STRUCTURAL MODIFICATIONS INVOLVED IN THE DISSOCIATION AND REASSOCIATION OF THE α AND β SUBUNITS OF OVINE LUTEINIZING HORMONE

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1. Introduction

The understanding of the molecular basis of gonadotropin hormone activity requires a knowledge of their structural properties. The amino acid sequence of the ovine luteinizing hormone (oLH) has been recently elucidated [1–3]. It is made of two subunits: the α subunit (LH-α), similar or identical in all the pituitary glycoprotein hormones (LH, FSH, TSH) and human chorionic gonadotropin (HCG), and the β subunit (LH-β) specific for each hormonal activity [4–6]. Both associated subunits are needed for the expression of the biological activity in the Parlow test [7, 8]. If conditions required for various states of association of LH subunits have already been investigated [8] there have been no studies aimed at defining the nature and the variation of the polypeptide chain folding in the different conditions used for dissociation or reassociation of the subunits. While this work was in progress, Jirgensons and Ward [9] published the circular dichroism (CD) spectra of oLH and its subunits at neutral and acid pHs but they did not explore other conditions of dissociation such as the urea treatment and made no attempt to correlate CD spectra to other physiochemical approaches of structural changes occurring during the dissociation processes. We found that these changes, specific for each dissociative condition, are time-dependent with probably a first order intermediary step. All the results so far obtained suggest that the loss of biological activity of LH-α and LH-β alone, or reassociated, can be related to modifications of the folding of their polypeptide chain as well as their state of association.

2. Materials and methods

oLH, LH-α and LH-β were prepared, respectively, according to Jutisz and Courte [10] and Papkoff and Samy [11] (see footnote*). All chemicals were reagent grade. 8 M urea solutions were purified on a Whatman DEAE cellulose column and made 0.1 M in NaCl. After dissolving the protein, the pH was adjusted to 6.9–7.0 with 0.1 N HCl. Acid treatment was performed either by adding 10 μl of N HCl to 1 ml of protein solution at pH 6.9–7.0 in 0.1 M NaCl (final pH 2.0) or by dissolving the protein in pH 1.3 buffer (0.05 M HCl, 0.05 M NaCl). All experiments were made at room temperature (20–22°).

Fluorescence and light-scattering [12] experiments were undertaken with laboratory-made instruments. Optical rotatory dispersion (ORD) and circular dichroism were performed with a Fica Spectropol and a Jouan Dichrograph II, respectively. The dichroism was expressed as mean residue ellipticity [θ] by taking a mean molecular weight per residue of 128 for oLH, 139 for LH-α and 119 for LH-β. Difference spectra were recorded with a Cary 15 or 14 spectrophotometer. Ultracentrifugation experiments were performed with a Spinco Model E analytical ultracentrifuge.

* They were obtained from the laboratoire de Physiologie Cellulaire, Collège de France, Paris (oLH batch number P 37 b titrating 1.95 NIH-LH-S 11).

3. Results and discussion

3.1. *Circular dichroism of oLH and of its subunits at neutral pH*

The CD spectra are presented in fig. 1a and 2. They are in good agreement with those recently published by Jirgensons and Ward [9] except that we failed to detect the 214 nm shoulder on the 205 nm band of LH-β.

The near UV bands clearly indicate the contribution of optically active side chain chromophores: either disulfide bridges (5 in LH-α for 96 amino acid residues and 6 in LH-β for 120 amino acid residues [3]) or tyrosine residues (5 in LH-α and 2 in LH-β). Both can be optically active [13, 14] and the precise assignment of these bands needs further elucidation. They are remarkably different for both subunits and the 235 nm band of LH-α is not detected in oLH which would suggest a somewhat different folding of LH-α in the native oLH. This is confirmed by the presence of the 197 nm band, not found in the native oLH.

