Interaction of N-myristoyldimyristoylphosphatidylethanolamine with dimyristoylphosphatidylcholine investigated by differential scanning calorimetry: Binary phase diagram

ARTICLE *in* BIOCHIMICA ET BIOPHYSICA ACTA · JUNE 2001

Impact Factor: 4.66 · DOI: 10.1016/S0005-2736(01)00317-0 · Source: PubMed

ELSEVIER

Biochimica et Biophysica Acta 1512 (2001) 22–26

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

www.bba-direct.com

Rapid report

Interaction of *N*-myristoyldimyristoylphosphatidylethanolamine with dimyristoylphosphatidylcholine investigated by differential scanning calorimetry: binary phase diagram

M. Ramakrishnan [a], Derek Marsh [b], Musti J. Swamy [a,*]

[a] *School of Chemistry, University of Hyderabad, Hyderabad 500 046, India*
[b] *Max-Planck Institut für biophysikalische Chemie, 37070 Göttingen, Germany*

Received 17 January 2001; received in revised form 28 February 2001; accepted 14 March 2001

Abstract

The temperature-composition phase diagram was derived for hydrated, binary mixtures of *N*-myristoyldimyristoylphosphatidylethanolamine (*N*-14 DMPE) and dimyristoylphosphatidylcholine by high sensitivity differential scanning calorimetry. Gel phase immiscibility was detected in mixtures containing up to 20 mol% *N*-14 DMPE and there was no evidence for compound formation between the two components. In the fluid phase nearly complete miscibility is indicated by the calorimetric data. These results are relevant to understanding the role of *N*-acylphosphatidylethanolamines in the stress combating responses of organisms and in their application to developing liposome-based drug delivery systems.

Keywords: *N*-Myristoyldimyristoylphosphatidylethanolamine; Differential scanning calorimetry; Dimyristoylphosphatidylcholine; Lipid membrane; Phase diagram; *N*-Acylphosphatidylethanolamine

N-Acylphosphatidylethanolamines (NAPEs) are naturally occurring, anionic phospholipids that are produced on acylating the amino function of the zwitterionic phospholipid, phosphatidylethanolamine [1]. NAPEs serve as precursors for *N*-acylethanolamines (NAEs), which act as endogenous ligands of the cannabinoid receptors, CB-1 and CB-2 [2]. In NAPEs isolated from natural sources, the *N*-acyl chains are usually long, i.e., comparable in length to the *O*-acyl chains [1]. Detailed biophysical studies have shown that, for NAPEs bearing long *N*-acyl chains, the *N*-acyl chains fold back into the hydrophobic interior of the membrane [3–6]. Spin label EPR and ATR-FTIR studies show that in NAPEs with matched *N*- and *O*-acyl chains the *N*-acyl chain is oriented parallel to the *O*-acyl chains and is located at approximately the same vertical position as the *sn*-2 chain [6,7]. The membrane content of both NAPEs and NAEs increases when the parent tissue is

Abbreviations: NAPE, *N*-acylphosphatidylethanolamine; NAE, *N*-acylethanolamine; DSC, differential scanning calorimetry; DMPC, dimyristoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; *N*-14 DMPE, *N*-myristoyldimyristoylphosphatidylethanolamine; DMPE, dimyristoylphosphatidylethanolamine; DPPE, dipalmitoylphosphatidylethanolamine; EPC, egg phosphatidylcholine; *N*-16 TPE, *N*-palmitoylphosphatidylethanolamine transphosphatidylated from EPC; ΔH_t, transition enthalpy

* Corresponding author. Fax: +91-40-301-2460 or 0145; E-mail: mjssc@uohyd.ernet.in

