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# A calorimetric study of phosphocholine membranes mixed with desmopressin and its diacylated prodrug derivative (DPP)

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**Abstract**

The influence of the water-soluble peptide, desmopressin (DDAVP) and its dipalmitoylated prodrug derivative (DPP) on the thermal behaviour of three different saturated phosphatidylcholine lipid membranes was investigated by differential scanning calorimetry. For lipid membranes composed of dimyristoyl, dipalmitoyl and distearoyl phosphatidylcholines the addition of DDAVP at concentrations of up to 10 mol% resulted in an insignificant change in the thermodynamic phase behaviour. In contrast, the dipalmitoylated DPP prodrug caused major changes on the lipid membrane phase behaviour manifested as a drastic decrease in the heat capacity peak height and a concomitant broadening of the main phase transition as well as a decrease in the transition enthalpy. In addition, the main phase transition temperature was slightly decreased and the pre-transition of the three phosphatidylcholines was abolished when DPP was present. 

*Keywords:* Differential scanning calorimetry; Acylated peptide; Peptide-membrane interaction

## 1. Introduction

With the recent advances in the biotech industry, an increasing number of new peptide and protein drug candidates have emerged for the treatment of various human diseases. In body fluids in general peptide and protein drugs are prone to chemical and enzymatic degradation. In addition, peptides and proteins are hydrophilic, high-molecular-weight substances and thus do not easily cross cell membranes. Therefore, the oral bioavailability of most peptide and protein drugs is very low. By increasing the lipophilicity of the peptide/protein drug it is possible to overcome some of the above mentioned problems (Wang et al., 1999). It has been shown that the linkage of an acyl chain to insulin can improve the stability in the small intestinal fluid (Asada et al., 1994) and also facilitate oral absorption (Asada et al., 1995). Additionally, by virtue of its sustained-release properties via binding to circulating serum albumin and/or tissue (e.g. lipid membranes), acylated insulin dramatically prolongs the circulation

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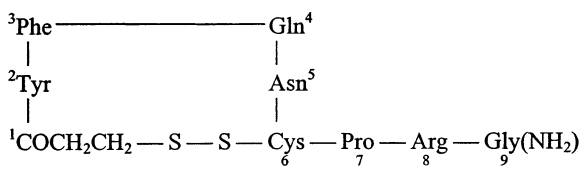
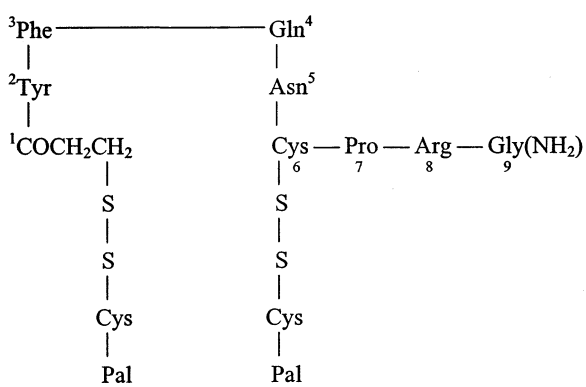
time and elevates the concentration in the vascular system (Kurtzhals et al., 1995; Markussen et al., 1996; Myers et al., 1997). The principle of fatty acid derivatisation has also been used to protract the action of glucagon-like peptide-1 by facilitating binding to serum albumin (Knudsen et al., 2000). Furthermore, human calcitonin (Fujita et al., 1996), Bowman–Birk protease inhibitor (Wang and Shen, 2000), tetragastrin (Tenma et al., 1993; Yodoya et al., 1994), and thyrotropin-releasing hormone (Yamada et al., 1992) have been chemically modified with fatty acids to improve intestinal absorption. Additionally, palmitoyl derivatives of interferon-α have been prepared for improved dermal delivery (Foldvari et al., 1998). Interestingly, the chemotherapeutic agent, paclitaxel, has been acylated in order to facilitate the incorporation into liposomal systems and thereby reducing the toxic side effect of the parent drug (Ali et al., 2000).