None of the characteristic bands of periodic or aperiodic structures in the far UV region are found for LH or its subunits. This could be due to a strong displacement of these bands or alternatively, and perhaps more likely, to a strong contribution of the disulfide bridges as for the disulfide-containing cyclic peptides Arginine vasotocin and 8-L-Ornithine vasopressin [15].

3.2. *Structural modifications observed in dissociating mediums of oLH and its subunits*

The complete dissociation of oLH into its subunits was checked in our experimental conditions either by sedimentation in the ultracentrifuge or by measurement of light-scattering.

oLH contains only tyrosine and phenylalanine as aromatic residues. Upon acidification at pH 2.0 the absorption bands of the tyrosine residues undergo a blue shift and show some hypochromicity: the minima of the difference spectrum are at 287.5 nm (circa 10% of the maximum absorbance at 275 nm) and at 278–280 nm. A perturbation of the phenylalanine residues can also be detected. This blue shift is completely reversible upon raising the pH to 7.0 although oLH starts to aggregate at this pH after exposure to pH 2.0 in our experimental conditions.

Among the two subunits, only LH-α gives rise to a difference spectrum at pH 2.0, the intensity of which

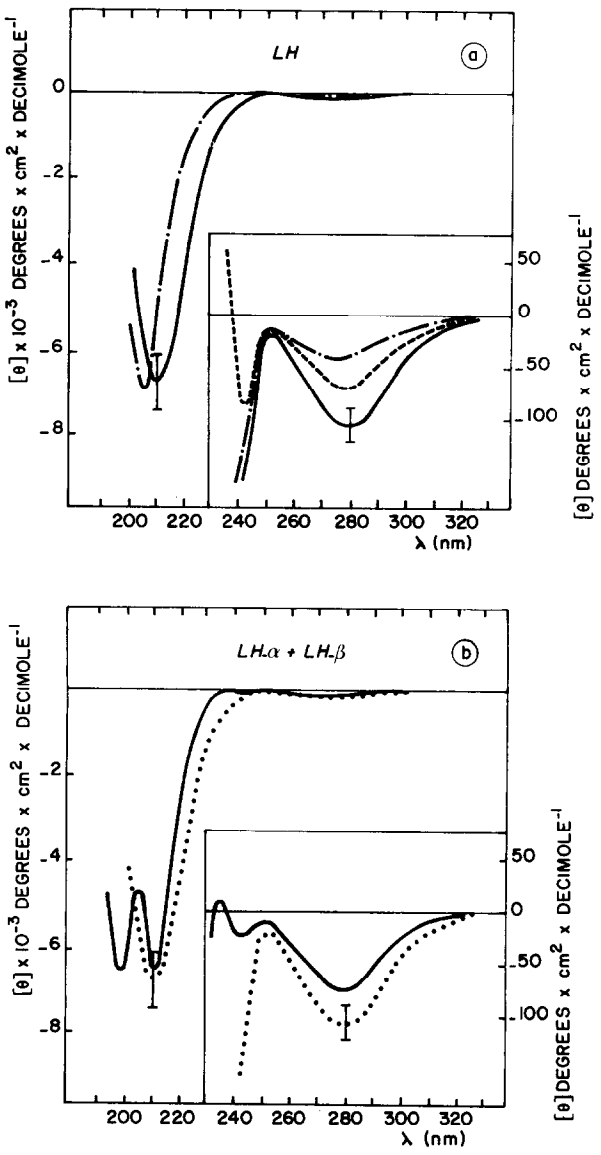

Fig. 1. (a) Circular dichroism spectra of ovine luteinizing hormone at neutral pH (——), pH 2 (—·—·—) and in 8 M urea (- - - -) at ionic strength 0.1 and at room temp. Estimated experimental errors at different wavelengths are indicated by a vertical line. Concentration range was between 0.1 to 2 mg/ml in cells of optical path from 0.01 cm up to 1 cm, except for urea solutions where concentration range was 1 to 18 mg/ml in cells of optical path from 0.001 to 1 cm. (b) Circular dichroism of recombined LH-α and LH-β at pH 7.0. The CD spectrum of native oLH (·····) has been represented for comparison.

