Amino-acid Sequence of a Melanophore-stimulating Peptide

A HIGHLY active melanophore-stimulating polypeptide has been isolated from hog posterior pituitary extracts¹. The amino-acid composition² of the active substance (MSH) is consistent with the view that it is an octadecapeptide containing the following amino-acid residues: Arg₁, Asp₂, Glu₃, Gly₄, His₅, Lys₆, Met₇, Phe₈, Pro₉, Ser₁₀, Try₁₁, Tyr₁₂.

In this preliminary communication we wish to report the complete amino-acid sequence of the peptide hormone as deduced from the results of 'stepwise' degradation studies on the intact molecule and from the characterization of peptide fragments isolated from enzymic digests. The methods used in this study may be summarized as follows:

Sequence studies on the intact octadecapeptide. The N-terminal sequence was investigated by a modification³ of Edman's phenylthiohydantoin method. 2.4 mgm. of the peptide hormone was used for this study, which was carried out as previously described^{3,4}. The phenylthiohydantoin derivative of the N-terminal amino-acid was obtained in 85–90 per cent yield and was identified chromatographically as that of aspartic acid. The peptide was afterwards submitted to ten successive applications of the degradation procedure, and the following N-terminal sequence was identified: Asp.Glu.Gly.Pro.Tyr.Lys.Met.Glu.His.(Phe?).

The C-terminal residue was identified as aspartic acid by the carboxypeptidase method⁵.

Studies on peptide fragments from enzymic digests

Action of trypsin. The peptide hormone (4.8 mgm.) was treated with trypsin (0.05 mgm.) at pH 8.0 and 30° for 30 min. The reaction mixture was submitted to ionophoresis on paper in a pyridine-acetic acid buffer (pH 6.5) for 3 hr. at a potential gradient of approximately 40 V/cm., and was resolved into four major and two minor components. The tryptophan and histidine residues were located by means of the Ehrlich and Pauli reagents respectively, and sample portions of each peptide component were separately hydrolysed and analysed for constituent amino-acids by two-dimensional chromatography on paper. The tryptic digest was found to contain the following peptides: *T*_{1a}, (Asp, Glu, Gly, Lys, Pro, Tyr), *T*_{1b}, (Asp, Glu, Gly, Pro, Tyr), *T*₂, (Arg, Asp, Glu, Gly, His, Lys, Met, Phe, Pro, Tyr), *T*₃, (Asp, Gly, Lys, Pro, Ser, Try), *T*_{4a}, (Arg, Glu, His, Met, Phe), *T*_{4b}, (Arg, Glu, His, Lys, Met, Phe). By the further action of trypsin, *T*₂ gave equimolar amounts of *T*_{1a} and *T*_{4a}, showing that these two peptides are part of the same sequence. *T*_{1b} and *T*_{4b} are minor components which, as shown below, are due to chymotrypsin contamination.

Action of chymotrypsin. The hormone (4.8 mgm.) was treated with chymotrypsin (0.05 mgm.) at pH 8.0 and 30° for 20 min. The digest was submitted to ionophoresis on paper in a pyridine-acetic acid buffer (pH 3.5) for 3 hr. at a potential gradient of 35–40 V/cm.; it was resolved into four major components which were analysed for constituent amino-acids as described above. The chymotryptic digest was found to contain the following peptides: *C*₁, (Asp, Glu, Gly, Pro, Tyr), *C*₂, (Arg, Asp, Gly, Lys,

Pro, Ser, Try), *C*₃, (Glu, His, Lys, Met, Phe), *C*₄, (Asp, Glu, Gly, His, Lys, Met, Phe, Pro, Tyr). By the further action of chymotrypsin *C*₄ gave rise to a mixture of *C*₁ and *C*₃, indicating that these two peptides belong to the same sequence, and *C*₂ was split into two new fragments, *C*₅, (Arg, Try) and *C*₆, (Asp, Gly, Lys, Pro, Ser).

Elucidation of complete amino-acid sequence. Peptides *T*_{1a}, *T*₃, *T*_{4a}, *C*₁, *C*₃, *C*₅ and *C*₆ were subjected to end-group analysis and sequence studies, from the amino end by means of the Léonis modification⁶ of the phenylthiohydantoin method, and from the carboxyl end by means of carboxypeptidase⁵. In this manner the complete structure of each peptide was determined, and the linkages connecting the various fragments ascertained from the overlapping sequences. The complete amino-acid sequence of the melanophore-stimulating hormone is thus established as follows:

The indicated sites of enzymic cleavage are seen to have occurred in accordance with the known specificities of trypsin and chymotrypsin.

The N-terminal decapeptide sequence determined directly on the intact hormone molecule is shown to be in complete agreement with the deduced sequence. Peptides *C*₁, *C*₃ and *T*₃ were also examined by partial acid hydrolysis (12 *N* hydrochloric acid at 37°) and end-group analysis using Sanger's fluorodinitrobenzene reagent⁷. All the di- and tri-peptide fragments which were obtained in this way could be unequivocally located in the indicated sequence.

It may be noted that the sequence Met.Glu.His.Phe.Arg.Try.Gly (positions 7–13) has also been shown to occur in the corticotropins (ACTH, positions 4–10)^{8,9}. This observation is of particular interest since it suggests a possible structural basis for the observed intrinsic melanophore-stimulating activity of the corticotropins^{8,10}, and recalls earlier evidence for the striking relationships between the structure and biological activities of the posterior pituitary hormones oxytocin and vasopressin¹¹.

Full experimental details of this work will be published elsewhere.

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