0005-2736/01/$ – see front matter
PII: S0005-2736(01)00317-0

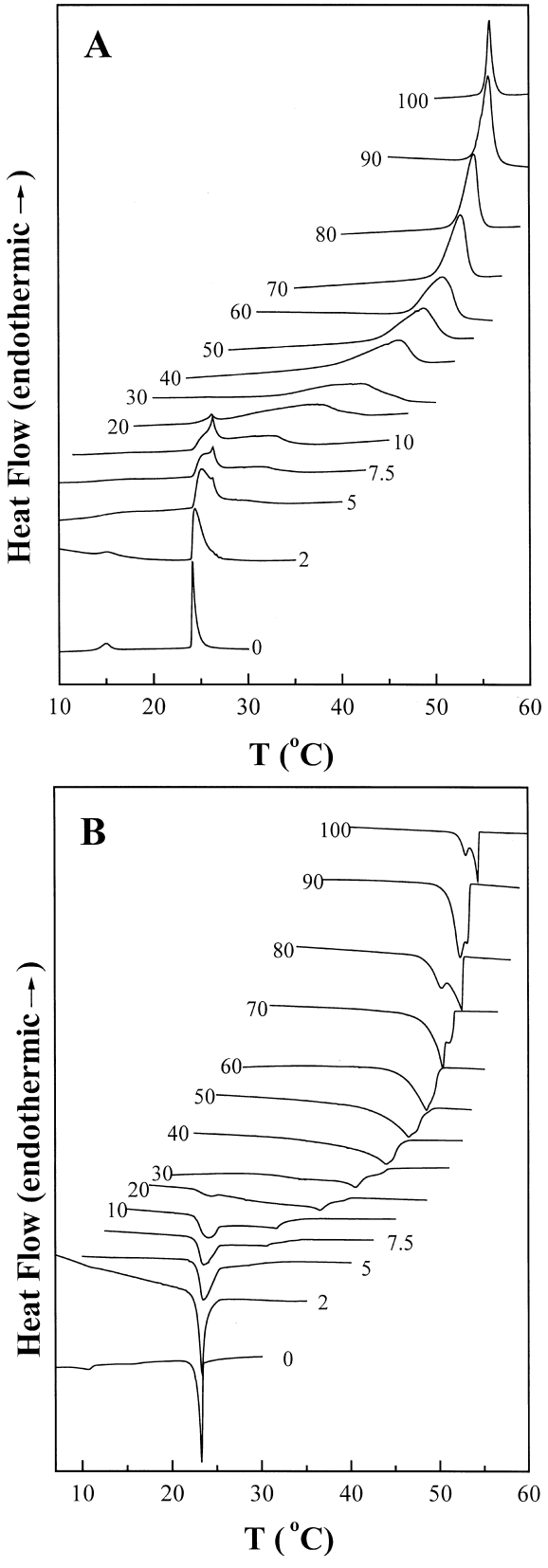

Fig. 1. Calorimetric heating (A) and cooling (B) scans of DMPC, *N*-14 DMPE and their mixtures at different compositions. Samples were dispersed in 1 M NaCl, 10 mM HEPES, and 1 mM EDTA, pH 7.4. Thermograms were recorded at a scan rate of 10°/h. The mol% of *N*-14 DMPE in each sample is indicated.
←

subjected to stress, such as injury in animals or dehydration in plants. This suggests that the increased production of NAPEs and NAEs might be related to the stress combating response of the parent organism [1,2]. Additionally, NAPEs have been shown to stabilize liposomes against leakage, indicating that they might be useful in developing liposomal formulations for drug delivery applications [8,9].

In light of the foregoing, it is important to characterize the physical properties of these two classes of compounds and investigate their interaction with other membrane constituents. Because of the high abundance of phosphatidylcholine in natural membranes and the dramatic increase in content of NAPEs in tissues subjected to stress, it is of special interest to investigate the interaction of NAPEs with phosphatidylcholines. The mixing behaviour of *N*-palmitoyl transphosphatidylated phosphatidylethanolamine (*N*-16 TPE) with egg phosphatidylcholine (EPC) has been reported earlier [10]. So far, however, no complete binary phase diagram has been established for mixing of NAPEs with phosphatidylcholines of defined chain composition. Here, we study the interaction of *N*-myristoyldimyristoylphosphatidylethanolamine (*N*-14 DMPE) with dimyristoylphosphatidylcholine (DMPC) in hydrated mixtures using differential scanning calorimetry (DSC). The temperature-composition binary phase diagram is constructed and the mixing behaviour of these two lipids is discussed.

