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PERTURBATION OF PHOSPHOLIPID MEMBRANES BY JUVENILE HORMONE

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Summary

The effects of juvenile hormone and its analogs Altozar 4E and ZR-777 5E on the phase properties of liposomes prepared from dipalmitoyl phosphatidylcholine (DPPC) have been examined by differential scanning calorimetry. Each of these compounds reduced the co-operativity of the gel to liquid-crystalline phase transition, which is manifest as a distinct broadening of the main transition endotherm, and split the transition into two distinguishable components centered at 34 and 37°C. However, there was no significant change in enthalpy of the main phase transition, suggesting that juvenile hormone and its analogs perturb the bilayer primarily in the vicinity of the phospholipid headgroups. Moreover, this perturbation does not appear to influence bilayer permeability since the osmotic swelling rates of liposomes prepared from either phosphatidylcholine or dipalmitoyl phosphatidylcholine that contained up to 33 mol% juvenile hormone were not significantly different from the swelling rates of corresponding liposomes containing no juvenile hormone. Splitting of the transition endotherm into two peaks appeared to be peculiar to compounds possessing juvenile hormone activity. A mixture of fatty acid methyl esters broadened the main transition of DPPC, but did not split the endotherm or shift the transition midpoint, and the insect hormone ecdysone had no discernible effect on the DPPC transition apart from eliminating the pretransition. The data indicate that juvenile hormone and its analogs can modulate the physical properties of phospholipid bilayers, and raise the prospect that some of the physiolog-

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Abbreviation: DPPC, dipalmitoyl phosphatidylcholine.

ical effects of this hormone may be achieved through its influence on the molecular organization of membrane lipid.

Introduction

Juvenile hormone is a sesquiterpenoid-like compound that is synthesized in and released from the corpora allata of insects [1]. Four structural analogs of of the hormone have been described [2—5] and these have been implicated in the regulation of a variety of morphogenetic, physiological and behavioural processes including inhibition of gene expression [6,7], egg maturation [8], diapause [9] and phase determination [10]. The mechanism(s) by which juvenile hormone effects these diverse actions is not known, although the suggestion of juvenile hormone-induced RNA specific for the synthesis of the yolk protein vitellogenin [11] indicates that at least some of the effects may be mediated through transcription of specific mRNA. In addition to its role in the induction of vitellogenin synthesis in the fat body, juvenile hormone further contributes to the process of egg maturation by facilitating the uptake of protein by developing oocytes. In *Rhodnius prolixus* this facilitation is achieved by the formation of large spaces between the cells of the follicular epithelium which overlie the vitellogenic oocytes [12]. These spaces appear in the presence of juvenile hormone but the effect is not mediated through the direct action of juvenile hormone on macromolecular synthesis [13]. Thus, an additional site and mechanism of action of juvenile hormone is required in order to explain certain effects of the hormone.

The lipophilic nature of juvenile hormone suggests the possibility that the molecule may interact with the lipid component of biological membranes, and the present study was undertaken to examine the possible effect that such interaction may have on the properties of lipid bilayers. Liposomes were prepared from L-α-dipalmitoyl phosphatidylcholine (DPPC), a major component of insect membranes [14], and the transition from gel to liquid-crystalline phase examined in the presence and absence of juvenile hormone by differential scanning calorimetry. Additional experiments involving spectrophotometric measurements of osmotic swelling were conducted to determine the effect(s) of juvenile hormone on the permeability of liposomes prepared from L-α-dipalmitoyl phosphatidylcholine or L-α-phosphatidylcholine.

Materials and Methods

Differential scanning calorimetry

Liposomes were prepared from DPPC and varying concentrations of juvenile hormone, juvenile hormone analogs, ecdysone and fatty acid methyl esters using the procedure of Demel et al. [15]. Lipid samples (2.5 μmol) dissolved in chloroform were dried under N_2 and residual solvent removed in vacuo. The lipid was resuspended in 40 μl of 40 mM Tris-acetate buffer, pH 7.0, containing 100 mM NaCl, and equilibrated at 45°C for 1 h. An aliquot of the suspension (15 μl) was transferred to Perkin-Elmer volatile sample pans for calorimetric analysis. Differential scanning calorimetry (DSC) was conducted using a Perkin-

Elmer DSC-2, and each sample was scanned between 5 and 55°C in both heating and cooling cycles at a rate of 5 K/min and a range of 2 mcal/s. Indium and water were used as calibration standards. The upper and lower limits of the phase transition were determined from cooling and heating scans, respectively, as the intercept between the predominant slope of the ascending major endothermic peak and the baseline (Fig. 1). The midpoint of the transition was estimated from heating scans only as the maximal endothermic peak. Enthalpy values were determined from the phase transition areas of heating scans only. The phospholipid content of each DSC sample was determined after opening the sample pan and dissolving the contents in chloroform-methanol (2 : 1, v/v). Phospholipid-P was determined using the method of Fiske and Subba Row as outlined by Dittmer and Wells [16].