Several naturally occurring membrane-associated proteins and peptides are acylated (Silvius, 1999). It is assumed that fatty-acid acylation is involved in the anchoring of proteins and peptides to membranes (Peitzsch and McLaughlin, 1993; McLaughlin and Aderem, 1995). The interaction of the naturally occurring acylated peptides and proteins with model lipid membranes has been studied extensively in order to obtain a deeper understanding of the interaction of the naturally acylated peptides with biological membranes. In the present study, we investigate quantitatively the physico-chemical effects of a diacylated desmopressin (DPP) derivative and the unmodified parent compound desmopressin (DDAVP) on model lipid bilayer membranes made of three different phophatidylcholines. The interactions of acylated prodrug derivatives with lipid membranes is of interest for several reasons, including the potential use of liposomes as a delivery system for acylated peptides and proteins, as well as for obtaining a deeper understanding of the mechanisms involved in the interaction of acylated peptides with cell membranes. By understanding the impact of the hydrophobic acyl chain anchor on the membrane binding, new ways to change the pharmacological effect and thereby the therapeutic profile of acylated drug compounds are likely to appear.




**DDAVP**




**DPP**

Fig. 1. Chemical structures of DDAVP and DPP. Each DPP molecule contains two palmitic acid moieties, which are linked to the peptide via two disulfide bonds.

DDAVP is a synthetic analogue of the neurohypophyseal peptide hormone vasopressin. DDAVP has a prolonged antidiuretic effect and it does not cause vasoconstriction and contraction of smooth muscles in the intestines or in the uterus as the natural peptide hormone does (Walse et al., 1998). The main use of antidiuretic peptides is in the treatment of diabetes insipidus of pituitary origin (Rang and Dale, 1991). The synthetic acylation method used for the acylation of DDAVP is believed to be reversible (Wang et al., 1999), enabling regeneration of DDAVP from DPP. Each DPP molecule contains two palmitic acid moieties, which link to the peptide via two disulfide bonds (Fig. 1). In this way DPP can be considered a prodrug, which is defined as a pharmacologically inactive derivative of a parent drug molecule that requires spontaneous or enzymatic transformation within the body in order to release the active drug (Krogsgaard-Larsen and Bundgaard, 1991). The reversible acylation con-

cept is quite appealing as the acylation improves the transport properties of peptides into and across cell membranes. Ideally, this is followed by a cleavage of the disulfide linkage and a regeneration of the active compound at the site of action. Wang et al. (1999) have recently compared the antidiuretic efficacy and biodistribution of DPP with that of the unmodified DDAVP. Interestingly, they report a 250-fold increase of the antidiuretic activity of the acylated DPP when administered subcutaneously in rats, most likely due to a slow elimination and prolonged tissue retention combined with the ability to regenerate DDAVP.

The physical interaction of the water-soluble DDAVP and the amphiphilic DPP with membranes can conveniently be investigated by studying the effect of these compounds on the phase transition behaviour of liposomal model membrane systems. The phase behaviour of liposomes is extremely sensitive to the mode of interactions between the drug and the lipid-bilayer surface as well as the hydrophobic interior. By using differential scanning calorimetry (DSC), results can be obtained of the transition temperature, the enthalpy of the transition and the transition width. These measurements provide bulk information on how the parent drug and the prodrug interact with lipid membranes, which can be further used to map out relevant physiological implications of such interactions. In the present study, we examine the effect of DDAVP and its diacylated prodrug derivative, DPP, on the phase transition behaviour of aqueous dispersions of dimyristoyl (DMPC), dipalmitoyl (DPPC) and distearoyl phosphatidylcholine (DSPC) membranes using DSC.

## 2. Materials and methods

### 2.1. Materials

DMPC (1,2-dimyristoyl-sn-glycero-phosphocholine), DPPC (1,2-dipalmitoyl-sn-glycero-phosphocholine), and DSPC (1,2-distearoyl-sn-glycero-phosphocholine) were obtained from Avanti Polar Lipids. Desmopressin, 1-deamino-D-Arg$^8$-vasopressin (DDAVP) was a gift from Ferring AB, Sweden, and dipalmitoylated desmopressin (DPP) was a gift from Dr Wei-Chiang Shen, University of Southern California School of Pharmacy, USA. All other chemicals were of reagent grade and used without further purification.

### 2.2. Liposome preparation

Stock solutions of DPP were prepared in dimethylformamide (DMF). Phospholipids and DDAVP were dissolved in a chloroform/methanol mixture (1:1). To obtain multilamellar liposomes, appropriate aliquots of each component were mixed in a test tube, and the organic solvent was evaporated under a stream of nitrogen. In order to remove all traces of organic solvent, the test tubes were placed under vacuum overnight. The dried lipid films were suspended in a phosphate buffer solution (pH 7.4) and placed for 1 h in a water bath 10° above the main phase transition temperature, $T_m$, ($T_{m\ \mathrm{DMPC}} = 23.9$ °C; $T_{m\ \mathrm{DPPC}} = 41.8$ °C; $T_{m\ \mathrm{DSPC}} = 55.0$ °C). The samples were vortexed several times during the hydration process.

