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Thermochimica Acta 424 (2004) 53–58
thermochimica acta
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The thermal effects of platinum(II) and palladium(II) complexes with 2-acetyl pyridine and pyridine-2-carbaldehyde *N*(4)-ethyl-thiosemicarbazones in membrane bilayers

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Received 16 January 2004; received in revised form 21 May 2004; accepted 26 May 2004
Available online 2 July 2004

Abstract

Platinum(II) and palladium(II) complexes with 2-acetyl pyridine and pyridine-2-carbaldehyde *N*(4)-ethyl-thiosemicarbazones, HAc4Et and HFo4Et respectively were synthesized and found to exhibit a cytotoxic potency in a very low micromolar range and to be able to overcome the cisplatin resistance of A2780/Cp8 cells. The biologically active complexes $Pd(Fo4Et)_2$ (**1**), $Pd(Ac4Et)_2$ (**2**), $Pt(Fo_4Et)_2$ (**3**) and $Pt(Ac4Et)_2$ (**4**) were tested for their perturbation in model membrane bilayers. The aim was to investigate if there is a possible relation between their mechanism of action in membranes with their biological activity. Indeed, it was found that complexes of deprotonated HAc4Et, (**2**) and (**4**), are more perturbing than complexes of deprotonated HFo4Et, (**1**) and (**3**).

Keywords: Platinum(II) and palladium(II) complexes; DSC; X-ray structure

1. Introduction

Differential scanning calorimetry is a fast and relatively inexpensive technique that allows the study of the thermotropic properties of the membranes in the absence and presence of bioactive molecules.

The presence of an additive in membrane bilayers affects the thermodynamic parameters that govern a thermogram such as the maximum of the main-phase transition or the pre-transition (T_m), the heat capacity of the peaks (C_p) and the line-width ($T_{m_{1/2}}$). The nature of the DSC thermograms can be understood if the total intermolecular effects (i.e. interfacial, hydrogen bonds and nonspecific hydrophobic and electrostatic interactions) between the additive and the phospholipid bilayers are considered.

Phospholipid bilayers are widely used as model membranes to study drug:membrane interactions since they have found to have similar dynamic properties as the biological ones [1–5]. Dipalmitoylphosphatidylcholine (DPPC) bilayers spontaneously form lipid bilayers in aqueous environments and have been utilized extensively to study drug:membrane interactions. These phospholipid bilayers are convenient to use because they undergo phase transitions close to ambient temperatures [6]. For some classes of pharmacologically important molecules (i.e. anesthetic steroids flavonoids, anti-inflammatory etc.) a relationship has been revealed between the degree these drugs affect the thermodynamic parameters described above (perturbation) and pharmacological activity [7–10].

Thiosemicarbazones (tsc's) have aroused considerable interest in chemistry and biology due to their antibacterial, antimalarial, antineoplastic and antiviral activities and are among the most potent inhibitors of ribonucleotide reductase (RR). The group of compounds catalyses the synthesis of deoxyribonucleotides from their ribonucleotide precursors and as such is responsible for maintaining a balanced supply of the deoxyribonucleotides required for DNA synthesis and repair. Strong positive correlation has been established between RR activity and the rate of replication of cancer cells [11]. The chemistry of transition metal complexes of tsc's has been receiving considerable attention

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doi:10.1016/j.tca.2004.05.031

