Acta Biochimica Polonica

Vol. 42 No. 1/1995

89–96

QUARTERLY

Aspects of self- and cross-association hydrophobicity in a single chain binary mixture. A computer study

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Received: 19 October, 1994, accepted: 31 January, 1995

Key words: single chain binary mixture, association phenomena, phase transitions, hydrophobic chain length

The differential scanning calorimetry (DSC) traces for binary mixtures of single chain amphiphiles present more than one transition temperature, while the traces for pure single chain amphiphiles have a single (or at most two) transition temperature(s). These secondary transition temperatures can appear owing to the selective association processes between the components of the lipid mixtures.

This paper presents an attempt to examine theoretically the association of single chain amphiphiles between (a) two identical molecules (self-association) and (b) two different molecules (cross-association). Association probabilities, the mean association probabilities and the overall ratio of efficiency, were evaluated for 36 binary mixtures of single chain amphiphiles. A test system for evaluation of differential effects of the hydrophobic chain length on the mean association probabilities was considered. Self-association of longer hydrophobic chains and cross-association phenomena show a high probability compared with the self-association of shorter hydrophobic chains. We found that "the efficacious length of association" in a binary mixture of single chain amphiphiles is 8 methylene groups.

Computer simulations regarding lipid-lipid interaction in lipid monolayers consisting of more than one type of lipid molecules approximate rather closely natural systems and should give information on the effect of these interactions in monolayers. With the exception of bacterial cell membranes, biological membranes contain the sterol, cholesterol, and the studies on theoretical approaches to mixed monolayers of phospholipids have been very actively pursued [1]. The behavior of lipid monolayers at the air-water interface is closely similar to that of the bilayers formed in aqueous dispersions of lipids. It has been reported that molecular clusters occur in lipid bilayers formed by binary mixtures [2].

In some binary lipid mixtures there occurs a separation of the molecular species, depending on the structure of the chains and the polar head groups. A unique phase transition was found by X-ray scattering on monolayers composed of one single chain amphiphile species [3], whereas DSC[1] thermograms for various lipid binary mixtures show that at least one secondary transition appears in addition to the main transition [4]. High-sensitivity DSC ther-

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[1] Abbreviations: DSC, differential scanning colorimetry; PC, phosphatidylcholine.

mograms have revealed that some of the mixed acyl phosphatidylcholines display main transition profiles which are actually consist of at least two individual transition peaks. This phenomenon has been observed with C(14):C(18)-PC, C(12):C(18)-PC, C(10):C(18)-PC, and C(14):C(16)-PC [4]. It has been suggested that these multiple peaks can arise from segregation of the bilayer into domains of randomly packed acyl chains or from packing of the hydrophobic chains into more than one interdigitated conformation within the gel state of these phosphatidylcholines.

Phase transition temperatures depend on the nature of the mixture components, especially on the length of hydrophobic chains [5–8]. These phase transitions are associated with transitions between different organization patterns, characteristic for liquid crystals [9]. There is no doubt that these phenomena are related to a selective association process of the components in binary phospholipid mixtures [10, 11]. In consequence they define the dynamic properties of monolayers and bilayers, especially the thickness fluctuations and appearance of the statistical pores [12–14]. Lipid molecules perform lateral and perpendicular movements on the bilayer surfaces. This points to a relation between the association processes and the existence of clusters and the dynamic features of lipid bilayer structure.

COMPUTING STRATEGY

Obviously, a binary mixture of single chain amphiphiles is characterized by the physical properties of its components: the length of hydrophobic chains (N_1,N_2), the cross section areas (a_1,a_2), the electric dipole moments of the polar head groups (p_1,p_2), and the mole fraction x_1 (or x_2, $x_1 + x_2 = 1$) of one of the components. The length of hydrophobic chains is given by the number of its C–C covalent bonds (number of methylene groups). The parameters concerning the shorter chain component will be denoted by index "1" and those concerning the longer chain component by index "2". In a binary mixture organized as a monolayer, two identical molecules can associate with the probabilities P_{11} and P_{22} (self-association probabilities), respectively, and two different molecules with the probability P_{12} (cross-association probability). These association probabilities can be calculated using the following model [10]:

