of its molecules are accessible for the quencher. It means that they are localized on the vesicle surface. During incubation f_α gradually decreases. This is the consequence of mTHPC molecules penetration deeply into lipid bilayer. According to our results one can conclude that the process of mTHPC embedding into lipid vesicles is very slow. Even after 24 hours of incubation fα is high as compared with those calculated for SULV loaded at the stage of preparation.

4 Conclusions

mTHPC localizes in hydrophobic region of lipid bilayer under equilibrium distribution. The rate of mTHPC incorporation is extremely low. It takes appoximately 20 – 30 h to reach equilibrium mTHPC distribution in lipid bilayer after its addition into suspension of SULV.

References

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