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Differential scanning calorimetric studies on the thermotropic phase behavior of dry and hydrated forms of *N*-acyltyramines

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ABSTRACT

In this paper, a homologous series of *N*-acyltyramine (NATA) – which is biosynthetic precursor for neuroactive lipids, *N*-acyldopamine – have been synthesized and their thermotropic phase transitions were characterized by differential scanning calorimetry (DSC). NATA undergoes a major sharp endothermic transition with the melting point of the hydrated samples occurs considerably at lower temperature compared to the dry samples. Thermodynamic parameters, transition enthalpy (ΔH_t) and transition entropy (ΔS_t), associated with the chain-melting phase transitions depends linearly on the number of carbon atoms. The contributions of each methylene unit to the transition enthalpy and entropy and the end contributions arising from the head group and the terminal group was determined and reported. These results are very important to understand the thermodynamics basis of NATA phase properties in other membrane lipids.

1. Introduction

Recent advancement in the field of lipodomics identified *N*-acyl derivatives of dopamine in the mammalian tissues. This belongs to an important class of endogenous signaling molecules, formed by an amide linkage with long chain fatty acids and catecholamines [1,2]. Although there are strong evidences for the existence of diverse group of fatty acid amides (FAAs) in the brain and other tissues, only a few have been identified to date [3]. The complete functions of these identified FAA are yet to be elucidated. Biochemical studies have reveled that FAA have numerous physiological functions in mammalian tissues. For example, *N*-arachidonoyldopamine and *N*-oleoyldopamine binds to transient receptor potential vanilloid 1 (TRPV1) with a potency similar to that of capsaicin, and produce thermal hyperalgesia [1,2]. The bio-synthesis of *N*-acyldopamine (NADA), a new family of multifunction lipid effectors of cannabinoid-vanilloid system, remains enigmatic. A proposed bio-synthesis involves several closely conjugated enzymatic stages proceed *via* condensation of free fatty acids with tyrosine and the subsequent conversion of *N*-acyltyrosine to *N*-acyldopamine [4]. An analogous pathway involves acylation of tyramine by an unknown enzyme. *N*-acyltyramine (NATA) easily undergoes oxidation by monophenol monoxygenase to NADA, whereas *N*-acyltyrosine undergoes hydrolysis under this condition [4]. The possibility of NADA bio-synthesis from corresponding NATA was further evidenced from the presence of *N*-acyltyramines in tissues where *N*-acyldopamines are located [5]. However, the bio-synthesis and the structure-activity relationship of these NATA in tissues are yet to be elucidated.

While significant amount of work has been contacted on *N*-acylethanolamine [6], *N*-acylamino acids [6,7], *N*-acyldopamines [8,9], there are no report on physiological roles and pharmacological potential of *N*-acyltyramines. *N*-Acetyltyramine has been identified in mammalian urine [10] and tyramine *N*-acylation activities were heterogeneously distributed throughout the brain [11]. Enzymes acetyl-CoA, arylamine *N*-acetyltransferase have been identified for the *N*-acetylation of the tyramines [11]. In mice model it has been demonstrated that *N*-acetyltyramine reduces the locomotors activity and prolonged the duration of barbiturate anesthesia [12]. Tyramines are primarily metabolized by an enzyme, monoamine oxidase (MAO) [13], whereas the corresponding *N*-acetyltyramines are not metabolized by MAO, suggesting different physiological role for *N*-acetyltyramines [11]. NATA are found to be better substrates for tyrosinase, an enzyme responsible for the production of melanin [14]. In view of their presence in mammalian system, and considered to be biosynthetic precursor for *N*-acyldopamine, it is crucial to carry out systematic studies on the properties of NATA. The knowledge of their phase behavior in aqueous dispersion in pure state as well as in mixtures with other

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membrane lipids is very important to delineate how they function *in vivo*. As the first step in this direction, a homologues series of *N*-acyltyramines with saturated acyl chains of even number of carbon atoms has been synthesized and studied by differential scanning calorimetry (DSC). The thermotropic phase behavior of dry and hydrated samples of NATA are obtained, analyzed and reported.