$$P_{11} = x_1^2 e_1/(x_1^2 e_1 + x_2^2 e_2 + x_1 x_2) \tag{1}$$

$$P_{12} = x_1 x_2/(x_1^2 e_1 + x_2^2 e_2 + x_1 x_2) \tag{2}$$

$$P_{22} = x_2^2 e_2/(x_1^2 e_1 + x_2^2 e_2 + x_1 x_2) \tag{3}$$

where the following notations are used:

$$e_1 = \exp[-(U_{12} - U_{11})/RT];$$
$$e_2 = \exp[-(U_{12} - U_{22})/RT] \tag{4}$$

$$U_{11} = -(p_1^2 N_A/(4\pi\varepsilon(2r_1)^3) + W_{11}) \tag{5}$$

$$U_{12} = -(p_1 p_2 N_A/(4\pi\varepsilon(r_1+r_2)^3) + W_{12}) \tag{6}$$

$$U_{22} = -(p_2^2 N_A/(4\pi\varepsilon(2r_2)^3) + W_{22}) \tag{7}$$

where p_1, r_1 and p_2, r_2 are the electric dipole moment and the radius of the polar head group of the amphiphiles from the shorter and the longer species, respectively. R is the gas constant and T is the absolute temperature. Also, U_{11}, U_{12}, U_{22} are the mole interaction energies between two neighbouring molecules that are identical (U_{11}, U_{22}) or different (U_{12}). These values determine the selective association between single chain amphiphiles, which results finally in the formation of structural inhomogeneities within the lipid aggregate. The first term in equations (5–7) accounts for the interaction between electric dipole moments; as dipole moments of lysophospholipids have no sterical restrictions, they are parallel to the surface of monolayer and can couple themselves in the most favourable energetic state. The second term in equations (5–7) is due to the van der Waals dispersion forces between hydrophobic chains.

These terms are given by the following formulas:

$$W_{11} = \sum_{i,k=1}^{N_1} \frac{A}{[4r_1^2 + (i-k)^2\lambda^2]^3} \tag{8}$$

$$W_{12} = \sum_{i=1}^{N_1}\sum_{k=1}^{N_2} \frac{A}{[(r_1 + r_2)^2 + (i-k)^2\lambda^2]^3} \tag{9}$$

$$W_{22} = \sum_{i,k=1}^{N_2} \frac{A}{[4r_2^2 + (i-k)^2\lambda^2]^3} \tag{10}$$

where $\lambda = 1.27$ Å is the length of the C–C covalent bond normally projected to bilayer.

At low temperatures, the monolayer is in the gel phase and the molecules are close enough so that it is correct to suppose that hydrophobic chains are parallel and all covalent bonds are in *trans* conformation. The calculus of van der Waals-London interaction energy was based on the assumption that each saturated hydrophobic chain consists of attraction centres located on the axis of the chain. Hamacker's constant was calculated in such a way that each attraction centre is equivalent to a methylene group. Within the limits of the second order perturbation theory, the hypothesis of local additivity of dispersion forces energies has been accepted [15]. In the case of cylinders of uniform polarizability and non-zero radius the approximate formula was given by Langbein [16]. Using this formula in the calculation of van der Waals-London dispersion energy, we have obtained the numerical result by about 4% higher than the value obtained from the Salem formula [15]. Moreover, since the difference between the cross molecule pair formation energy and the identical molecule pair formation energy appears in computation of the association probabilities, the effect of this approximation on the association probabilities will be considerably diminished. Therefore, we can assume that the conclusions concerning the association probabilities are valid even if the nonadditivity of dispersion forces is not considered.

The distance between any two attraction centres (the methylene group near the polar head group and the methyl end on the longer chain) is less than 1000 Å, therefore the retardation effects have not been considered [16]. At the same time, the distance between the nearest attraction centres ($\cong 7.14$ Å) is far greater than the dimensions of attraction entities (atoms, covalent bonds), and London's law ($W = -A/d^6$) is valid, where $A = 5.6 \times 10^3$ Å^6 J mol^{-1}. In this paper all our computations have been made on the assumption of equimolar ratios of single chain amphiphiles ($x_1 = x_2 = 1/2$).