DMPC and DMPE were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Myristic acid was from Sigma (St. Louis, MO, USA) and oxalyl chloride was a product of Fluka (Buchs, Switzerland). *N*-14 DMPE was synthesized by acylation of DMPE as described earlier [11]. Samples for differential scanning calorimetry were made by dissolving the two lipids in dichloromethane and mixing appropriate aliquots of the two solutions to give the desired ratio. The solvent was then evaporated by gently blowing dry nitrogen gas over the sample, and the

Table 1
Phase coexistence widths (ΔT) and calorimetric enthalpies, ΔH_t for hydrated dispersions of *N*-14 DMPE/DMPC binary mixtures

N-14 DMPE/DMPC (mol/mol)	ΔT (°C)	ΔH_t (kcal/mol)
0:100	3.5	5.4
2:98	5.5	4.6
5:95	12.5	5.4
7.5:92.5	13.8	5.3
10:90	14.6	6.2
20:80	19.7	6.9
30:70	17.0	6.5
40:60	14.0	6.6
50:50	12.0	6.8
60:40	8.5	6.9
70:30	6.5	6.9
80:20	5.3	6.0
90:10	5.0	5.9
100	3.5	8.4

Buffer: 1 M NaCl, 10 mM HEPES, 1 mM EDTA, pH 7.4.

final traces of solvent were removed by vacuum desiccation. The dry lipid mixture was hydrated with 500 μl of 10 mM HEPES buffer (pH 7.4) containing 1 mM EDTA and 1 M NaCl (HBS) at approx. 65°C and then subjected to five cycles of freeze-thawing. DSC experiments were carried out on a Hart Scientific 4207 heat flow differential scanning calorimeter, essentially as described earlier [12]. The samples were placed in 1.5 ml Hastelloy ampoules with screw top lids and calorimetric scans were recorded at a rate of 10°/h. Transition enthalpies were evaluated by integrating the area under each peak using the software supplied by the instrument manufacturers.

Heating and cooling thermograms of various hydrated binary mixtures of *N*-14 DMPE and DMPC are given in Fig. 1A and B, respectively. The transition enthalpies obtained from the thermograms are given in Table 1. From Fig. 1A, it can be seen that the gel-liquid crystalline phase transition of *N*-14 DMPE is centred at 55.2°C and is consistent with the literature value [11]. The main chain melting phase transition of DMPC, centred around 24°C, is also consistent with literature values [13]. The pretransition of DMPC broadens progressively with increase in content of *N*-14 DMPE, although the onset temperature does not change. It is totally abolished at 10 mol% of the latter. The chain melting transition also broadens, without appreciable increase in the onset temperature, up to 20 mol% *N*-14 DMPE. This suggests immiscibility in the gel phase for mixtures containing 0–20 mol% of *N*-14 DMPE, although the slight upward trend in the onset temperature might indicate a very limited mixing. As the fraction of *N*-14 DMPE is increased further, however, the thermograms gradually become narrower and the onset of the chain melting transition shifts rapidly to higher temperatures.

The cooling scans in the high concentration region of *N*-14 DMPE display two closely spaced 'transitions'. This feature was also observed for hydrated

