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NONIDEAL LIPID MIXING IN PHOSPHATIDYLCHOLINE/PHOSPHATIDIC ACID SYSTEMS. EVIDENCE FROM MODEL CALCULATIONS ON PHASE DIAGRAMS

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Abstract Lateral mixing behaviour in layered structures of lipid mixtures of DMPC/DMPA (dimyristoylphosphatidylcholine/dimyristoylphosphatidic acid) and DTPC/DTPA (ditridecanoylphosphatidylcholine/ditridecanoylphosphatidic acid) is examined by evaluating phase diagrams using statistical mechanical methods. Results predict substantial differences in the mixing behaviour of the chemically quite similar lipid systems.

INTRODUCTION

Elucidation of lateral lipid organization, especially of deviations from ideal mixing behaviour is of major importance in order to get an insight into rules governing processes in biological membranes as well as in artificial lipid systems comprising lipid mixtures.

Phase diagrams of binary lipid mixtures have been derived from calorimetric investigations on the gel to liquid crystalline phase transition. In order to obtain quantitative data on lipid mixing behaviour, experimental results were subjected to evaluation procedures using the two

theoretical approaches of regular solution theory and quasichemical approximation.[1,2]

THEORETICAL

In order to draw conclusions on nonideality of lateral lipid mixing, an appropriate theoretical description of phase diagrams is to be achieved (cf. Lee[3]). For any given temperature between the transition temperatures T_{m1}, T_{m2} of the pure components of a binary lipid mixture (mole fractions X_1 and $X_2 = 1-X_1$) the chemical potentials of the mixed system in the gel phase (subscript s) and the liquid crystalline phase (subscript l) will be equal. For the existence of at least partial miscibility in both phases we get for each of the two components (i = 1,2)

$$RT \ln (a_{si}/a_{li}) = \Delta H_i (1 - T/T_{mi})$$

and even more simple for the case of gel phase immiscibility

$$RT \ln a_{li} = \Delta H_i (1 - T/T_{mi})$$

where transition enthalpies are denoted by ΔH_i, R is the gas constant, T the absolute temperature. In order to account for some nonideality in lipid mixing, an appropriate choice of activities $a_i = X_i f_i$ has to be introduced (dropping subscripts s and l for convenience). Accordingly activity coefficients f_i are defined[3,4] for the theoretical approaches of ideal miscibility:

$f_1 = f_2 = 1$

regular solution theory:

$$RT\ln f_1 = \varsigma X_2^2 \; ; \quad RT\ln f_2 = \varsigma X_1^2$$

quasichemical approximation:

$$f_1 = ((1 - \nu X_2)/X_1)^{Z/2}$$

$$f_2 = ((1 - \nu X_1)/X_2)^{Z/2}$$

The nonideality parameter ς is attributed to the differences in interaction energies between respective nearest neighbour pairs $\varsigma = Z\,(2\,U_{12} - U_{11} - U_{22})$, with Z being the coordination number. The nonideality parameter ν is related to the mole fractions of different kinds of nearest neighbour pairs[5] comprising like molecules $X_{(11)} = (Z/2)\,(X_1 - \nu X_1 X_2)$; $X_{(22)} = (Z/2)\,(X_2 - \nu X_1 X_2)$ and unlike molecules $X_{(12)} = Z\,\nu X_1 X_2$.

From values of the nonideality parameters, conclusion can be drawn on the existence of homogeneous (single phase) or heterogeneous mixing. Conditions for the stability of a single phase are given by[3,4]

$$\varsigma < RT/2X_1X_2; \quad \nu > (Z-2)/(Z-1)$$

Moreover, the molar free energy of mixing in a binary lipid mixture is given for the theoretical approaches considered, according to

$$\Delta G = RT(X_1 \ln X_1 + X_2 \ln X_2) + RT(X_1 \ln f_1 + X_2 \ln f_2)$$

EXPERIMENTAL

Lipid mixtures of DMPC/DMPA and DTPC/DTPA have been examined for their gel to liquid-crystalline

phase transition using high sensitivity scanning microcalorimetry.

Dispersions of multilamellare lipid aggregates were prepared in the concentration range $1 - 6 \cdot 10^{-3}$ mol/l.