RESULTS

Association probabilities were calculated for 36 binary mixtures of single chain amphiphile molecules and are displayed in a triangular matrix. Each element of the triangular matrix contains hydrophobic chain length of the two components. The elements of the matrix are: (N_1, N_2) with $N_1 < N_2 \leq 24$, $N_1 \varepsilon$ [8, 10,..., 22]; $N_2 \varepsilon$ [N_1+ 2, 24]. All binary mixtures consist of an even number of carbon atoms. Cross section area of the first component polar group is $a_1 = 40$ Å^2 and that of the second component is $a_2 = 60$ Å^2. The cross section area of the polar head groups can be evaluated for particular physical conditions [17]. Polar head groups are electrically neutral. Values of 5, 10 ,15 ,..., 45 D were selected for the electric dipole moment of each component.

Generally, the electric dipole moment of amphiphile molecules depends strongly on the aqueous medium composition which can modify both the fixed effective electrical charges of the electric dipole moments and the distance between them. For this reason, a set of values for the electric dipole moments was chosen so that the real values for particular molecules fall within this interval.

A simple transition from two-dimensional handling of theoretical data to three dimensions could uncover some interesting relationships between the probability of association and the length of the hydrophobic chain. Figures 1–3 show the self-association probability of the shorter acyl chain, the longer acyl chain and the cross-association probability in the case when p_1 = 5 D and p_2 = 5 D. For constant N_1 the increase in the longer acyl chain has an opposite effect on either of the two self--association probabilities (P_{11} increases and P_{22} decreases), while the cross-association probability varies in the same sense as P_{11}. The increase in the length of the shorter acyl chain (when N_2 is constant) can be favourable to the self-association of the longer chain (Fig. 3), but not for that of the shorter component (Fig. 2). In this situation the cross-association probability varies in the same way as that of the shorter hydrophobic chain. These findings are relevant and show an interesting aspect of the selective association within a binary mixture: insertion of a methylene group into the shorter hydrocarbonic chain has opposite effects on each of the three association probabilities, P_{11}, P_{12} and P_{22} as compared with the insertion of a methylene group into the longer hydrocarbonic chain. Another prominent feature is that for a given set (N_1, N_2) the cross-association probability (P_{12}) and the self-association probability of the longer acyl chain (P_{22}) have high

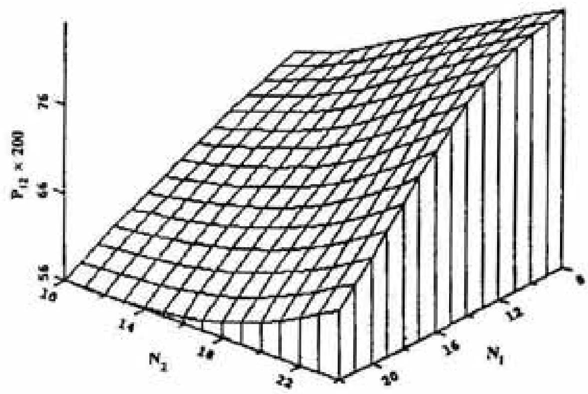

Fig. 1. A three dimensional view of the cross-association probability P_{12} as a function of the hydrophobic chain length of the two components of a binary lipid mixture.
The electric dipole moments of the polar head groups were: $p_1 = 5$ D, $p_2 = 5$ D. The cross-association probabilities were multiplied by 200.

values compared with the self-association probability of the shorter acyl chain (P_{11}).

Two possibilities compete to moving the effective electrical charges of the electric dipole moments: (a) the electric dipole moment is made up by a succession of covalent bonds and (b) the appearance of a screening effect of the electric charges produced by other molecules from the neighboring medium. An immediate consequence would be that the electric dipole moments of the two components in a given mixture cannot be taken as a constant. Therefore, a more adequate parameter describing appropriately enough the selective processes of association in a single chain binary lipid mixture is the mean association probability over the electric dipole moments of the two molecules as variables.