### 2.3. Differential scanning calorimetry

Heat capacity curves were obtained using a N-DSC II differential scanning calorimeter (Calorimetry Sciences Corp., Provo, Utah). The scan rate was 30 °C/h for both up and down scans. The lipid concentration was 3 mM and the samples were scanned six times. The samples were equilibrated for 1 h at the starting temperature and all results shown are in the up-scan mode. Data was analysed using ORIGIN software, and all the heat-capacity curves have been baseline-corrected.

## 3. Results and discussion

The influence of the DPP and the unmodified DDAVP on the thermodynamic phase behaviour of DMPC, DPPC and DSPC multilamellar bilayers has been investigated by DSC. The effect of

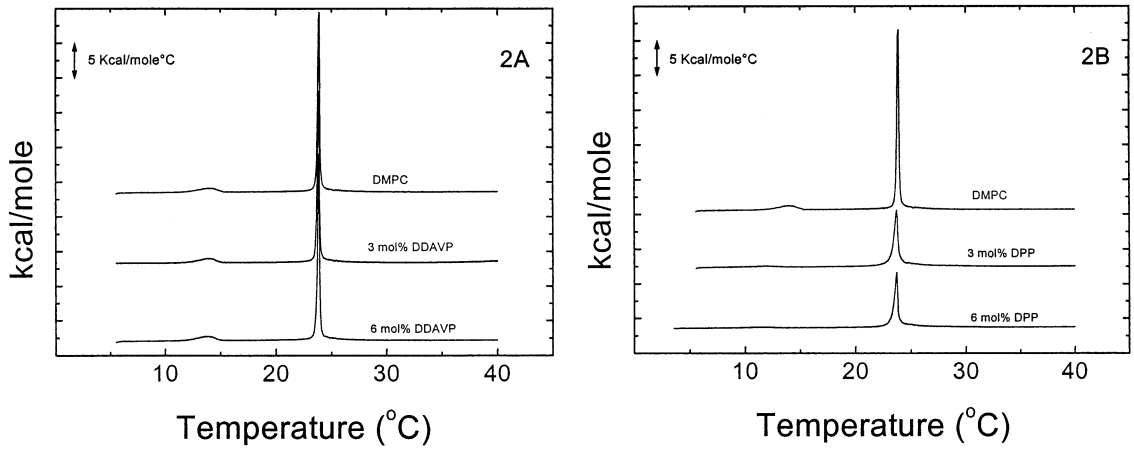



Fig. 2. DSC thermograms obtained at a scan rate of 30 °C/h of multilamellar suspensions of DMPC liposomes in the presence of increasing concentrations of DDAVP (A) and DPP (B).

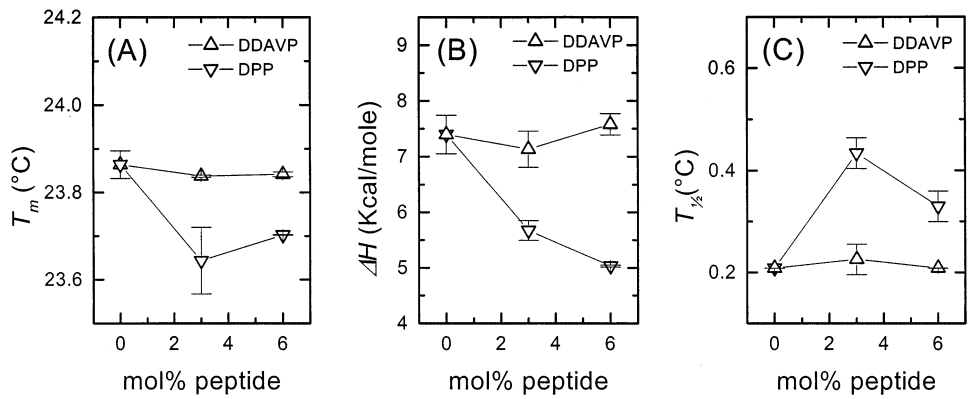



Fig. 3. (A) Variation of the transition temperature, $T_m$, (B) the transition enthalpy, $\Delta H$, and (C) the width of the transition, $T_{\frac{1}{2}}$, of suspensions of multilamellar DMPC liposomes containing DDAVP (△) and DPP (▽). The error bars represent the standard deviation of three consecutive up-scans.