2. Materials and methods

2.1. Materials

Tyramine, decanoyl chloride, lauroyl chloride, myristoyl chloride and palmitoyl chloride were obtained from Sigma–Aldrich, USA. All other reagents were of analytical grade obtained from local suppliers. All solvents were distilled and dried prior to use.

2.2. Synthesis of N-acyltyramines

N-Acyltyramines were synthesized by simple condensation reaction between fatty acid chloride and tyramine. Briefly, 200 mg of tyramine were dissolved in 5 ml of dichloromethane and tetrahydrofuran (2:1, v/v). Approximately, 1 mole equivalent of the acid chloride and triethylamine were then added in an ice-bath, under constant stirring. After all the reagent was added, the reaction was allowed to continue for 24 h at room temperature. The completion of the reaction was judged by thin layer chromatography on silica gel (solvent system: hexane/ethylacetate 70/30, v/v). Then, the solution was washed successively with double distilled water, dilute hydrochloric acid and dilute bicarbonate solution. The organic layer was collected and evaporated to dryness under reduced pressure. The resultant residue was purified by flash chromatography (eluent: hexane/ethylacetate 70/30, v/v). The obtained yields ranged between 55 and 60% for different NATA. The NATAs obtained were characterized by TLC, melting point, FTIR, ^{1}H NMR, ^{13}C NMR, elemental analysis and LC–MS (details in supporting information).

2.3. Differential scanning calorimetry

DSC studies were carried out on a Perkin-Elmer PYRIS Diamond differential scanning calorimeter on NATA that were obtained by recrystallization from dichloromethane/acetone mixture at −20 °C. About 1–3 mg of dry NATA were weighed accurately into aluminum sample pans, covered with an aluminum lid and sealed by crimping. Reference pans were prepared similarly, but without any sample in them. Heating and cooling scans were performed from room temperature (*ca.* 25 °C) to about 110 °C at a scan rate of 2.0 °C/min, and each sample was subjected to three heating scans and two cooling scan.

DSC studies of hydrated NATAs were carried out on a VP DSC microcalorimeter from MicroCal (Northampton, MA). Samples of NATAs (4–5 mg) were weighed accurately in aluminum pans and transferred into clean, dried glass test tubes. Each sample was dissolved in ∼300 μL of chloroform, and a thin film of the lipid was obtained by blowing a gentle stream of dry nitrogen gas over the solution. Final traces of the solvent were removed by vacuum desiccation for about 4–5 h. Then the thin film was then hydrated with 1.0 mL of double distilled water and the hydrated sample was subjected to 5–6 cycles of freeze–thawing with occasional vortexing in order to obtain a homogeneous suspension, which was used for DSC studies. Each sample was subjected to three heating scans and two cooling scans. While the lower end of the scan was set as 10 °C the upper end was set between 85 and 110 °C depending on the main, chain-melting phase transition temperature of the sample. All scans were performed at a scan rate of 1°/min.

For studies with both dry and hydrated samples, transition enthalpy (ΔH_t) for each transition was determined by integrating the area under the transition. In all cases only the first heating scan is considered for further analysis. Transition entropies (ΔS_t) were determined from the transition enthalpies assuming a first order transition according to the equation [15]:

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

where T_t is the transition temperature determined from the peak of the thermogram. ΔH_t values are taken at this temperature in order to calculate the corresponding ΔS_t values.

3. Results and discussion

3.1. Synthesis and characterization of N-acyltyramines

N-Acyltyramines of even acyl chainlengths (n = 10–16) have been synthesized in fair yields by the reaction of the corresponding acid chlorides with tyramine. NATA were purified by column chromatography and recrystallization (from DCM/acetone at −20 °C), were found to be pure by thin layer chromatography. The IR spectra of the purified NATA shows an absorption band corresponding to the amide I and amide II at 1637 and 1548 cm^{-1}, respectively (Fig. S1). Further the structures of the NATA were characterized by ^{1}H NMR (Fig. S2) and ^{13}C NMR (Fig. S3) spectroscopy. In addition, purity of the NATA was analyzed by LC–MS (Fig. S4) and elemental analysis. The results obtained from these methods are fully consistent with structures of NATA and suggest that they are of high purity.