Buffering solution contained 0.1 mol/l NaCl, 0.01 mol/l HEPES (pH 5.2 $\pm$ 0.2) and 0.001 mol/l EDTA.

Dispersions were subjected to a procedure of incubation at temperatures above and below the main transition including a freeze-thawing cycle, prior to measurement.

Experimental details will be reported elsewhere (manuscript in preparation).

For the pure lipid components, temperatures of the main transition and transition enthalpies have been determined as follows:

24.0 °C and 29.8 kJ/mol for DMPC; 53.5 °C and 27.8 kJ/mol for DMPA; 14.2 °C and 21.8 kJ/mol for DTPC; 42.3 °C and 18.4 kJ/mol for DTPA.

Interestingly, for DTPC, a pretransition has not been found.

PHASE DIAGRAMS

Phase diagrams have been constructed from onset and completion temperatures of the calorimetric heating scans after correction for the widths of transition of the pure lipid components. Mole fractions of corresponding components of phosphatidic acid are denoted by X_{PA}.

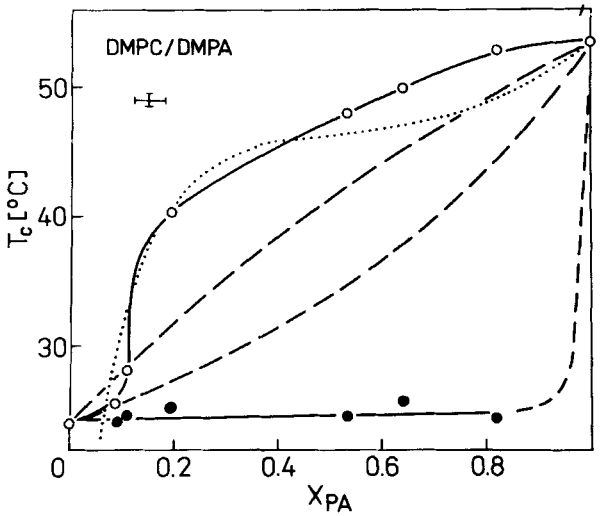

FIGURE 1. Phase diagram of DMPC/DMPA (full line and dashed line giving one possible completion of the solidus), theoretical phase diagram assuming ideal mixing (dashed line) and theoretical liquidus assuming gel phase immiscibility and ς_L = 4.9 kJ/mol.

Comparison of the experimental phase diagram of DMPC/DMPA and theoretical phase diagrams, clearly indicates strong deviation from ideal mixing behaviour. From the flatness of the solidus, to a good approximation, gel phase immiscibility is to be expected. Simulation of the phase diagram under this assumption and a single "best fit value" of ς_L = 4.9 kJ/mol, however, reasonably agrees with the experimental values only up to about 50% DMPA.

From comparison of phase diagrams in Figure 2, mixing behaviour of DTPC/DTPA, obviously, is far away from ideal miscibility. On the other hand, DTPC/DTPA mixing appears to differ appreciably from the chemically quite similar DMPC/DMPA system.

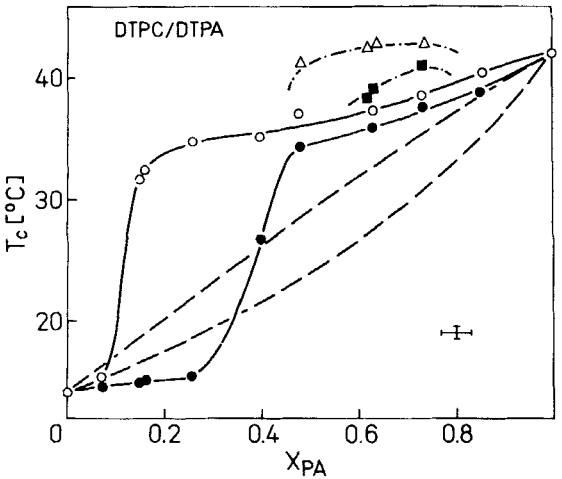

FIGURE 2. Phase diagram of DTPC/DTPA (full line) and theoretical phase diagram assuming ideal mixing (dashed lines), for lines connecting rectangular and triangular symbols see text.

