ELSEVIER

Biochimica et Biophysica Acta 1329 (1997) 302–310

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Differential scanning calorimetric studies on the thermotropic phase transitions of dry and hydrated forms of *N*-acylethanolamines of even chainlengths

M. Ramakrishnan, V. Sheeba, Sneha Sudha Komath, Musti J. Swamy *

School of Chemistry, University of Hyderabad, Hyderabad-500 046, India

Received 22 April 1997; accepted 21 May 1997

Abstract

N-acylethanolamines (NAEs) have attracted the attention of researchers in the last two decades due to their occurrence in biological membranes under conditions of stress as well as under normal conditions. Differential scanning calorimetric studies have been carried out on dry and hydrated samples of a homologous series of *N*-acylethanolamines containing saturated acyl chains of even number of carbon atoms ($n = 8$–20). In both cases a major sharp endothermic transition was observed which occurs at the melting point for the dry NAEs whereas for the hydrated samples it occurs at considerably lower temperatures. The enthalpies and entropies corresponding to this transition could be fitted, in each case, to a straight line suggesting that the transition enthalpy and transition entropy consist of a fixed component from the polar head group and the terminal methyl group, whereas the contribution of the methylene groups, $(CH_2)_n$, is linearly proportional to the number of carbon atoms in it. The contributions of each methylene unit to the transition enthalpy and transition entropy of NAEs were found to be $\Delta H_{inc} = 0.82\ (\pm 0.02)$ and $0.96\ (\pm 0.06)$ kcal mol^{-1}, and $\Delta S_{inc} = 2.01\ (\pm 0.06)$ and $2.37\ (\pm 0.17)$ cal mol^{-1} K^{-1}, respectively, for the dry and hydrated samples of NAEs, whereas the end contributions arising from the head group and the terminal methyl group were determined to be $\Delta H_o = -0.10\ (\pm 0.26)$ and $-0.52\ (\pm 0.82)$ kcal mol^{-1} and $\Delta S_o = 2.12\ (\pm 0.71)$ and $3.1\ (\pm 2.3)$ cal mol^{-1} K^{-1}, respectively, for the dry and hydrated samples of NAEs. These results are relevant to an understanding of the thermodynamics of the phase properties of NAEs in membranes. © 1997 Elsevier Science B.V.

Keywords: *N*-acylethanolamine; Fatty acid; Phase transition; Transition enthalpy; Transition entropy; Chainlength dependence

1. Introduction

N-acylethanolamines (NAEs) have been found to accumulate in the infarcted areas of canine myocardium and ischemic brain tissue at concentrations of up to 400–500 nmol/g of wet tissue [1,2]. Biochemical studies have revealed that NAEs have interesting biological properties which include inhibition of the permeability dependent Ca^{2+} release from rat heart and liver mitochondria [3], stimulation of Ca^{2+}–Mg^{2+}-ATPase activity [4] and inhibition of

Abbreviations: NAE, *N*-acylethanolamine; NAPE, *N*-acylphosphatidylethanolamine; DSC, differential scanning calorimetry; T_t, transition temperature; ΔH_t, transition enthalpy; ΔS_t, transition entropy; ΔH_{inc}, incremental value of enthalpy per CH_2 unit; ΔS_{inc}, incremental value of entropy per CH_2 unit; ΔH_o, end contributions to the transition enthalpy; ΔS_o, end contributions to the transition entropy

* Corresponding author. Fax: +91-40-3010145; E-mail: mjssc@uohyd.ernet.in

PII S0005-2736(97)00120-X

lipid peroxidation in membranes [5]. Additionally, *N*-oleoylethanolamine has been demonstrated to be a potent inhibitor of ceramidase [6], whereas *N*-arachidonylethanolamine (anandamide) has been found to be associated with the cannabinoid receptor as its endogenous ligand [7]. Anandamide has also been shown to inhibit gap–junction conductance and intercellular calcium signalling in striatal astrocytes [8], as well as to reduce sperm fertilising capacity by inhibition of the acrosome reaction [9].

The metabolism of *N*-acylethanolamines has been investigated in considerable detail (see, for recent reviews, Refs. [10,11]) and as a result we now know that the NAEs can be degraded by *N*-acylethanolamine amidohydrolase to the free fatty acid and ethanolamine [3,12–14]. This is true for anandamide also as a specific anandamide amidohydrolase activity which exhibits high substrate specificity was identified in rat brain microsomes [15]. Additionally, it is known that NAEs may be produced in biological media both by the condensation of the corresponding fatty acid with ethanolamine [16–18] as well as by the degradation of *N*-acyl phosphatidylethanolamines (NAPEs) [10,11,18–20], which are also found to accumulate in animal tissues under conditions of stress or injury. However, since the former biosynthetic activity is the reverse reaction of the NAE amidohydrolase that catalyses NAE degradation, it has been suggested that the latter pathway, rather than the former, meets the requirements and conditions for the biosynthesis of various species of NAEs [11,19]. Currently, efforts are being directed towards the purification of enzymes that are involved in the biosynthesis and catabolism of NAEs and NAPEs in order to characterise them in greater detail [21–24].

