An Unsymmetric Monolayer Vesicle Membrane

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A monolayer vesicle membrane with a (large) thiosuccinic acid and a (small) sulphonic acid head group is orientated in such a way that ⩾98% of the large head groups are on the outside and, correspondingly, ⩾98% of the small head groups are inside.

Macrocyclic amphiphiles with two long alkyl chains and two head groups of differing size make possible the synthesis of fully unsymmetric cell membranes. The basis of this hypothesis is that pronounced curvature in monolayer membranes will only occur when wedge-shaped molecules aggregate in such a way that the thin end (or small head group) forms the smaller inner surface of a cell (vesicle, liposome). The broad end of the molecular wedge provides the outside surface. A wedge-shaped amphiphile (**1**) has been synthesized from a bis-maleic acid macrotetrolide and stepwise addition of thiosuccinic acid and sodium bisulphite.[1]

The large head group is of about the same length (*ca.* 10 Å) and width (*ca.* 5 Å)[2] as the narrow part of the macrocycle, since both are succinic acid derivatives connected *via* a sulphur bridge. The sulphonic acid on the other end of the macrocycle may be taken as a disk with a diameter of 6 Å.[3]

The ratio of surface areas of both head groups is *ca.* 2:1 if the succinic acid is oriented parallel to the membrane surface. It may, however, rotate about the C–S bonds, independently of the hydrophobic part. Its effective surface is much smaller if it is inclined towards the layer plane.[4]

Sonication of (**1**) at pH 4 gives microvesicles with an average diameter of 200 Å. We assume that (**1**) produces monolayer membranes with a thickness of *ca.* 20 Å. The ratio of inner to outer surface is *ca.* 5:3, and a strong orientation of head groups would be predicted. Proof of the supposed

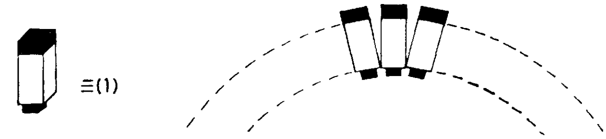
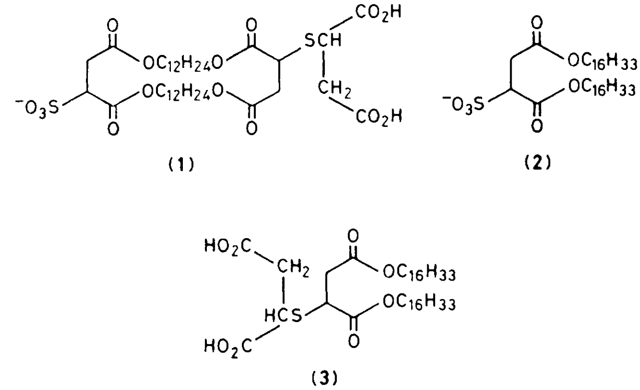


Figure 1. Scheme of an unsymmetric monolayered vesicle membrane.



