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ROLE OF HISTIDINE RESIDUES IN OVINE LUTROPIN: EFFECTS ON STEROIDOGENIC ACTIVITY

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SUMMARY: The modification of histidine residues of ovine pituitary lutropin by rose bengal sensitized photooxidation has been investigated. The destruction of an average of one histidine out of six lead to 90% loss of biological activity as examined by the in vitro steroidogenic response in the rat Leydig cell essay. Further modification of an average 2 - 3 histidine residues reduced the biological activity to less than 1% of the native lutropin. The modified lutropin was incapable of inhibiting the native lutropin induced steroidogenesis. Gel filtration experiments and polyacrylamide disc gel electrophoresis patterns indicated that no dissociation of the molecule into subunits occurred. This is the first report on the essentiality of the histidine residue for the activity of lutropin.

INTRODUCTION

Ovine lutropin is a glycoprotein hormone consisting of two non-identical subunits (1). The amino acid sequence of both the subunits (α and β) have been determined (2,3). Neither subunit possesses any tryptophan. Previous reports from this laboratory demonstrated that the biological and chemical properties of lutropin were sensitive to the number and specific groups modified when reacted with periodate (4), tetranitromethane (5) and citraconic and maleic anhydrides (6). Other modification studies on ovine lutropin have been recently summarized by Ward (7). Although extensive studies have been reported on the role of histidine in various enzymatic proteins (8,9), no studies have thus far been reported on hormonal proteins.

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The present report describes the effects of histidine modification by rose bengal sensitized photooxidation on the physicochemical and biological properties of lutropin. Ovine lutropin possesses a total of six histidine residues equally distributed in the two subunits. The results reported here show that modification of but a single histidine residue of the six present in both subunits results in a profound effect on the biological activity of the hormone.

MATERIALS AND METHODS

Highly purified ovine lutropin was isolated from frozen pituitary glands by a method described earlier (10). The two subunits (oLHα and oLHβ) were prepared by the countercurrent distribution procedure (11) and further purified by gel filtration on columns of Sephadex G-100 in 0.05 M NH_4HCO_3.

The modification of histidine residues was carried out by the rose bengal (Eastman) sensitized photooxidation method (8,9). A sample with dye to protein ratio 1:100 (w/w) in 0.03 M ammonium bicarbonate (pH 7.9) was irradiated at 22°C. by a 300 watt projector lamp (tungsten filament) for variable time periods essentially as previously described (12,13). Immediately after irradiation the dye was removed from the lutropin sample by filtering through a DEAE cellulose column equilibrated with 0.03 M ammonium bicarbonate and lyophilized. Under these conditions ovine lutropin is unadsorbed.

Amino acid analysis of the samples were carried out according to the procedure of Spackman et. al. (14), in a Beckman Model 119C analyzer attached to a computing integrator. Samples were hydrolyzed in constant boiling HCl (1 mg/ml), in evacuated and sealed tubes at 110°C. for 20h. The performic acid oxidation of the samples was accomplished by dissolving the samples (1 mg/ml) in pre-formed performic acid (1 ml of 30% H_2O_2 and 9 ml formic acid at room temperature for 1h) and maintaining the solution at 0° for 1h as described by Li(15). The solution was diluted with 5 vols. of water and lyophilized.

Sephadex G-100 gel filtration was carried out on a 2.5 x 30 cm column in 0.05 M ammonium bicarbonate. Polyacrylamide disc gel electrophoresis in 7.5% acrylamide at pH 8.3 was done essentially as described (12).

The biological activity of the various samples was determined by a modified in vitro steroidogenic method which employs a suspension of rat testis interstitial cells (17).

