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Hormone–Phospholipid Interaction: a Possible Hormonal Mechanism of Action in the Control of Membrane Permeability

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Abstract

Changes, proportional to the concentration of added gibberellic acid, in both width and location of the proton resonance peak of the nine equivalent protons of the terminal methyl groups of phosphatidyl choline (lecithin), have allowed calculation of the dissociation constant of the lecithin–gibberellic acid complex in $CDCl_3$. The existence of such a complex supports previous findings of an interaction of gibberellic acid with liposomes in aqueous media.

Introduction

Most explanations of the initial biochemical or biophysical event triggered by plant or animal hormones have foundered because of a lack of conclusive evidence of a reaction between the hormone and the postulated receptor. Recently we presented evidence of an effect of the plant hormone, gibberellic acid (GA_3), on the permeability of model membranes (liposomes) composed of natural plant components (Wood and Paleg 1972). In addition, we postulated that GA_3 might produce some or all of its *in vivo* responses through a similar effect. In this report we present results of an n.m.r. study which demonstrate an interaction between the hormone and one of the normal components of plant membranes, phosphatidyl choline (lecithin), derived from a natural plant source.

Materials and Methods

The lecithin was purified from a crude lipid extract of soybeans (Sigma, lot 97B1460) by alumina and silicic acid column chromatography. The fatty acid composition of the purified lecithin, determined by g.l.c. (on HIEFF-IBP packing) of the methylated fatty acids after saponification with 30% KOH in 90% aq. methanol for 15 min at 55°C, was palmitic 10·0, stearic 3·8, oleic 7·6, linoleic 71·2, linolenic 6·2% with only minor quantities of other acids present. A molecular weight of 800 was adopted for the purpose of preparing lecithin solutions.

GA_3 was obtained from I.C.I. and was 97% pure, the remainder being GA_4 and GA_7.

The lecithin was dissolved in deuterochloroform to a final concentration of 30 mg/ml. Proton magnetic resonance spectra of lecithin and GA_3 in $CDCl_3$ were recorded on a Varian DA-60-IL spectrometer operating at 60 MHz. Chemical shifts were measured relative to internal tetramethylsilane by frequency counting with an accuracy of $\pm 0{\cdot}1$ Hz. Line widths were measured on the 50 Hz range at a sweep rate of 0·2 Hz/s. The values recorded are the average of at least three measurements.

Results and Discussion

The 60 MHz proton magnetic resonance spectrum of lecithin (0·025 molal) in $CDCl_3$ showed discreet, identifiable resonances for the *N*-trimethylamino group (singlet, δ 3·37 p.p.m., width at half height $3{\cdot}0 \pm 0{\cdot}1$ Hz) and olefinic protons (multi-

plet, δ 5·35 p.p.m.) as well as complex band envelopes characteristic of aliphatic $-(CH_2)_n-$ and $-CH-O-$ protons.

On addition of GA_3 the resonance corresponding to the trimethylamino group broadened and was shifted to higher magnetic field. Both effects were proportional to the concentration of GA_3. The smaller, broadening effects of GA_3 on the $-CH_2-$ and $=CH-$ resonances were analysed, and the regressions were found to differ significantly from zero slope at the 5% level. Table 1 presents the data for peak location and width

Table 1. Effect of GA_3 on the position and half width of three phosphatidyl choline proton n.m.r. peaks

GA_3 added (as % of wt. of lecithin)	$N(CH_3)_3$ signal		$-CH_2-$ signal		$=CH-$ signal	
	Location (Hz)	Half width (Hz)	Location (Hz)	Half width (Hz)	Location (Hz)	Half width (Hz)
0	202·4	3·0	75·3	1·43	321·0	1·46
0·5	201·6	2·75	75·2	1·36	321·0	1·42
1·0	201·6	2·80	75·2	1·42	321·1	1·35
2·0	201·4	2·96	75·2	1·42	321·1	1·32
5·0	201·1	2·95	75·3	1·50	321·1	1·56
7·5	200·9	3·18	75·1	1·39	321·1	1·39
10·0	200·7	3·48	75·3	1·64	321·1	1·35
15·0	199·7	3·56	75·1	1·55	321·0	1·35
20·0	199·3	4·02	75·2	1·38	321·1	1·66
25·0	198·9	4·12	75·2	1·60	321·0	1·51
30·0	198·6	4·15	75·2	1·70	321·0	1·57
50·0[A]	198·2	4·40	75·3	1·72	320·9	1·78
10·0[B]	202·3	2·90	75·1	1·38	320·9	1·45