largely because of their pharmacological properties [12]. The combination of tsc's with agents like platinum(II) or palladium(II) that damage DNA produces synergistic inhibition of tumor growth and may lead to improvements in the effectiveness of cancer chemotherapy [13–15]. The complexes of palladium(II) with 2-acetylpyridine 4*N*-ethyl thiosemicarbazone, HAc4Et, were investigated against Leukemia P388 [16]. A high correlation between potency for *Sister* Chromatid Exchange, (SCE) induction, effectiveness in cell division delay ($P < 0.01$) in normal human lymphocytes in vitro and in vivo established antitumour activity in P388 leukemia bearing mice was found. All the complexes of *palladium*(*II*) were less cytotoxic and almost, all were found more effective than the parent ligand, HAc4Et, acting synergistically [16]. The complexes of platinum(II), with HAc4Et were found to exhibit a cytotoxic potency in a very low micromolar range, and are able to overcome the cisplatin resistance of A2780/Cp8 cells; these cells are characterized by a marked intracellular glutathione content and a reduced cisplatin uptake with respect to the parental A2780 cells [17]. These complexes may be endowed with important anticancer properties since they elicit IC50 values in the μM range as does the clinically used drug *cis*-DDP (*cis*-diamminedichloro-platinum(II)), and, moreover, they display cytotoxic activity in tumor lines resistant to *cis*-DDP. *cis*-DDP has for a long time been of major significance in cancer therapy. There are two major limitations to *cis*-DDP therapy; the toxic side effects and the acquired resistance [18]. The goal of reducing toxic side effects, while maintaining therapeutical efficacy, can be accomplished by improving the solubility of the complexes, by slowing down degradation processes through shielding of the platinum with bulky ligands, and by increasing membrane permeability with more lipophilic ligands.

The aim of this study was to investigate any existing relationship between the biological activity of the complexes $Pd(Fo4Et)_2$ (**1**), $Pd(Ac4Et)_2$ (**2**), $Pt(Fo_4Et)_2$ (**3**) and $Pt(Ac4Et)_2$ (**4**) and their thermal effects related to membrane perturbation by DSC.

2. Materials and methods

2.1. Materials

Dipalmitoyl-glycero-sn-3-phosphatidylcholine (DPPC) was obtained from Avanti Polar Lipids Inc., AL, USA. Solvents were purified and dried according to standard procedures. For the platinum(II) compounds a stock $[PtCl_4]^{2-}$ solution was prepared by dissolving of $PtCl_2$ (2.66 g, 10 mmol) in conc. HCl under reflux, filtering to remove a turbidity of undissolved material, neutralizing with Na_2CO_3 and diluting with distilled water up to 250 ml (pH = 6.0–6.5) to yield a solution of 0.04 M $[PtCl_4]^{2-}$.

2.2. Preparation of the complexes

Solvents were purified and dried according to standard procedures. The heterocyclic thiosemicarbazones, pyridine-2-carbaldeheyde HFo4Et, HAc4Et, 4*N*-ethyl thiosemicarbazone, 2-acetyl pyridine 4*N*-ethyl thiosemicarbazone, were prepared as described by Klayman [19].

- **1** [Pd(Fo4Et)2]. To a solution of HFo4Et (3.2 mmol) in methanol (10 mL) was added a solution of K_2PdCl_4 (1.5 mmol) in distilled water (10 mL). The pH of the solution was adjusted to 8.0–9.0 by the addition of aqueous 1.0 M NH_3 and the reaction mixture was stirred for 5 h at room temperature at constant pH. The powder was filtered off, washed with cold methanol and ether and dried in vacuo over silica gel, finally redried at 70 °C in vacuo over P_4O_{10} d.p. 165–166 °C, yield 50%. IR: $\upsilon = 3444$ br [ν(OH)], 3284 and 3121 [ν(NH)], 1587, 1555sh, 1539 [ν(C=N)], 769 749 [ν(C=S)], 741 [ν(C–S)], 437 and 418 [ν(Pd–N)], 389, 375 cm^{-1} [ν(Pd–S)]; UV-Vis for **1** (DMF) λ/nm (ε/L mol^{-1} cm^{-1}) 479 (1650), 380 (8730) and 324 (68000). Elemental analyses are consistent with $C_{18}H_{22}N_8S_2Pd$ (Found: C, 41.8; H, 4.3; N, 21.3; S, 12.1;. Calcd: C, 41.5; H, 4.2; N, 21.5; S, 12.3 %).
- **2** [$Pd(Ac4Et)_2$] was prepared according to published procedure by reaction of HAc4Et and K_2PdCl_4 [20]. D.p. 192–193 °C. Elemental analyses were consistent with the stoichiometry with $C_{20}H_{26}N_8S_2Pd$ (Found: C, 44.1; H, 4.3; N, 20.3; S, 12.0;. Calcd: C, 43.8; H, 4.8; N, 20.4; S, 11.7 %).
- **3** [$Pt(Fo4Et)_2$] and **4** [$Pt(Ac4Et)_2$] were prepared according to published procedure by reaction of HAc4Et and $[PtCl_4]^{2-}$ [17]. D.p. 182 and 185 °C for **3** and **4** respectively. Elemental analyses were consistent with the stoichiometry $C_{18}H_{22}N_8S_2Pt$ (Found: C, 35.6; H, 4.0; N, 18.7; S, 10.6; Pt; 31.5. Calcd: C, 35.3; H, 3.6; N, 18.3; S, 10.5; Pt, 31.8%) and $C_{20}H_{26}N_8S_2Pt$ (Found: C, 37.4; H, 4.5; N, 17.5; S, 9.8; Pt; 30.3. Calcd: C, 37.7; H, 4.11; N, 17.6; S, 10.0; Pt, 30.4%) for **3** and **4** respectively.

