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Short communication

Interaction of merocyanine 540 with charged membranes

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Abstract

In this work, phospholipid liposomes were used to investigate the influence of lipid negative charge on the interaction of merocyanine 540 (MC540) with model membranes. Liposomes were prepared from a mixture of neutral dimyristoyl lecithin (DMPC) and negatively charged dimyristoyl phosphatidic acid (DMPA). A strong dependence between the presence of charges on the membrane and dye association was found. The affinity of the dye to liposomes was decreased with an increasing content of DMPA in liposomes. Changes in absorption spectra of MC540 suggest that the decrease in affinity of MC540 to charged membranes is accompanied by a hypsochromic solvatochromic shift and changes in monomer/dimer equilibrium of MC540 incorporated in the membrane.

Keywords: Merocyanine 540; Charged liposomes; Absorption spectroscopy

1. Introduction

The fluorescent dye merocyanine 540 (MC540) preferentially photosensitizes leukemia cells, lymphoma cells [1] and enveloped pathological viruses [2]. It has been shown that the highly susceptible cells to MC540-sensitized photoinactivation tend to bind more dye and to saturate their dye-binding sites more rapidly than MC540-resistant cells. Therefore, the preferential binding of a sensitizer to the malignant species is suggested to be a key factor in the efficiency of MC540-sensitized photodynamic impact [3].

MC540 incorporates to the outer leaflet of the phospholipid bilayer of a cellular membrane [4]. Considering this, the difference between high and low affinity binding should reflect differences in composition and/or structural organization of the membrane. In model experiments with liposomes, it has been proposed that MC540 resides in membrane slightly above domain of the glycerol backbone of phospholipids and it has been shown that MC540 binding is very sensitive to lipid packing of phospholipid bilayer [5]. Due to the presence of a negative charge on the MC540 molecule, localized charges on the surface of membrane may play an important role in the process of incorporation of MC540 into the membrane. Up to now, investigations have shown that there is some correlation between binding of MC540 and the presence of the charged structure on the membrane [6,7]. The aim of our work was to determine the influence of the lipid negative charge on the interaction of MC540 with membranes.

2. Experimental

MC540 was purchased from Molecular Probes (the Netherlands) and was used without further purification. The dye stock solution of MC540 of 10^{-3} M was prepared in bidistilled water and kept in the dark until use. A mixture of two synthetic phospholipids, 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dimyristoyl-sn-glycero-3-phosphatidic acid (DMPA) from Fluka (Germany), was used in the preparation of small unilamellar liposomes by sonicating their water dispersion (14×10^{-6} mol of DMPA–DMPC mixture in 1 ml) in a temperature-controlled sonicating bath ($T = 25$ °C) for 1 h. The molar ratio of both lipids DMPA/DMPC in the suspension was 0:100, 10:90 and 20:80, respectively. The liposome suspension of molar concentration of 14×10^{-3} M was used as stock solution. The bidistilled water, pH 6.2 ± 0.1, was used in all sample preparations.

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Liposomes and MC540 at different lipid/MC540 molar ratios (in the interval from 20:1 to 450:1) were incubated for 10 min. The binding of MC540 to the liposomes were determined by recording and analyzing absorption spectra of the dye. The absorption spectra were recorded by a Fluorolog 3–11 (Yvon Jobin, USA). All measurements were performed at the temperature of 25 °C and the samples were protected from the light.

3. Results and discussion

Fig. 1 shows the visible absorption spectra of MC540 in DMPC liposomes with various contents of DMPA and for different lipid/MC540 molar ratios. In the aqueous solution of MC540 (Fig. 1), two absorption maxima, namely at 500 nm (a dimer peak) and at 533 nm (a monomer peak) were observed. Titration of water solution of MC540 by liposome suspensions caused changes in the spectral appearance. In the pure DMPC liposomes, a new absorption band became resolvable at 568 nm. The original water peak at 500 nm was reduced, whereas the peak at 533 nm changed its intensity by variation of lipid amount in the sample (Fig. 1). Two isosbestic points were also created at about 546 and 595 nm. According to Sikurova et al. [8], the present data can be interpreted as the incorporation of MC540 into the lipid membrane and the peaks at 568 and 533 nm can be assigned to monomer and dimer forms of MC540, respectively, bound in the lipid membrane. Absorbance values (Figs. 1 and 2) were enhanced up to the saturation level at a lipid/dye molar ratio of about 100:1 which indicates that almost all dye were associated with liposomes at lipid/dye molar ratios over 100:1.