RESULTS AND DISCUSSION

When ovine lutropin was irradiated in the presence of rose bengal, a specific destruction of histidine was observed as

TABLE I
Amino Acid Composition of Lutropin Derivatives

Amino Acid	Native Lutropin[b]		Lutropin Derivatives[c]	
			I	II
His	6.2	(6)	5.1	1.8
Lys	13.2	(12)	12.1	11.7
Arg	12.0	(11)	11.4	11.3
Asp	12.3	(11)	12.2	13.0
Thr	17.1	(16)	16.1	16.5
Ser	14.7	(14)	14.2	15.0
Glu	17.6	(14)	15.6	20.0
Pro	30.4	(28)	28.4	29.0
Gly	14.4	(11)	13.7	14.8
Ala	17.6	(15)	15.7	19.0
½Cys[a]	20.8	(22)	20.5	18.0
Val	15.7	(13)	13.2	14.1
Met[a]	7.2	(7)	6.6	6.5
Ile	7.0	(7)	7.2	6.8
Leu	16.2	(14)	14.7	15.3
Tyr	6.9	(7)	7.2	6.7
Phe	8.6	(8)	8.8	8.8

[a]Determined as cysteic acid and methionine sulfone after performic acid oxidation

[b]Values in parentheses are theoretical (Ref. 1)

[c]Derivatives I and II are treated 1 and 10 minutes respectively with light in the presence of rose bengal

shown in Table 1. One minute light treatment caused 15% destruction of the total histidine residues and this corresponded to an average of one histidine residue per molecule of lutropin. On continued illumination for 10 mins, 70% of the total histidine was destroyed. The probability of conversion of

methionine to methionine sulfoxide in these experiments does not appear to be likely since reports from other laboratories have clearly demonstrated that methionine is not oxidized under the conditions we have employed (18). No significant changes in the tyrosine or cystine content was noticed even though these residues are known to be susceptible by rose bengal sensitized photo-oxidation under different conditions (8,9).

The rate of loss of steroidogenic activity of the hormone correlated with the destruction of histidine residues in lutropin as shown in Table II. The results indicate that an average loss of one histidine residue per molecule is sufficient to destroy 90% of the biological activity. Destruction of three or more histidines (6-30 min treatment) results in total inactivation. The rate of histidine destruction appears to have biphasic kinetics, i.e., an initial rapid phase followed by a slower phase. This may be due to a differential accessibility of various histidine residues within the lutropin molecule.

Modified preparations of lutropin examined by disc gel electrophoresis gave stained patterns that were closely similar to the native hormone with no evidence of dissociation into subunits. Similarly, gel filtration of Sephadex G-100 showed a single, symmetrical peak eluting with a Ve/Vo of 1.55. Native lutropin elutes with a Ve/Vo of 1.64. The above experiments suggest that the loss of steroidogenic activity is not due to the dissociation of the molecule into subunits. Furthermore, other bioassays with photoxidized lutropin in which 2-3 histidine residues were destroyed, indicates that this molecule is unable to competitively inhibit the native lutropin induced steroidogenic response. This observation suggests that this modified form of lutropin lacks the ability to bind to the target site.

TABLE II

Correlation between the destruction of histidine with the loss of steroidogenic activity of lutropin.

Light Treatment (min)	Remaining Residues of Histidine	Steroidogenic Activity (Percent)
Control	6.0	100
1.0	5.1	12
2.0	4.7	6
6.0	2.4	1
10.0	1.8	<1
20.0	1.5	<1
30.0	0.43	<1

Although the exact site of histidine modification has not been localized, a preliminary experiment involving the preparation of subunits from a modified preparation in which 1-2 histidine residues were destroyed showed that 2.5 histidine residues were present in the α-subunit, and only 1.7 residues in the β-subunit, respectively. Such differences were more pronounced when modification was carried out on individual subunits. The time required to destroy one histidine residue in α-subunit, is adequate to modify an average of 2.5 histidine residues in the β-subunit. Thus, these results point to the preferential destruction of histidine in the β-subunit. Future studies will establish the precise site of histidine modification in the molecule and the importance of this region of the molecule, in subunit-subunit interaction, in interaction with the target site, in expression of physiological response, and in immunorecognition.

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