2.3. Differential scanning calorimetry

Appropriate amounts of the phospholipid with HAc4Et or HFo4Et or their complexes with Pd or Pt were dissolved in spectroscopic grade chloroform. The solvent was then evaporated by passing a stream of O_2-free nitrogen over the solution at 50 °C and the residue was placed under vacuum (0.1 mmHg) for 12 h. For measurements this dry residue was dispersed in appropriate amounts of bidistilled water by vortexing. After dispersion in water (50% w/w), portions of the samples (ca. 5 mg) were sealed in stainless steel capsules (7.54 mm diameter and 2.79 mm height) obtained from Perkin-Elmer. Thermograms were obtained on a Perkin-Elmer DSC 7 calorimeter. Prior to scanning, the samples were held above their phase transition temperature for 1–2 min to ensure equilibration. All samples were

scanned with a scanning rate of 2.5 °C/min. The temperature scale of the calorimeter was calibrated using indium ($T_m = 156.6$ °C) as standard sample.

3. Results and discussion

3.1. Preparation of complexes

The complexes of Pd(II) and Pt(II) were prepared from the reaction of ligands, HFo4Et, HAc4Et (Fig. 1) and the appropriate metal salt in aqueous methanol solutions in basic solution, pH 8–9 and molar ratio 1:2, and according to the reactions (1)–(2)

$$[MCl_4]^{2-} + 2HFo4Et + 2NH_4OH \xrightarrow{CH_3OH/H_2O} M(Fo4Et)_2 + 2Cl^- + 2NH_4Cl + 2H_2O \quad (1)$$

$$[MCl_4]^{2-} + 2HAc4Et + 2NH_4OH \xrightarrow{CH_3OH/H_2O} M(Ac4Et)_2 + 2Cl^- + 2NH_4Cl + 2H_2O \quad (2)$$

where M = Pt(II) and Pd(II).

The stoichiometry of the complexes indicates that Pd(II) and Pt(II) are connected with the deprotonated form (anion $Fo4Et^-$ and $Ac4Et^-$) in the complexes $[M(L)_2]$. The complexes are moderately soluble in H_2O and alcohols and more soluble in polar solvents, such as DMF and DMSO. The structure of the complex **4** has been solved [22] (Fig. 2). The platinum atom is in a square planar environment surrounded by two *cis* nitrogen atoms and two *cis* sulfur atoms. The ligands are not equivalent, one being tridentate with (N,N,S) donation, the other being monodentate using only the sulfur atom to coordinate to the metal. It might be possible that there is a weak 'agostic' bond between Pt and the amido-nitrogen, and its attached hydrogen, the metal-hydrogen distance being 2.755 Å. The crystal packing is determined by π–π, and Pt–C interactions.

