Vol. 133, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 27, 1985 Pages 337-342

AMINO ACID SEQUENCE OF *MANDUCA SEXTA* ADIPOKINETIC HORMONE ELUCIDATED BY COMBINED FAST ATOM BOMBARDMENT (FAB) / TANDEM MASS SPECTROMETRY[1]

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Received October 14, 1985

Combination of Fast Atom Bombardment Tandem Mass Spectrometry with Amino Acid Analysis assigns the amino acid sequence of the *Manduca sexta* adipokinetic hormone as pGlu-Leu-Thr-Phe-Thr-Ser-Ser-Trp-GlyNH_2. Similarities and differences with other invertebrate hormones and with mammalian glucagon are discussed.

Peptide hormones control a wide range of physiological, biochemical and developmental processes in insects. In spite of their key role the primary structures of only a few insect peptide hormones have been elucidated up to now and among these are the following: Proctolin from cockroaches (1) which causes contraction of the hindgut; an adipokinetic hormone (AKH I) (2) from locusts which mobilizes lipids from fat body during flight; two different adipokinetic hormones (AKH II) from *Schistocerca gregaria* and from *Locusta migratoria* (3) which are likely to have

[1]Presented in part at the 1. GBF-MS workshop **New Trends in Molecular Biological Mass Spectrometry,** Gesellschaft für Biotechnologische Forschung, Braunschweig (F.R.G.), 19 - 20 September, 1985 and in part at the International Conference on **Novel Techniques in Protein Micro-Sequence Analysis,** Max Planck Institut für Molekulare Genetik, Berlin (F.R.G.), 2 - 3 October, 1985.

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Abbreviations: AKH, adipokinetic hormone; AKH II-L, adipokinetic hormone II from *Locusta migratoria*; AKH II-S, adipokinetic hormone II from *Schistocerca gregaria*; CC, cardioacceleratory peptide; FAB, Fast Atom Bombardment; M, myoactive peptide; MI, metastable ion; MSMS; Tandem Mass Spectrometry; RPCH, red pigment concentrating hormone.

0006-291X/85 $1.50

similar functions as AKH I; two myoactive peptides M I and M II from cockroaches (4,5) which are identical with both the cardioacceleratory peptides CC I and CC II isolated from cockroaches (6) and with the hypertrehalosemic hormones of cockroaches (6,7). M I is most likely to be identical with neurohormone D (8). In addition the N-terminus of the silkworm prothoracicotropic hormone is known (9).

All these hormones, except proctolin and prothoracicotropic hormone, appear to belong to the same peptide family and were all isolated from corpora cardiaca . Similar to AKH in locusts, AKH of *Manduca sexta* controls the mobilization of lipids during flight as shown recently (Schulz and Ziegler, in preparation). It is a novel **nona**peptide having an amino acid composition only slightly different from that of the other locust AKHs (10). Moreover extracts from *Manduca sexta* corpora cardiaca are active in locusts, although high concentrations are needed (11). This indicates that both peptides might belong to the same family of peptides.

Our interest in the understanding of the control of energy metabolism in *Manduca sexta* (12) and our wish to compare this peptide with other members of this family prompted us to sequence this nonapeptide. As the N-terminus is blocked we have used mass spectrometry to determine the sequence of *Manduca sexta* AKH. In recent papers (13,14) we have outlined a novel strategy for the sequencing of N-terminal blocked peptides, particularely pGlu containing peptides, by using Fast Atom Bombardment, FAB (15), as ionization technique, and Tandem Mass Spectrometry, MSMS (16), for the actual sequencing of mass selected ions