Table 1 lists the mean probabilities of self-association (h_{11} and h_{22}) and cross-association (h_{12}) for some binary mixtures. They are given by the formula:

$$h_{ij} = \frac{\int_5^{45}\int_5^{45} P_{ij}\,dp_1 dp_2}{\int_5^{45}\int_5^{45} dp_1 dp_2} \qquad (11)$$

Most of single chain amphiphiles possess an electric dipole moment of the polar head group ranging between 5 D and 45 D [10, 11]. Therefore, we considered it appropriate to calculate the above parameter integrating formula (11) between 5 D and 45 D.

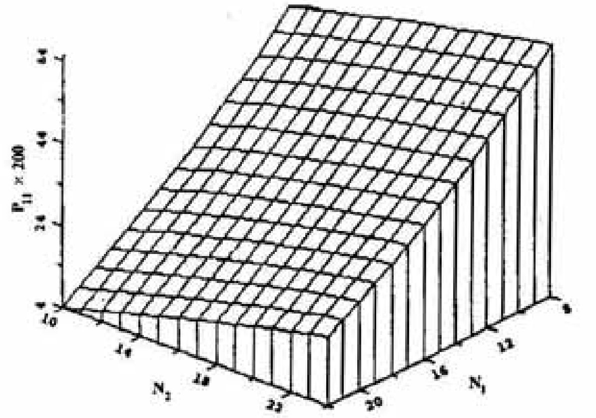

Fig. 2. A three dimensional view of the self-association probability P_{11} of the shorter component as a function of the hydrophobic chain length of the two components of a binary lipid mixture.
Other conditions as in Fig.1.

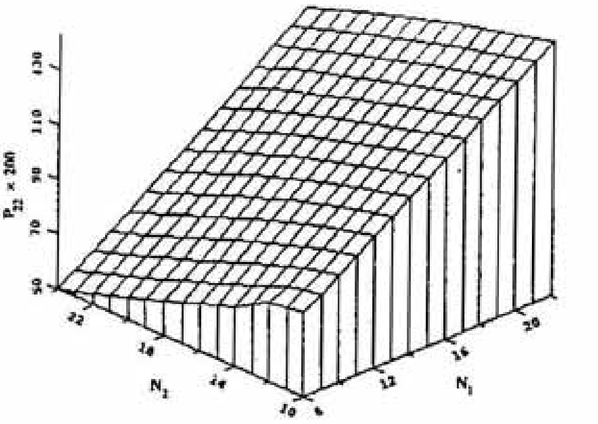

Fig. 3. A three dimensional view of the self-association probability P_{22} of the longer component as a function of the hydrophobic chain length of the two components of a binary lipid mixture.
Other conditions as in Fig.1.

From the data presented in Table 1 we can easily see the relationships between mean association probabilities:

$$h_{11}(N_1, N_2) = h_{11}(N_1 + 2, N_2 + 8) \qquad (12)$$

where N_1 and N_2 are the number of carbon atoms of the shorter and the longer component, respectively. The data point to the existence of an equivalence between the increase in the shorter chain length by one methylene group and the increase by four methylene groups of the longer chain length. Similarly, for the mean self-association probability h_{22} we have found

Table 1

Mean association probabilities for 21 binary mixtures.

The upper, middle and lower rows denote, respectively, h_{11}, h_{12} and h_{22} (for example, 8:10 is the binary lipid mixture for which $N_1 = 8$ and $N_2 = 10$).

8:10	8:12	8:14	8:16	8:18	8:20	8:22	8:24
0.2119 0.3529 0.4351	0.2234 0.3630 0.4153	0.2318 0.3756 0.3928	0.2402 0.3898 0.3687	0.2495 0.4043 0.3459	0.2577 0.4186 0.3236	0.2654 0.4324 0.3018	0.2731 0.4460 0.2810
10:12	10:14	10:16	10:18	10:20	10:22	10:24	
0.1884 0.3513 0.4602	0.1987 0.3618 0.4393	0.2076 0.3758 0.4164	0.2161 0.3910 0.3927	0.2243 0.4064 0.3691	0.2323 0.4214 0.3460	0.2401 0.4365 0.3235	
12:14	12:16	12:18	12:20	12:22	12:24		
0.1666 0.3483 0.4845	0.1762 0.3595 0.4642	0.1849 0.3743 0.4409	0.1928 0.3903 0.4166	0.2006 0.4068 0.3925	0.2079 0.4355 0.3687		