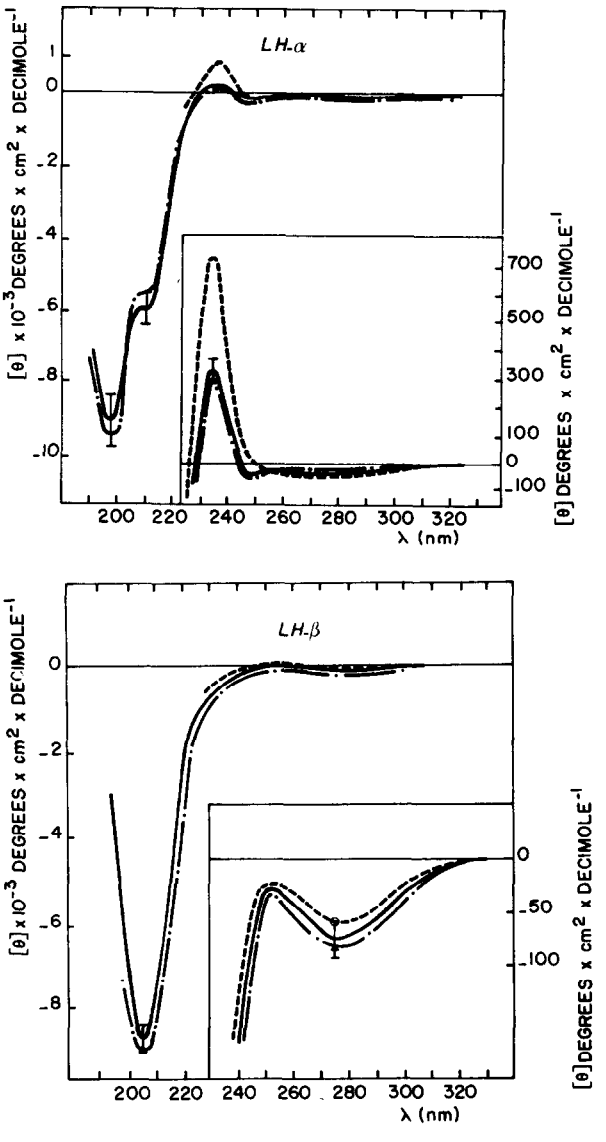

Fig. 2. Circular dichroism spectra of LH-α and LH-β of ovine luteinizing hormone at neutral pH (——), pH 2 (–·–·–) and in 8 M urea (- - - -) at ionic strength 0.1 and at room temperature. Estimated experimental errors are indicated by a vertical line. Concentration range was between 0.4 to 3 mg/ml in cells of optical path from 1 to 0.001 cm.

is less than the one observed with native oLH. This gives another piece of evidence for a slightly different folding of the separated LH-α. When recombined with LH-β at pH 7.0 the amplitude of the difference spec-

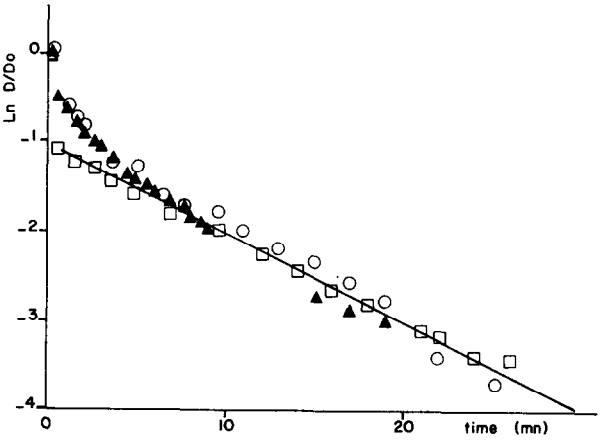

Fig. 3. Structural changes with time of oLH upon acidification at pH 2. ○: scattered light at an angle of 90° (2.1 mg/ml). ▲: variation of absorbance at 287.5 nm (1.7 mg/ml). □: emission of fluorescence at 350 nm (excitation at 280 nm) 0.14 mg/ml.

trum at pH 2.0 is regained almost entirely. As the reassociation of LH-α and LH-β does not produce a difference spectrum this would suggest that the pertrubed tyrosine residues of LH-α would not be located at the area of association of the two subunits; that is to say. they are not a probe for the dissociation itself.

