

DIMERS OF MEROCYANINE 540 III. IN DIMYRISTOYL LECITHIN LIPOSOMES

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Electronic absorption spectroscopy was applied to study the occurrence and equilibrium of different coloured species of merocyanine 540 (M-540) in dimyristoyl lecithin (DML) liposomes. The M-540 absorption spectra show two isosbestic points (537 nm, 595 nm) which confirm the existence of two coloured species - monomers and dimers. The molar extinction coefficients of the pure monomer $\epsilon_M = 151100 \text{ mol}^{-1} \text{ l cm}^{-1}$ and pure dimer $\epsilon_D = 5400 \text{ mol}^{-1} \text{ l cm}^{-1}$ were determined at absorption maximum 568 nm. Distribution of M-540 monomers and dimers bound to DML liposomes was quantitatively determined and the monomer $\rightleftharpoons$ dimer equilibrium constant $K = 234.06 \text{ } \mu\text{mol/l}$ was calculated.

Introduction

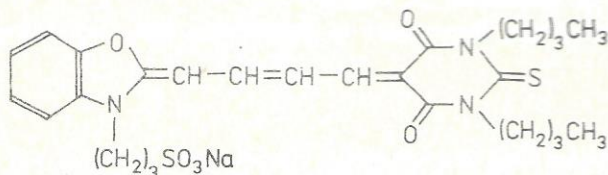
Merocyanine 540 (M-540) is a fluorescent anionic dye which appears to incorporate itself into membranes /14/, where it can serve as a reporter molecule for the state of the membrane potential in nerve cells /1/, mitochondria /4/ and cardiac cells /6/. It was shown to bind preferentially to fluid, cholesterol-free domains of the outer leaflet of the cell membrane /3, 15/. Binding of M-540 to cell membranes under certain conditions is cell type-specific. High-affinity binding sites for this dye abound on electrically excitable cells, leucemic cells and some classes of immature blood cells /2,7,8,11/. The changes of physical state of membrane give rise to dimerization of M-540. The dye shows a potential-dependent equilibrium between monomers and dimers /9/. WAGGONER and GRINVALD /13/ published calculated absorption spectra of the pure monomer and pure dimer for M-540 bound in egg phosphatidylcholine vesicles.

In our preceding paper /10/ monomer-dimer distribution and the equilibrium constant for large concentration range of M-540 in egg lecithin liposomes were stated. In this paper we have the aim to establish monomer-dimer distribution for M-540 in dimyristoyl lecithin (DML) liposomes. Absorption spectra of M-540 in DML have not yet been published and the pure monomer or pure

dimer spectrum is not known either.

Materials and methods

In our experiments merocyanine 540 (M-540) was used as a fluorescent probe (see structure below). This dye was obtained from Eastman Kodak, Co., and was used without further purification.



A phospholipid employed in our experiments was synthetic lecithin obtained from Fluka AG: L- β , γ -dimyristoyl- α -lecithin (DML). This lecithin was employed in the preparation of the "alcohol" liposomes according to the standard method [12]. The M-540 concentrations in these lecithin dispersions were in the range of $(1.5 \times 10^{-6} - 99.5 \times 10^{-6})$ mol/l. The samples were protected from light. All sample preparations and measurements were carried out at the temperature of $(25 \pm 1)^\circ\text{C}$.

The absorption spectra were recorded by spectrophotometer SPECORD M 40 (Carl Zeiss, Jena, GDR) within the 350-750 nm region.

Results and discussion

FIG. 1 illustrates the visible absorption spectra of M-540 in DML liposomes for M-540 concentrations ranging within the extent of $(1.5 - 99.5) \times 10^{-6}$ mol/l and constant DML concentration (2.87×10^{-3}) mol/l. The spectra show two absorption peaks at 568 ± 1 nm and at 531-534 nm. With increasing M-540 concentration, the following changes of the spectra were detected: i/ The peak at shorter wavelengths was blue-shifted from 534 nm to 531 nm with increasing dye concentration. ii/ With increasing concentration the molar extinction coefficient ϵ at 534 nm enhanced compared to a slight decrease at 568 nm. These concentration dependent changes characterize the M-540 aggregation process. The presence of two isosbestic points (537 nm; 595 nm) in absorption spectra (FIG. 1) reflects the existence of two various dye species (monomers and dimers). Higher aggregates were not observed in the concentration range used. No absorption peak corresponding to absorption of M-540 molecules in water was observed. The longer wavelength band can be attributed to dye monomers and the shorter

