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Molecular structure, supramolecular organization and thermotropic phase behavior of *N*-acylglycine alkyl esters with matched acyl and alkyl chains

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ABSTRACT

N-Acylglycines (NAGs), the endogenous single-tailed lipids present in rat brain and other mammalian tissues, play significant roles in cell physiology and exhibit interesting pharmacological properties. In the present study, a homologous series of *N*-acylglycine alkyl esters (NAGEs) with matched chains were synthesized and characterized. Results of differential scanning calorimetric studies revealed that all NAGEs exhibit a single sharp phase transition and that the transition enthalpy and entropy show a linear dependence on the *N*-acyl and ester alkyl chain length. The structure of *N*-myristoylglycine myristyl ester (NMGME), solved by single-crystal X-ray diffraction, showed that the molecule adopts a linear geometry and revealed that the structure of *N*-myristoyl glycyl moiety in NMGME is identical to that in *N*-myristoylglycine. The molecules are packed in layers with the polar functional groups of the ester and amide functionalities located at the center of the layer. The crystal packing is stabilized by N—H···O hydrogen bonds between the amide C═O and N—H groups of adjacent molecules as well as by C—H···O hydrogen bonds between the amide carbonyl and the methylene C—H adjacent to the ester carbonyl of neighboring molecules as well as between ester carbonyl and methylene group of the glycine moiety of adjacent molecules. Powder X-ray diffraction studies showed a linear dependence of the *d*-spacings on the acyl chain length, suggesting that all NAGEs adopt a structure similar to the packing exhibited in the crystal lattice of NMGME.

1. Introduction

Lipids, the amphiphilic molecules are the major constituents of the cell membranes and membranes of organelles, and play significant roles in a variety of physiological processes related to cell signaling, health and diseases, and energy storage (Finkelstein et al., 2014; Spiegel et al., 1996; Fernandis and Wenk, 2007). In view of this, Lipid Maps Lipidomics Gateway has been initiated to bring together all the information on lipids, from plant and animal tissues, including humans (Cotter et al., 2006; Fahy et al., 2007; http://www.lipidmaps.org/data/structure/index.html). Recent reports suggested that *N*-acyl amino acids and *N*-acyl neurotransmitters (NAANs) are present in mammalian tissues and may play potentially important roles in physiological processes (Connor et al., 2010). Since NAANs are significant molecules present in the membranes of cells of mammalian tissues, it is important to explore their structure, molecular organization, and interaction with other membranes lipids as well as membrane proteins. In this direction, in previous work, we have reported the structure, molecular packing and thermotropic phase behavior of four different NAANs namely *N*-acyldopamines, *N*-acylglycines, *N*-acylserotonins, and *N*-acylalanines (Reddy et al., 2013, 2014; Reddy and Swamy, 2015; Sivaramakrishna et al., 2015).

Among NAANs, *N*-acylglycines (such as *N*-palmitoylglycine and *N*-stearoylglycine), isolated from rat brain and other peripheral mammalian tissues, have been found to exhibit interesting biological and pharmacological properties (Connor et al., 2010; Hanuš et al., 2014; Tan et al., 2009, 2010). Reaction of the carboxyl moiety of *N*-acylglycines (NAGs) with long chain alcohols would yield *N*-acylglycine alkyl esters (NAGEs). NAGEs with matched *N*-acyl and *O*-alkyl chains have been investigated to evaluate their ability to enhance the permeability of stratum corneum – which is the outermost layer of the skin – which in turn is expected to facilitate transdermal drug delivery. These studies have revealed that *N*-lauroylglycine lauryl ester – the NAGE with 12 C-atoms in the *N*-acyl and *O*-alkyl chains enhanced the permeability by