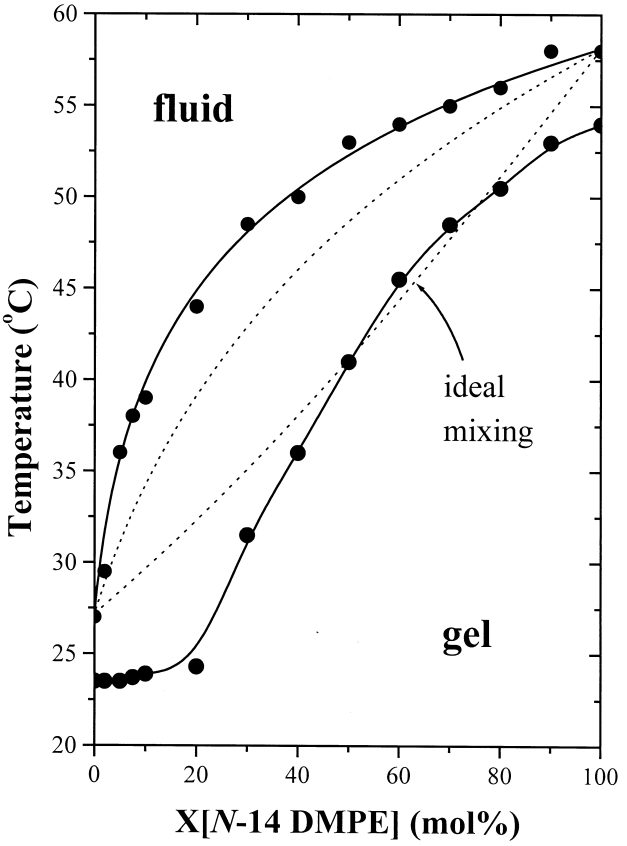

Fig. 2. Binary phase diagram of *N*-14 DMPE/DMPC mixtures dispersed in 1 M NaCl, 10 mM HEPES, and 1 mM EDTA, pH 7.4, deduced from the phase boundaries established from the endothermic transitions shown in Fig. 1A. The solidus and fluidus points were determined from the onset and completion of change in the excess heat capacity, respectively. The dotted lines represent the solidus and fluidus phase boundaries predicted for ideal mixing. See text for more details. The solid line for the fluidus has the form for ideal mixing but with the transition enthalpy of one component used as an arbitrary fit parameter. The solid line for the solidus is a B-spline that serves only to guide the eye.

dispersions of NAPEs (with matched acyl chains) alone, including those of *N*-14 DMPE [11]. It is likely that this irreversibility may be related to the packing of the *N*-acyl chain in the interior of the gel phase membrane, although the precise mechanism is not yet clear.

The binary phase diagram constructed from the heating thermograms of the hydrated mixtures is shown in Fig. 2. The region of gel phase immiscibility is clearly seen from the horizontal solidus line that extends up to mole fractions of *N*-14 DMPE of $X \approx 0.2$. For comparison, the phase diagram calculated for ideal mixing (see e.g. [14]) is also included in Fig. 2, shown by dotted lines. In addition to the pronounced deviations from ideal mixing associated with gel phase immiscibility, limited deviations from ideal mixing are also seen in the fluid phase. However, at high mole fractions of *N*-14 DMPE, the observed phase boundaries approach more closely those predicted for ideal mixing. Although the progression of the solidus and fluidus boundaries of the phase diagram from 30:70 to 100:0 mol/mol of *N*-14 DMPE/DMPC curve in a way that is reminiscent of the formation of a high melting compound, there is no evidence for an isothermally melting compound of non-vanishing DMPC content. Any putative 'compound' therefore must have a composition close to that of pure *N*-14 DMPE.

Of the binary phase diagrams known for phospholipid mixtures [13], that for DMPC mixed with dipalmitoylphosphatidylethanolamine (DPPE) [15] most resembles the phase diagram obtained here for admixture with *N*-14 DMPE. This is significant because both the transition temperature ($T_t \approx 60$–63°C) and the transition enthalpy ($\Delta H_t \approx 8.5$ kcal/mol) of DPPE (see [13]) are relatively close to those of *N*-14 DMPE. Compared with DMPC/DPPE mixtures – apart from the difference in transition temperature of the higher melting component – the major difference is that *N*-14 DMPE mixes better with DMPC in the gel phase than does DPPE. Gel phase immiscibility extends only up to mole fractions $X \leq 0.2$ of *N*-14 DMPE (Fig. 2), whereas with DPPE immiscibility continues up to $X \approx 0.4$–0.5 of the higher melting component [15]. For mixtures of DMPC with distearoylphosphatidylcholine (DSPC, which has a transition temperature close to that of *N*-14 DMPE, but a significantly higher transition enthalpy), the region of gel phase immiscibility extends much further up to mole fractions $X \approx 0.6$–0.7 of DSPC.

The interaction of *N*-16 TPE with EPC was investigated earlier by DSC [10] but for a very limited number of mixtures. Because of the *O*-acyl chain heterogeneity in these lipids, the region of gel-fluid coexistence is extremely broad even for the single components ($\Delta T \approx 38$°C). Therefore, only the peak transition temperatures were reported and a phase diagram was not recorded. Nevertheless, it was concluded that mixing was non-ideal, although there was no clear indication for gel phase immiscibility. Lack of the latter probably arises because of the chain heterogeneity. In addition, our experiments were carried out at high ionic strength (buffer containing 1 M NaCl) to suppress electrostatic effects of the negatively charged NAPE. Electrostatic repulsion will tend to favour mixing with a zwitterionic lipid.

Taken overall, therefore, *N*-14 DMPE mixes better with DMPC than does DPPE which exhibits comparable thermodynamics of chain melting. This preferentially better mixing of the NAPE with phosphatidylcholines (much better also than that of DSPC with DMPC) supports the idea that NAPEs can provide a membrane-compatible reservoir for storing NAEs. The latter are then only mobilized to exert their functional role under conditions of membrane (or tissue) stress [1,2]. We have shown previously [6] that the NAPEs are membrane-compatible at the molecular level. The present findings on the thermodynamic mixing properties further reinforce the conclusions of this former work.