[A] Solubility of GA_3 exceeded. [B] Methyl gibberellate.

for the protons' signal from $N(CH_3)_3$, $-CH_2-$ and $=CH-$ groups. Included in the table is a value for a sample of the methyl ester of GA_3 prepared from the same batch of GA_3 in the concentration series. It is quite inactive in influencing the polar head group of lecithin and produced no alteration of the n.m.r. spectrum. Fig. 1 demonstrates the shift in the location of the signal of the quaternary nitrogen of the choline moiety as increasing fractions of the lecithin are bound by the added hormone.

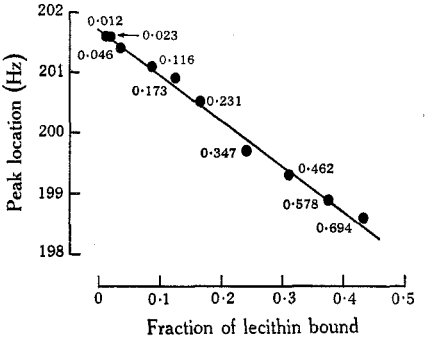

Fig. 1. Change in location of the n.m.r. peak of the trimethyl group protons of phosphatidyl choline as the proportion of lecithin complexed increases with increasing hormone (GA_3) levels. The amount of lecithin was held constant (30 mg) and various amounts of GA_3 (molar ratios of GA_3 : lecithin as indicated on figure) were added. Molecular weight of GA_3 = 346, molecular weight of lecithin assumed to be 800. Slope of regression line $-7·4 \pm 0·2$; $r = -0·996$.

We interpret these changes as indicative of complex formation between lecithin and GA_3 in which the primary association involves an electrostatic attraction between the positively charged trimethylamino group of lecithin and the carboxyl group of GA_3.

Restricted rotational mobility of the trimethylamino group, olefinic, and aliphatic protons in the complex leads to a broadening of the resonances through a decrease in spin–spin relaxation time (Jardetzky 1964). The upfield shift of the trimethylamino resonance may be ascribed to a reduction of the effective positive charge at the trimethylamino group by ion-pair formation in the complex (Jackman and Sternhell 1969).

Under conditions of rapid exchange, which we assume to hold for complex formation in the present case, the observed n.m.r. spectrum of lecithin is the weighted average of the spectra of the complexed and uncomplexed forms. From the variation of the chemical shift and half width of the trimethylamino resonances with GA_3 concentration, the dissociation constant ($K_D = 8{\cdot}8 \pm 1{\cdot}0 \times 10^{-3}$ molal), change in chemical shift on complex formation ($-7{\cdot}4 \pm 0{\cdot}2$ Hz), and increase in half width on complex formation ($1{\cdot}9 \pm 0{\cdot}2$ Hz) were calculated by the exact method of Sykes (1969) assuming a 1 : 1 association between lecithin and GA_3:

$$\text{lecithin} + GA_3 \rightleftharpoons \text{complex}.$$

The value of the dissociation constant indicates a strong association of the same order of magnitude as that observed for phosphatidyl serine and L-epinephrine (Hammes and Tallman 1971) and considerably stronger than that reported for DDT and lecithin (Tinsley *et al.* 1971).

One cannot, of course, draw direct conclusions concerning the reaction of GA_3 and phospholipid in aqueous systems from our results in deuterochloroform. However, we have previously shown that GA_3 will interact with lipid dispersions (liposomes) in an aqueous environment (Wood and Paleg 1972) and we have here attempted to study the possible mechanism by which GA_3 reacts with a key component (lecithin) of such liposomes. It seems clear that the association primarily involves the hydrophilic quaternary nitrogen head group of the lecithin and the carboxyl group of GA_3.

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