Osmotic swelling experiments

Liposomes of either phosphatidylcholine or DPPC were prepared in 79 mM Tris-acetate buffer, pH 7.0, containing 50 mM KCl. Phosphatidic acid was added at a concentration of 4 mol% relative to phosphatidylcholine or DPPC to give the bilayers a net negative charge permitting entrapment of buffer. Osmotic swelling experiments were conducted as described [17] in a Beckman Acta III spectrophotometer equipped with thermostatically controled cuvette holders. A 50-μl aliquot of the liposome preparation was rapidly injected into a cuvette containing 2.5 ml of 50 mM aqueous glycerol and a fast-running stirring bar. The change in absorbance was followed at 450 nm for about 10 s with a chart recorder. Experiments with phosphatidylcholine liposomes were conducted at 30°C, whereas those with DPPC liposomes were carried out at 45°C. Injections were made through a polyethylene cannula running down one corner of the cuvette to a point just above the stirring bar.

Initial swelling rates were calculated according to the equation:

$$\frac{d(1/A)\%}{dt} = \frac{\Delta A_t}{\Delta t} \cdot \frac{100}{A_0}$$

where ΔA_t is the change in absorbance over time t and A_0 is the maximal initial absorbance.

Chemicals

Fatty acid methyl esters (mixture of stearate, oleate, linoleate and linolenate methyl esters), L-α-dipalmitoyl phosphatidylcholine, L-α-phosphatidylcholine (from bovine liver), phosphatidic acid and the juvenile hormone (type III) used for differential scanning calorimetry were obtained from the Sigma Chemical Company, St. Louis, MO.

The juvenile hormone (type III) used for osmotic swelling experiments and ecdysone were obtained from Calbiochem-Behring Corporation, La Jolla, CA.

The juvenile hormone analogs, Altozar 4E and ZR-777 5E were generously provided by Zoecon Corporation, Palo Alto, CA.

Results

As DPPC liposomes were heated through 41°C, the phospholipid underwent a gel to liquid-crystalline phase transition with an enthalpy of 8.3 kcal/mol of

TABLE I

PHASE PROPERTIES OF DIPALMITOYL PHOSPHATIDYLCHOLINE LIPOSOMES CONTAINING JUVENILE HORMONE, JUVENILE HORMONE ANALOGS, ECDYSONE, AND FATTY ACID METHYL ESTERS

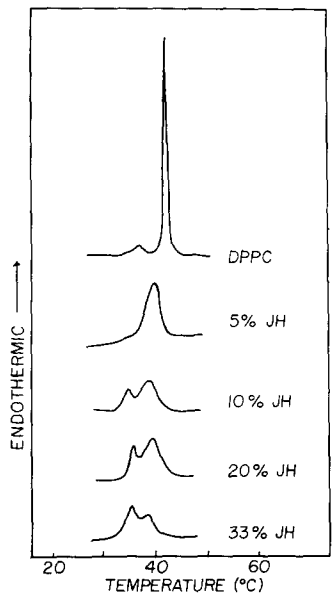
Lower limits and enthalpies were determined from heating scans. Upper limits were determined from cooling scans. Percentages are all mol/mol. Standard errors for the enthalpy measurements are indicated; n = 3.

	Properties of the phase transition		
	Lower limit (°C)	Upper limit (°C)	Enthalpy (kcal/mol P)
DPPC control	41	42	8.3 ± 0.54
5% juvenile hormone	37	40.5	6.8 ± 0.53
10% juvenile hormone	33	40.5	7.2 ± 0.69
20% juvenile hormone	33	40.5	7.7 ± 0.51
33% juvenile hormone	33	40.5	8.2 ± 0.63
20% Altozar 4E	28	37	9.1 ± 0.55
20% ZR-777 5E	28	40	8.6 ± 0.61
20% ecdysone	41	43	8.0 ± 0.46
12% fatty acid methyl esters	38	46	8.7 ± 0.44

phospholipid (Fig. 1; Table I). The addition of 5 mol% juvenile hormone eliminated the pretransition, broadened the main transition, largely by decreasing the temperature of its onset, and shifted the midpoint of the main transition downward (Fig. 1; Table I). At 10 mol%, juvenile hormone further reduced the onset temperature to 33°C resulting in more extensive broadening of the transition, and split the main transition into two distinguishable components with midpoints at 34 and 37°C (Fig. 1). Increasing the proportion of juvenile hormone through 20 to 33 mol% did not cause any further broadening (Fig. 1; Table I), and juvenile hormone did not significantly alter the transition enthalpy at any of the concentrations tested (Table I).