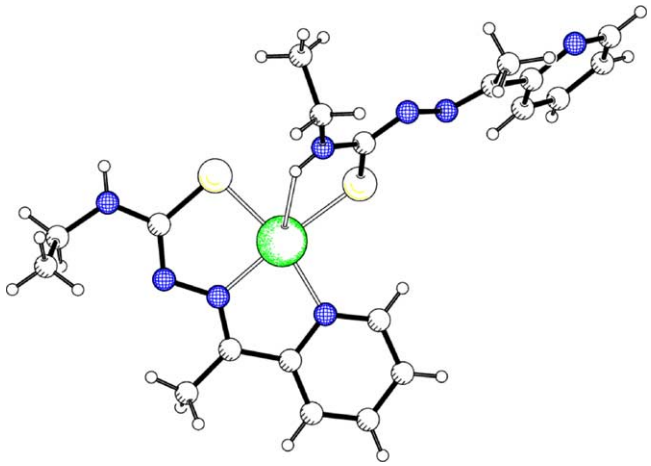

Fig. 2. Structural representation of **4** [22].

3.2. Differential scanning calorimetry

Fully hydrated DPPC bilayers show a characteristic thermogram consisting of a broad low enthalpy transition at 35.3 °C and a sharp enthalpy main transition at 41.2 °C. The DPPC bilayer exists in the gel phase (L'_β) for temperatures lower than 33 °C, and in the liquid crystalline phase for temperatures higher than 42 °C (L'_α). In between 33 and 42 °C the phospholipid bilayer exists in P'_β or ripple phase [5,6,23]. The obtained DSC scan of fully hydrated

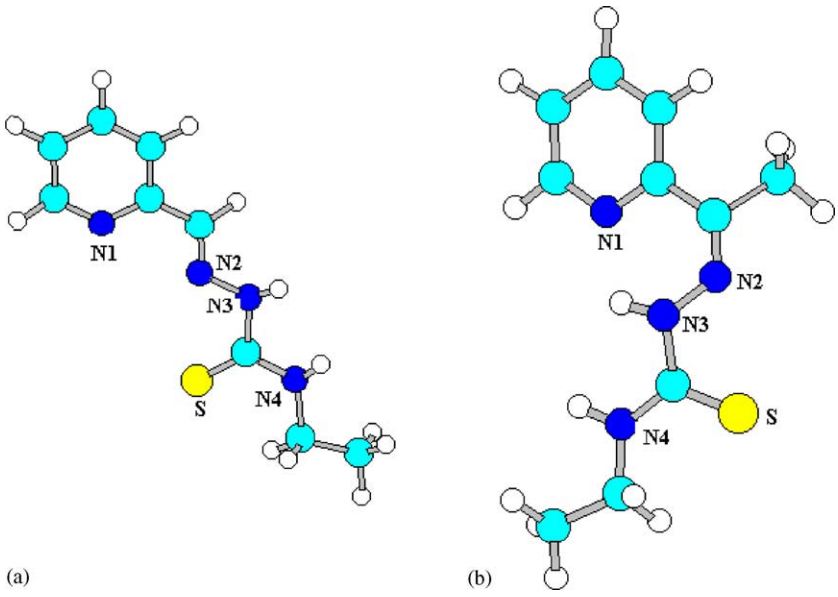

Fig. 1. (a) The fully PM3 optimized geometry of the neutral HFo4Et ($\Delta H_f = 102.2$ kcal mol^{-1}; dipole moment = 6.85 Debye) in the gas state. (b) The fully PM3 optimized geometry of the neutral Hac4Et ($\Delta H_f = 90.0$ kcal mol^{-1}; dipole moment = 7.68 Debye) in the gas state (Hyperchem 6.0) [21].