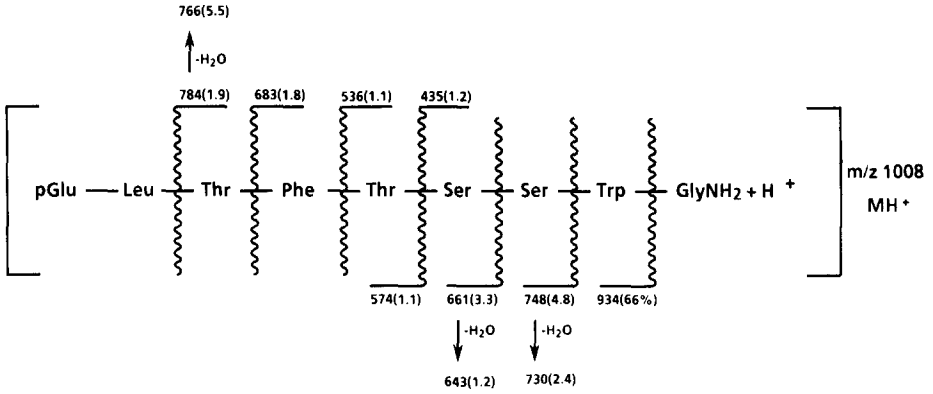
Materials and Methods

The rearing conditions of the tobacco hornworms used in the experiments were described earlier (10). The extraction and isolation of the AKH from corpora cardiaca has been reported previously (17).

The conditions for recording the FAB mass spectrum were identical with those described for the sequencing the locust AKH I using the same strategy (13). For recoding the metastable ion (MI) decomposition spectrum the instrument was set up to full transmission in order to achieve better sensitivity.

Results

Amino acid analysis from hydrolysates in hydrochloric acid and methane sulfonic acid yields the amino acid composition as Glx, Gly, Leu, Phe, 2x Ser, 2x Thr and Trp, which together with the signals at m/z 1008 (MH^+) and 1030 ($M + Na^+$) in



Scheme 1: Fragment ions of unimolecular decompositions of the mass selected metastable (MH^+) ion m/z 1008 of *Manduca sexta* AKH. Further sequence unspecific ions were observed at m/z 990 (100%, loss of H_2O from (MH^+)), 972 (6.2%, consecutive losses of two H_2O), 963 (3.5%, loss of CH_3NO), and 878 (3,3%, loss of C_9H_8N from the Trp side chain).

the FAB mass spectrum of an approximately 2 nmol sample (in glycerol) of the peptide gives a molecular weight of 1007 Dalton. Although some structure-indicative fragments appear in the FAB mass spectrum, a sequence assignment could not be achieved because of poor signal-to-noise ratio. MSMS, however, is known to increase significantly the signal-to-noise ratio mainly because it reduces chemical noise (16,18,19). In fact, the metastable ion decomposition spectrum of the mass selected (MH^+) ion, m/z 1008, contains all information necessary for a complete sequencing. The signals (given together with their relative abundances in Scheme 1) arise from the peptide sample and are not due to the glycerol matrix as shown in a control experiment. Interpretation of the signals is straightforward. The most abundant sequence-indicative fragment ion at m/z 934 is only compatible with loss of $GlyNH_2$ which defines the C-terminal amino acid at position 9 to be Gly; as the N-terminus must contain the pGlu unit, the sequence of *Manduca sexta* AKH reads pGlu-Leu-Thr-Phe-Thr-Ser-Ser-Trp-$GlyNH_2$. All attempts to arrive from the amino acid analysis and MS data sequences, which are different from that we suggest, failed.

Discussion

Manduca sexta AKH belongs to the same peptide family as the locust AKHs, M I and M II from *Periplaneta americana*, and the red pigment concentrating hormone (RPCH) from *crustacea* (20). *Manduca sexta* AKH is a blocked peptide like

TABLE 1: Amino acid sequences of related invertebrate peptide hormones and of the partial sequence 3 - 12 of glucagon

AKH I	pGlu - Leu - Asn - Phe - Thr - Pro - Asn - Trp - Gly - $ThrNH_2$
AKH II - S	pGlu - Leu - Asn - Phe - Ser - Thr - Gly - $TrpNH_2$
AKH II - L	pGlu - Leu - Asn - Phe - Ser - Ala - Gly - $TrpNH_2$
M I	pGlu - Val - Asn - Phe - Ser - Pro - Asn - $TrpNH_2$
M II	pGlu - Leu - Thr - Phe - Thr - Pro - Asn - $TrpNH_2$
RPCH	pGlu - Leu - Asn - Phe - Ser - Pro - Gly - $TrpNH_2$
Manduca sexta AKH	pGlu - Leu - Thr - Phe - Thr - Ser - Ser - Trp - $GlyNH_2$
Glucagon (3 - 12)	- Gln - Gly - Thr - Phe - Thr - Ser - Asp - Tyr - Ser - Lys -

