## SHORT COMMUNICATIONS

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### Free energy of mixing of phospholipids and cholesterol at the air–water interface

Experiments performed with mixed monolayers show a condensing effect of cholesterol on lecithin at air–water interfaces[1–3]; this effect could be the result of some specific interaction between these molecules or simply a geometrical molecular rearrangement not necessarily involving a specific interaction energy.

The present investigation was carried out to evaluate the free energy of mixing at air–water interfaces of the binary systems: cholesterol–phospholipid, cholesterol–hexadecanol, cholesterol–hexadecylamine and cholesterol–palmitic acid as a measure of the tendency for interaction between the components.

An automatic recording surface balance previously described[4] was used for obtaining the surface pressure ($\pi$) – area curves. The synthetic phospholipids used were : $\beta,\gamma$-dipalmitoyl-DL-$\alpha$-lecithin and $\beta,\gamma$-dipalmitoyl-DL-$\alpha$-cephalin obtained from Mann Laboratories; 1-hexadecanol and hexadecylamine were obtained from Fluka; palmitic acid from Fisher; cholesterol from Schuchardt. All these lipids gave, in preliminary experiments, limiting areas per molecule in accordance with the accepted values. Inorganic reagents were A.R. grade. The subphase was, in all cases, a phosphate buffer (pH 7.2, ionic strength 0.15) made up with sodium chloride. Deionized water, freshly distilled with alkaline permanganate was used throughout. Purified petroleum ether was used for the preparation of the spreading lipid solutions with the addition of ethanol (20%, v/v) for phospholipids; 1 ml of the solvent spread on 1000 cm$^2$ produced less than 0.5 dyne/cm surface pressure when the surface was reduced to 100 cm$^2$.

Mixed monolayers were obtained by spreading, at 0 dyne/cm surface pressure, the corresponding petroleum ether solutions of the lipids. Compression curves were obtained at a rate of 4 cm/min. All experiments were performed in an air-conditioned room at 20° (± 1°). The excess free energy of mixing ($\Delta G_M^E$) was calculated from the compression curves, using the equation:

$$\Delta G_M^E = \int_0^\pi (\delta_{12} - N_1\delta_1 - N_2\delta_2)\mathrm{d}\pi$$

where $\delta_{12}$, $\delta_1$ and $\delta_2$ represent the area of the mixed monolayer and the areas of the respective pure components; $N_1$ and $N_2$ being the respective molar fractions[5].

The integrals $I_{12} = \int_0^\pi \delta_{12}\mathrm{d}\pi$; $I_1 = \int_0^\pi \delta_1\mathrm{d}\pi$; $I_2 = \int_0^\pi \delta_2\mathrm{d}\pi$, were separately evaluated from the corresponding compression curves setting the upper limit of the integration at 40 dyne/cm. The free energy of mixing was calculated[4] from the equation:

$$\Delta G_M = \Delta G_M^E + \Delta G_M^I$$

in which $\Delta G_M^I = (RTN_1 \ln N_1 + RTN_2 \ln N_2)$ is the free energy of mixing for an ideal system. Each value represented in Table I is the average of at least five separate experiments.

TABLE I

FREE ENERGY OF MIXING ($\Delta G_M$, cal/mole) AT AIR–WATER INTERFACE FOR MIXTURES OF CHOLESTEROL WITH PHOSPHOLIPID OR $C_{16}$ HYDROCARBON CHAIN COMPOUNDS

Temperature, 20°; subphase: phosphate buffer (pH 7.2; $I$ 0.15). Integration limits: 0 and 40 dyne/cm. Figures in parentheses correspond to the integrals between 0 and 27.5 dyne/cm.