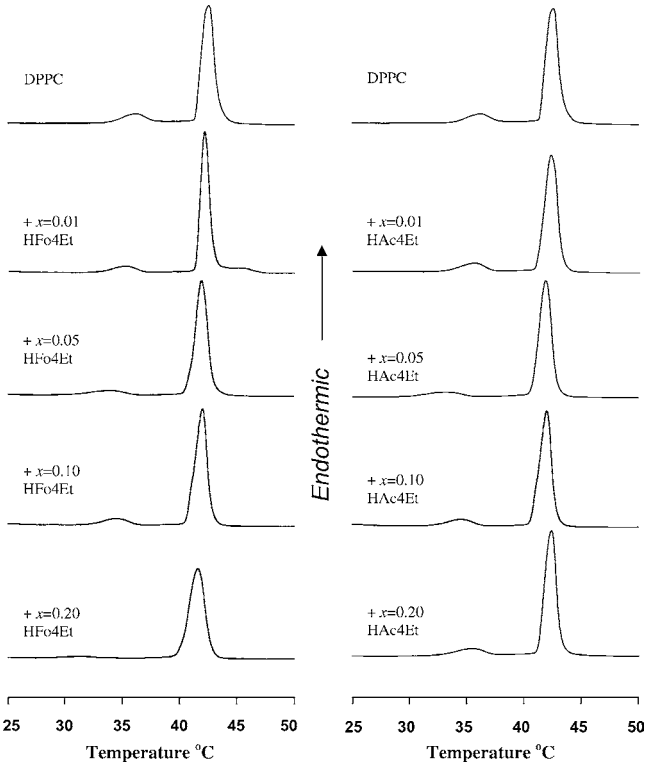

Fig. 3. DSC scan of DPPC bilayers containing different concentrations of the ligands HFo4Et (left) and HAc4Et (right).

Table 1
Diagnostic DSC parameters of the free ligands HAc4Et and HFo4Et or in a complex form with Pt or Pd in DPPC bilayers

Sample	ΔH (kcal/mol)	$T_{m_{1/2}}$	T_m
DPPC	7.6	1.2	41.2
DPPC + $x = 0.01$ HAc4Et	7.3	0.9	41.0
DPPC + $x = 0.05$ HAc4Et	7.3	1.3	40.8
DPPC + $x = 0.1$ HAc4Et	7.1	1.2	40.8
DPPC + $x = 0.2$ HAc4Et	7.1	1.6	40.4
DPPC + $x = 0.01$ HFo4Et	7.5	1.3	41.1
DPPC + x = 0.05 HFo4Et	7.4	1.3	40.7
DPPC + x = 0.1 HFo4Et	7.4	1.4	40.9
DPPC + $x = 0.2$ HFo4Et	7.3	1.2	41.0
DPPC + $x = 0.01$ Pd $Pd(Ac4Et)_2$	7.6	1.3	41.1
DPPC + $x = 0.05$ Pd $Pd(Ac4Et)_2$	7.5	1.4	40.8
DPPC + $x = 0.1$ $Pd(Ac4Et)_2$	7.5	1.8	40.7
DPPC + $x = 0.2$ $Pd(Ac4Et)_2$	7.4	6.6	40.4
DPPC + $x = 0.01$ $Pd(HFo4Et)_2$	7.4	1.2	41.0
DPPC + $x = 0.05$ $Pd(HFo4Et)_2$	7.8	1.2	40.2
DPPC + $x = 0.1$ $Pd(HFo4Et)_2$	7.7	1.5	39.3
DPPC + $x = 0.2$ $Pd(HFo4Et)_2$	8.0	1.6	39.9
DPPC + $x = 0.01$ $Pt(Ac4Et)_2$	7.6	1.1	39.0
DPPC + $x = 0.05$ $Pt(Ac4Et)_2$	7.8	2.8	37.4
DPPC + $x = 0.1$ $Pt(Ac4Et)_2$	8.0	5.4	39.8
DPPC + $x = 0.2$ $Pt(Ac4Et)_2$	7.5	6.6	40.6
DPPC + $x = 0.01$ $Pt(HFo4Et)_2$	7.4	1.2	41.3
DPPC + $x = 0.05$ $Pt(HFo4Et)_2$	7.5	1.3	40.4
DPPC + $x = 0.1$ $Pt(HFo4Et)_2$	7.6	1.6	39.8
DPPC + $x = 0.2$ $Pt(HFo4Et)_2$	7.8	1.8	39.9

DPPC multibilayers shows a pre-transition centered at 35 °C and a peak maximum at 41.2 °C. The main-phase transition is accompanied by several structural changes in the lipid molecules as well as systematic alterations in the bilayer geometry, but the most prominent feature is the *trans-gauche* isomerization-taking place in the acyl chain conformation. The average number of *gauche* conformers indicates the effective fluidity, which depends not only on the temperature, but also on perturbations due to the presence of a drug molecule intercalating between the lipids.

