

INSECT GLUCAGON-LIKE PEPTIDES: EVIDENCE FOR A HIGH MOLECULAR WEIGHT FORM IN MIDGUT FROM *MANDUCTA SEXTA* (L.)

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Abstract—Extracts of midgut from the tobacco hornworm *Manduca sexta* were examined for the presence of glucagon-related peptides. Although antisera specific for glucagon of molecular weight 3500 were not reactive with gut material, glucagon-like immunoreactivity was readily detected with an antiserum that reacts with high molecular glucagon-like peptides. Gel filtration of a partially purified extract identified the major immunoreactive component as a peptide of approximate molecular weight 15,000. It is concluded that the hornworm midgut, like its mammalian counterpart, contains a larger glucagon-like peptide which shows highly selective immunoreactivity.

Key Word Index: Glucagon-like peptide, immunoassay, midgut, tobacco hornworm, *Manduca sexta*

INTRODUCTION

THE IDENTIFICATION of glucagon-like peptides in extracts of mammalian gastrointestinal tissue was first reported by UNGER *et al.* (1961). Although early studies using so-called pancreas-specific and non-specific antisera tended to emphasize differences between the pancreatic and intestinal glucagons (SAMOLS *et al.*, 1966; EISENTRAUT *et al.*, 1968; ASSAN and SLUSHER, 1972), it now appears that the two are related chemically as well as immunologically (MOODY *et al.*, 1977; JACOBSEN *et al.*, 1977; TAGER and MARKESE, 1979). Recent studies suggest that the larger intestinal forms represent intermediates in the conversion of proglucagon to glucagon and that pancreas-specific antisera do not recognize glucagon-related peptides which have peptidyl extensions at their carboxyl-termini (MOODY *et al.*, 1977; TAGER and MARKESE, 1979; CONLON *et al.*, 1979). It has previously been reported that neurosecretory tissue and haemolymph from the tobacco hornworm, *Manduca sexta* L. contain a peptide (molecular weight 3500) which is reactive with pancreas-specific glucagon antisera (TAGER *et al.*, 1975, 1976; KRAMER *et al.*, 1980). This hormone-like peptide also possesses the expected intraspecific biological activity in promoting glycogenolysis (TAGER *et al.*, 1976). In order to extend the comparison of vertebrate and invertebrate glucagons, the insect gut has been examined for the

presence of immunoreactive peptides. This report identifies a large glucagon-like peptide in the midgut of the tobacco hornworm and shows that it is immunologically related to vertebrate intestinal glucagon.

MATERIALS AND METHODS

Insects. *Manduca sexta* eggs were obtained from Dr. J. P. REINECKE (Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Fargo, North Dakota), and larvae were reared on a standard agar-based diet (BELL and JOACHIM, 1976) at 28°C and 60% relative humidity with a 16-hr light; 8-hr dark photoperiod. Fifth instar larvae (5–8g) were anesthetized by cooling and midguts were dissected by a standard technique (SCHNEIDERMAN, 1967). The midguts were flushed free of contents with 0.15 M NaCl and were immediately frozen on dry ice and lyophilized.

Tissue extraction and analysis. Finely pulverized, freeze-dried tissue (307 midguts, 30 g) was homogenized in 150 ml of 0.1 M HCl prepared in 60% ethanol. The homogenate was centrifuged and the supernatant fluid was collected and adjusted to pH 6 using 6 M NH₄OH. The solution was then diluted with 2 vol of ethanol and 4 vol of diethyl ether. After the mixture had incubated at 4°C for 18 hr, the precipitate was collected by centrifugation at 5000 g for 30 min at 4°C and was dissolved in 5 ml of 3 M acetic acid. This sample was applied to a 2.5 cm i.d. × 90-cm column of BioGel P-30‡ previously equilibrated with the same solvent and 3-ml fractions were collected. Aliquots (0.2 ml) of the fractions were dried at 21°C under vacuum and the residues were dissolved in the previously described buffer prior to radioimmunoassay (TAGER *et al.*, 1976). The pancreas-specific antiglucagon serum (previously designated R-1), the carboxyl-terminus-specific antiglucagon serum (previously designated CTS) and the centrally directed§ antiglucagon serum (previously designated C-1 or NS) were used as described (TAGER *et al.*, 1977; TAGER and MARKESE, 1979).

