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Distribution of Merocyanine 540 in Phospholipid Membranes

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The interaction of the fluorescent photosensitizer merocyanine 540 (MC-540) with model phospholipid membranes was studied. Two different-colored species (monomers and dimers) of MC-540 were registered in phospholipid liposomes. Variations in both phospholipid composition (DMPC, DPPC, POPC, egg PC) and temperature (15–60°C) resulted in changes in the MC-540 monomer–dimer distribution. The values of the monomer–dimer equilibrium constant of MC-540 in egg PC ($K = 14.8$ μM), in POPC ($K = 26.7$ μM), and in DMPC ($K = 271.0$ μM) were determined at the temperature of 23 ± 2°C. Suppression of MC-540 association with phospholipid bilayers was provoked by the addition of albumin to a liposome suspension. Albumin was observed to compete very successfully with lecithins containing saturated fatty acid chains (DPPC, DMPC), while only a weak competition of albumin with unsaturated lecithins (POPC, egg PC) for binding MC-540 molecules was registered.

KEY WORDS: Merocyanine 540; phospholipid membranes.

INTRODUCTION

Merocyanine 540 (MC-540) is a lipophilic fluorescent dye which was reported to bind preferentially to electrically excitable cells, certain classes of normal immature blood cells, leukemia and neuroblastoma cells, lipid-enveloped viruses, and virally infected cells [1–4]. At the present time this dye is used clinically for the purging of autologous bone marrow grafts [5] and preclinically for the inactivation of enveloped viruses in blood products [6]. In spite of this important application in medicine, neither the mechanism of its action nor the variations in affinity of different types of cells for dye molecules have been adequately explained. Serum albumin was shown to be one of the major modulators of MC-540 affinity to biological membranes. However, the components of plasma membranes that compete with albumin binding sites for MC-540 molecules have not been identified [7]. Since phospholipids are the main building components of membranes, it is important to clarify binding properties of MC-540 to various phospholipids and to compare them with binding to albumin.

This study was designed to investigate the effects of lecithin composition, temperature, and presence of albumin on MC-540 incorporation into phospholipid bilayers.

MATERIALS AND METHODS

Merocyanine 540 was obtained from Eastman Kodak, Co. Two synthetic lecithins, dimyristoyl-L-α-phosphatidylcholine (DMPC) and dipalmitoyl-L-α-phosphatidylcholine (DPPC), obtained from FLUKA AG were used in our experiments. We also used natural 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC) and natural egg lecithin (egg PC) prepared at the Charkow State University. All phospholipids were employed in the

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preparation of "injection" liposomes according to the standard method [8]. These liposomes were stained with MC-540 at a lipid:dye molar ratio of 20:1–43,000:1. Bovine albumin obtained from IMUNA Co. was added to liposome suspensions before staining with MC-540 at a lipid:albumin molar ratio of 20:1–250:1.

The interaction of MC-540 with phospholipid bilayers was determined by absorption and fluorescence spectroscopy. Absorption spectra were recorded by a SPECORD M 40 (Carl Zeiss) spectrophotometer. The corrected fluorescence spectra were obtained on an LS 5 (Perkin–Elmer) spectrofluorimeter. The temperature was controlled by circulating thermostated water through temperature-controllable cuvette holders.

RESULTS AND DISCUSSION

The incorporation of MC-540 into model membranes of various lecithin compositions (egg PC, POPC, DMPC, DPPC) was investigated. The lecithins chosen differ in their fatty acid chains. DPPC and DMPC are fully saturated phospholipids, while POPC and egg PC contain unsaturated hydrocarbon chains. The absorption spectra of MC-540 in the all lecithins showed two peaks, at

569 and 534 nm for MC-540 in DPPC,
568 and 532 nm for MC-540 in DMPC,
568 and 531 nm for MC-540 in POPC, and
567 and 531 nm for MC-540 in egg PC.

These spectra exhibited concentration-dependent changes corresponding to the MC-540 aggregation process. The longer-wavelength band can be attributed to dye monomers, and the shorter-wavelength band to dimers. The fluorescence spectra of MC-540 in all lecithins showed only one peak (excitation at 550 nm), corresponding to the fluorescent monomers of MC-540 at

595 nm for MC-540 in DPPC,
594 nm for MC-540 in DMPC,
595 nm for MC-540 in POPC, and
592 nm for MC-540 in egg PC.

Higher dye aggregates were not observed in these samples. Thus, for the molar concentration of MC-540 bound to liposomes (c_L), the following equation is valid:

$$c_L = c_M + 2c_D$$

where c_M and c_D are concentrations of monomers and dimers, respectively. From the spectral data the values of the monomer–dimer equilibrium constant K,

$$K = \frac{c_M^2}{c_D}$$

were stated at the temperature of 23 ± 2°C:

$K = 271.0 \pm 36.5$ μM for MC-540 in DMPC,
$K = 26.7 \pm 2.4$ μM for MC-540 in POPC, and
$K = 14.8 \pm 0.4$ μM for MC-540 in egg PC.

These data showed that monomer–dimer equilibrium is shifted toward dimers in unsaturated lecithins (POPC, egg PC).

Temperature-induced spectral changes of MC-540 bound to lecithin liposomes were observed and quantitatively evaluated for MC-540 in DMPC liposomes in terms of monomer–dimer distribution changes. The dissociation process of MC-540 dimers into monomers with increasing temperature was registered. Concentrations of monomers and dimers and corresponding dissociation constants for the temperature range of 15–40°C were calculated (Table I).

Temperature-dependent spectral changes of MC-540 in egg PC and POPC liposomes did not confirm simple monomer–dimer distribution changes, unlike MC-540 in DMPC. In addition, there were at least three colored species of MC-540 in POPC and four dye species in egg PC with different responses to the heating.

In the next experiments the influence of serum albumin on binding of MC-540 to lecithin liposomes was investigated. Absorption and fluorescence spectra of MC-540 in aqueous albumin solutions revealed the formation of a new absorption band (at 556 nm) and fluorescent band (at 572 nm) corresponding to MC-540 associated to albumin. The addition of albumin to liposome suspensions resulted in noteworthy spectral changes of MC-540. Analysis of these spectra revealed that the spectrum of MC-540 in albumin–liposome suspensions can be considered a superposition of the spectrum of MC-540 bound to lecithin and the spectrum of MC-540 bound to albumin. Thus, the total dye concentration in albumin–liposome suspensions can be expressed by the following equation:

Table I. Temperature-Dependent Changes in the Dissociation Constant K of MC-540 (0.05 mM) in DMPC (2.95 mM) Liposomes

	t (°C)									
	15	17	19	21	23	25	28	31	35	40
K (μM)	35	45	79	161	271	328	418	629	913	1518

$$c = c_L + c_A$$

where c is the total dye concentration, and c_L and c_A are the concentrations of the dye bound to liposomes and albumin, respectively. The distribution of MC-540 between liposomes and albumin was shown to be determined by the type of phospholipid used. In the case of DMPC and DPPC liposomes stained with MC-540 (5.1 μM), the presence of 30 μM albumin induced the association of more than 90% of MC-540 to albumin, whereas for POPC and egg PC liposomes, binding of more than 90% dye was observed for albumin concentrations of more than 110 μM. These data showed that albumin competed very successfully with lecithins containing saturated fatty acid chains (DPPC, DMPC) and competed weakly with unsaturated lecithins (POPC, egg PC) for binding dye molecules. Different cells' sensitivity toward MC-540 in the presence of albumin could be related to various abundances of saturated and unsaturated fatty acid chains in the membrane lipid composition.

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