In addition to the interest generated in them by their occurrence in biological membranes under conditions of stress, *N*-acylethanolamines have also elicited considerable interest due to their pharmacological and medicinal properties. It has been found that the NAEs exhibit antiinflammatory, antianaphylactic, antiviral and antibacterial properties (reviewed in Ref. [10]).

While a significant amount of work has been carried out on the biological, medicinal and pharmacological properties of NAEs, studies on their physical properties and their interaction with other membrane components have been relatively scarce. In one study, Epps and Cardin [25] investigated the interaction of *N*-oleoylethanolamine with phosphatidylcholine vesicles and showed that it decreases the phase transition temperature of dipalmitoylphosphatidylcholine multilamellar vesicles. More recently, Ambrosini et al. [26] investigated the effect of *N*-oleylethanolamine and *N*-lauroylethanolamine on the phase behaviour of egg phosphatidylethanolamines using X-ray diffraction and differential scanning calorimetry (DSC) and suggested that the NAEs may stabilise the bilayer structure of the lipid membranes. Apart from these two studies, to the best of our knowledge, there have been no reports on the physical properties and phase behaviour and interaction of these compounds with other membrane components such as phospholipids. In view of their occurrence in biological membranes and the possible medicinal uses of these compounds, it is important to carry out systematic studies on the properties of NAEs and to investigate their phase behaviour in aqueous dispersion in pure state as well as in mixtures with other membrane lipids. As the first step in this direction we have performed differential scanning calorimetric studies on a homologous series of NAEs with saturated acyl chains in order to characterise the phase transitions of dry and hydrated forms of *N*-acylethanolamines and the results obtained are reported here.

2. Experimental

2.1. Materials

Octanoic acid, decanoic acid, lauric acid, myristic acid, palmitic acid and arachidic acid were obtained from Aldrich Chemical, USA. Stearic acid was purchased from Sisco Research, Mumbai. Oxalyl chloride was a product of E. Merck, Germany. All other reagents were of reagent grade and were obtained from local suppliers. Solvents were distilled and dried prior to usage.

2.2. Synthesis of N-acylethanolamines

The fatty acids were converted to the corresponding acid chlorides by treating with 4 mol equivalents of oxalyl chloride at room temperature for 2 h under

nitrogen atmosphere [27]. After the reaction was completed, the excess oxalyl chloride was removed under a stream of dry nitrogen gas. *N*-acylethanolamines were synthesised by the drop-wise addition of one mole equivalent of the acid chloride in dichloromethane to a solution of 5 mol equivalents of freshly distilled 2-ethanolamine in dichloromethane cooled in an ice-bath, under constant stirring. After all the reagent was added, the reaction was allowed to continue for another 30 min and the solution was washed successively with double distilled water, dilute HCl and dilute bicarbonate solution and then the solvent was evaporated under a stream of dry nitrogen gas.

The *N*-acylethanolamines of longer acyl chain-lengths (C-10 to C-20) were purified by recrystallisation from acetone. For this, the product obtained in the previous step was dissolved in a small volume of dichloromethane, about 10 vol of acetone were added to it and the solution was kept in a freezer overnight. The precipitated *N*-acylethanolamines were recovered by filtration. *N*-octanoylethanolamine was purified by column chromatography on silica gel. The column was packed in 5% ethylacetate in hexane and the sample was loaded onto the column. Elution was done with increasing concentrations of ethylacetate and *N*-octanoylethanolamine eluted at 70% ethylacetate. The final yields obtained were usually in the range of 60–80% and the products were found to be pure by TLC on silica gel (solvent: CH_2Cl_2/MeOH/NH_4OH: 65/25/4, v/v). The IR spectra of the purified *N*-acylethanolamines showed resonances in the carbonyl stretching region at 1643 and 1559 cm^{-1}, corresponding to the amide I and amide II bands, respectively, whereas bands corresponding to carbonyl resonances of free fatty acid, acid chloride and ester were absent.

2.3. Differential scanning calorimetry

Differential scanning calorimetric studies were performed on a Perkin-Elmer DSC-4 differential scanning calorimeter equipped with a data station. The calorimeter was calibrated with a sample of indium. Aluminium pans were used for dry NAEs whereas stainless steel ampoules were used for measurements with aqueous dispersions. For measurements with dry samples, 1–3 mg of the appropriate NAE was weighed into the sample pan, covered with an aluminium lid and sealed by crimping. Reference pans were prepared without any sample in them. Hydrated NAE samples were prepared in the following way. Solid *N*-acylethanolamines (1–3 mg) were weighed into large volume stainless steel sample pans, 30 μl of distilled H_2O was added to each pan, the pan was covered with the lid and sealed by crimping. Reference pans were prepared with only water in them. Hydration was achieved by heating the sealed pans in the DSC equipment to about 120°C. DSC measurements were made after cooling the pans to room temperature. Heating and cooling scans were made from room temperature (ca. 25°C) to 130°C at a scan rate of 2.5°/min or 1.5°/min and each sample was scanned three times upwards and two times downwards. Transition enthalpies were determined by integrating the peak area under the transition curve. Transition entropies were determined from the transition enthalpies assuming a first order transition according to the expression [28]:

$$\Delta H_t = T_t \cdot \Delta S_t \quad (1)$$

3. Results

3.1. Differential scanning calorimetry of dry N-acylethanolamines

Differential scanning calorigrams for the heating and cooling scans of solid–fluid phase transitions of *N*-stearoylethanolamine are shown in Fig. 1 as representative examples. There are three peaks in the first heating scan and the third peak which is centered around 102.5°C coincides with the capillary melting point of the compound and therefore this transition corresponds to the melting of the sample, that is, the solid–liquid phase transition. The other two transitions which occur at temperatures below the main melting transition must therefore correspond to transitions in the solid phase. While all the NAEs studied here ($n = 8$–20) show a major transition that corresponds to the capillary melting of the sample only some of them display the additional transitions below the main melting transition. Thus, all the NAEs with acyl chains of 14–20 carbon atoms display two smaller transitions before the main melting transition

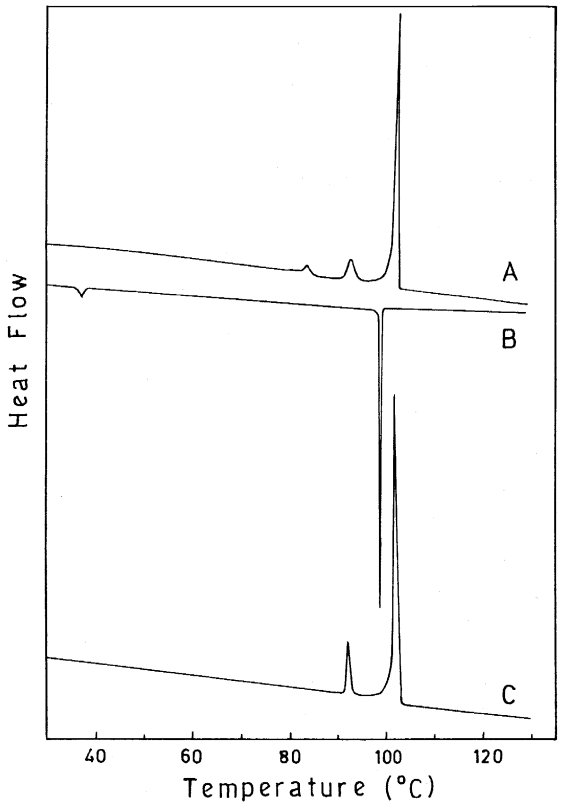

Fig. 1. Differential scanning calorigrams of dry *N*-stearoylethanolamine. A, first heating scan; B, cooling scan performed immediately after the first heating scan; C, second heating scan recorded immediately after the cooling scan. Subsequent heating and cooling scans were identical to B and C, respectively. Upward curves are endotherms and downward curves are exotherms. Scan rate: 2.5°/min.

in the first heating scan. *N*-decanoylethanolamine (C-10) and *N*-lauroylethanolamine (C-12) display only one additional transition that is rather broad and occurs at a lower temperature than the melting transition in the first heating scan. The calorigrams obtained for *N*-octanoylethanolamine show a single sharp endothermic transition that is associated with the melting of the sample and no additional transitions were observed for this compound either before or after the main melting transition. When the samples were subjected to further cycles of heating and cooling after the first heating and cooling scans, it was observed that the NAEs having acyl chainlengths of 8–14 C-atoms give only a single sharp transition corresponding to the melting of the sample, whereas the NAEs having acyl chainlengths of 16–20 C-atoms give an additional transition before the main melting transition. The half width of the main melting transition is in the range of 0.5–1.0°C for the *N*-acylethanolamines investigated in this study.

3.2. Differential scanning calorimetry of N-acylethanolamines in the presence of excess water

Differential scanning calorigrams of hydrated *N*-stearoylethanolamine for heating and cooling scans are shown in Fig. 2 as representative examples. In contrast to the dry sample of this lipid which showed two minor transitions and a major chain-melting phase transition, the hydrated sample shows only a single transition which corresponds to the chain-melting phase transition. All the other NAEs with even chainlengths in the range 8–20 C-atoms also show a single transition in both the heating and cooling scans.