3.2. Differential scanning calorimetry

Fig. 1 shows a differential scanning calorigrams of saturated NATA of even acyl chainlengths. The heating thermograms presented in Fig. 1 shows endothermic solid to fluid phase transition. Except *N*-decanoyltyramine and *N*-dodecanoyltyramine, all other NATA shows two peaks with major transition matching the capillary melting point of the compound. The minor transitions are reproducible for samples obtained from different batches, indicating the presence of solid-solid phase transition with possible polymorphism. However, the cooling scans gave broad peaks, with the mid-point of the transition centered at several degrees lower as compared to the heating thermograms (Fig. S5). This suggests that during cooling, some of the NATA does not return back to its original structural form. The reason for this not known with certainty, but could be related to difficulty in effectively packing the *N*-acyl chain in the solid phase. However, upon immediate second heating, the major chain-melting transition reappears with an enthalpy identical to that observed in the first heating (Fig. S4). Nevertheless there was a small decrease in the transition enthalpies of minor peaks. These calorimetric data provide strong evidence for the presence of a metastable state [16]. Therefore, in all cases first heating scan is consider for further analysis, and the total area under the major transitions was integrated to get the transition enthalpies. The phase transition temperatures, T_t, were estimated from the peak positions at highest the maximal heat flow, whereas the transition entropy, ΔS_t were calculated from the transition enthalpy using Eq. (1). The transition temperatures (T_t), transition enthalpies (ΔH_t) and transition entropies (ΔS_t) obtained from the first heating thermograms presented in Table 1.

DSC of NATA in the presence of excess of water shows an endothermic solid to fluid phase transition. Except *N*-decanoyltyramine and *N*-dodecanoyltyramine, all NATA shows two peaks with major transition lower than the dry sample, indicating that the hydration results in reducing the chain melting transition temperature. In general, the hydration of polar

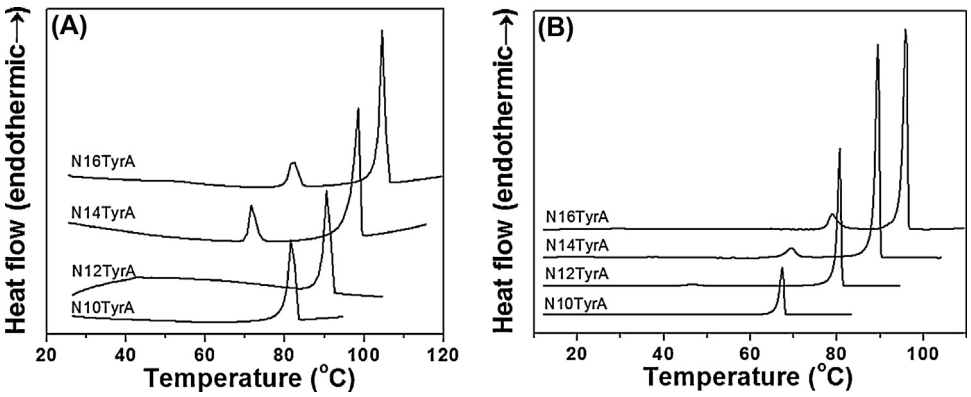

Fig. 1. Differential scanning calorigrams of *N*-acyltyramines (A) dry and (B) hydrated states. The number of carbon atoms in the acyl chain is indicated against each thermogram. Scan rate was 1.0 °C/min.

Table 1
Transition temperatures (T_t), transition enthalpies (ΔH_t), and transition entropies (ΔS_t) of *N*-acyltyramines in dry and hydrated states.

