THE N-TERMINUS OF OX GROWTH HORMONE

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1. Introduction

Ox pituitary growth hormone possesses two N-terminal residues (phenylalanine and alanine) [1], and therefore two types of polypeptide chain. The molecular weight of the hormone has been found in recent studies to be 20–26,000 [2–7], and if a value in this range is accepted there are about 0.5 moles of each N-terminal residue/mole of protein. The presence of a total of only one mole of N-terminal acids/mole of protein suggests that there cannot be a covalent link between the polypeptide chains (cf. the branched chain model proposed in [8]), and such a conclusion is supported by studies on the amino acid sequence of ox growth hormone [6,9,10], which suggest unbranched chains, each containing about 200 amino acid residues. However, attempts to separate the alanyl and phenylalanyl chains have been unsuccessful. In this paper studies on the N-terminal sequences of ox growth hormone are described, which indicate that, near the N-terminus anyway, the two chains differ only by the presence or absence of a single alanine residue.

The N-terminal sequences were investigated in two ways:

(1) determination of the N-terminal sequence using the "dansyl"-Edman technique.

(2) investigation of peptides derived from the N-terminus by cleavage after methionine residues with cyanogen bromide.

2. Experimental

Ox growth hormone was prepared by a chromatographic method [7]. It contained about 0.4 μmoles of alanine and 0.4 μmoles of phenylalanine as N-terminal amino acids in 20 mg of protein (i.e. in 1 μmole of protein if the molecular weight is 20,000).

Stepwise degradation of growth hormone was based on the "dansyl"-Edman technique of Gray [11]. 2 mg of growth hormone and 20 μl of phenylisothiocyanate were reacted in 0.2 ml of buffer "M" (containing 60 ml N-ethyl morpholine, 1.5 ml acetic acid, 500 ml ethanol and 438 ml water; by volume [12]) at 37° for 2½ h and then dried in an evacuated desiccator and extracted with 2 × 1 ml benzene. The dried residue was dissolved in 0.5 ml trifluoroacetic acid, stood at 20° for 1 h and an aliquot equivalent to 0.2 mg of growth hormone was then removed for N-terminal analysis by the "dansyl"-method [13]. The remainder of the peptide was dried and put through another cycle of the degradative procedure to determine the next residue. The procedure was repeated for 5–6 cycles, after which the results became difficult to interpret.

The aliquot taken for "dansylation" was reacted with 40 μl 0.1 M $NaHCO_3$ and 40 μl "dansyl" chloride (2.5 mg/ml in acetone) for 3 h at 20°. "Dansyl"-protein was hydrolysed with 0.1 ml 6 N HCl (sealed tube, 110°) for 4 or 16 h (the shorter hydrolysis time gives better recoveries of some "dansyl"-amino acids, especially "dansyl"-proline [13]).

"Dansyl"-amino acids were separated and identified by high voltage paper electrophoresis [13] or by chromatography on polyamide sheets [14].

For cleavage of growth hormone with cyanogen bromide, 100 mg of protein, 50 mg of cyanogen bromide and 10 ml of 75% formic acid were incubated at 20° for 16 h. The mixture was dried to about 1 ml on a rotary evaporator, diluted to 15–20 ml with

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water, and then freeze dried. Determination of the N-terminal residues of the material so treated indicated that lysine, arginine, and serine had been exposed in addition to the phenylalanine and alanine originally present. The reaction products were separated into soluble and insoluble fractions by extraction with pH 6.5 buffer (10% pyridine, 0.3% acetic acid, 89.7% water; by volume). Most of the N-terminal phenylalanine and alanine now proved to have passed into the soluble fraction, which was therefore examined further to obtain peptides derived from the N-terminus.

The soluble fraction was submitted to gel filtration on Sephadex G-15 in 50% acetic acid, and the retarded peptides (which had N-terminal alanine and phenylalanine) were further fractionated by high voltage paper electrophoresis (pH 2; 2% formic acid, 8% acetic acid, 90% water; by volume) and recovered by elution with the same buffer (after staining a guide strip for detection).

Amino acid analyses were performed with a Beckman amino acid analyser, Model 120C. N-terminal groups and sequences of peptides were determined by the "dansyl" and "dansyl"-Edman methods [11,13].

3. Results and Discussion

3.1. *"Dansyl"-Edman degradation*

The "dansyl"-Edman degradation of ox growth hormone is complicated by the fact that two chains are being degraded at once. The new N-terminal amino acids exposed after each cycle of the Edman reaction are indicated in table 1. These results could arise from the degradation of two very similar chains, one of which had an extra residue at the N-terminal end. The proposed sequences are shown in fig. 1, where they are compared with the N-terminal sequence of human growth hormone [15]. Ox and human growth hormones are clearly homologous in this region, though the human hormone has only a single type of chain, beginning with phenylalanine.

Table 1
Edman degradation of ox growth hormone.

Number of Edman steps	Residues detected
0	Phe, Ala
1	Pro, Phe
2	Ala, Pro
3	Met, Ala
4	Ser, Met
5	Leu, Ser

3.2. *Cyanogen bromide cleavage*

The results of the Edman degradation suggest that there is a methionine residue near the N-terminus of the molecule, which should be susceptible to cleavage by cyanogen bromide. When the products of cyanogen bromide cleaved growth hormone were examined, two small peptides were obtained (by electrophoresis of the fraction retarded on Sephadex G-15) one with N-terminal alanine (CN-1a), the second with N-terminal phenylalanine (CN-1b). These had the following amino acid compositions:

CN-1a: Pro (1.0), Ala (1.9), Phe (1.0), Homoserine + Homoserine lactone (0.7) [and a trace of Ser (0.1)].