increasing amounts (3 and 6 mol%) of DDAVP and DPP on the thermotropic phase transition behaviour of DMPC is illustrated in Fig. 2A and B, respectively. The multilamellar liposomes of DMPC show as expected a pre-transition at 14.1 °C and a main phase transition at 23.9 °C (Marsh, 1990). The phase transitions of DMPC lipid membranes are unaltered by the presence of 3 and 6 mol% of the water-soluble peptide, DDAVP. In contrast, the presence of DPP in DMPC liposomes at concentrations of 3 and 6 mol% DPP has a significant influence on the thermodynamic phase behaviour. This is most clearly seen as a decrease of the peak height of the main transition peak and the disappearance of the pre-transition. In Fig. 3A, which shows the main phase transition temperature, $T_m$, as a function of mol% DDAVP and DPP, it is seen that the transition temperature for DMPC liposomes is rather constant when DDAVP is present whereas a small decrease in the transition temperature is seen when DPP is present. The transition enthalpy, $\Delta H$, of the main phase transition is shown in Fig. 3B for DMPC liposomes incorporated with DDAVP and DPP. The presence of DDAVP in DMPC does not influence the transition en-

thalpy, whereas the transition enthalpy is dramatically decreased at 3 and 6 mol% DPP. The width at half maximum, $T_{\frac{1}{2}}$, of DMPC liposomes, shown in Fig. 3C as a function of mol% peptide, is not influenced by the presence of DDAVP whereas the transition width is increased when DPP is incorporated into the DMPC liposomes.

In Fig. 4A and B, the effect of increasing amounts of DDAVP and DPP on the thermodynamic phase behaviour of DPPC liposomes is shown. DPPC alone showed a pre-transition at 35.7 °C and a main phase transition at 41.8 °C. Fig. 5A shows that the main phase transition temperature remains constant for DDAVP/DPPC mixtures, whereas a slight decrease in the transition temperature is seen when DPP is present. Fig. 5B shows that addition of DDAVP (3 and 6 mol%) does not influence the transition enthalpy of DPPC liposomes. However, the addition of DPP to DPPC liposomes caused a significant decrease in the transition enthalpy of DPPC and an increase in the width at half maximum of the transition peak, $T_{\frac{1}{2}}$, as shown in Fig. 5C.

Fig. 6 shows DSC calorimetric curves for DSPC containing DDAVP (A) and DPP (B) in 3 and 6 mol%. Pure DSPC liposomes show a pre-transition at 50 °C and a main transition at 55 °C. In addition, the sub-main transition

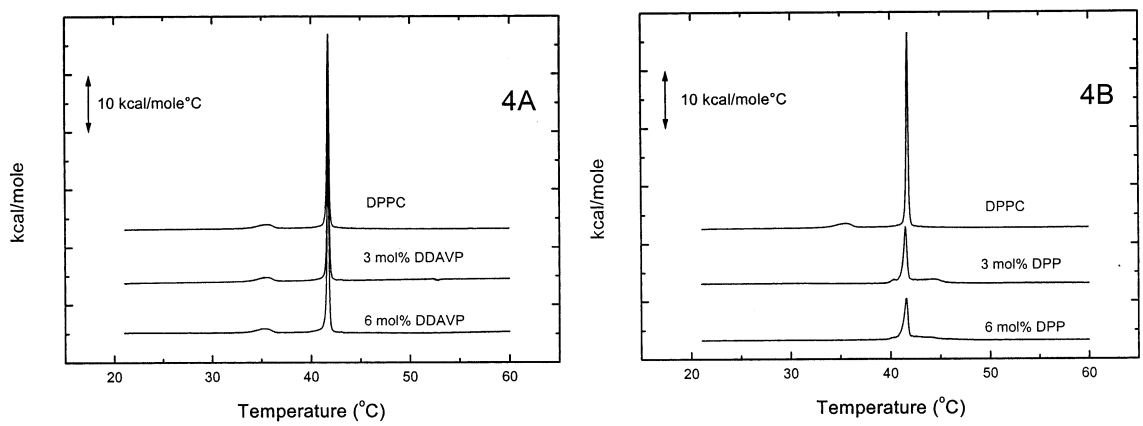



Fig. 4. DSC thermograms obtained at a scan rate of 30 °C/h of multilamellar suspensions of DPPC liposomes in the presence of increasing concentrations of DDAVP (A) and DPP (B).