Acyl chain length (n)	Dry NATA			Hydrated NATA		
	T_t (°C)	ΔH_t (kcal mol^{-1})	ΔS_t (cal mol^{-1} K^{-1})	T_t (°C)	ΔH_t (kcal mol^{-1})	ΔS_t (cal mol^{-1} K^{-1})
10	81.47	5.08	14.32	68.49	1.55	4.55
12	91.39	6.69	18.38	80.78	4.46	12.60
14	98.05	10.43	28.13	89.95	6.08	16.77
16	103.76	11.85	31.46	96.01	8.25	21.65

head group reduces the chain melting transition temperature. Similar observations were reported for the aqueous dispersion of phosphotidylcholine [17], phosphotidylethanolamine [17] *N*-biotinylphosphotidylethanolamine [18], *N*-acylethanolamines [19], *N*-acylphosphotidylethanolamine [20] and *N*-acyldopamines [8].

3.3. Chain length dependence of transition enthalpy and transition entropy

The chain length dependence of transition enthalpy and transition entropy for the chain-melting phase transitions of the various NATAs investigated in this study, both for the dry samples and the hydrated dispersions are reported in Table 1. Fig. 2 shows that both transition enthalpy and transition entropy are approximately linear with respect to chainlength (n). The data could be fit well to expressions 2 and 3 given below [21]:

$$\Delta H_t = (n-2)\Delta H_{inc} + \Delta H_0 \quad (2)$$

$$\Delta S_t = (n-2)\Delta S_{inc} + \Delta S_0 \quad (3)$$

where ΔH_0 and ΔS_0 are the end contributions to the transition enthalpy and transition entropy, respectively, arising from the terminal methyl group and the polar head group region of NATA. ΔH_{inc} and ΔS_{inc} are the incremental values of transition enthalpy and transition entropy per CH_2 group. Theoretically, ΔH_{inc} gives a measure of cohesiveness of acyl chain packing. The linear chain length dependence of transition enthalpy and transition entropy suggests that the structure, molecular packing and intramolecular interaction of the different NATAs in the solid state are likely to be very similar. From the linear least square analysis, the incremental values and end contributions of transition enthalpy and entropy were determined and reported in Table 2. For comparison, data for the corresponding NAEs [22] and NADAs [8] are reported in Table 2. The values of ΔH_{inc} for solid-to-liquid phase transition of NATA in the dry state are higher than the ΔH_{inc} in the hydrated state. Similar observation was found in NAE [20]. Nevertheless, ΔH_{inc} found in the case of NATA, both in dry state and hydrated state, are significantly higher than those obtained for NAE. It is likely that the acyl chains in the solid phase and aqueous dispersion of NATA are more ordered than the respective solid phase and aqueous dispersion of NAE. This difference in the ΔH_{inc} of NATA and NAE possibly arises from the hydrophobic effect and π-stacking of the aromatic rings of the phenolic moiety. The observed negative end contributions of enthalpy, ΔH_0, and entropy, ΔS_0, is an indicative of the hydrophobic effect playing a role in packing the NATAs. The more negative ΔS_0 for hydrated samples as compared to the dry samples indicate that the water molecules around the phenolic moiety plays a significant role in packing the aqueous dispersion of NATAs. On

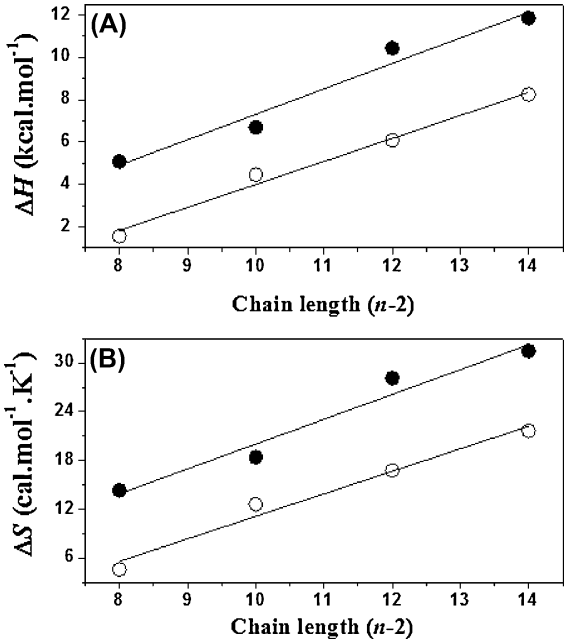

Fig. 2. Chain length dependence of transition enthalpies (A) and transition entropies (B) of *N*-acyltyramines. Data obtained from heating scans of dry (●) and hydrated (○) samples of NATA are shown. Solid line represents linear least-squares fit of the data.