The splitting of the main DPPC transition into two components was specific for compounds displaying juvenile hormone activity. Two juvenile hormone analogs, Altozar 4E and ZR-777 5E again broadened the main transition by lowering the onset temperature, in this case to 28°C (Table I), and split the main transition into two peaks with midpoints at 29 and about 35°C. Again, there was no significant change in enthalpy (Table I). A mixture of fatty acid methyl esters containing stearate, oleate, linoleate, and linolenate methyl esters, none of which display juvenile hormone activity, had markedly different effects on the phase properties of DPPC liposomes. For example, the methyl esters not only failed to split the transition, but broadened it without influencing its midpoint by both reducing the lower limit and increasing the upper limit (Fig. 2; Table I). Ecdysone, another insect hormone, eliminated the pretransition but appeared to have little other effect (Fig. 2; Table I).

To determine whether the association of juvenile hormone with phospholipids alters the bilayer permeability, liposomes prepared with and without juvenile hormone, and containing 79 mM Tris acetate and 50 mM KCl, were rapidly injected into a hypotonic solution of 50 mM glycerol. This resulted in an initial rapid rise in the absorbance at 450 nm, reflecting an increase in tur-



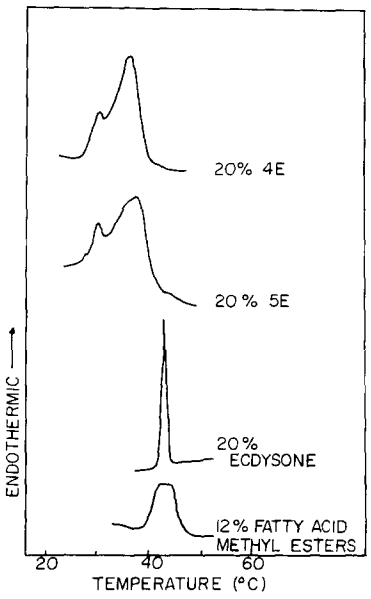


Fig. 1. Differential scanning calorimetry heating scans of dipalmitoyl phosphatidylcholine liposomes containing 0 to 33 mol% juvenile hormone.

Fig. 2. Differential scanning calorimetric heating scans of dipalmitoyl phosphatidylcholine liposomes containing Altozar 4E, ZR-777 5E, ecdysone and fatty acid methyl esters.

bidity, and was followed by a time-dependent decrease in absorbance that represented osmotic swelling (Fig. 3). This decrease was linear for 3 to 5 s. Initial swelling rates were calculated from this linear portion and expressed as a

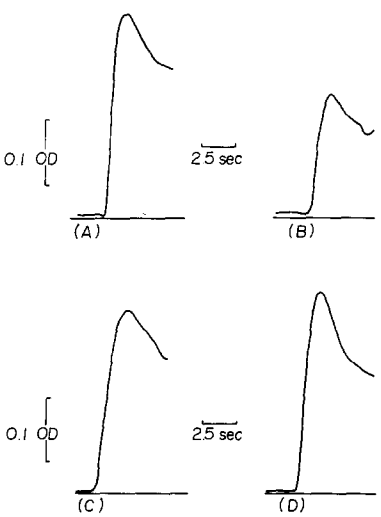


Fig. 3. Typical swelling profiles for liposomes prepared from DPPC (A), DPPC with 33 mol% juvenile hormone (B), phosphatidylcholine (C), and phosphatidylcholine with 33 mol% juvenile hormone (D). All liposomes contained 4 mol% phosphatidic acid.

TABLE II

INITIAL SWELLING RATES ($d(1/A)\%/dt$) OF LIPOSOMES PREPARED FROM EITHER DIPALMITOYL PHOSPHATIDYLCHOLINE OR PHOSPHATIDYLCHOLINE CONTAINING JUVENILE HORMONE

Both control and juvenile hormone-treated liposomes contained 4 mol% phosphatidic acid. Standard errors of the means are shown. The number of trials is indicated in parentheses.