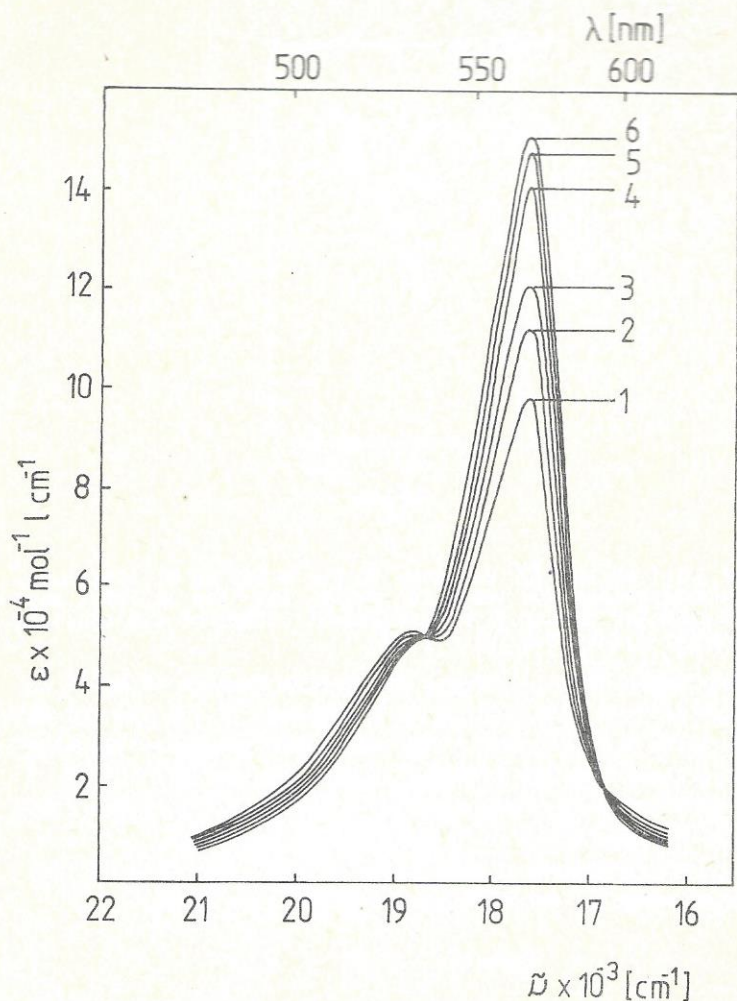


FIG. 1 Visible absorption spectra of M-540 in suspension of DML liposomes. Suspension contained constant DML concentration (2.87 mmol/l) and varying M-540 concentration: 1/ 99.50 $\mu\text{mol/l}$; 2/ 53.00 $\mu\text{mol/l}$; 3/ 31.02 $\mu\text{mol/l}$; 4/ 10.50 $\mu\text{mol/l}$; 5/ 1.85 $\mu\text{mol/l}$; 6/ 1.50 $\mu\text{mol/l}$

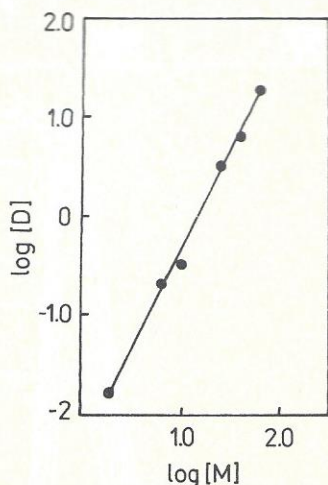


FIG. 2 Plot of $\log [D]$ vs. $\log [M]$ for M-540 in EL liposomes. The concentrations are taken in $\mu\text{mol/l}$. The line is least square fitted with the correlation coefficient 0.998 and its equation is: $\log [D] = 2.01 \log [M] - 2.38$

ter wavelength band to dimers. Absorption spectra of M-540 in DML have not yet been published. A decrease of the dye concentration below $1.5 \times 10^{-6} \text{ mol/l}$ does not alter the M-540 spectrum, in the case of freshly prepared samples. So the absorption spectrum of freshly prepared M-540 solutions at infinite dilution can be considered to be a monomeric spectrum.