Table 2
Incremental values (ΔH_{inc}, ΔS_{inc}) of chainlength dependence and end contributions (ΔH_0, ΔS_0) to phase transition enthalpy and entropy of *N*-acyltryamines.

Thermodynamic parameters	Dry fatty acid amides			Hydrated fatty acid amides		
	[a]NAEs	NATAs	[b]NADAs	[a]NAEs	NATAs	[b]NADAs
ΔH_{inc} (kcal mol^{-1})	0.82	1.21	0.95	0.95	1.09	1.42
ΔH_0 (kcal mol^{-1})	−0.10	−4.72	1.01	−0.52	−6.86	−10.55
ΔS_{inc} (cal mol^{-1} K^{-1})	2.01	3.05	2.27	2.37	2.77	3.76
ΔS_0 (cal mol^{-1} K^{-1})	2.12	−10.57	7.22	3.1	−16.62	−26.99

[a] For comparison thermodynamic values are adopted from ref. [20].
[b] For comparison thermodynamic values are adopted from ref. [9].

the other hand, the end contribution from enthalpy and entropy of the aqueous dispersion of NATAs is less negative compared to *N*-acydopamine [see Table 2]. This could arise due to the presence of catechol moiety in NADA, which are expected to form water mediated hydrogen bond between the catechol moieties (2H-bond). It is well known that, each hydrogen bond contributes 5–6 kcal mol^{-1} for the enthalpy of stabilization [22]. The additional stabilization from H-bond, might impose geometrical constrain in π-stacking the catechol moieties, results in more negative end contribution from the enthalpy and entropy. Whereas NATA differs from NADA by one —OH group in the ortho-position. NATA contains one —OH group, therefore, it is expected to have only one H-bond between the phenolic moieties of NATAs. Therefore, the imposed geometrical constrain in forming π-stacking in the NATAs might be less compared to the NADA. As a result ΔH_0, and ΔS_0 is less negative for NATA. Furthermore, the gel-to-fluid transition temperature decreases upon hydration of NATAs was >8°, whereas the corresponding transition for NADA was ∼5° and for NAE was >15°. Collectively, these observation clearly suggest that the acyl chains are less ordered in the aqueous dispersion of NATA than NADA, whereas the acyl chains of NATAs are more tightly packed than the NAE, and the most likely factor responsible for this are hydrophobic effect and π-stacking. Based on the above analysis, we suggest that the chain packing in the fatty acid amides might be in the following order, NAE < NATA < NADA. The results also illustrate that how slight modification in the polar head group region of FAAs can significantly affect principles underlying the molecular packing in FAAs.

3.4. Chain length dependence of transition temperature

The transition temperature T_t, determined from the DSC studies, both for the dry samples of NATA as well as their dispersion in water are listed in Table 1. It can be seen that transition temperatures of the NATA increase with increasing chain length. However, the magnitude of the change in transition of both dry and hydrated samples decreases steadily with increase in the chain length (Fig. 3). Similar trends were observed for the aqueous dispersion of phosphotidylcholine, phosphotidylethanolamine [17], *N*-biotinyl phosphotidylethanolamine [18], *N*-acylethanolamines [19], *N*-acylphosphotidylethanolamine [20] and *N*-acyldopamines [8]. As the acyl chain length increases, the total contribution from $(CH_2)_n$ dominates the transition enthalpy and transition entropy, and results in negligible end contribution from transition enthalpy and transition entropy. Therefore, at infinite acyl chain length, Eqs. (2) and (3) can be reduced to [21]:

$$\Delta H_t = (n-2)\Delta H_{inc} \quad (4)$$

$$\Delta S_t = (n-2)\Delta S_{inc} \quad (5)$$

Then the transition temperature for infinite chain length will be given by $T_t^{\infty} = \Delta H_{inc}/\Delta S_{inc}$. From Table 2, the values of T_t^{∞} for dry and hydrated samples have been estimated as 394.3 and 391.6 K, respectively.