Especially for high mole fractions of DTPA, miscibility seems to be greatly improved in the gel state. Interestingly, in this concentration range, equilibrium conditions in lipid mixing are reached only after long term refrigerator storage of samples. Rectangular and triangular symbols in Figure 2 refer to freshly prepared samples, whereas circular symbols, incorporated into the phase diagram, refer to reproducible results for the same samples after about 5 weeks. The reasons for this behaviour cannot be accounted for, yet.

MODEL CALCULATIONS

Non ideality parameters were evaluated by applying a point by point calculation from the smoothed

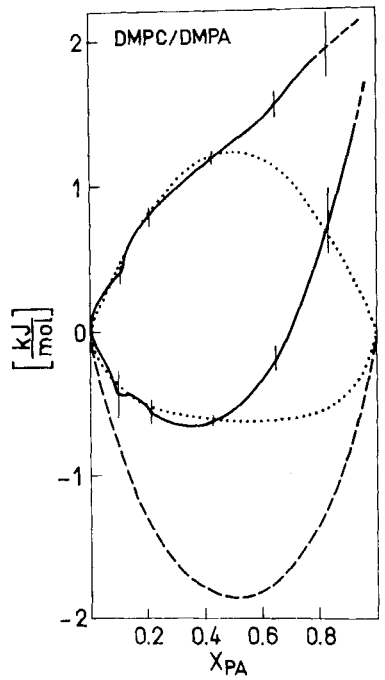

FIGURE 3. Molar free energy of mixing (lower branches) and enthalpic contribution describing nonideality (upper branches) for liquid crystalline phase of DMPC/DMPA according to regular solution theory (full lines). Dotted lines refer to ϱ_L = 4.9 kJ/mol, the dashed line to ideal mixing.

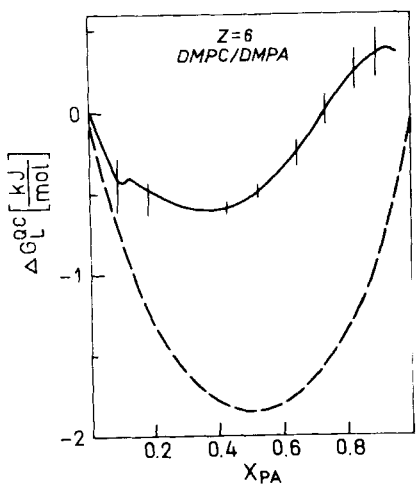

FIGURE 4. Molar free energy of mixing for liquid crystalline phase of DMPC/DMPA according to quasi-chemical approximation (full line). The dashed line corresponds to ideal mixing.

experimental curves of phase diagrams.
Both methods used, yield essentially the same results concerning lipid mixing behaviour, in most of the concentration range considered. It should be pointed out, however, that there may arise considerable differences for extreme mixing ratios, as displayed, for example, by Figures 3 and 4 for DMPA mole fractions higher than about 0.8.
As the value of molar free energy of mixing has to return to zero for pure DMPA, description of mixing behaviour by the method of quasichemical approximation appears to be more advantageous, at least for extreme mixing ratios. Another fact that makes quasichemical approximation superior to regular solution theory is given by the possibility of providing a quantitative estimate of mole fractions of different nearest neighbour molecular pairs, thus also characterizing short-range lateral lipid distribution.

RESULTS AND DISCUSSION

For the reasons just outlined, results of model calculations are depicted in Figures 5 and 6 in terms of the nonideality parameter of quasichemical approximation.

Calculations on DMPC/DMPA were done assuming gel phase immiscibility. As can be seen from Figure 5, the nonideality parameter for the liquid crystalline phase of DMPC/DMPA strictly decreases with increasing mol fraction of DMPA. It is always smaller than one, corresponding to nonideal mixing

with a tendency towards demixing. For mole fractions up to about 0.65 of DMPA, however, single phase stability is preserved. For higher portions of DMPA even the region of heterogeneous mixing is entered, corresponding to a separation into different phases. Results assuming hexagonal (Z = 6) or square (Z = 4) plane lattice do not differ significantly in this respect. For that reason we will restrict ourselfes on considering hexagonal lattices when stepping to the next system.