some equivalence between different single chain binary mixtures:

$$h_{22}(N_1,N_2) = h_{22}(N_1 + 2,N_2 + 4) \tag{13}$$

The above relationships suggest that the addition of one methylene group to the first component corresponds to the addition of two such groups to the second component to produce the same effect of the mean self-association probability of the longer chain. As concerns the mean cross-association probability h_{12} we have obtained a similar effect on identical enlargement of both the shorter and the longer acyl chain.

If we define the overall ratio of efficiency as the ratio between the number of carbon atoms added to the short hydrophobic chain (DN_1) and the number of carbon atoms added to the long hydrophobic chain (DN_2) that modifies the mean association probability to the same extent we can conclude that the values of this parameter are as follows:

$$K_{ij} = \frac{DN_1}{DN_2} = \begin{cases} 1/4 & \text{for } i = 1 = 1 \\ \cong 1 & \text{for } i = 1,\ \ j = 2 \\ 1/2 & \text{for } i = j = 2 \end{cases} \tag{14}$$

In order to get appropriate information on effectiveness of the selectivity between hydrophobic chains we have investigated the differential mean association probabilities by adding two methylene groups to either the short acyl chain or the long acyl chain. For the reason of simplicity we have accepted the following notation:

$$Dh_{22}^{10} = h_{22}(N_1 + 2,N_2) - h_{22}(N_1,N_2) \tag{15}$$

$$Dh_{22}^{01} = h_{22}(N_1,N_2 + 2) - h_{22}(N_1,N_2) \tag{16}$$

It is more suitable to express the differential mean self-association probabilities of the longer hydrophobic chain (that have a higher value) as a function of the length of one of the acyl chains or the difference between them. Figure 4 shows the differential self-association probability (Dh_{22}^{10}) of the longer chain for a group of binary mixtures. The abscissa represents the length of the shorter hydrophobic chain. The positive values of Dh_{22}^{10} suggest that the addition of a methylene group to the shorter hydrophobic chain is energetically favourable for association of two longer hydrophobic chains. The highest probability of such a process is reached for the binary lipid mixture C(12):C(20) and the behaviour of the curves is biphasic. A more interesting aspect of Dh_{22}^{10} is that the maximum value for a given length of the longer hydrophobic chain (N_2) respects the following rule: $DN = N_2 - N_1 \doteq 8$, the only exception being the mixture C(14):C(24). In a binary mixture the self-association of the longer chains is a process which occurs with high probability and this is most favourable for "efficacious length of association" of van der Waals dispersion forces. On increasing the longer hydrophobic chain we will obtain an opposite effect (Dh_{22}^{01}). Therefore, the self-association of the longer hydrophobic chain is made impossible by insertion of two methylene groups to the longer chain. In Fig. 5 we can find $DN = N_2 - N_1 = 6$ methylene groups as the "critical