3.3. Transition temperatures

The transition temperatures (T_t) determined from the DSC studies, both for the dry samples of NAEs as well as their dispersions in water, are listed in Table 1. It can be seen that transition temperatures of the NAEs increase with increasing chainlength though

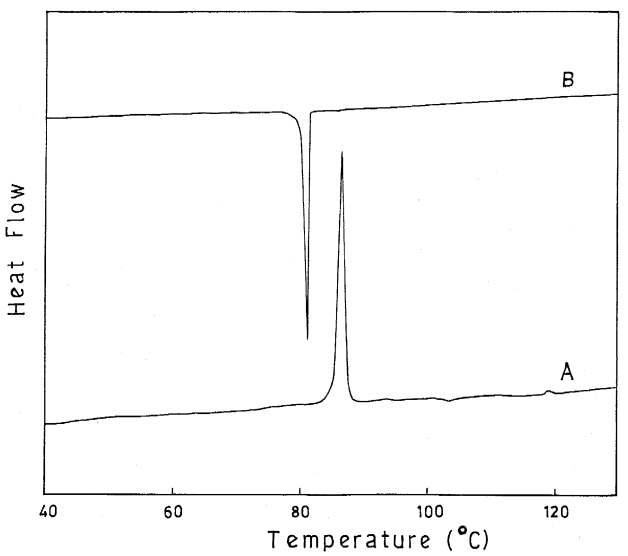

Fig. 2. Differential scanning calorigrams of *N*-stearoylethanolamine in presence of excess water. A, heating scan; B, cooling scan. Upward curve is endotherm and downward curve is exotherm. Scan rate: 2.5°/min.

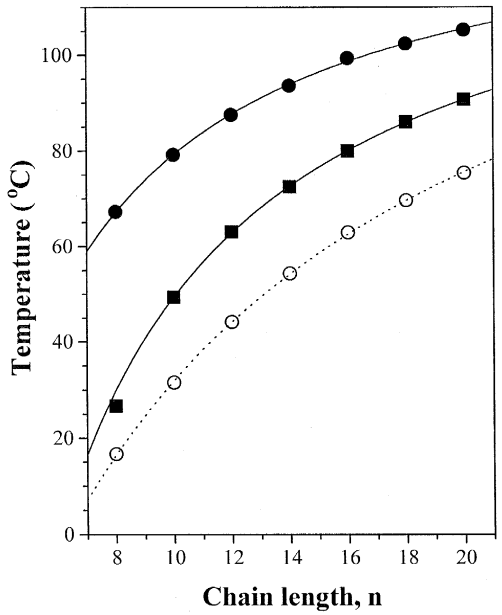

Fig. 3. Chainlength dependence of the transition temperatures of *N*-acylethanolamines and fatty acids of even chainlengths. Data obtained from heating scans of dry (●) and hydrated (■) samples of NAEs are shown. Transition temperatures of fatty acids (○), taken from literature [21], are also shown for the sake of comparison. The solid lines and the dotted line are nonlinear least square fits of each data set to Eq. (4). The data for *N*-octanoylethanolamine in water was omitted from the least squares analysis.

the change in transition temperatures decreases steadily with increase in the chainlength. This can be recognised more readily from Fig. 3 where the transition temperature of NAEs is plotted as a function of the acyl chainlength, *n*. For the sake of comparison, transition temperatures of the parent fatty acids (dry samples), obtained from literature [29], are also shown in Fig. 3.

3.4. Transition enthalpies and entropies

The transition enthalpies and transition entropies obtained from the heating scans for the main chain-melting phase transition of the various *N*-acylethanolamines investigated in this study, both for the dry samples and the hydrated dispersions, are reported in Table 1. It was observed that the transition enthalpy values obtained from the cooling scans (data not shown) are usually in close agreement with those obtained from the heating scans. The values for the transition entropies were calculated assuming a first order transition. The data given are the average values obtained from the calorigrams recorded at scan rates of 2.5°/min and 1.5°/min.

4. Discussion

Interest in *N*-acylethanolamines has been increasing in the last several years after the demonstration that *N*-arachidonylethanolamine binds to the cannabinoid receptor as its endogenous ligand [7]. Since the major pathway for the biosynthesis of NAEs is by the enzymatic degradation of *N*-acylphosphatidylethanolamine (NAPE) and because NAPEs have been shown to be present in biological membranes in significant quantities under certain conditions of stress, there is a considerable interest in this class of compounds as well [10,11,19]. In order to develop structure–function relationships for these two classes of compounds it is important to study their phase behaviour in aqueous dispersions and to characterise the thermodynamics that govern their phase properties as well as to characterise the structures formed

Table 1
Transition temperatures (T_t), transition enthalpies (ΔH_t) and transition entropies (ΔS_t) for the phase transitions of *N*-acylethanolamines