| | *Molar fraction* | | | | |
|---|---|---|---|---|---|
| | *0.20* | *0.33* | *0.50* | *0.67* | *0.80* |
| $\beta,\gamma$-Dipalmitoyl-DL-$\alpha$-lecithin | −330 (−210) | −480 (−450) | −630 (−460) | −510 (−460) | −300 (−300) |
| $\beta$-$\gamma$-Dipalmitoyl-DL-$\alpha$-cephalin | −430 (−300) | −440 (−250) | −640 (−430) | −430 (−310) | −340 (−220) |
| Hexadecylamine | (−240) | (−350) | (−390) | (−340) | (−280) |
| 1-Hexadecanol | −180 (−220) | −260 (−290) | −370 (−380) | −250 (−280) | −200 (−230) |
| Palmitic acid | −380 (−330) | −460 (−430) | −450 (−430) | −350 (−350) | −280 (−280) |
| Ideal system $\Delta G_M^I = RT\,(N_1 \ln N_1 + N_2 \ln N_2)$ | −290 | −370 | −400 | −370 | −290 |

The significance of cholesterol–phospholipid interaction in the structure of some biological membranes has been emphasized in experimental[1–3] and theoretical[6–8] investigations. By analysis of molecular models the existence of cholesterol–lecithin and cholesterol–sphingomyelin equimolecular complexes has been postulated which form the basic framework of myelin membranes. The stability of these complexes has been attributed to attractive Van der Waals forces between the methylene groups of the hydrocarbon chains of phospholipids and those of cholesterol, *plus* the hydrogen bonding of the cholesterol hydroxyl with the oxygen atom of the phosphatidic acid moiety of the phospholipid[7].

The free energy of mixing for a binary system is a measure of the tendency for its components to undergo a dissolution process. It can be seen from Table I that the most negative values for all the mixtures studied correspond to the system: cholesterol–$\beta,\gamma$-dipalmitoyl-DL-$\alpha$-lecithin and cholesterol–$\beta,\gamma$-dipalmitoyl-DL-$\alpha$-cephalin, when its components are present in equimolecular amounts at the interface; the $\Delta G_M$ values are roughly 200 cal/mole more negative than those corresponding to the ideal systems or to the cholesterol–hexadecanol and cholesterol–palmitic acid systems at 0.5 molar fraction.

The conclusion can be drawn then, that the tendency for dissolution of these phospholipids in equimolecular amounts of cholesterol is greater than corresponding tendency for dissolution of hexadecanol or palmitic acid in equimolecular amounts of cholesterol at the air–water interface. This greater tendency for dissolution could originate from the existence of some kind of interaction between cholesterol and these phospholipids.

If the only condition for the interaction between cholesterol and the phospholipids were the coexistence of the specific sites postulated, having in mind the relative composition of the lipids of myelin[7], one would expect the same deviation at 0.5 and 0.67 molar fraction for the system cholesterol–palmitic acid, in which the cholesterol has the possibility for interaction with the methylene groups of the $C_{16}$ chain of

palmitic acid and through the hydroxyl group with the oxygen atom of the carbonyl group. This not being the case it seems that other conditions must be fulfilled.

The figures in parentheses in Table I represent the $\Delta G_M$ values obtained using as integration limits 0 and 27.5 dyne/cm; it can be seen that the differences between these values and those corresponding to the integration limits 0 and 40 dyne/cm are small for the cholesterol–hexadecanol, and cholesterol–palmitic acid mixtures. On the contrary, for the systems cholesterol–phospholipid the difference of the $\Delta G_M$ values obtained by changing the integration limits is remarkable; between the limits 0 and 27.5 dyne/cm the $\Delta G_M$ values are very close to those of the ideal system.

Van der Waals forces exert the maximum attractive effect between methylene groups of long-chain hydrocarbons when the distance between the centers of the carbon atoms is 4.1 Å, being practically zero for distances greater than 6 Å. Below 4.1 Å the effect changes abruptly, becoming an increasing repulsive effect[7].

The difference between the $\Delta G_M$ values for both pairs of integration limits in the cholesterol–phospholipid systems could be interpreted as indicative that a closed packed configuration is necessary for the enhancement of the tendency to mutual dissolution. As this closed packed configuration has no effect on the $\Delta G_M$ values for the cholesterol–$C_{16}$ hydrocarbon chains compounds, in spite of being real systems, it may be concluded that some geometrical molecular arrangement characteristic of of the phospholipid molecules is necessary in order to increase the tendency for mutual dissolution between cholesterol and the phospholipid.

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