3.2.1. Incorporation of HFo4Et in DPPC bilayers

The presence of HFo4Et in DPPC bilayers at low concentration of 1:99 molar ratio ($x = 0.01$) affects only marginally the thermal scan by broadening the pre-transition (Fig. 3). At higher concentration of 5:95 molar ratio ($x = 0.05$) it further causes broadening of the pre-transition. At $x = 0.10$ and 0.20 the effect is surprisingly depressed. Thus, the pre-transition is shifted towards higher temperatures. T_m and ΔH were not affected significantly by the presence of HFo4Et (see Table 1).

3.2.2. Incorporation of HAc4Et in DPPC bilayers

In contrast to HFo4Et, increase of the HAc4Et concentration resulted in a progressive effect. More particularly, at $x = 0.01$ HAc4Et caused slight broadening of the pre-transition, at $x = 0.05$ caused further broadening and at $x = 0.20$ almost obliterated it (Fig. 3). The lowering of the main-phase transition although not very significant (<1 °C) it paralleled with increasing concentration of HAc4Et. ΔH remained almost constant in all preparations.

3.2.3. Complexation of HFo4Et with Pd

When HFo4Et was complexed with Pd ($Pd(HFo4Et)_2$) and was incorporated in DPPC bilayers its effects were augmented (Fig. 4). At $x = 0.01$ the complex affected the DPPC bilayers as HFo4Et. At higher concentration, however, the presence of complex in DPPC bilayers resulted in the obliteration of the pre-transition. The obliteration of the pre-transition was obvious at concentrations with $x = 0.05$. T_m and ΔH were remained almost constant in all preparations (see Table 1).

3.2.4. Complexation of HAc4Et with Pd

Similarly, when HAc4Et was complexed with Pd ($Pd(Ac4Et)_2$) and was incorporated in DPPC bilayers its effects were augmented (Fig. 4). Already, from $x = 0.01$ the complex almost caused abolish of the pre-transition while when was not in complex form only broadened it slightly. When $x = 0.01$ was used pre-transition was almost barely observed and the main-phase transition was significantly broadened. It is evident from these results that complexation of ligands HAc4Et and HFo4Et with palladium resulted in the enhancement of the membrane perturbing effects.

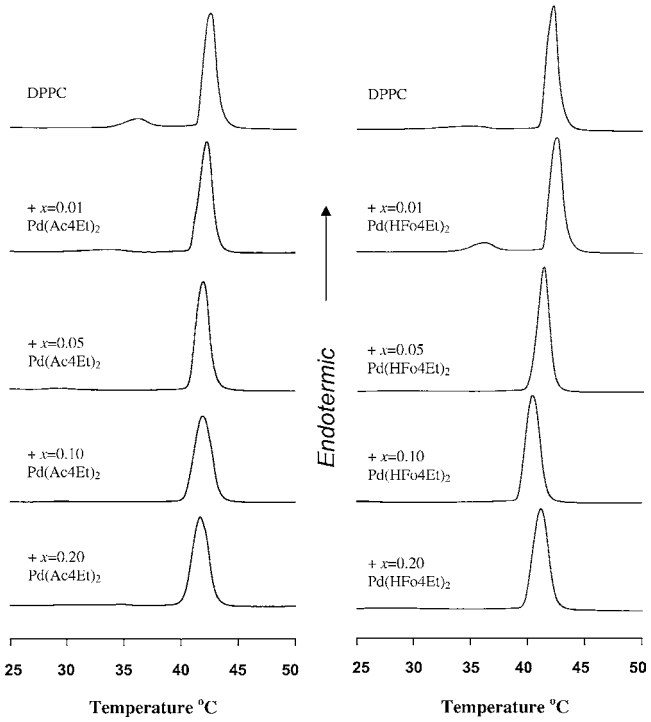

Fig. 4. DSC scan of DPPC bilayers containing different concentrations of the complexes $Pd(Ac4Et)_2$ (left) and $Pd(HFo4Et)_2$ (right).