Number of C-atoms (*n*)	T_t (°C)		ΔH_t (kcal/mol)		ΔS_t (cal/mol/K)	
	dry	hydrated	dry	hydrated	dry	hydrated
8	66.6	26.7	4.90	1.73	14.42	5.77
10	79.3	49.4	6.55	7.14	18.59	22.14
12	87.9	63.0	7.65	9.01	21.19	26.80
14	93.9	72.5	9.64	10.39	26.26	30.06
16	99.7	80.0	11.53	13.67	30.92	38.71
18	102.5	86.0	12.98	14.68	34.07	40.88
20	105.3	90.7	14.54	16.45	38.42	45.21

by them. Previous electron microscopic and ^{31}P-NMR studies have shown that NAPEs form lamellar structures in gel and fluid phases and that *N*-acylation of PE in general stabilises the bilayer form [30,31]. DSC, ^{31}P-NMR and FTIR studies have shown that when the *N*-acyl chain is longer than 10-C atoms, it interacts with the hydrophobic interior of the membrane [31–33]. For NAPEs having matched *N*- and *O*-acyl chains the transition temperatures are shown to be almost identical to those of the parent diacyl PEs [30–33]. Similar studies on NAEs are, however, lacking. In the present study we have investigated the phase transitions of a homologous series of NAEs by differential scanning calorimetry and the results obtained are discussed here.

4.1. Chainlength dependence of transition enthalpy and transition entropy

The chainlength dependence of the transition enthalpy, ΔH_t, and transition entropy, ΔS_t, for the dry and hydrated samples of *N*-acylethanolamines, are given in Fig. 4A and B, respectively. Data for the corresponding fatty acids (dry samples), obtained from literature [34], are also given for comparison. It is seen that in the chainlength region of 8–20 C-atoms, for all the NAEs with even number of C-atoms, both ΔH_t and ΔS_t exhibit an essentially linear dependence. The data may be fit to the expressions [29]:

$$\Delta H_t = (n-2)\Delta H_{inc} + \Delta H_o \quad (2)$$

$$\Delta S_t = (n-2)\Delta S_{inc} + \Delta S_o \quad (3)$$

where ΔH_o and ΔS_o are the end contributions to the transition enthalpy and transition entropy, respectively, arising from the terminal methyl group and the head group region. ΔH_{inc} and ΔS_{inc} are the incremental values of transition enthalpy and transition entropy per CH_2 group. A linear least square analysis of the chainlength dependent values of the transition enthalpy and transition entropy of the different NAEs yielded the values of ΔH_{inc} and ΔS_{inc} as 0.82 ($\pm$0.02) and 0.95 ($\pm$0.06) kcal mol^{-1}, and, 2.01

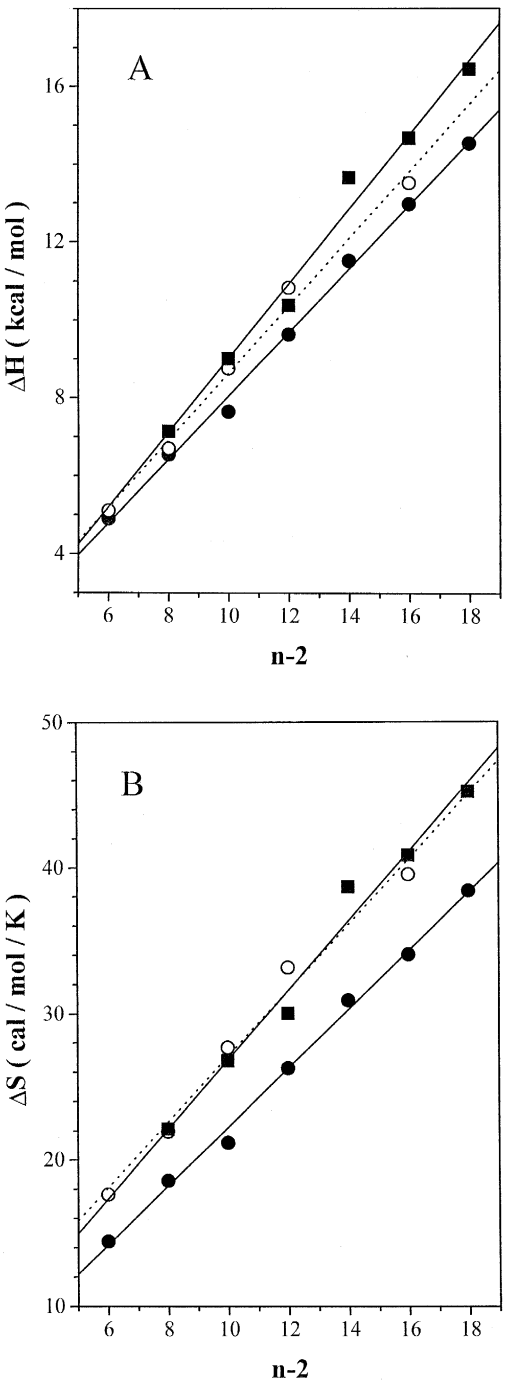

Fig. 4. Chainlength dependence of the transition temperatures of *N*-acylethanolamines and fatty acids of even chainlengths. Data obtained from heating scans of dry (●) and hydrated (■) samples of NAEs are shown. Transition temperatures of fatty acids (○), taken from literature [21], are also shown for the sake of comparison. The solid lines and the dotted line are nonlinear least square fits of each data set to Eq. (4). The data for *N*-octanoylethanolamine in water was omitted from the least squares analysis.

