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PORCINE LUTROPIN : A STUDY OF THE ASSOCIATION OF ITS SUBUNITS BY HYDROGEN-DEUTERIUM EXCHANGE

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SUMMARY

Hydrogen-deuterium exchange of lutropin and its α and β subunits has been studied by infrared spectroscopy. It appears that lutropin exhibits a large dynamic accessibility to the solvent. The association of the subunits leads to the shielding of only eight peptide protons that were fully exposed to the solvent in the free subunits. The conformational dynamics of lutropin subunits does not seem to be diminished upon their binding in the active dimer.

INTRODUCTION

The dimeric nature of glycoprotein hormones is unique among hormones : one of their subunits (α) is common to all of them while the other one (β) is hormone-specific; neither of the isolated subunits exhibits any biological activity and the formation of the α - β dimer is required for the expression of the activity specified by the β-subunit (for a recent review, see 1).

Three models can be proposed to explain the appearance of biological activity upon binding of the subunits :

a) the site of interaction of the hormone with its receptor ("active site") is borne by the hormone-specific β-subunit which takes its active three dimensional organization upon binding to the common α-subunit, serving as a "template".

b) the potential "active sites" are all borne by the common α-subunit which active foldings are specifically directed by the β-subunits.

c) the "active site" is formed by the association of polypeptide portions from both subunits.

In order to test these possibilities we have been studying, as a model, the dependence of biological activity on the structure of porcine lutropin

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(luteinizing hormone, LH). This hormone has a molecular weight of 28 000, each subunit being half this figure. However the α-subunit polypeptide chain is shorter (90 residues) than the β (119 residues) but bears about twice as much polysaccharides. In addition both subunits are extremely rich in disulfide bridges (2-4).

By far ultra-violet circular dichroism spectroscopy we have shown that upon binding there was some increase in the proportion of β-structure at the expense of aperiodic structure (5). However, this result could no be taken as a proof of the induction of the active conformation of one subunit by the other. Since a structural induction must be accompanied by a diminished conformational flexibility isotopic hydrogen exchange appeared well suited for such a study.

MATERIALS AND METHODS

Porcine lutropin and its subunits, purified as described by Hennen et al., (6) has been kindly given by these authors. Heavy water of 99.7 % deuterium enrichment was provided by the Commissariat à l'Energie Atomique (C.E.A. Saclay, France).

Hydrogen-exchange kinetics were monitored by infrared spectroscopy according to the method of Blout et al., 1961 (7) following the previously described experimental procedure (8,9). A Perkin-Elmer 180 spectrometer equipped with matched thermostated CaF_2 cells of 0.1 mm pathlength was used in double beam mode with buffered ^2H_2O in the reference beam. The sample solutions were prepared by dissolving lyophilised proteins (6-7 mg) in 400 µl of the appropriate ^2H_2O buffer at zero time. The exchange rate of peptide hydrogens was followed by measurements of the time course of the amide II band absorption decrease. The percentage of unexchanged peptide hydrogens was estimated from the relative absorbancies of amide II (A_{II}) and amide I (A_I) bands (7). A ratio value of 0.45 was used for undeuterated proteins (7,8,10,11). The background of the amide II band arising from the side-chains and carboxyl groups contributions was determinated, for each exchange experiment, after complete deuteration of the peptide groups. Because of the high content of disulfide bridges in lutropin, this was achieved in one experiment by a three-hour heating at 60°C in the presence of sodium dodecyl sulfate and dithiolthreitol. However identical final A_{II}/A_I ratio by heating the hormone were obtained without these reagents. Consequently, total deuteration was achieved in all other experiments by a three-hour heating at 60°C at the end of the kinetic.

The experimental data were interpreted with the generally accepted EX_2 exchange mechanism (12) according to which exchange occurs only when peptide hydrogens are freely exposed to the solvent with a first-order rate constant k_0. The first-order rate constant k for a given peptide hydrogen is equal to $\rho\ k_0$ where ρ is its solvent exposure coefficient (13). The distribution of the ρ values for a protein is representative of its conformational dynamics. Kinetics were plotted as a function of log ($k_0 t$) so that the data obtained at different pH and temperatures (where k_0 values are different) could be compared.