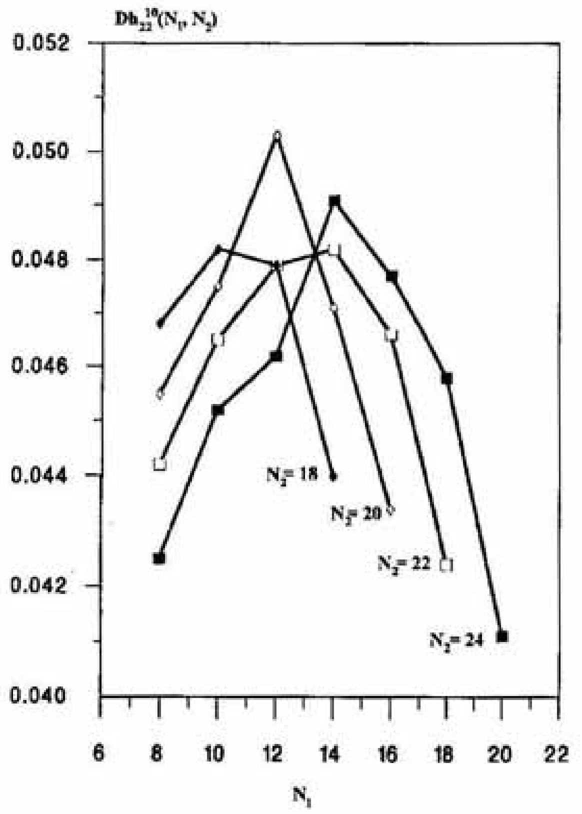

Fig. 4. Differential mean self-association probabilities Dh_{22}^{10} for insertion of two methylene groups to the shorter hydrophobic chain versus the length of the shorter component of a binary lipid mixture.

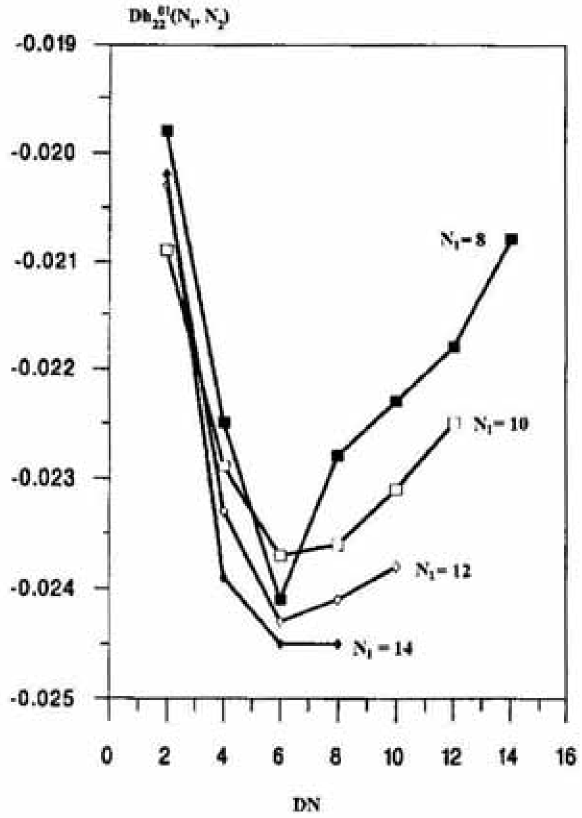

Fig. 5. Differential mean self-association probabilities Dh_{22}^{01} for insertion of two methylene groups to the longer hydrophobic chain versus the length difference between the two components (DN = $N_2 - N_1$).

length of dispersion" of van der Waals dispersion forces for a single chain amphiphile. Here, dispersion means the "non-association process" and not the London's component of the van der Waals forces. On comparing Fig. 4 and Fig. 5 we can see that the effect of the methylene addition to the shorter acyl chain on the mean self-association probability h_{22} ($Dh_{22}^{10} \sim 0.045$) is higher than the effect of methylene addition to the longer acyl chain on the mean self-association probability h_{22} ($Dh_{22}^{01} \sim 0.023$). This finding is obviously correlated with formula (11). Therefore, the change of the energy interacting through van der Waals dispersion forces between two components of different length is more affected when a CH_2-group is added to the shorter hydrophobic chain than when it is added to the longer one.

We found that the simultaneous insertion of two methylene groups to either of the two hydrophobic chains results in an increase of the self-association of the longer hydrophobic chain and a decrease in the self-association of the shorter hydrophobic chain. The binary mixtures C(8):C(14), C(10):C(16), C(12):C(18) and C(14):C(20) have this property to oppose with the highest probability for the self-association process of the longer chain by increasing the second component.