The dramatic increase in the content of NAPEs when the parent tissue is subjected to stress is interpreted as a stress combating response of the organism [1,2]. Also, NAPEs have been demonstrated to stabilize liposomes against contents leakage, especially in the presence of serum components [8,9]. The present study, therefore, provides thermodynamic characterization of the membrane interactions of NAPEs that are relevant to these aspects, both of biological function and of potential biotechnological application.

This work was supported in part by a research grant from the Department of Science and Technology, Government of India to MJS. MRK thanks the Council of Scientific and Industrial Research, Government of India, for a senior research fellowship.

References

[1] H.H.O. Schmid, P.C. Schmid, V. Natarajan, *N*-Acylated glycerophospholipids and their derivatives, Prog. Lipid Res. 29 (1990) 1–43.
[2] H.H.O. Schmid, P.C. Schmid, V. Natarajan, The *N*-acylation-phosphodiesterase pathway and cell signalling, Chem. Phys. Lipids 80 (1996) 133–142.
[3] S. Akoka, C. Tellier, C. LeRoux, D. Marion, A phosphorus magnetic resonance and a differential scanning calorimetry study of the physical properties of *N*-acylphosphatidylethanolamines in aqueous dispersions, Chem. Phys. Lipids 46 (1988) 43–50.
[4] D. Lafrance, D. Marion, M. Pézolet, Study of the structure of *N*-acyldipalmitoylphosphatidylethanolamines in aqueous dispersion by infrared and Raman spectroscopies, Biochemistry 29 (1990) 4592–4599.
[5] J.C. Domingo, M. Mora, M.A. de Madariaga, The influence of *N*-acyl chain length on the phase behaviour of natural and synthetic *N*-acylethanolamine phospholipids, Chem. Phys. Lipids 75 (1995) 15–25.
[6] M.J. Swamy, M. Ramakrishnan, B. Angerstein, D. Marsh, Spin-label electron spin resonance studies on the mode of anchoring and vertical location of the *N*-acyl chain in *N*-acylphosphatidylethanolamines, Biochemistry 39 (2000) 12476–12484.
[7] C.P. Lafrance, J.E. Blochet, M. Pézolet, *N*-Acylphosphatidylethanolamines: effect of the *N*-acyl chain length on its orientation, Biophys. J. 72 (1997) 2559–2568.
[8] J.C. Domingo, M. Mora, M.A. de Madariaga, Incorporation of *N*-acylphosphatidylethanolamines into egg phosphatidylcholine vesicles: characterization and permeability properties of the binary system, Biochim. Biophys. Acta 1148 (1993) 308–316.
[9] M. Mercadal, J.C. Domingo, M. Bermudez, M. Mora, M.A. De Madariaga, *N*-Palmitoylphosphatidylethanolamine stabilizes liposomes in the presence of human serum: effect of lipidic composition and system characterization, Biochim. Biophys. Acta 1235 (1995) 281–288.
[10] J.C. Domingo, M.A. De Madariaga, Molecular organization of hydrated dispersions of *N*-acylethanolamine phospholipids and mixtures with phosphatidylcholine, Colloids Surf. 115 (1996) 97–105.
[11] M.J. Swamy, D. Marsh, M. Ramakrishnan, Differential scanning calorimetry of chain-melting phase transitions of *N*-acylphosphatidylethanolamines, Biophys. J. 71 (1997) 2556–2564.
[12] M.J. Swamy, B. Angerstein, D. Marsh, Differential scanning calorimetry of thermotropic phase transitions in vitaminylated lipids: aqueous dispersions of *N*-biotinyl phosphatidylethanolamines, Biophys. J. 66 (1994) 31–39.
[13] D. Marsh, Handbook of Phospholipid Bilayers, CRC Press, Boca Raton, FL, 1990.
[14] G. Cevc, D. Marsh, Phospholipid Bilayers. Physical Principles and Models, Wiley-Interscience, New York, 1987, 442 pp.
[15] E.J. Shimshick, H.M. McConnell, Lateral phase separation in phospholipid membranes, Biochemistry 12 (1973) 2351–2360.