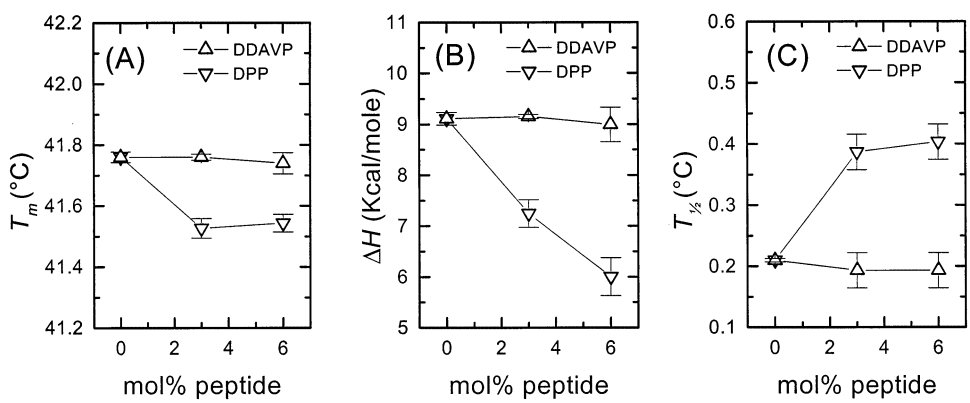



Fig. 5. (A) Variation of the transition temperature, $T_m$, (B) the transition enthalpy, $\Delta H$, and (C) the width of the transition, $T_{\frac{1}{2}}$, of suspensions of multilamellar DPPC liposomes containing DDAVP (△) and DPP (▽). The error bars represent the standard deviation of three consecutive up-scans.

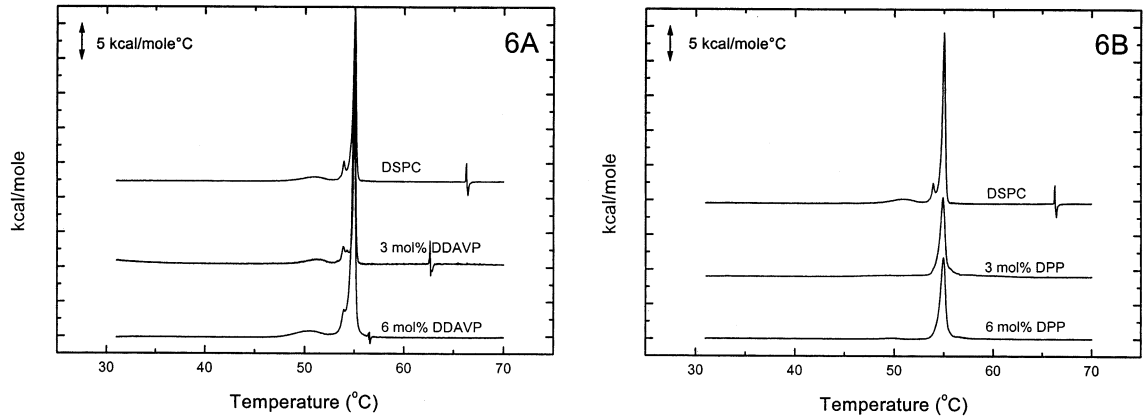



Fig. 6. DSC thermograms obtained at a scan rate of 30 °C/h of multilamellar suspensions of DSPC liposomes in the presence of increasing concentrations of DDAVP (A) and DPP (B).

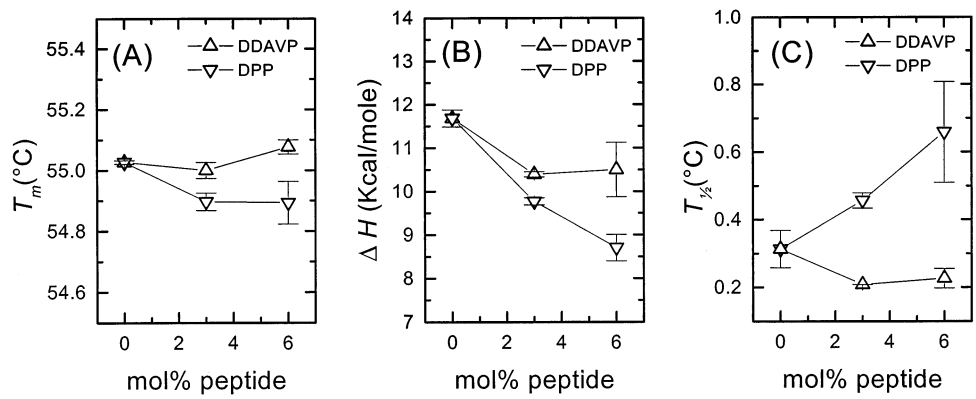



Fig. 7. (A) Variation of the transition temperature, $T_m$, (B) the transition enthalpy, $\Delta H$, and (C) the width of the transition, $T_{\frac{1}{2}}$, of suspensions of multilamellar DSPC liposomes containing DDAVP (△) and DPP (▽). The error bars represent the standard deviation of three consecutive up-scans.