When the whole amount of M-540 dye is bound to lecithin, the following equation for molar concentration is valid:

$$[M-540] = [M] + 2[D] \quad , \quad (1)$$

where $[M-540]$ is total dye concentration, $[M]$ and $[D]$ are concentrations of monomers and dimers, respectively. The values of $[M]$ and $[D]$ can be calculated /15/ from the data of optical densities OD in case we know the absorption coefficients of monomers ϵ_M and dimers ϵ_D :

$$OD = \epsilon_M [M] + \epsilon_D [D] \quad (2)$$

TAB. 1 Titration of DML (2.87×10^{-3} mol/l) liposomes with M-540. [M-540] - total molar concentration of the dye; [M] and [D] - the computed concentration of M-540 monomers and dimers, respectively; OD_{568} - the measured optical density at the monomeric maximum 568 nm, K - the computed monomer $\rightleftharpoons$ dimer equilibrium constant

[M-540] 10^6 mol/l	OD_{568} cm^{-1}	[M] 10^6 mol/l	[D] 10^6 mol/l	K 10^6 mol/l
1.50	0.227	1.500	0.000	-
1.85	0.275	1.819	0.015	220.68
7.20	1.029	6.803	0.198	233.74
10.50	1.440	9.850	0.325	298.53
31.02	3.726	24.544	3.238	186.04
53.00	6.030	39.670	6.665	236.12
99.50	9.750	63.890	17.804	229.27

$$Av.K = 234.06 \times 10^{-6} \text{ mol/l}$$

$$SD = 36.52 \times 10^{-6} \text{ mol/l}$$

We have determined the molar extinction coefficient of the pure monomer $\epsilon_M = 151100 \text{ mol}^{-1} \text{ l cm}^{-1}$ and pure dimer $\epsilon_D = 5400 \text{ mol}^{-1} \text{ l cm}^{-1}$ at 568 nm according to the method of WEST and PEARCE /15/ from the pure monomer spectrum and from the family of absorption curves. The concentrations of [M] and [D] calculated in such a way are stated in TAB. 1 together with the value of monomer $\rightleftharpoons$ dimer equilibrium constant K, defined by the following equation:

$$K = \frac{[M]^2}{[D]} \quad (3)$$

In case the [M] and [D] values fulfil the mass law for monomer $\rightleftharpoons$ dimer equilibrium, according to equation (3) the dependence $\log [D]$ vs. $\log [M]$ should be linear with a slope of two. Using our data we have plotted the stated dependence (FIG. 2). The equation of the straight line with the slope of two is fulfilled by the data for all M-540 concentrations used and so these values conform to the mass law for monomer $\rightleftharpoons$ dimer equilibrium and the values of $K = 234.06 \times 10^{-6} \text{ mol/l}$ are constant within a standard deviation of $36.52 \times 10^{-6} \text{ mol/l}$.

References

- /1/ COHEN, L. B., SALZBERG, B. M., DAVILA, H. V., ROSS, W. N., LANDOWNE, D., WAGGONER, A. S., WANG, C. H., J. Membrane Biol. 19 (1974) 1
- /2/ EASTON, T. G., VALINSKY, J. E., REICH, E., Cell 13 (1978) 475
- /3/ HUMPHRIES, G. M. K., LOVEJOY, J. P., Biochem. Biophys. Res. Com. 111 (1983) 768
- /4/ KINNALLY, K. W., TEDESCHI, H., MALOFF, B. L., Biochem. 17 (1978) 3419
- /5/ ROSS, W. N., SALZBERG, B. M., COHEN, L. B., DAVILLA, H. V. Biophys. J. 14 (1974) 983
- /6/ SALAMA, G., MORAD, M., Science 191 (1976) 485
- /7/ SCHLEGEL, R. A., PHELPS, B. M., WAGGONER, A., TERADA, L., WILLIAMSON, P., Cell 20 (1980) 321
- /8/ SIEBER, F., MEAGHER, R. C., SPIVAK, J. L., Differentiation 19 (1981) 65
- /9/ SIEBER, F., CRAIG, A., KREUGER, G. J., SMITH, R. E., ASH, R. C., Blood 68 (1986) 292 a
- /10/ ŠIKUROVÁ, L., HABAN, I., FRANKOVA, R., Studia biophysica 128 (1989) 163
- /11/ VALINSKY, J. E., EASTON, T. G., REICH, E., Cell 13 (1978) 487
- /12/ VLADIMIROV, Yu., DOBRETISOV, G. E., Fluorescentnyje zondy v issledovanii biologicheskikh membran, Nauka, Moskva 1980
- /13/ WAGGONER, A. S., GRINVALD, A., Ann. N. Y. Acad. Sci. 303 (1977) 217
- /14/ WAGGONER, A. S., Ann. Rev. Biophys. Bioeng. 8 (1979) 47
- /15/ WEST, W., PEARCE, S., J. Phys. Chem. 69 (1965) 1894
- /16/ WILLIAMSON, P. L., MASSEY, W. A., PHELPS, B. M., SCHLEGEL, R. A., Moll. Cell. Biol. 1 (1981) 128

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