The fluorescence emission spectrum of oLH at pH 7.0 (maximum of excitation at 278–280 nm) has a maximum at 325–330 nm which is shifted to 310–320 nm upon acidification at pH 2.0.

In acid conditions the CD spectra of LH-α and LH-β are almost unchanged, all the variations observed are within experimental errors (fig. 2). On the contrary, the far UV band of oLH is shifted to the shorter wavelengths and the near UV band is significantly lowered in intensity and undergoes a small blue shift (fig. 1a). The ORD trough of native oLH at 225 nm ($[R']_{225}$ = 8,300 ± 500, cross-over point at 201 ± 1 nm) at pH 7.0 is shifted to 220 nm at pH 2.0, with no significant change in amplitude ($[R']_{220}$ = 8,100 ± 500). These results are close to those already reported [9]. They would also suggest a slightly different folding from the native state of the separated subunits LH-α and LH-β.

In 8 M urea solution, a new positive band appears at 234 nm in the CD spectrum of oLH while the negative band at 280 nm is slightly decreased and the far UV band shifted to shorter wavelengths (fig. 1a).

Clearly the structural modifications of oLH in 8 M urea are distinct from those observed at acid pHs. Similar results are obtained with LH-α where the 234 nm band increases but no modification of the CD spectrum can be detected with LH-β (fig. 2). Again the LH-β subunit, which is responsible for the specificity of hormonal activity, seems to have a more stable folding than LH-α.

3.3. *Effect of time on the structural modification*

By gel filtration the dissociation of oLH has already been reported to be a slow process [8]. Again, upon acidification, the full development of the difference spectrum, the shift of the fluorescence emission, or the decrease of scattered light (related to the dissociation) are time-dependent processes. A logarithmic plot of the amplitude variations with time (shown in fig. 3) is biphasic. At first inspection the whole process can be considered as a "two-first-order-reactions" process, the slowest reaction having a rate constant of k = 0.1 min^{-1} at 20°. This rate is found to be the same with all the methods tested and for a wide range of concentrations (fig. 3). The simplest scheme which would fit the data would be the rapid formation of an intermediate, corresponding for instance to a partial unfolding of the polypeptide chains, followed by a slow process leading to a form able to dissociate. Further experiments are needed to ascertain such a mechanism.

3.4. *Reassociation of LH-α and LH-β*

At pH 7.0, 20° and 0.1 M NaCl the process of reassociation is very slow: after mixing of the two subunits (1 mg/1 ml of each subunit) the sedimentation coefficient is close to the value found for the dissociated oLH at pH 2.0, i.e. $S_{20,w}$ = 1.6; it increases only slowly with the time of contact to 1.65 S after 6 hr, 2.0 S after 24 hr and 2.2 S after 48 hr. In our experimental conditions native oLH (2 mg/ml) at pH 7.0, μ = 0.1, has a sedimentation coefficient $S_{20,w}$ of 2.4. Although after 48 hr of contact 90% of the subunits can be considered as reassociated, the CD spectrum of the mixture is both different from the native CD of oLH (see fig. 1b) and the calculated CD from the two subunits. No other evolution was found after 15 days of contact.

The difficulty to regain the original folding of the subunits must be paralleled to the lack of complete recovery of biological activity observed by various authors [6–8]. This would suggest that a correct folding of the polypeotide chains is required for the complete hormonal activity of oLH.

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