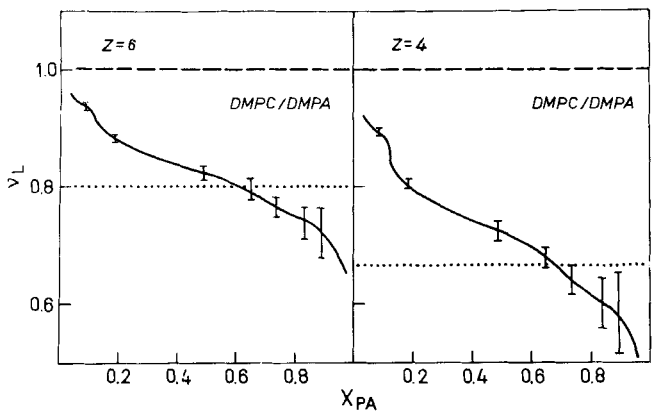

FIGURE 5. Nonideality parameter of quasi-chemical approximation for the liquid crystalline phase of DMPC/DMPA. Dashed lines correspond to ideal mixing, dotted lines to the limits of single phase stability.

For the DTPC/DTPA system (Figure 6) remarkably different results are obtained. In the liquid crystalline phase homogeneous mixing is established with a certain tendency towards demixing, in the whole concentration range covered. The same holds for the gel phase of mixtures containing up

to about 30 % of DTPA. For higher portions of DTPA, lipid mixing is drastically improved, favouring even unlike molecule pair formation in the approximate range of 45 to 80 % of DTPA.

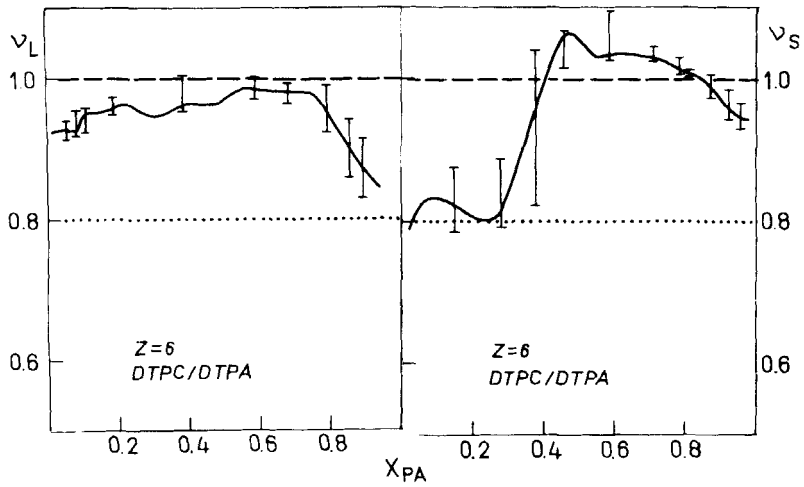

FIGURE 6. Nonideality parameter of quasi-chemical approximation for the liquid crystalline (subscript L) and gel phase (subscript S) of DTPC/DTPA. Dashed and dotted lines like in Figure 5.

Results on DMPC/DMPA agree quite reasonably with previous results of Galla et al.[6] who observed phase separation in the liquid crystalline phase of dipalmitoylphosphatidylcholine/dipalmitoylphosphatidic acid mixtures. On the contrary, in DTPC/DTPA phase separation doesn't occur at all. Moreover, complete gel phase immiscibility is exhibited by the DMPC/DMPA system, while homogeneous mixing is observed in the gel phase of DTPC/DTPA, even favouring unlike molecule pair formation.

Such a kind of "supermiscibility" in the gel phase is reported here for the first time for lipid mixtures.

Considering the similarity regarding their chemical structures, the drastic differences in the mixing behaviour of the two systems under study are quite likely to arise from differences in phase behaviour of the pure lipid components in the gel phase. In phosphatidic acids, acyl chains are found to be tilted with respect to the normal of the layer surface[7], depending on temperature and surface charge density. On the other hand, in DMPC a pretransition is observed resulting in a change of acyl chain packing from tilted to parallel with respect to the normal[8]. In DTPC such a pretransition is lacking.

Presumably, such differences in acyl chain packing have a significant bearing upon phase behaviour. Additional experimental evidence appears to be necessary to decide on this question.

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