CN-1b: Pro (1.0), Ala (1.0), Phe (1.0), Homoserine + Homoserine lactone (0.8).

The sequences of these two peptides were determined by "dansyl"-Edman degradation:

CN-1a: Ala-Phe-Pro-Ala-Homoserine lactone

CN-1b: Phe-Pro-Ala-Homoserine lactone

CN-1a and CN-1b can clearly derive from the two N-terminal sequences proposed above (fig. 1) by cleavage after methionine (residue 5 of the alanyl chain, residue 4 of the phenylalanyl chain) and conversion of methionine to homoserine or its lactone. The results from cyanogen bromide cleavage thus confirm the idea that the two chains of ox growth hormone are very similar near the N-terminus, differing only by the presence or absence of a single alanine residue. Studies on the primary structure of the remainder of the ox growth hormone molecule [6,9,10] suggest that the rest of the chains may also be very similar, and possibly, identical.

Fellows [16] has also investigated the products of cyanogen bromide cleavage of ox growth hormone, but reported only the presence of a peptide equivalent to CN-1b, which by itself cannot explain the two N-terminal residues of the parent hormone. Parcells and Li [17] studied peptides obtained from dinitrophenyl-

Ox growth hormone { Phenylalanyl chain : H_2N-Phe-Pro-Ala-Met-Ser-Leu . . . . . ; Alanyl chain : H_2N-Ala-Phe-Pro-Ala-Met-Ser-Leu . . . . . }

Human growth hormone : H_2N-Phe-Pro-Thr-Ile-Pro-Leu . . . . . .

Fig. 1. N-terminal sequences proposed for the two chains of ox growth hormone. The N-terminal sequence of human growth hormone [15] is included for comparison.

ated growth hormone, and obtained two N-terminal sequences which do not agree well with the sequences given here.

3.3. *Origin of the two growth hormone chains*

It is not yet clear how the two chains of ox growth hormone arise. Removal of the N-terminal alanine by an enzyme during isolation is a possible explanation, but if this is the case the fact that the two chains occur in nearly equal proportions after several quite different purification procedures is surprising. Possibly enzymic degradation occurs in the gland before isolation, perhaps during activation of a growth hormone precursor. Alternatively the difference could have a genetic origin, due either to the presence of two alleles within the population of animals from which the glands are obtained, or to duplication of the gene which codes for growth hormone.

There is strong evidence that under many conditions ox growth hormone tends to aggregate, and that the predominant form of the hormones in some circumstances is a dimer. This explains earlier molecular weight determinations, which gave a value of about 45,000 [8,18]. Whether the alanyl and phenylalanyl chains are distributed in a random fashion among such dimers, or whether specific dimers (containing either dissimilar chains or similar chains) are favoured, is not yet clear.

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References

[1] C.H.Li and L.Ash, J. Biol. Chem. 203 (1953) 419.
[2] P.Andrews, Nature 209 (1966) 155.
[3] P.Andrews and S.J.Folley, Biochem. J. 87 (1963) 3P.
[4] J.M.Dellacha, M.A.Enero and I.Faiferman, Experientia 22 (1966) 16.
[5] G.J.Ellis, E.Marler, H.C.Chen and A.E.Wilhelmi, Federation Proc. 25 (1966) 348.
[6] J.A.Santomé, J.M.Dellacha and A.C.Paladini, in: Growth Hormone, eds. A.Pecile and E.E.Müller (Excerpta Medica Foundation, Amsterdam, 1968), p. 29.
[7] M.Wallis and H.B.F.Dixon, Biochem. J. 100 (1966) 593.
[8] C.H.Li, Advan. Protein Chem. 11 (1956) 101.
[9] J.A.Santomé, C.E.M.Wolfenstein, M.Biscoglio and A.C. Paladini, Arch. Biochem. Biophys. 116 (1966) 19.
[10] M.Wallis, Biochem. Biophys. Acta 115 (1966) 423.
[11] W.R.Gray, in: Methods in Enzymology, Vol. 11, ed. C.H.Hirs (Academic Press, New York, 1967) p. 469.
[12] W.Konigsberg, in: Methods in Enzymology, Vol. 11, ed. C.H.Hirs (Academic Press, New York, 1967) p. 461.
[13] W.R.Gray, in: Methods in Enzymology, Vol. 11, ed. C.H.Hirs (Academic Press, New York, 1967) p. 139.
[14] K.R.Woods and K.T.Wang, Biochim. Biophys. Acta. 133 (1967) 369.
[15] C.H.Li, W-K.Liu and J.S.Dixon, J. Am. Chem. Soc. 88. (1966) 2050.
[16] R.E.Fellows, in: International Symposium on Growth Hormone, eds. A.Pecile, E.E.Müller and F.C.Greenwood, Excerpta Medica International Congress Series No. 142 (1967) p. 25.
[17] A.J.Parcells and C.H.Li, J. Biol. Chem. 233 (1958) 1140.
[18] C.H.Li and K.O.Pedersen, J. Biol. Chem. 201 (1953) 595.