Table 2
Incremental values (ΔH_{inc}, ΔS_{inc}) of the chainlength dependence and end contributions (ΔH_o, ΔS_o) to the phase transition enthalpy and entropy of dry and hydrated samples of *N*-acylethanolamines and dry samples of fatty acids

Lipid	ΔH_{inc} (kcal/mol)	ΔH_o (kcal/mol)	ΔS_{inc} (cal/mol/K)	ΔS_o (cal/mol/K)
NAEs (Hydrated)	0.95 ($\pm$0.06)	−0.52 ($\pm$0.82)	2.37 ($\pm$0.17)	3.1 ($\pm$2.3)
NAEs (Dry)	0.82 ($\pm$0.02)	−0.10 ($\pm$0.26)	2.01 ($\pm$0.06)	2.12 ($\pm$0.71)
Fatty acids	0.862 ($\pm$0.047)	0.017 ($\pm$0.51)	2.25 ($\pm$0.16)	4.6 ($\pm$1.8)

($\pm$0.06) and 2.37 ($\pm$0.17) cal mol^{-1} K^{-1}, respectively, for the dry and aqueous dispersions [1]. The end contributions were determined to be $\Delta H_o = -0.1$ ($\pm$0.26) and -0.52 ($\pm$0.82) kcal mol^{-1}, and, $\Delta S_o = 2.12$ ($\pm$0.71) and 3.1 ($\pm$2.3) cal mol^{-1} K^{-1}, respectively, for the dry and hydrated samples of the NAEs. These values as well as the corresponding values for the fatty acids obtained from a similar analysis of the thermodynamic data available in the literature [30] are presented in Table 2. The end contributions i.e., values of ΔH_o and ΔS_o, both for the NAEs and the fatty acids, are rather small and therefore do not contribute significantly to the overall transition enthalpy and transition entropy. The significance of the incremental values of transition enthalpy and transition entropy is discussed below.

A linear chainlength dependence of the transition enthalpy and transition entropy may be expected for the homologous series of *N*-acylethanolamines (dry samples), provided the structures of the NAEs in the solid state and in the liquid state do not change significantly with chainlength. From the linearity of the data in Fig. 4A and B, it appears that the structures of the dry NAEs in the solid state as well as in the liquid state for the chainlength range of 8–20 C-atoms are rather similar. This suggests that packing of the acyl chains of NAEs in the solid phase is similar in the chainlength range of 8–20 C-atoms. Similarly, for the NAEs in the presence of water it can be said that the structures in the gel phase and that in the fluid phase are similar in the chainlength range of 10–20 C-atoms. The structure of *N*-octanoylethanolamine appears to be different from the other NAEs since the values of transition enthalpy and transition entropy for this compound do not fit with the linear chainlength dependence of these calorimetric parameters observed with the rest of the NAEs investigated in the present study. From Eq. (4) the phase transition temperature of this compound in the presence of water could be predicted to be about 30°C (see below); however, the only transition observed for the aqueous dispersion of this compound is seen at 26.7°C, which is considerably lower than the T_t value predicted from Eq. (4). Additionally, the hydrated sample of *N*-octanoylethanolamine is optically clear, whereas the hydrated dispersions of the other NAEs are turbid. These observations suggest that the structure of *N*-octanoylethanolamine in the presence of water is different from that of the other NAEs. Further studies are required to delineate the differences in the structures of *N*-octanoylethanolamine and the other NAEs in aqueous dispersion.

The values of ΔH_{inc} for the solid-to-liquid phase transitions of dry NAEs and free fatty acids are comparable and are significantly higher than those obtained for the gel-to-fluid transitions in aqueous dispersions of saturated diacyl phospholipids which are extensively studied (see Table 2 in Ref. [35]) [2]. It is likely that this difference arises both from the differences in the gel phase structures of the phospholipids and the structures in the solid phase of NAEs and fatty acids where the acyl chains in solid NAEs and fatty acids are likely to be more ordered than the lipid acyl chains in the hydrated gel phases of the phospholipids as well as from the nature of the fluid phase in the aqueous dispersions of phospholipids where the hydrophobic lipid acyl chains are expected to be more ordered than those of the pure NAEs in the fluid phase.

[1] The data corresponding to the hydrated *N*-octanoylethanolamine were omitted from the least-square analysis because the ΔH_t and ΔS_t values obtained for this sample deviated significantly from the least square fits obtained for the data from the other NAEs.

[2] The values given in this reference correspond to two CH_2 units coming from the two acyl chains of these diacyl phospholipids. Therefore, for a proper comparison the values given in this reference should be halved.

