EFFECT OF PHOSPHOLIPID ON CONFORMATION OF OVINE LUTEINIZING HORMONE AS TESTED BY CIRCULAR DICHROISM

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1. Introduction

It has recently been demonstrated that phospholipase treatment of the plasma membrane from bovine corpora lutea [1] or a soluble gonadotropin receptor from rat testes [2] markedly decreases the ability to bind human chorionic gonadotropin or luteinizing hormone. Accordingly, it was of interest to examine the interaction of phospholipid with luteinizing hormone or its subunits, as measured by circular dichroism.

Interactions of the zwitterionic phospholipids have been studied principally with the apoprotein moieties of serum lipoproteins (e.g. [3–6]). These interactions are accompanied by a transition to a more helical form of the protein, similar to that observed with proteins in the presence of the simpler amphiphile, sodium dodecyl sulfate [4,7,8].

In the present study we demonstrate that interaction of phospholipid with oLH* or one of its subunits is accompanied by significant conformational changes, but the appearance of a more helical form is not observed.

* *Abbreviations used:* oLH, ovine luteinizing hormone; oLHα, oLHβ, the subunits of ovine luteinizing hormone; CD, circular dichroism.

2. Materials and methods

The oLH [9] and its α and β subunits [10] were prepared as described previously (loc. cit.). The proteins were dissolved either in 0.1 M NH_4OH, pH 9.9–10.0, 0.1 M NaCl, or 0.01 M sodium phosphate, pH 7.0 or 8.1.

The phospholipid was a synthetic DL-(2,3-dihexadecyl)-glycerylphosphoryl-choline, M.A., purchased from Mann Research Labs, New York (Lot S1369). A weighed amount of the phospholipid was suspended in 0.01 M sodium phosphate, pH 7.0 or 8.1 and sonicated at 60 W for 150 sec. This yielded a sligtly opalescent colloidal solution. Some experiments were performed with phospholipid bilamellar vesicles prepared from egg yolk [11]. The vesicles were fractionated on Sepharose 4B in 0.1 M NaCl + 0.01 M Tris solution, pH 7.4, as described by Huang [11].

The protein solution was mixed with the lipid dispersion, the mixtures left at room temperature (24–25°C) for 20–22 hr and the CD measurements made in a Durrum–JASCO improved CD–SP dichrograph as described previously [4,8]. The results are expressed as specific dichroism $\Delta\epsilon^{1\%}_{1\,cm}$, i.e. as CD calculated for 1% protein in 1 cm optical path. The base lines in the recordings were those of the phospholipid dispersions without sample and were equivalent to buffer alone.

3. Results and discussion

We first examined the isolated subunits, oLHα and oLHβ. A phospholipid effect on the tertiary structure of the beta subunit was observed, as shown in fig. 1. However, this CD effect was manifested only in a rather narrow pH zone, 9.1–10.0. Curve 1 in fig. 1 represents the oLHβ without lipid at pH 9.3 and curve 2 shows the effect of the lipid under the same conditions. At lower pH (6.8–8.5), the CD curve of the hormone subunit in the absence of the lipid was practically identical to curve 1, thus at these pH values the lipid had no measurable effect on the conformation. The pH zone 10.0 to 11.2 has not been studied, but at pH 11.2, the band centered near 250 nm vanished (curve 3) and the CD of the lipid-containing oLHβ solution was identical with that of the oLHβ by itself. Similar experiments with the oLHα subunit at pH 9.1–10.0 showed no lipid effect on conformation (weak positive CD at 270–300 nm and a relatively strong positive CD band at 240–250

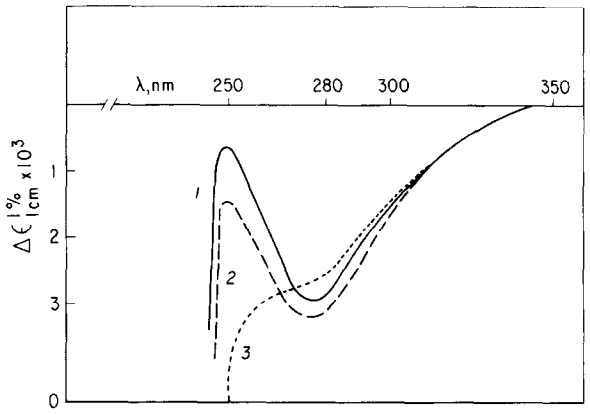

Fig. 1. Circular dichroism of oLHβ-subunit in the absence and presence of DL-(2,3-dihexadecyl)-glyceryl-phosphoryl choline in the 240–350 nm spectral zone. Curve 1, 0.1% oLHβ in 0.005 M sodium phosphate + 0.03 M NH_4OH, pH 9.3, no phospholipid. Curve 2, 0.1% oLH in the same solvent but with 0.1% dispersion of sonicated phospholipid, pH 9.3. Curve 3, 0.1% oLH at pH 11.2 with or without lipid dispersion. All solutions were tested in the same 1.0 cm optical path cuvette. The sensitivity scale setting in all runs was 5.10^{-5} dichroic differential optical density per 1 cm on the chart. The reproducibility in terms of $\Delta\epsilon^{1\%}_{1\ cm} \times 10^3$ was better than ± 0.1.