	Initial swelling rates	
	Control	33 mol% juvenile hormone
Phosphatidylcholine	15.76 ± 2.22 (8)	17.22 ± 1.80 (7)
Dipalmitoyl phosphatidylcholine	10.82 ± 1.30 (10)	13.84 ± 1.32 (11)

percentage of the initial absorbance to normalize for any differences in liposomal size among experiments [17]. The swelling rates were not significantly different ($\alpha = 0.05$) in the presence or absence of 33 mol% juvenile hormone for liposomes prepared from either DPPC or phosphatidylcholine (Table II).

Discussion

Several natural components of membranes including sterols [18—20], proteins [21,22] and inorganic cations [21,23] as well as certain membrane-active drugs [24] are known to influence the phase properties of lipid bilayers. Cholesterol, for example, typically broadens the main transition of pure phospholipids, reduces the transition enthalpy and, at concentrations in excess of 33 mol%, completely eliminates the gel to liquid-crystalline transition. These observations collectively indicate that the sterol associates stoichiometrically with the fatty acid side chains, thereby withdrawing them from the co-operative lipid phase [18]. Calcium and magnesium ions increase the transition temperature and enthalpy of negatively charged phospholipid vesicles presumably by associating with the charged phospholipid head-groups [21,23]. Some proteins and the drug chlorpromazine also broaden the main transition of phospholipids by lowering its onset temperature without influencing the transition enthalpy, and this pattern of change in phase behaviour has been interpreted as reflecting shallow perturbation of the bilayer primarily in the region of the phospholipid headgroups [21,22,24].

It is clear from the present study that juvenile hormone and its analogs also broaden the gel to liquid-crystalline phase transition of DPPC without significantly altering its enthalpy. This would suggest that the hormone and its analogs reduce the co-operative behaviour of the phospholipid by perturbing near the surface of the bilayer. Moreover, the splitting of the main transition observed at the higher concentrations of hormone is a unique feature of the perturbation by juvenile hormone and its analogs, and is not induced by other hydrophobic compounds tested. The data also suggest that the association of the hormone with the bilayer gives rise to distinct populations of phospholipid molecules having different physical properties. Such modulation of bilayer properties may be important in explaining some of the physiological effects of

juvenile hormone, since several lines of evidence implicate cell membranes as possible sites of action for the hormone. For example, juvenile hormone increases the permeability of membranes containing gating ion translocators [25], an observation that has been attributed to a possible rearrangement of phospholipid molecules within the membrane bilayer. In the same study, juvenile hormone had no effect on permeability in the absence of these translocators, which is consistent with our observation that the hormone does not alter the permeability of liposomes.

Juvenile hormone might also inhibit key membrane-bound enzymes (e.g. adenyl cyclase) and thereby modulate physiological activity [26]. The ability of membrane phospholipids to modulate the activities of membrane-bound enzymes is well established [27—29]. For example, the activities of several ATPases have recently been shown to be reduced by the presence of lipophilic anaesthetics [30,31]. In one case, the anaesthetic agents were also found to fluidize and lower the phase transition temperature of membrane lipids [31]. Juvenile hormone is known to stimulate succinate oxidation, inhibit NAD-linked oxidations in isolated insect mitochondria [32] and to stimulate ATPase activity in imaginal discs of *Drosophila melanogaster* [33]. Juvenile hormone has also been shown to stimulate Na^+/K^+-dependent ATPase in follicle cells of *Rhodnius prolixus* [34]. In addition, the volume changes that occur in follicle cells in response to juvenile hormone are ouabain-sensitive, indicating possible modulation of ATPase activity by the hormone.

The present study demonstrates that juvenile hormone can partition into phospholipid and thus raises the prospect that alterations in membrane enzyme activities and permeability attributable to the hormone may reflect its ability to modulate the physical properties of lipid bilayers. The concentrations of juvenile hormone tested were well above documented haemolymph levels. However, the haemolymph titer of the hormone does not indicate the amount of hormone that is present in the target cells or, indeed, that may be associated with the cell membrane. The lipophilic nature of juvenile hormone suggests that it may partition into membranes and thus attain concentrations in situ similar to those that have been shown in the present study to cause perturbation of lipid bilayers in vitro. Thus an additional possible site of action of insect juvenile hormone(s) may be membrane lipid.

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