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MELANOCYTE-STIMULATING HORMONES FROM SHEEP PITUITARY GLANDS

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SUMMARY

The melanocyte-stimulating hormones, α- and β-MSH were isolated from sheep pituitary glands. Ovine α-MSH was found to have the same amino acid sequence as α-MSH from other animals. Three varieties of β-MSH from sheep were studied. One appeared to be identical with bovine β-MSH.

INTRODUCTION

Melanocyte-stimulating hormones (α- and β-MSH) from a number of animal species have been studied[1-18]. It is of interest that the structure of α-MSH is the same in all species while β-MSH varies slightly from one species to another. In addition, more than one type of β-MSH occurs in the pituitary gland of a given species. Although the presence of α-MSH in sheep pituitary glands was often cited, its amino acid sequence had not been determined previously. In this paper we wish to report experiments in which we found that sheep α-MSH is identical to all known α-MSH's. Also, we shall describe the isolation of three β-MSH fractions. The most prominent one is probably the same as bovine β-MSH.

EXPERIMENTAL

Materials

Frozen, whole sheep pituitary glands, purchased from Cornish Laboratories in Berkeley, Calif. (U.S.A.), were lyophilized and ground to a powder in a Waring blendor. The resultant pituitary powder was stored at −10°. Crystalline trypsin and chymotrypsin were purchased from Mann Research Laboratories, Inc., New York, N.Y.; and purified carboxypeptidase was obtained from Worthington Biochemical Corporation, Freehold, N.J.

Isolation

α- and β-MSH were isolated from sheep pituitary glands by the method used to obtain these peptides from monkey pituitaries[18]. OXF-1 was the only starting material for CM-cellulose chromatography.

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Sequence determination

Following procedures identical to those described before, we determined the sequence of sheep α-MSH[18]. NH_2- and COOH-terminal amino acids of peptide fragments that resulted from tryptic and chymotryptic digestion of sheep α-MSH were obtained by conventional methods.

RESULTS

Chromatography of sheep pituitary material on a CM-cellulose column separated β-MSH, α-MSH and ACTH (Fig. 1). Yields from 100 g of lyophilized sheep pituitary powder were approx. 4 mg β-MSH, 3 mg α-MSH and 20 mg ACTH. Two less active fractions possessing chromatographic properties similar to those of β-MSH also were obtained. The biological activities of sheep α-MSH and β-MSH were $3 \cdot 10^9$ and $1 \cdot 10^9$ MSH units/g, respectively. The less active β-MSH fractions had potencies of $2\text{–}4 \cdot 10^8$ MSH units/g. The potency of sheep α-MSH was the same as that of pig α-MSH prepared in the same manner.

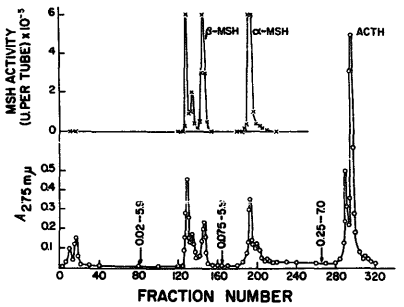

Fig. 1. CM-cellulose column chromatography of oxycellulose-purified sheep pituitary material OXF-1. Conditions of chromatography: Approx. 500 mg of OXF-1 was fractionated on a 15 × 290 mm column which had been equilibrated with 0.005 M ammonium acetate at pH 5.9. The column was developed at room temperature by stepwise introduction of ammonium acetate buffers of the following concentrations and pH values: 0.005 M (pH 5.9), 0.02 M (pH 5.9), 0.075 M (pH 5.9) and 0.25 M (pH 7.0). All buffers contained 0.03% toluene as a preservative. The effluent was collected in 5-ml fractions at a flow rate of 25–30 ml/h. The lower part of the diagram gives the absorbancy of the fractions and the upper part the MSH activity of selected fractions. The arrows on the diagram indicate points of buffer change.

The homogeneity of our preparation was tested by paper electrophoresis and enzymic digestion. On a paper electrophoretogram of the intact hormone, a faint tail trailing after the main spot of α-MSH could be observed. This indicated the possibility of heterogeneity. Minute quantities of extraneous impurities also were detected by "finger printing" techniques[19] with two-dimensional paper electrophoresis and paper chromatography. Using this procedure with a chymotryptic digest of sheep α-MSH, we observed several faint ninhydrin positive spots in addition to those corresponding to pig α-MSH. However, these minor peptide fragments were absent when a preparation

which had been further purified by paper electrophoresis was submitted to chymotryptic digestion. It yielded only the peptide fragments identical with those of pig hormones. Apparently these minor fragments were derived from extraneous impurities and could therefore be disregarded in assigning the amino acid sequence of sheep α-MSH.