RESULTS AND DISCUSSION

The position of the amide I band in proteins is known to be indicative of the content of periodic structures (14-15). The absorption maximum of porcine lutropin at 1636 cm^{-1} at pH 5.1 and 7.0 shows, in agreement with our previous C.D. measurements (5), the presence of a relatively high content in β-conformation in the native hormone. There is no significant displacement of the amide I band for the free subunits (α : 1638 cm^{-1}; β : 1636 cm^{-1}) relative to the native hormone. As expected, the increase in aperiodic structure revealed by circular dichroism upon dissociation of the subunits is too slight to be qualitatively detected by infrared spectroscopy.

The conformational dynamics of porcine lutropin.

Fig.1 shows the exchange curves for porcine lutropin and its subunits as plots of the percentage of unexchanged peptide hydrogens (X) versus log ($k_o t$).

Exchange data of lutropin at pH 5.1 and 7.0 fall on one continuous curve. According to the EX_2 exchange mechanism this indicates that no conformational change occurs in this pH range. It is noteworthy that as much as 60% of the peptide hydrogens exhibit ρ values higher than 10^{-1}, and only 10% present ρ values lesser than 10^{-4}. The comparison of the distribution of ρ values for lutropin with that for other proteins reported in the literature shows that lutropin exhibits a large accessibility to the solvent. It must be stressed that important conformational dynamics does not involve lability of the activity as evidenced, for example, by the fast exchange of peptide hydrogens of lysosyme (16), the activity of which is very stable, even in the presence of denaturant (17).

The association of the lutropin subunits.

In Fig.1 it can be seen that peptide hydrogens of the α-subunit are much more accessible to the solvent than those of the β-subunit and those of the native hormone. Thus, according to the EX_2 mechanism, its structure must be much more flexible than that of the β-subunit. This result is in agreement with the observation of Pierce et al. (18) that the five disulfide bridges of bovine lutropin α-subunit were reduced very rapidly even in the absence of denaturant.

The very high conformational dynamics of the lutropin α-subunit rules out the possibility that it can serve as a "template" to induce the active conformation of the hormone-specific β-subunit.

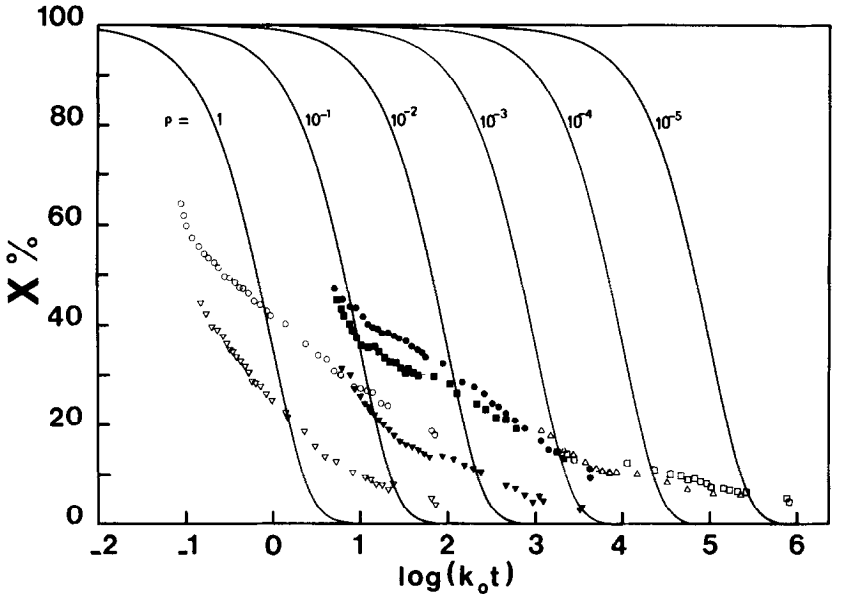

Figure 1. Percentage of unexchanged peptide hydrogens in lutropin (■ pH 5.1, □ pH 7.0), α-subunit (▽ pH 3.6, ▼ pH 5.3) and β-subunit (⊙ pH 3.6, ● pH 5.4, Δ pH 7.7) as a function of log (k_0t). (—) Exchange curves of hypothetical polypeptides characterized by the ρ values indicated on the figure.