Abbreviations: DAE, *N*-, *O*-diacylethanolamine; DSC, differential scanning calorimetry; FTIR, fourier transform infrared; ΔH_o, end contribution to transition enthalpy; ΔH_{inc}, incremental contribution of each CH_2 group to transition enthalpy; ΔH_t, transition enthalpy; NAANs, *N*-acyl amino acids and *N*-acyl neurotransmitters; NAE, *N*-acylethanolamine; NAG, *N*-acylglycine; NAGE, *N*-acylglycine alkyl ester; NMG, *N*-myristoylglycine; NMGME, *N*-myristoylglycine myristyl ester; NMR, nuclear magnetic resonance; PXRD, powder X-ray diffraction; ΔS_o, end contribution to transition entropy; ΔS_{inc}, incremental contribution of each CH_2 group to transition entropy; ΔS_t, transition entropy; T_t, transition temperature

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~12.5 fold, suggesting that NAGEs may potentially be useful in formulating transdermal drug delivery systems (Vavrova et al., 2003). It is also interesting to note that NAGEs are structural isomers of *N*-, *O*-diacylethanolaminines (DAEs), which are reported to be present in the plasma and blood cells and investigated in detail by biophysical approaches (Balvers et al., 2013; Tarafdar and Swamy, 2009; Kamlekar et al., 2010; Tarafdar et al., 2012).

In order to utilize NAGEs for biomedical applications such as in developing transdermal drug delivery systems, it is important to characterize them with respect to structure, thermotropic phase transitions and interaction with other lipids/amphiphiles that are used in the formulation of the delivery systems. As a first step in this direction, in the present study, we have synthesized *N*-acylglycine alkyl esters by the esterification of *N*-acylglycines, and characterized the NAGEs thus obtained by spectroscopic methods. Thermotropic phase behavior of dry NAGEs was investigated by differential scanning calorimetry (DSC) and the crystal structure of *N*-myristoylglycine myristyl ester (NMGME) was solved by single-crystal X-ray diffraction. Results of powder X-ray diffraction (PXRD) measurements on the homologous series of NAGEs showed that the *d*-spacings exhibit a linear dependence on the chain length of the acyl/alkyl chains, which suggests that all the compounds in the homologous series have a similar packing arrangement in the solid state. These studies are relevant for understanding the structure-activity relationship of these lipids and their interaction with other membrane constituents such as phospholipids and membrane proteins.

2. Materials and methods

2.1. Materials

Long-chain fatty acids and fatty alcohols, p-toluene sulfonic acid, and glycine were purchased from Sigma-Aldrich (USA). Oxalyl chloride was obtained from Spectrochem (Mumbai, India). Solvents and other chemicals used were of analytical grade and purchased locally. Milli-Q water was used in all experiments.

2.2. Synthesis of N-acylglycine alkyl esters

N-Acylglycine alkyl esters (NAGEs) were synthesized by a minor modification of a reported procedure for the synthesis of *N*-hexadecanoylserine (Zhou and Kohn, 1990). Briefly, glycine alkyl ester hydrochloride (1 mmol), prepared by the esterification of glycine with fatty alcohol in presence of p-toluene sulfonic acid (Minakuchi et al., 2012), was dissolved in 4 ml of water, followed by the addition of chloroform (12 ml) and sodium bicarbonate (2 mmol) with vigorous stirring. To this mixture, fatty acid chloride (1.1 mmol) in chloroform (4 ml), prepared by the reaction of fatty acid with oxalyl chloride (Ramakrishnan et al., 1997), was added and the reaction mixture was kept under stirring for about 3–4 h at room temperature. The organic phase was washed successively with saturated sodium chloride solution, 0.1 N HCl, and twice with water. The solution was dried over anhydrous sodium sulphate, filtered, and evaporated to dryness under reduced pressure. Recrystallization of the residue from hexane containing traces of acetone at −20 °C, yielded *N*-acylglycine alkyl ester (NAGE) in 80–85% yield. The NAGEs thus obtained were analyzed by capillary melting point, FT-IR, ^{1}H- and ^{13}C NMR spectroscopy. IR spectra (KBr pellet) were recorded on a Thermo Scientific NICOLET 380 FTIR spectrometer, whereas ^{1}H- and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker Avance NMR spectrometer at 400 MHz and 100 MHz, respectively.