DISCUSSION

We have seen that there are three possibilities of association between components of a binary lipid mixture consisting of single chain amphiphiles: (a) between two identical molecules of either the first or the second component (self-association) and (b) between two different molecules (cross-association). The association probabilities for 36 binary mixtures were analysed with respect to the acyl chain length. A three dimensional representation was found to be most suitable for this analysis. The most important conclusion is that for certain values of the electric dipole moments there are molecular couples which have a maximum stability with respect to the changes in the external electrolytic medium. This fact confirms cluster formation and stability, equivalent to the existence of micrononhomogeneities within lipid bilayers [10]. On the other hand, there are very unstable molecular associations, and this affects the reproductibility of some phase transitions measurements [4]. Generally, an increase of the electric dipole moment or the chain length of one component of a binary lipid mixture diminishes the self-association probability between the molecules of that component, but increases the self-association probability for the other. Moreover, the cross-association probability is high for any binary mixture of single chain amphiphiles.

The association processes of two single chain amphiphiles may be considered as formation of special double-chain lipid with a large headgroup and a dipole moment which, sometimes, can be much smaller than either of the two individual electrical dipole moments. Even when both chains of a phospholipid molecule are of equal length the molecule can adopt such a conformation that its chains appear to be unequal. This is often observed in the crystalline state of the phospholipid bilayers using X-ray diffraction [9]. Therefore, the process of selective association of single chain amphiphiles in a binary mixture could explain some problems regarding the aggregation of the lipid molecules into supermolecular structures: either micelles or planar and spherical bilayers.

As concluding remarks we can say that the selective association of single chain amphiphiles is characterized by the following features:

- i. addition of a methylene group to the shorter component has an opposite effect on each of the three association probabilities (P_{11}, P_{12} and P_{22}) then has the addition of a methylene group to the longer component;
- ii. the mean cross-association probabilities (h_{12}) and the mean self-association probabilities of the longer hydrophobic chain (h_{22}) have high values. We consider that the self-association of the longer chain and cross-association phenomena are most probably responsible for the secondary phase transitions of the binary lipid mixtures;
- iii. the effect of the methylene group of the shorter hydrophobic chain on the mean self-association probabilities h_{11} and h_{22}, is four times and twice more as large, respectively, as the effect of a methylene group of the longer hydrophobic chain;
- iv. self-association of the longer hydrophobic chain is favoured by the increase of the shorter hydrophobic chain. The maximum points for the binary mixtures C(10):C(18); C(12):C(20) or C(14):C(22) points to the difference of 8 methylene groups as "efficacious length of association" given by the van der Waals dispersion forces for single chain amphiphiles;
- v. the critical length of dispersion for the self-association process of the longer hydrophobic chain is 6 methylene groups.

It should be added that: (a) the association probability P_{11} is drastically diminished by the increase in the longer hydrocarbonic chain; (b) the dispersion forces affect much more the dependence of P_{12} on the electric dipole moment of each component than in the case of self-association probabilities.

On analysing formulas (1–7) we can observe that the amphiphile molecule association by one of the three ways discussed is determined by the relative magnitude of the interaction energy between two different molecules, and between two identical molecules, and not by the absolute value of interaction energy between two molecules in each of the possible pairs. In other words, neither the dipole interaction energy, nor the interaction energy through dispersion forces between two different or identical molecules play the leading role in the association processes. Unfortunately, there is a lack of experimental results concerning the characteristics of binary lipid mixtures consisting of single chain amphiphiles, especially those related to the electric dipole moment of the mixed components. Since the effective electric charges of single chain amphiphiles and the distance between them are determined by the properties of external aqueous medium we think that the mean association probabilities describe appropriately enough the selective association from lipid mixtures.

There are some dipole moment values (those for which an association probability gives the maximum or minimum value) for which the associated amphiphiles are resistant to changes of the dipole moments. At the same time there are dipole moment values for which the association process is very sensitive to any change of them. Taking into account that the magnitude of the electric dipole moment of polar head group may be influenced by the external aqueous medium, it follows that, in some physical conditions, the internal bilayer dynamics must be correlated with the molecular dynamics of the external aqueous medium. A mixture of fatty acid and lysophosphatidylcholine forms planar bilayers even though the separate components form micelles [6]. An immediate consequence is that, in a mixture of lysophospholipids, the pairs formed are likely to consist of two phospholipid chains with a double polar head group, which are fitted for the outside monolayer of liposomes.

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