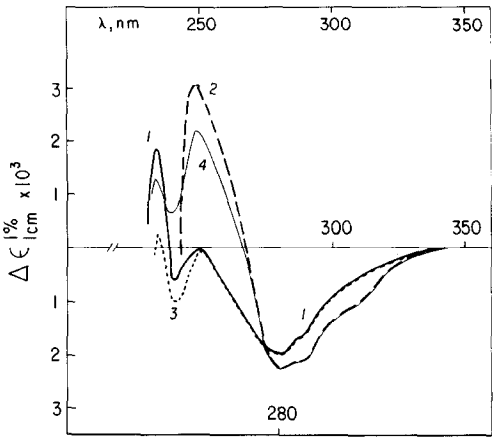

Fig. 2. Circular dichroism of the oLH in the absence and presence of DL-(2,3-dihexadecyl)-glyceryl-phosphoryl choline or egg yolk lipid vesicles. Curve 1, 0.1% oLH in 0.005 M sodium phosphate + 0.05 M NH_4OH, pH 6.7–9.3 in the absence of phospholipid. Curve 2, 0.1% oLH with 0.1% dispersion of sonicated phospholipid, pH 9.3. Curve 3, 0.1% oLH in 0.005 M phosphate + 0.1% sonicated lipid, pH 7.6. Curve 4, 0.04% oLH with equal amount of egg yolk lipid vesicles in 0.05 M NaCl + 0.005 M Tris + NaOH→pH 9.3. All solutions were tested in the same 1.0 cm optical path cuvette. The sensitivity scale setting was either 5.10^{-5} or 2.10^{-5} dichroic differential optical density per 1 cm on the recorder chart. The reproducibility in terms of $\Delta\epsilon^{1\%}_{1\ cm} \times 10^3$ was approximately ± 0.15.

nm). Similarly, neither oLHα nor oLHβ subunit CD could be modified by the lipid in the far-UV. There was no indication of α-helix formation, as it has been observed in such interactions with the protein moieties of lipoproteins [5] or myelin proteins [12]. This inability of the lipid to modify the polypeptide chain backbone conformation of the oLH subunits may be caused by two factors: 1) the rigidity of the macromolecule which is crosslinked by eleven disulfide bridges [10,13,14], and 2) by the high content of antihelix amino acids [13,14]. The presence of carbohydrate [13,14] also is a factor deterring helix formation [4].

The effect of the synthetic phospholipid then was tested on solutions of native oLH. The results are shown in fig. 2. Curve 1 is that of the oLH without lipid and curve 2 shows the lipid effect on the side chain chromophores under the same conditions. Significant differences were observed in the 230–

350 nm spectral zone; as in the case of oLHβ subunit, the phospholipid effects were most pronounced in solutions at pH 9.1–9.9. In neutral solution (curve 3), a slight difference in the CD was observed at 230–245 nm (Compare curve 1).

To examine whether the CD effects were attributable to the type of phospholipid, phospholipid vesicles prepared from egg yolk [11] were substituted for the synthetic phospholipid. Fig. 2, curve 4, shows the effect observed. The effect at pH 9.3 was similar to that with the synthetic phospholipid, but the vesicles had no effect at neutral pH (7.4) either with or without a sonication treatment. It was also found that neither the synthetic nor natural lipid mixture could affect the main chain conformation of oLH as measured by CD in the far-UV. The phospholipid effect was considerably augmented in the native LH (fig. 2) over that of the β-subunit (fig. 1).

The near-UV CD spectra of native oLH and its subunits have been analyzed in some detail [15–18]. In this region the CD spectra are those of the tyrosine and disulfide chromophores. The latter are characterized by the broad CD band at 250–320 nm tapering off gradually at 330–350 nm [18,19]. The negative band has a fine structure indicative of the tyrosine chromophores and overlapping the broad and weaker disulfide CD band. The phospholipid effect was most decisive near 250 nm causing a strong enhancement of the band centered at 248–250 nm. Curve 2, fig. 2 shows that this band is a composite one, most likely involving the tyrosine and disulfide chromophores; and the shoulder on the long wavelength side of it might be indicative of phenylalanine effects. The enhancement of both positive bands may be due to a transition of some of the chromophores from more to less polar microenvironment caused by the hydrophobic chains of the bound lipid.

The pH range in which the phospholipid effect on oLH is most readily detected suggests an ionization of tyrosine hydroxyl groups may be involved. As a working hypothesis we suggest adjacent acidic and basic amino acids in the β-subunit [14] attract the basic and acidic functional groups on the phospholipid, respectively, as has been proposed in the molecular theory of protein–phospholipid interaction to form serum lipoproteins [20]. In the latter case a pronounced helical formation in the protein chain is favored, but in the case of luteinizing hormone such a transition is precluded (vide supra). Instead, another type of interaction is detected by CD as in curves 2 and 4, fig. 2, for which both subunits must be present. We suggest a specific amino acid sequence may be involved in this interaction with the β-subunit since the α-subunit alone, as well as other proteins (e.g. soybean trypsin inhibitor, Bence-Jones protein), did not show the phospholipid effect. Moreover, the monofunctional amphiphile, sodium dodecyl sulfate, produces no such augmentation effect (Jirgensons, unpublished results).

It is known that the LH subunits do not bind specifically to the hormone receptor for oLH and that the intact hormone is required [1,21]. Hormone binding to the isolated receptor decreases at the pH where we observe the maximal CD effect [2], so we do not believe this result signifies a role of an ionized tyrosine in the receptor site interaction, but rather that one or more of the tyrosines in the α-subunit are behaving as a reporter group when in the ionized form. We place this interaction of the hydrophobic portion of the phospholipid with the α-subunit because the β-subunit has only two tyrosine residues [14] and these have been shown to be masked in the intact oLH [22,23]. However, the β-subunit contains more hydrophobic amino acid residues than the α-subunit, and the designation of which tyrosines are actually involved will require more extensive study.

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