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CHARGE TYPE AND TRANS-MEMBRANE TRANSPORT OF FLUORESCENT PROBES IN SURFACTANT VESICLES

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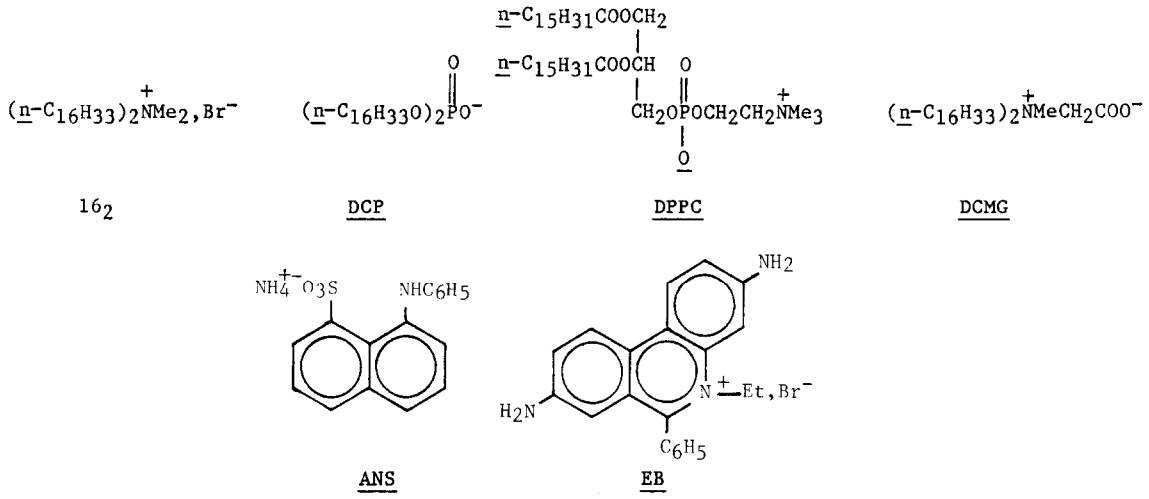
Summary. We describe the effects of charge type on the trans-membrane transport of anionic and cationic fluorescent probes in cationic, anionic, and zwitterionic surfactant vesicles.

To what extent does the charge type of surfactant vesicles or liposomes determine the rate of trans-membrane transport of organic ionic fluorescent probes?[1] This question is significant because of the central role played by surfactant assemblies in the study of membrane mimetic reactions.[2] Studies bearing on this problem have been carried out with bilayer-coated nylon capsules[3a] and with photoactivated carrier permeants in phosphatidylcholine liposomes.[3b] However, time-resolved kinetic studies of more complicated permeants in simple synthetic vesicles are few.[1,3c] Here we report initial results from a study of four vesicular surfactants and two probes that help shape an answer to our initial question.

The surfactants and probes are illustrated in Chart I. Cationic dihexadecyldimethylammonium bromide (16_2),[1,4] anionic dicetyl phosphate (DCP, Sigma),[2d] and zwitterionic dipalmitoylphosphatidylcholine (DPPC, Sigma)[1] were all readily available. Zwitterionic N,N-dicetyl-N-methylglycinate (DCMG) was synthesized by quaternization of N,N-dicetyl-N-methylamine with ethyl bromoacetate (refluxing ethanol, 21 days, recryst. 2X from ether), followed by saponification (0.11 M NaOH in refluxing 97% aqueous EtOH, 48 hrs), neutralization (HCl), leaching with hot $CHCl_3$, and recrystallization (2X, EtOAc). We thus obtained DCMG, mp ~76°C, in 58% overall yield based on the tertiary amine. Satisfactory spectral and analytical data were obtained for DCMG. The fluorescent probes were commercially available: 1-anilino-8-naphthalene sulfonate (ANS, Aldrich) and ethidium bromide (EB, Sigma).

All vesicle solutions were prepared by sonication[5] at 55-60°C, cooled to ambient temperature, and passed through 0.8 μM Millex PF filters before use. Sonicated vesicles of

Chart I



16_2 DCP DPPC DCMG

ANS EB

16_2, DPPC, and DCP have been well-characterized,[1,2d] but we include a brief characterization of the novel DCMG vesicles. Dynamic light scattering gave the hydrodynamic diameter as ~1750 Å, considerably larger than 16_2 (~400 Å), or DPPC (~500 Å) vesicles prepared under comparable conditions.[1] A sharp phase transition was observed by differential scanning calorimetry at 27°C (ΔH = 5.5 kcal/mol) in $1x10^{-3}$ M solutions of vesicular DCMG. For comparison, small 16_2 vesicles exhibit a major phase transition at ~25°C, whereas the T_c of DPPC vesicles is considerably higher (37°, 41°C).[1]