It is interesting to note that the incremental values of the transition enthalpy and transition entropy for the NAEs in the absence and in the presence of water differ considerably. Particularly, it is to be noted that the values of ΔH_{inc} are somewhat larger in the presence of water than for the dry samples. However, a knowledge of the structures of the phases formed by these molecules below and above the phase transition temperatures in the absence and in the presence of water is necessary before these differences can be rationalised.

4.2. Chainlength dependence of transition temperatures

From the data presented in Table 1, it can be seen that the transition temperatures of the NAEs increase with increasing chainlength, both in dry samples as well as when they are fully hydrated. The magnitude of the change in the transition temperature, however, decreases steadily as the chainlength increases and this is clearly seen when the values of T_t are plotted against the number of C-atoms, *n*, in the acyl chain (Fig. 3). This is similar to the trends observed for the transition temperatures of fatty acids in the solid phase [29] as well as for the gel-to-fluid phase transition temperatures observed for the aqueous dispersions of phosphatidylcholines [36], phosphatidylethanolamines [36] and *N*-biotinyl phosphatidylethanolamines [35].

From the data presented in Table 2 it is possible to calculate the transition temperature for an infinite chainlength of the NAE since, as the chainlength increases, the contribution of the $(CH_2)_n$ part of the molecule will dominate the transition enthalpy and transition entropy values and therefore the Eqs. (2) and (3) can be reduced to:

$$\Delta H_t = (n-2)\Delta H_{inc} \quad (2a)$$

$$\Delta S_t = (n-2)\Delta S_{inc} \quad (3a)$$

The transition temperature then will be given by $T_t^{\alpha} = \Delta H_{inc}/\Delta S_{inc}$. From Table 2 the values of T_t^{α} for the dry samples of NAEs and fatty acids can be estimated as 408.0 and 383.1 K, respectively. For the hydrated dispersions of NAEs, the value of T_t^{α} is estimated as 400.8 K.

The chainlength dependence of the transition temperatures of both NAEs and the corresponding fatty acids was fitted to the equation predicted from the linear dependence of the transition enthalpy and transition entropy given in Eqs. (2) and (3) [36,37]:

$$T_t = \Delta H_t/\Delta S_t = (\Delta H_{inc}/\Delta S_{inc})\left[1-(n_o - n'_o)/(n-n'_o)\right] \quad (4)$$

where n_o $(= -\Delta H_o/\Delta H_{inc})$ and n'_o $(= -\Delta S_o/\Delta S_{inc})$ are the chainlengths at which the transition enthalpy and transition entropy, respectively, extrapolate to zero. It can be seen from Fig. 3 that the transition temperatures of both the NAEs and the fatty acids are described accurately by Eq. (4). Additionally, from the fitting parameters, the transition temperature at infinite chainlength of the dry and hydrated samples of *N*-acylethanolamines, T_t^{α}, have been estimated to be 408.4 K with χ^2 of 0.2 K and 408.5 K with χ^2 of 0.3 K, whereas the T_t^{α} value estimated for the fatty acids is 425.2 K with χ^2 of 0.12 K. The values estimated from the fitting parameters for the dry and hydrated NAEs are in good agreement with the values of 408.0 and 400.8 K predicted from the linear regression of the calorimetric data.

5. Conclusions

A linear dependence has been observed in the thermodynamic parameters, ΔH_t and ΔS_t, associated with the chain-melting phase transitions of a homologous series of *N*-acylethanolamines in the dry state as well as in the presence of excess water, in the present study. The incremental values of transition enthalpy (ΔH_{inc}) contributed by each CH_2 unit in the hydrated dispersions of NAEs are slightly larger than the corresponding values obtained for the solid-to-liquid phase transition of the dry NAEs. However, the values of ΔH_{inc} for NAEs, both in the dry state as well as in the hydrated dispersions are considerably larger than the values of ΔH_{inc} obtained for the aqueous dispersions of phospholipids. These differences can be rationalised only after the structures of the phases formed by NAEs below and above the chain-melting phase transitions, both in the absence and in the presence of water, are known.

Acknowledgements

This work was supported by a research grant (SR/OY/C-15/93) from the Department of Science and Technology, Government of India to MJS. VS and SSK were supported by a Junior Research Fellowship and a Senior Research Fellowship, respectively, from the University Grants Commission, Government of India.