(Jørgensen, 1995) appears just below the main phase transition temperature. As for DMPC and DPPC liposomes the thermodynamic phase behaviour of DSPC liposomes is undisturbed by the presence of DDAVP. When DPP is present the pre-transition is abolished, and the main phase transition temperature range is increased and the peak height is decreased in a concentration dependent manner. The addition of both peptides does not affect the transition temperature significantly as shown in Fig. 7A. Fig. 7 furthermore reveals that the transition enthalpy is lowered and an increase in $T_{\frac{1}{2}}$ is observed when DPP is present in DSPC liposomes.

The addition of DDAVP to all three studied phosphocholine membrane systems causes only minor changes in the thermodynamic phase behaviour, most likely because DDAVP predominantly is found in the aqueous phase of the liposome suspensions and consequently is not affecting the cooperative melting behaviour of the acyl chains of the phosphocholine lipids constituting the membrane. In contrast, the addition of DPP to the phosphocholine membranes causes a dramatic effect on the thermodynamic phase behaviour. The effects of DPP on the thermodynamic behaviour of the three phospholipid are qualitatively similar. The main effect of DPP on

the lipid membrane phase transition properties is a decrease in the transition enthalpy, $\Delta H$, and an increase in the width of the heat capacity peak. Likewise, a minor decrease in the phase transition temperature, $T_m$, is observed. The addition of DPP to all three phospholipid membranes also causes the pre-transition to disappear. The lowering of the enthalpy and the broadening of the transition may indicate that DPP associates and penetrates into the interior of the lipid bilayer thereby causing a change in the cooperative melting behaviour of the lipids probably due to a disruption in the acyl chain packing. We also tried to incorporate the peptides in a concentration of 10 mol% (data not shown). As is the case of lower concentrations of DDAVP, no particular effect of increasing DDAVP concentrations on the thermodynamic phase behaviour of the three phosphatidylcholines studied was observed. When DPP was added in 10 mol% to lipid membranes of DMPC, DPPC and DSPC a sedimentation of DPP was observed when the lipid film was hydrated with the buffer solution. This is most probably due to a saturation of the lipid membranes with the acylated peptide. Noticeable, the liposomes kept their integrity for high concentrations of DPP. We also tried to add DPP from the water phase, by mixing a liposome suspension with DPP. In that case, the phase behaviour of the DPPC lipids was undisturbed, indicating that the DPP molecules aggregate and presumably form micelle-like structures at high concentrations. This may lead to a slow kinetics for the interaction of DPP with the lipid membrane. Interestingly, it has been shown that acylated peptides with acyl chains of 14 carbon atoms or more induce formation of micelle-like structures (Pool and Thompson, 1998).

## 4. Conclusion

From the present study, it appears that DDAVP do not interact with phospholipid membranes at concentrations up to 10 mol%, most probably because DDAVP is situated in the aqueous phase of the multilamellar liposome preparations. The calorimetric results for the diacylated DPP derivative, however, reveal that this prodrug derivative can be incorporated into liposomes in up to 6 mol%. At higher concentration (10 mol%), the lipid membranes become saturated and a major part of the DPP molecules aggregates and form micelle-like structures. The incorporation of DPP into DMPC, DPPC and DSPC causes qualitatively similar perturbations in the thermodynamic phase behaviour, manifested as a decrease in the transition enthalpy and a broadening of the transition as well as a minor downwards temperature shift of the peak position.

The basic thermodynamic results obtained in the present study provide indirect information on the drug loading capacity, the preferred location of the parent and acylated drugs as well as on the strength of the interaction of the peptide drugs with lipid bilayer membranes composed of saturated phospholipids. These observations might advantageously be used to guide the design of peptide-loaded liposomal drug delivery systems, where in particular a proper balance between the drug transporting properties and the ability of the liposomal system to deliver the active compounds at the site of action are of great importance. Furthermore, the results can also assist in obtaining a deeper understanding of the membrane-anchoring of naturally acylated peptides and proteins in biological systems.

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