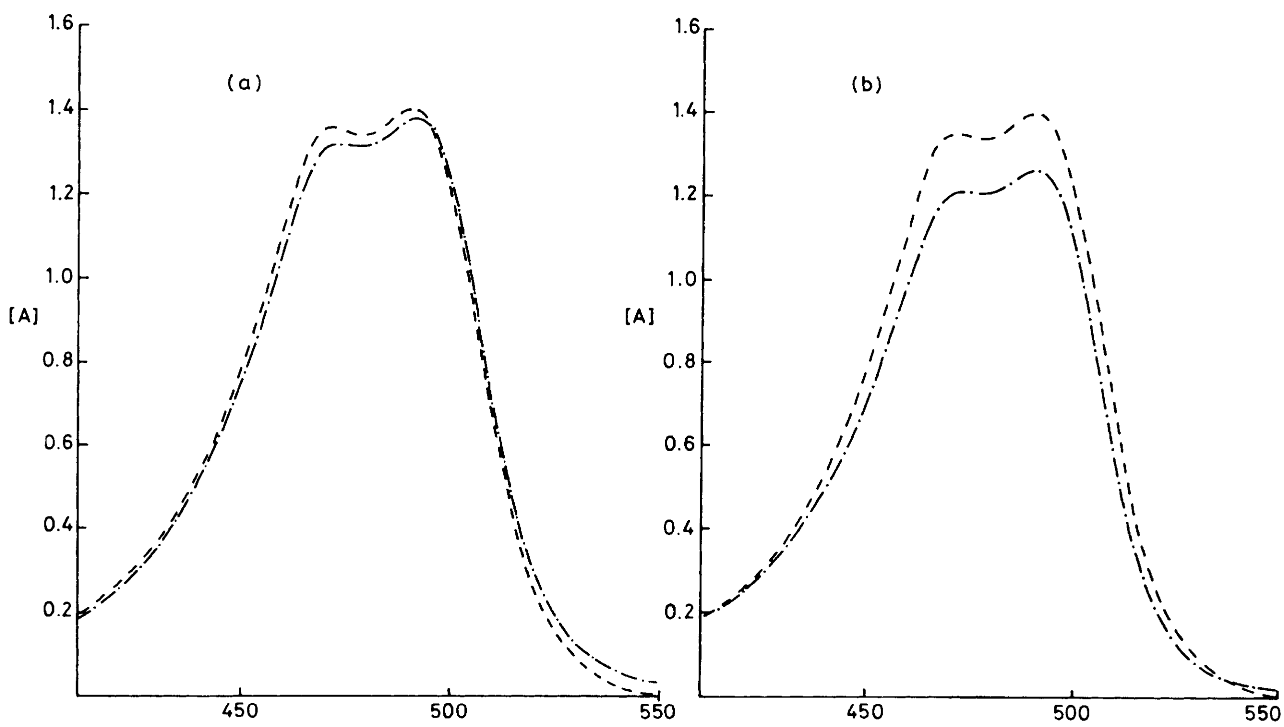


Figure 2. (a) Acridine orange (1 μmol in 15 ml) in hydrochloric acid at pH 2 (- - - - -) and after addition of 3.5 μmol of vesicular (**1**) (-·-·-·-·-); the lower extinction is caused by dilution; (b) as (a) except that 10% of (**2**) was added when the vesicle was prepared. This corresponds to *ca.* 5% of sulphonate groups on the outer surface.

unsymmetric arrangement, succinic acid outside and sulphonic acid inside, came from the exploitation of the metachromatic effect.[5,6]

Bilayer membrane vesicles were made from dicetyl esters of succinic acid containing sulphonic acid (**2**) or thiosuccinic acid head groups (**3**). At pH 7 both vesicles showed strong spectral changes when their aqueous solutions were added to solutions of acridine orange or methylene blue. At a 1 : 10 ratio of dye: compounds (**2**) or (**3**) the absorption at 490 nm was diminished by *ca.* 60%. At pH 2 the effect of sulphonic acid (**2**) vesicles was still the same, whereas the succinic acid derivative (**3**) was protonated and its vesicles had no effect on the acridine orange spectrum. Mixed vesicles containing 95, 90, or 80% of thiosuccinic acid (**3**) and 5, 10, or 20% of sulphonic acid (**2**) were then prepared. Their metachromatic effect was proportional to the amount of sulphonic acid present. The monolayer membrane vesicles made from (**1**)† were then examined under the same conditions as the bilayered vesicles. *No* metachromatic effect was observed up to ratios of (**1**): acridine orange of 3 : 1 (Figure 2a). When 10% of the sulphonic acid (**2**) was added to the same vesicle a decrease in the 490 nm band was again observed (Figure 2b).

† Characterized by electron microscopy and by entrapment experiments with acridine orange and methylene blue. A solution (pH = 2) of the dye (10^{-2} M) and (**1**) (10^{-3} M) was sonicated. The extracellular dye was removed by chromatography on Amberlite IRC-50. The entrapped dyes exhibited strong metachromatic effects and did not react with dithionite.

We consider these results to provide proof that none, or less than 2%, of the sulphonic acid head groups of (**1**) is on the outer vesicle surface. To our knowledge this is the first report of the synthesis of a fully unsymmetric vesicle membrane. Such membranes should be useful for the construction of molecular arrangements which allow vectorial reactions, such as charge separation,[7]† if head groups are introduced which can act as electron or proton donors and acceptors.[8] To the carboxylic acid head groups dye-stuffs may be bound covalently; the sulphonic acid group can fix cationic counterions selectively.

Received, 12th November 1982; Com. 1296

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