Melting points of NAGEs were determined using a Superfit (Mumbai, India) melting point apparatus. In brief, powdered samples were loaded into glass capillaries with a sealed end and the melting points were estimated visually with the aid of a magnifying lens, which was incorporated into the apparatus.

2.3. Differential scanning calorimetry

DSC studies were carried out using a Perkin-Elmer PYRIS Diamond differential scanning calorimeter. Samples of dry NAGEs (1–2 mg) were weighed accurately and taken into aluminium sample pans covered with aluminium lids and closed by crimping. Reference pans were made 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 1.5°/min and each sample was subjected to three heating and two cooling scans. In each case, only the first heating scan was used for further analysis. Transition enthalpies (ΔH_t) were estimated by integrating the area under the transition curve. Transition entropies (ΔS_t) were calculated from the transition enthalpies by assuming a first order phase transition according to the following equation (Marsh, 1990):

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

Where T_t is phase transition temperature and ΔH_t values at this temperature were used to calculate the corresponding ΔS_t values.

2.4. Single crystal X-ray diffraction

Colorless, thin plate-like crystals of *N*-myristoylglycine myristyl ester (NMGME) were grown from dichloromethane containing traces of methanol. X-Ray diffraction data were recorded at 100 K on a Bruker SMART APEX CCD area detector system equipped with a graphite monochromator and using Mo-K_α (λ = 0.717073 Å) radiation obtained from a fine-focus sealed tube. The minimum resolution of X-ray diffraction measurements is 0.84 Å. Data reduction was done using Bruker SAINTPLUS program. Absorption correction was applied using SADABS program and refinement was done using SHELXTL program (Sheldrick, 2008). Mercury 2.3 and Diamond softwares were used for making molecular packing and hydrogen bonding diagrams, respectively.

2.5. Powder X-ray diffraction

Powder X-ray diffraction patterns of NAGEs were recorded on a Bruker SMART D8 Advance X-ray diffractometer (Bruker-AXS, Karlsruhe, Germany) using Cu-K_α X-radiation (λ = 1.5406 Å) at 40 kV and 30 mA at 25 °C. Diffraction patterns were collected over the 2θ range of 2–50° at a scan rate of 0.8°/min. Samples ground to fine powders with the help of a mortor and pestle were used for recording the diffraction data. Origin 7.0 software was used for overlaying the experimental PXRD patterns.

3. Results and discussion

3.1. Characterization of N-acylglycine alkyl esters

In this study, a homologous series of *N*-acylglycine alkyl esters with matched acyl and alkyl chains have been synthesized by esterification of glycine with alcohols of varying alkyl chain lengths followed by condensation with the fatty acid chlorides of same acyl chain length. The synthesized compounds were purified by recrystallization from hexane containing traces of acetone and characterized by FTIR, ^{1}H- and ^{13}C NMR spectroscopy. Representative FTIR and ^{1}H NMR spectra of *N*-myristoylglycine myristyl ester are shown in Fig. S1 and Fig. S2, respectively. FTIR spectra of NAGEs exhibited absorption bands at ~1633–1650 cm^{-1} (amide-I), ~1542–1556 cm^{-1} (amide-II), ~1734–1749 cm^{-1} (ester carbonyl), ~2915–2923 cm^{-1} and ~2841–2851 cm^{-1} (C—H stretching), ~1236–1249 cm^{-1} (C—O stretching) and 3326–3338 cm^{-1} (N—H stretching). The NAGEs also exhibited split bands corresponding to C—H bending (scissoring mode, ~1458–1469 cm^{-1}) and rocking (~717–725 cm^{-1}), which is consistent with O⊥ chain packing (Di and Small, 1995). The FTIR data corresponding to the individual NAGEs are listed in Table S1.

^{1}H NMR spectra of NAGEs exhibited resonances at 2.24–2.27 δ (2H,