References

[1] D.E. Epps, P.C. Schmid, V. Natarajan, H.H.O. Schmid, Biochem. Biophys. Res. Commun. 90 (1979) 628–633.
[2] V. Natarajan, P.C. Schmid, H.H.O. Schmid, Biochim. Biophys. Acta 878 (1986) 32–41.
[3] D.E. Epps, J.W. Palmer, H.H.O. Schmid, D.R. Pfeiffer, J. Biol. Chem. 257 (1982) 1383–1391.
[4] D.E. Epps, F. Mandel, A. Schwartz, Cell Calcium 3 (1982) 531–543.
[5] N.L. Parinandi, H.H.O. Schmid, FEBS Lett. 237 (1988) 49–52.
[6] M. Sugita, M. Willams, J.T. Dulaney, H.W. Moser, Biochim. Biophys. Acta 398 (1975) 125–131.
[7] W.A. Devane, L. Hanus, A. Breuer, R.G. Pertwee, L.A. Stevenson, G. Griffin, D. Gibson, A. Mandelbaum, A. Etinger, R. Mechoulam, Science 258 (1992) 1946–1949.
[8] L. Venance, D. Piomelli, J. Glowinski, C. Giaume, Nature 376 (1995) 590–594.
[9] H. Schuel, E. Goldstein, R. Mechoulam, A.M. Zimmerman, S. Zimmerman, Proc. Natl. Acad. Sci. USA 91 (1994) 7678–7682.
[10] H.H.O. Schmid, P.C. Schmid, V. Natarajan, Prog. Lipid Res. 29 (1990) 1–43.
[11] H.H.O. Schmid, P.C. Schmid, V. Natarajan, Chem. Phys. Lipids 80 (1996) 133–142.
[12] V. Natarajan, P.V. Reddy, P.C. Schmid, H.H.O. Schmid, Biochim. Biophys. Acta 664 (1981) 445–448.
[13] P.C. Schmid, P.V. Reddy, V. Natarajan, H.H.O. Schmid, J. Biol. Chem. 258 (1982) 9302–9306.
[14] V. Natarajan, P.C. Schmid, P.V. Reddy, H.H.O. Schmid, J. Neurochem. 42 (1984) 1613–1619.
[15] F. Desarnaud, H. Cadas, D. Piomelli, J. Biol. Chem. 270 (1995) 6030–6035.
[16] V. Natarajan, P.C. Schmid, H.H.O. Schmid, Biochim. Biophys. Acta 878 (1986) 32–41.
[17] M. Colodzin, N.R. Bachur, H. Weissbach, S. Udenfriend, Biochem. Biophys. Res. Commun. 10 (1963) 165–170.
[18] N.R. Bachur, S. Udenfriend, J. Biol. Chem. 241 (1966) 1308–1313.
[19] T. Sugiura, S. Kondo, A. Sukagawa, T. Tonegawa, S. Nakane, A. Yamashita, Y. Ishima, K. Waku, Eur. J. Biochem. 240 (1996) 53–62.
[20] V. Di Marzo, A. Fontana, H. Cadas, S. Schinelli, G. Simino, J-C. Schwartz, D. Piomelli, Nature 372 (1994) 686–691.
[21] P.C. Schmid, M.L. Zuzarte-Augustin, H.H.O. Schmid, J. Biol. Chem. 260 (1985) 14145–14149.
[22] K.D. Chapman, T.S. Moore, Plant Physiol. 102 (1993) 761–769.
[23] R.S. McAndrew, B.P. Leonard, K.D. Chapman, Biochim. Biophys. Acta 1256 (1995) 310–318.
[24] N. Ueda, Y. Kurahashi, S. Yamamoto, T. Tokunaga, J. Biol. Chem. 270 (1995) 23823–23827.
[25] D.E. Epps, A.D. Cardin, Biochim. Biophys. Acta 903 (1987) 533–541.
[26] A. Ambrosini, E. Bertoli, P. Mariani, F. Tanfani, M. Wozniak, G. Zolese, Biochim. Biophys. Acta 1148 (1993) 351–355.
[27] S. Akoka, C. Tellier, C. LeRoux, D. Marion, Chem. Phys. Lipids 46 (1988) 43–50.
[28] D. Marsh, CRC Handbook of Lipid Bilayers. CRC Press, Boca Raton, 1990, 387 pp.
[29] K. Larsson, In: F.D. Gunstone, J.L. Harwood, F.B. Padley (Eds.), The Lipid Handbook. Chapman and Hall, London, 1986, pp. 321–384.
[30] J.L. Newman, D.L. Stiers, W.H. Anderson, H.H.O. Schmid, Chem. Phys. Lipids 42 (1986) 249–260.
[31] S. Akoka, C. Tellier, C. LeRoux, D. Marion, Chem. Phys. Lipids 46 (1988) 43–50.
[32] J.C. Domingo, M. Mora, M.A. de Madariaga, Chem. Phys. Lipids 75 (1995) 15–25.
[33] D. Lafrance, D. Marion, M. Pézolet, Biochemistry 29 (1990) 4592–4599.
[34] CRC Handbook of Chemistry and Physics, 70th ed., 1990. CRC Press, Boca Raton, Florida, pp. C666–C670.
[35] M.J. Swamy, B. Angerstein, D. Marsh, Biophys. J. 66 (1994) 31–39.
[36] D. Marsh, Biochim. Biophys. Acta 1062 (1991) 1–6.
[37] D. Marsh, In: G. Pifat, J.N. Herak (Eds.), Supramolecular Structure and Function. Plenum Press, New York, 1982, pp. 127–178.