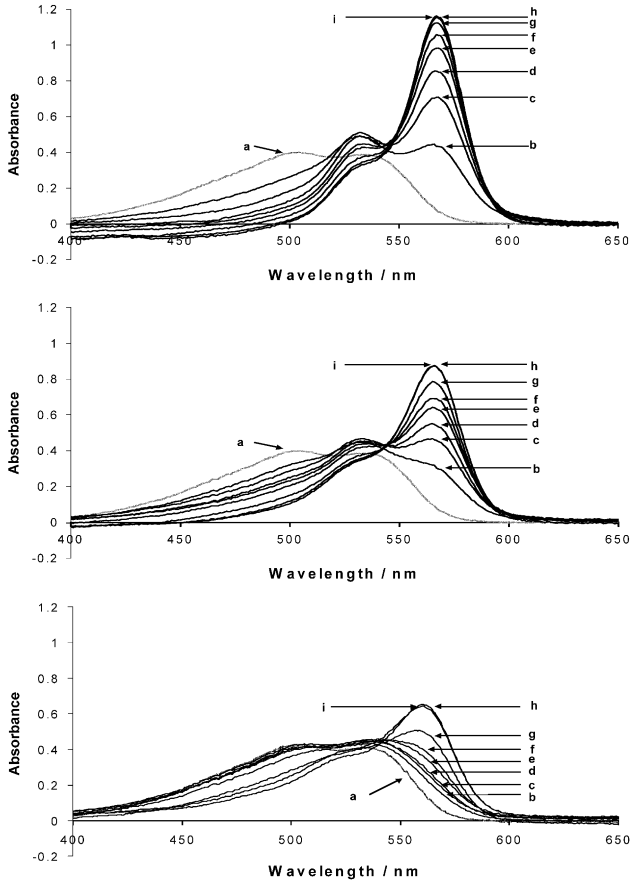

Fig. 1. Visible absorption spectra of MC540 in the suspension of DMPC liposomes containing 0% mol (I), 10% mol (II) and 20% mol (III) of DMPA at various lipid/dye ratio: (a) aqueous solution of MC540 1×10^{-5} mol l^{-1}, (b) 10:1, (c) 20:1, (d) 30:1, (e) 50:1, (f) 70:1, (g) 140:1, (h) 280:1 and (i) 440:1.

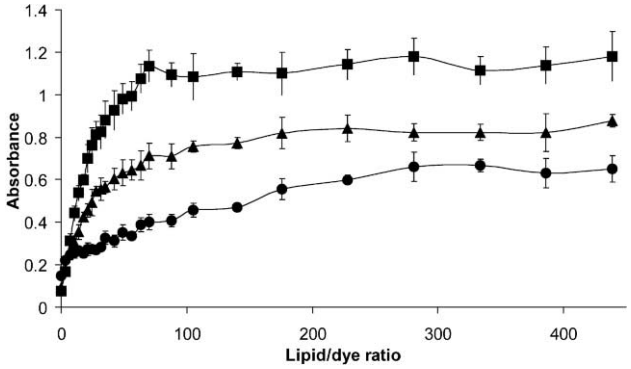

Fig. 2. Absorbance of MC540 monomers bound to the liposomes with various content of DMPA at different lipid/dye ratio: (■) 0% mol DMPA, (▲) 10% mol DMPA and (●) 20% mol DMPA.

The presence of DMPA in liposomes caused changes in the shape of MC540 spectra. With an increase of DMPA concentration in the liposome suspension, the absorption maxima of the membrane-bound MC540 monomer were reduced, and a blue shift of its position to 566 nm at 10% mol of DMPA and to 560 nm at 20% mol of DMPA occurred. This observation suggests that the presence of negative charges on the membrane strongly suppress the occurrence of MC540 monomers in a lipid membrane. In addition, the recorded blue shift of monomer peaks in the spectra may indicate the localization of MC540 monomer in a less hydrophobic region of membrane [9]. MC540 molecules fully associated with liposomes were observed at a higher lipid/dye molar ratio—about 150:1 for 10% mol DMPA and about 270:1 for 20% DMPA, which indicates a decrease of the MC540 affinity to charged liposomes (Figs. 1 and 2). An increase in the intensity of absorbance in the region near 530 nm (Fig. 1) may suggest formation of new colored species (e.g., aggregates) of MC540 in presence of DMPA.

4. Conclusions

Results of study suggest that the electrostatic repulsion between negative charges present on MC540 molecules and on liposomes prevents the penetration of MC540 into the hydrophobic core of membrane and decreases occurrence of monomers of the dye bound to the liposomes.

Acknowledgements

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