The intensity ratio of the fluorescence emission peaks III (385 nm) to I (375 nm) of $5x10^{-5}$ M pyrene in $1x10^{-3}$ M vesicular DCMG reflects the pyrene's microenvironment.[6] The experimental value for DCMG, 0.82, is intermediate between those of 16_2 (0.78) and DPPC (0.88).[1] The polarity of pyrene's microenvironment in these vesicles is therefore bracketed by methanol (III/I = 0.75)[6] and ethanol (0.91).[6]

The intensity ratio of pyrene excimer fluorescence (475 nm) to monomer fluorescence (392 nm) is considered to report on the medium's microviscosity.[7] With $1x10^{-3}$ M vesicular surfactant and $5x10^{-5}$ pyrene, I_{475}/I_{392} was 1.19 for DCMG and 0.81 for DPPC. The significantly higher pyrene excimer/monomer fluorescence in DCMG suggests that these zwitterionic vesicles offer less resistance to pyrene translation than DPPC liposomes.

Anionic ANS has been used as a dynamic fluorescent probe of permeation in lecithin[8] and 16_2 vesicles.[1] Although cationic EB exhibits markedly enhanced fluorescence upon electrostatic binding to anionic sites of polymeric nucleic acids, and further enhancement

after intercalation into less polar sites of double stranded nucleic acid helices,[9] EB does not appear to have been used as a time-dependent fluorescent probe. We studied the time-dependent enhancement of fluorescence attending the stopped-flow mixing of ANS or EB with the four vesicular surfactants of Chart I; each combination of probe and vesicle corresponds to a different charge/charge interaction.

We measured[1] the half-times in sec for the enhancement of probe fluorescence intensity. The intensity/time correlations were not strictly first-order,[8] but half-times could be determined with precisions of ±2.4%. These half-times are inversely related to the rate of trans-membrane transport of the probe.[1,8]

Between 15° and 40°C, the anionic probe ANS does not bind to anionic DCP vesicles, although it crosses rapidly ($\tau_{1/2}$ = 0.20 and 0.07 sec, respectively) into vesicles of cationic 16_2. Its transport into zwitterionic DPPC vesicles, however, occurs only above ~38°C (T_c), and even then with $\tau_{1/2}$ = 4.5 sec at 40°C, ~65 times more slowly than into 16_2.[1] Is this primarily an electrostatic effect? Probably not, for transport of ANS into (albeit charge-reversed) zwitterionic DCMG is also rapid: $\tau_{1/2}$ ranges from 1.4 sec at 12°C to 0.58 sec at 40°C, with a minimum of 0.31 sec at 25.5°, near T_c of the vesicle (27°C).[10] Additionally, cationic EB does not cross into zwitterionic DPPC vesicles, although it slowly ($\tau_{1/2}$ = 260 and 371 sec at 25° and 40°) permeates the charged-reversed zwitterionic DCMG vesicles.

It is possible that the long chains of DPPC, which are vicinally anchored to a glycerol backbone, are optimally spaced for efficient bilayer packing, and provide a more effective barrier to probe molecule transport than the geminally anchored chains of DCMG. The latter arrangement, of necessity, creates "clefts" in the vesicular surface that may afford entries for the probes.

Nevertheless, the influence of electrostatics is superimposed upon the architectural effects. For example, $\tau_{1/2}$ (25°) for transport of anionic ANS into zwitterionic DCMG increases upon doping the vesicles with anionic DCP (from 0.31 sec in the absence of DCP to 0.53 sec at 17 mol-% DCP), but decreases upon doping with cationic 16_2 (from 0.31 sec to 0.14 sec at 9 mol-% 16_2). Also, cationic probe EB (λ_{max} in H_2O, 480 nm) is excluded from cationic 16_2 vesicles (λ_{max} remains at 480 nm) and from zwitterionic DPPC (λ_{max}, 480 nm), although it is strongly bound by anionic DCP vesicles (λ_{max}, 520 nm)[11] and shows time-dependent fluorescence enhancement in zwitterionic DCMG (λ_{max}, 490 nm, λ_{em}, 620 nm).

The present results illustrate the cooperative effects of surfactant architecture and charge in determining vesicle/probe interactions and attendant dynamic phenomena. They should prove useful in the fine-tuning of fluorescent probes for various model and real membranes.

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(11) The T_c of DCP vesicles is 66°C.[12] The lack of time dependence in the strong fluorescence enhancement which attends the binding of EB to DCP vesicles at 25° or 41°C is due either to very rapid (<1 msec) permeation or to the absence of permeation (surface binding only) below T_c.

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