The non-continuity of curves at pH 3.6 and 5.4 shows that both subunits undergo a conformational destabilization upon decrease of pH from 5.4 to 3.6. This transition has also been recently evidenced by proton magnetic resonance (19). No clear-cut analysis of the exchange of the hormone at pH 3 could be made as ultracentrifugation and radio-hormone receptor assay (data not shown) have shown that porcine lutropin was only half dissociated at this pH. At lower pH (1.1) the agregation of at least one of the two subunits hindered the determination of hydrogen exchange kinetics of pH-dissociated lutropin.

In order to point out the effect of the association of subunits on the hydrogen exchange kinetics we have compared in Fig.2, the number of unexchanged peptide hydrogens in the native lutropin and the sum of those in the two isolated subunits as a function of log (k_0t).

The difference between the two curves can be interpreted either as an overall stabilization of the conformation of the subunits in the native hormone and/or as the specific shielding of discrete regions forming the binding area of the subunits. Although both possibilities can occur at the same time it appears that the two curves are nearly parallel and that the difference in the number of unexchanged peptide hydrogens is equal to

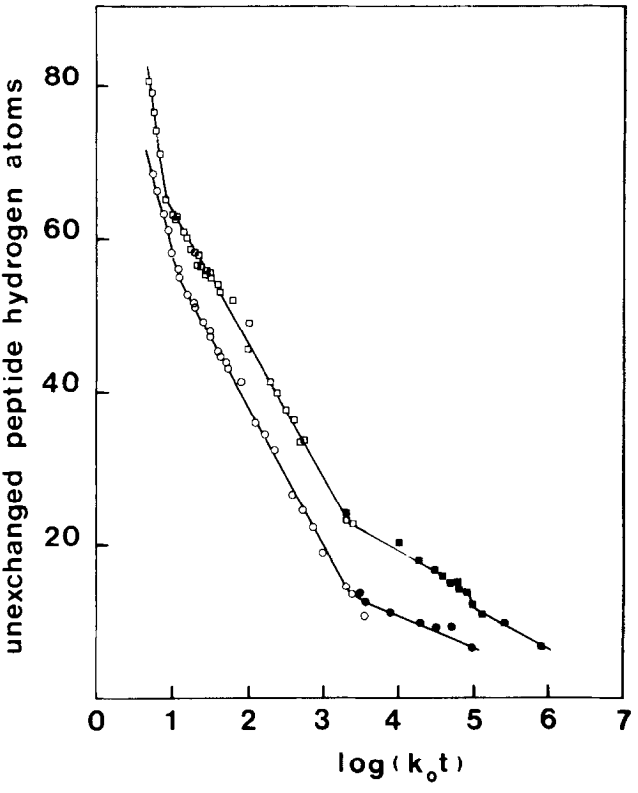

Figure 2. Comparison of the sum of the numbers of unexchanged peptide hydrogens in the isolated subunits (o pH 5.3-5.4, ● pH 7.7) with that of lutropin (□ pH 5.1, ■ pH 7.0).

about eight over the whole kinetics. This vertical displacement of the curve due to the binding of subunits strongly suggests that there is very little structural stabilization of their conformation if any, but rather shielding of eight peptide groups that were fully exposed to solvent in the free subunits.

This report shows that the appearance of biological activity upon the formation of quaternary structure is not due to any induction of the active conformation of one subunit by the other but to the juxtaposition of essential amino-acids residues from both subunits forming the "active site" of the hormone. The demonstration that residues of the common α-subunit are indeed involved in the "active site" of the hormone (20, 21) strengthens this view.

In previous work it has been shown (5, 22, 23) that there was a slight increase in β-conformation at the expense of aperiodic structure upon

binding of lutropin subunits. From the present data, we suggest that this increase in β-structure could take place in the binding area of subunits.

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