the other members of this family of invertebrate peptides. It is unusual being a **nona**peptide as the other members are either **deca**peptides like AKH I (2) or **octa**peptides like M I and M II (4) and AKH II from *Locusta migratoria* and from *Schistocerca. gregaria* (3). The hypertrehalosemic factors from the stick insect, *Carausius morus*, which according to their amino acid composition are also members of the same family, are also **nona**peptides (21). For seven members of this family the amino acid sequences are now known. In four of them (AKH I, AKH II-S, AKH II-L and RPCH) the first four amino acids from the N-terminus are identical and in the other three (*Manduca. sexta* AKH, M I and M II) conservative exchanges were found. All the peptides have in position five a Thr or Ser which, again, is a conservative replacement. Position six shows the highest variability with four different amino acids found; these include nonpolar amino acids and amino acids with uncharged polar side chains. Position seven again shows only conservative exchanges. In position eight all peptides have Trp and two peptides have at position nine a Gly (Table 1). If we assume that the original peptide had a Ser in position seven, then all the differences found in the amino acid sequences of these peptides, can be accounted for by the mutation of single bases in the genetic code. The strongest resemblance between *Manduca sexta* AKH and any other member of the family is seen with M II (4,6).

Experiments with corpora cardiaca extracts may show that this similarity in structure is correlated with their biological activity. Extracts from corpora cardiaca of *Manduca sexta* have higher activity in the bioassay for glycogen phosphorylase activation in cockroaches (unpublished results) than in locusts (11). Similarities between M II (CC II) and glucagon (position 3 through 11) have been pointed out (6). The similarity between glucagon and *Manduca sexta* AKH is even stronger (Table 1). Glucagon-like substances have been detected by immunochemical methods in *Manduca sexta* (22-25). Glucagon itself, in high concentration (50 nmoles/kg), has been reported to have a glycogenolytic activity (23), while very high concentrations (5000 nmoles/kg) were needed to activate fat body glycogen phosphorylase (26). It seems as if the similarity in structure between *Manduca sexta* AKH and glucagon could explain these findings, but there are obvious discrepancies. Tager have isolated from the corpora cardiaca of *Manduca sexta* a glucagon-like peptide with more than 4 times the weight of *Manduca sexta* AKH. While this larger peptide could be a precursor of AKH, it is surprising that they did not find AKH (22). The presence of a glucagon-like substance has been demonstrated by immunohistochemical methods (25). This substance was found in two cell groups of the brain but not in the corpora cardiaca. The reaction was best with antisera specific for the N-terminus of glucagon. Similarities between *Manduca sexta* AKH and glucagon are located close to the N-terminus. It is reported that fibers from these brain cells reach the corpora cardiaca and although no glucagon-like material was found in the fibers, these authors assume that the glucagon-like material detected in the corpora cardiaca (23) is located in fibers coming from the brain. Therefor AKH or a precursor could be made in these cells within the brain. However, the information now avaliable from the study of locusts shed some doubt on this interpretaion. In locusts, AKH is synthesized in intrinsic neurosecretory cells of corpora cardiaca (27,28) and *Manduca sexta* corpora cardiaca also contain intrinsic neurosecretory cells (Hoff and Ziegler, unpublished results).

With the sequence of *Manduca sexta* AKH now known, it will be possible to get cDNA and also enough synthetic peptide to make antibodies. With these powerful tools it should then be possible to solve some of the questions mentioned

above and to tackle successfully problems concerning hormonal regulation of energy metabolism in *Manduca sexta*.

Acknowledgments

The authors would like to thank Dr. M. Kanost (Queen's University, Kingston Canada) and Dr. Karl Bauer (Technische Universität Berlin, F.R.G.) for critical reading of the manuscript. This work was supported by grants of the Deutsche Forschungsgemeinschaft to R.Z. (Zi 135/7-5), and H.S. (Sonderforschungsbereich 9, Projekt A9), and the Fonds der Chemischen Industrie.

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