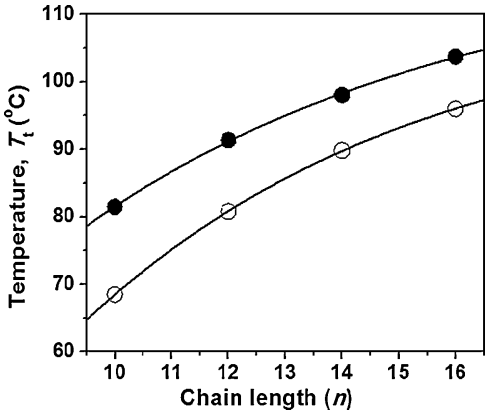

Fig. 3. Chain length dependence of phase transition temperatures of NATA in the dry (●) and hydrated (○). Solid line corresponds to non-linear least-squares fit of each data set to Eq. (6).

It has been shown for a number of diacyl lipids as well as for single chain amphiphiles, the chain length dependence of transition temperature could be fitted with Eq. (6), provided the transition enthalpy and transition entropy follows linear dependence [6,23].

$$\Delta T_t = \frac{\Delta H_t}{\Delta S_t} = \left(\frac{\Delta H_{inc}}{\Delta S_{inc}}\right)\left[1 - \frac{(n-n_0')}{n-n_0'}\right] \quad (6)$$

where $n_0 = (-\Delta H_0/\Delta H_{inc})$ and $n_0' = (-\Delta S_0/\Delta S_{inc})$ are the values of chain length (n) at which the transition enthalpy and transition entropy extrapolate to zero. It can be seen from Fig. 3, that the transition temperature of the entire series of both dry and hydrated NATAs could be satisfactorily fit by non-linear least squares method to Eq. (6). Additionally, from the fitting parameters, the transition temperature at infinite chainlength of the dry and hydrated samples of *N*-acyltyramines, T_t^{∞}, have been estimated as 400.7 and 388.9 K, respectively. The values estimated from the fitting parameters for the dry and hydrated NAEs are in reasonable agreement with the T_t^{∞} values predicted from the linear regression of the calorimetric data.

4. Conclusions

In this paper, a homologous series of biological important *N*-acyl tyramines have been synthesized and their thermotropic phase behavior of dry and hydrated states was studied by differential scanning calorimetry. A linear dependence has been observed in the thermodynamic parameters, ΔH_t and, ΔS_t, associated with the chain-melting phase transitions in the dry state as well as in the hydrated state. The incremental transition enthalpies, ΔH_{inc}, and entropies, ΔS_{inc}, per CH_2 group are more than the NAEs, indicating that chain packing in the gel and/or fluid phase differs between *N*-acyltyramines and NAEs. This also implies that the acyl chains are tightly packed in the NATAs, presumably due to the hydrophobic/π-stacking interactions from the non-polar moieties in the head group region of NATAs. The end contribution of entropy, ΔS_0, is also

negative for dry and hydrated samples, suggesting that hydrophobic effect playing a role in the packing of NATAs. However, the values of ΔH_{inc} and ΔS_{inc} of the hydrated dispersion of NATAs are considerably less than the aqueous dispersion of NADAs, suggesting that the acyl chains are tightly packed in the aqueous dispersion of NADAs. These results suggest that the head groups (phenolics of NATAs and catechol of NADAs) plays major role in determining the chain packing. In summary, our study shows that how a slight modification in the polar head group region of FAAs can significantly affect principles underlying the molecular packing in FAAs. Currently, we are investigating the miscibility of NATA with other lipids, such as phosphatidylchloine and phosphatidylethanolamine. These results are expected to provide more evidence for their role in biological processes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tca.2014.03.030.

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