3.2.5. Complexation of HFo4Et with Pt

HFo4Et when complexed with Pt ($Pt(HFo4Et)_2$) exerted similar effects as with Pd (Fig. 5). More specifically, at the concentration of $x = 0.01$ caused a significant broadening of the pre-transition while at higher concentrations caused abolishment of it and broadening of the main-phase transition.

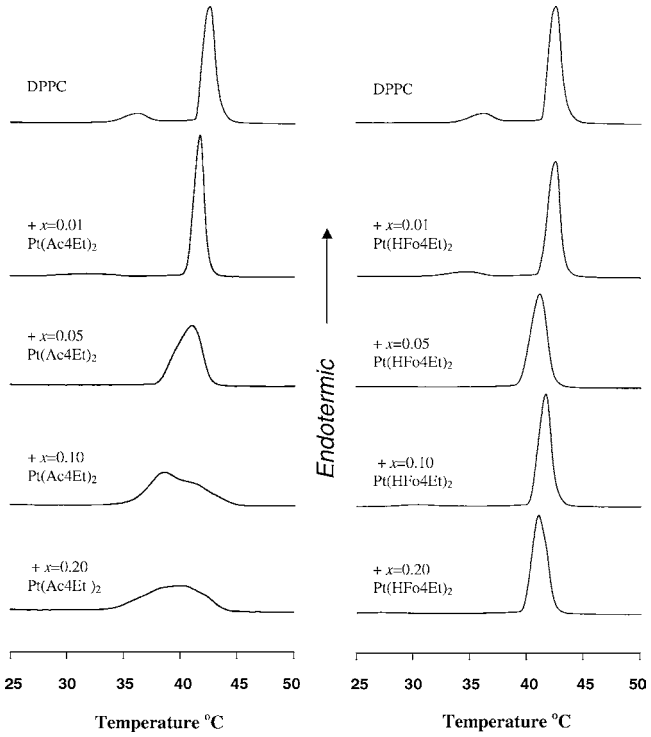

Fig. 5. DSC scan of DPPC bilayers containing different concentrations of the complexes $Pt(Ac4Et)_2$ (left) and $Pt(HFo4Et)_2$ (right).

3.2.6. Complexation of HAc4Et with Pt

HAc4Et when complexed with Pt ($Pt(Ac4Et)_2$) exerted more significant effects than when it is complexed with Pd. The presence of $x = 0.01$ $Pt(Ac4Et)_2$ caused abolishment of the pre-transition. At higher concentration of $x = 0.05$ the incorporation of the complex resulted in significant broadening of the main-phase transition. At $x = 0.1$ the observed peak was significantly broadened consisting of different components. The same applies when $x = 0.2$ is used. The quality of these thermal scans may be interpreted as a reversible transition from a vesicular suspension to an extended peak bilayer network. In particular, Schneider and his collaborators published an article in which they combine calorimetry, viscosity and electron microscopy methods to explain similar thermal profile [24,25]. They state that these structural transitions arise from two effects: (i) the enhanced membrane elasticity accompanying the lipid state fluctuations on chain melting and (ii) solvent-associated interactions (including electrostatics) that favor a change in membrane curvature. Other authors explain similar thermal behavior of bioactive molecules as the "inherent inhomogeneity" of the membrane bilayer. Thus, this preparation may contain domains consisting mainly of pure DPPC bilayers and other domains rich with the ligand [26,27].

4. Conclusions

HAc4Et is a more perturbing agent than HFo4Et as it is evident that causes the most significant broadening of the pre- and main-phase transitions and lowering of the main-phase transition temperature at identical concentrations. When both ligands are complexed they augment their perturbing effects in membrane bilayers. Overall, deprotonated HAc4Et complexes $Pd(Ac4Et)_2$ and $Pt(Ac4Et)_2$ are more perturbing than complexes $Pd(HFo4Et)_2$ and $Pt(HFo4Et)_2$. $Pt(Ac4Et)_2$ is the most perturbing complex observed.

The different perturbation observed between the drugs under study may related to their possible differences in drug delivery and consequently in part explains their distinct bioactivity.

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