Sheep α-MSH did not possess free NH_2- and COOH-terminal groups, as determined by Edman degradation and carboxypeptidase digestion. The amino acid composition and the NH_2- and COOH-terminal groups of peptide fragments from chymotryptic digestion are shown in Table I. The fact that peptide Ch-1 was negative to ninhydrin reagent and that the carboxypeptidase digestion released only tyrosine as the COOH-terminal amino acid indicated a blocked α-amino group on the seryl residue of this fragment. Thus peptide Ch-1 must be derived from the NH_2-terminal sequence of the parent molecule. The highly basic property of Ch-5 and the lack of a free COOH-terminal residue suggested an amide group on the COOH-terminus of this peptide. It therefore must have been derived from the COOH-terminal portion of the intact hormone. The amino acid sequences of peptides Ch-2 and Ch-5 were established by partial acid hydrolysis (Table II). Assignment of positions for these two peptides in the complete sequence of sheep α-MSH was based on the information supplied by tryptic digestion (Fig. 2. Table III). The nature of the NH_2-terminal blocking group has not been determined. It probably is an acetyl group as in pig α-MSH.

TABLE I

PEPTIDES FROM CHYMOTRYPTIC DIGESTION OF SHEEP α-MSH

Peptide	*COOH-terminal*	*NH_2-terminal*	*Total hydrolysate*	*Partial sequence*	*Remarks*
Ch-1	Tyr		(Tyr, Ser)	R–Ser. Tyr	Ninhydrin reaction negative
Ch-2	Phe		(His, Ser, Glu, Met, Phe)	(His, Ser, Glu, Met, Phe)	
Ch-3	Try and Arg		(Arg)	Arg. Try*	Positive Ehrlich reaction for tryptophan
Ch-4			(Arg, Lys, Gly, Pro, Val)		Positive Ehrlich reaction for tryptophan
Ch-5	None	Gly	(Lys, Gly, Pro, Val)	Gly(Lys, Pro, Val)NH_2	Highly basic

* Sequence derived from known specificity of chymotrypsin.

TABLE II

PARTIAL ACID HYDROLYSIS OF PEPTIDES Ch-2 AND Ch-5

Fragments are numbered in the order of increasing basicity.

Fragment	*Peptide Ch-2*	*Fragment*	*Peptide Ch-5*
7	His–Phe	3	Gly–(Pro, Lys)
8	His	6	Pro–Val-NH_2
4	Glu–His	2	Lys–Pro–Val
2	Met–Glu	5	Lys
3	Ser–Met–Glu	1	Gly–Pro–Val
5	Ser–Met–Glu–His–Phe		
6	Ser–Met Phe		
	Ser–Met–Glu–His–Phe		Gly–Lys–Pro–Val-NH_2

Three fractions of β-MSH were obtained from the CM-cellulose chromatographic fractionation (Fig. 1). The most active fraction appeared to be homogeneous and was submitted to structural studies. Edman degradation revealed a partial NH_2-terminal sequence of Asp–Ser–Gly–Pro–Tyr–Lys–Met–Glu–His–Phe. Tryptic and chymotryptic digestions also gave peptide fragments consistent with an amino acid sequence identical to that of bovine β-MSH. However, neither of the less active components showed any structural similarity to pig β-MSH. Because of the scarcity of these minor components, as well as their possible heterogeneity, we did not study them further.

TABLE III

PEPTIDES FROM TRYPTIC DIGESTION OF SHEEP α-MSH

Peptide	*Total hydrolysate*	*Remark*
T-1	(Tyr,Ser,Met,Glu,His,Phe,Arg)	
T-2	(Gly,Lys,Pro,Val)	Positive Ehrlich reaction for tryptophan

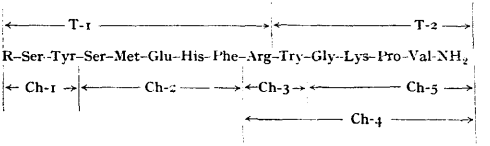

Fig. 2. Amino acid sequence of sheep α-MSH. The fragments from tryptic and chymotryptic digestions are indicated by the symbols T and Ch respectively.

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