‡ (Mention of a proprietary product does not constitute an endorsement by the U.S. Department of Agriculture over others that may be suitable.)

§ (The term *centrally directed* is meant here to define an antiglucagon serum which reacts with pancreatic glucagon (molecular weight 3500) and also high molecular weight forms that have extensions at the amino- and carboxyl-termini, c.f. TAGER and MARKESE, 1979.)

RESULTS AND DISCUSSION

Preliminary experiments undertaken to validate our extraction procedure showed that an ethanol-ether precipitate obtained from midgut tissue of the tobacco hornworm, but not one obtained from a corresponding amount of fat body, contained a peptide that reacted with an antiserum specific for glucagon related peptides. In order to remove salts or other material potentially interfering in the radioimmunoassay, a partially purified extract of midgut was gel-filtrated on BioGel P-30 with a typical result shown in Fig. 1. The void volume of the column occurred at fraction 33 and the salt volume at fraction 135. The high adsorbance seen in the latter part of the profile is due to an unidentified brown pigment which was present in the sample. As shown in the figure, radioimmunoassay using the centrally directed antiserum revealed a major peak of glucagon-like material centred at fraction 59. Application of protein standards to the same column indicated that this immunoreactive peptide has a molecular weight of approximately 15,000. The degree of heterogeneity suggested by the smaller amounts of material present in fractions 40-50 and 70-90 is reminiscent of that seen in extracts of mammalian intestine (TAGER and MARKESE, 1979). The yield of immunoreactive material (approximately 0.2 ng-equivalents of glucagon equivalents per gram of fresh tissue) was only 1% of that usually obtained from mammalian intestine (MOODY, 1972; TAGER, and MARKESE, 1979). This estimate should be regarded as a lower limit, however, since the affinity of the antibody for the insect peptide may be low.

To examine the structural and immunological characteristics of the midgut glucagon-like peptide in greater detail, aliquots of the fractions shown in Fig. 1

were subjected to radioimmunoassay using two pancreas-specific antiglucagon sera. No immunoreactive material could be detected in the gel-filtrated gut extract using antiserum R-1, which reacts only with glucagon of molecular weight 3500, or using antiserum CTS, which reacts selectively with the carboxyl-terminal undecapeptide of glucagon. These findings are in accord with the known specificities of these antisera in mammalian tissues: whereas both tissue-specific and non-specific antisera react with glucagon of molecular weight 3500, only the latter react with higher molecular weight forms. Furthermore, the preponderance of these larger peptides in midgut of *M. sexta* is consistent with the size distribution of glucagon-related peptides in the mammalian intestine (MOODY, 1972; TAGER and MARKESE, 1979).

Thus, it is evident that a high molecular weight glucagon-like component predominates in the midgut of *M. sexta*, whereas a low molecular weight form predominates in neurosecretory tissue and haemolymph (TAGER *et al.*, 1975, 1976; KRAMER *et al.*, 1980). As is the case for the mammalian intestinal peptide (FALOONA and UNGER, 1974) the physiological function of the midgut glucagon-like peptide in the hornworm is unknown. It is likely that the insect peptide of molecular weight 15,000, like its mammalian counterpart, represents a precursor of the lower molecular weight hormone and that its structure is represented by that of the hormone extended both at its carboxyl and amino-termini (MOODY *et al.*, 1977; TAGER and MARKESE, 1979). Although the midgut peptide has not yet been isolated, the present results identify physical and immunological similarities in glucagon-related peptides from the the gut of *M. sexta* and the intestines of higher vertebrates. It would appear, then, that the evolutionary origin of glucagon-

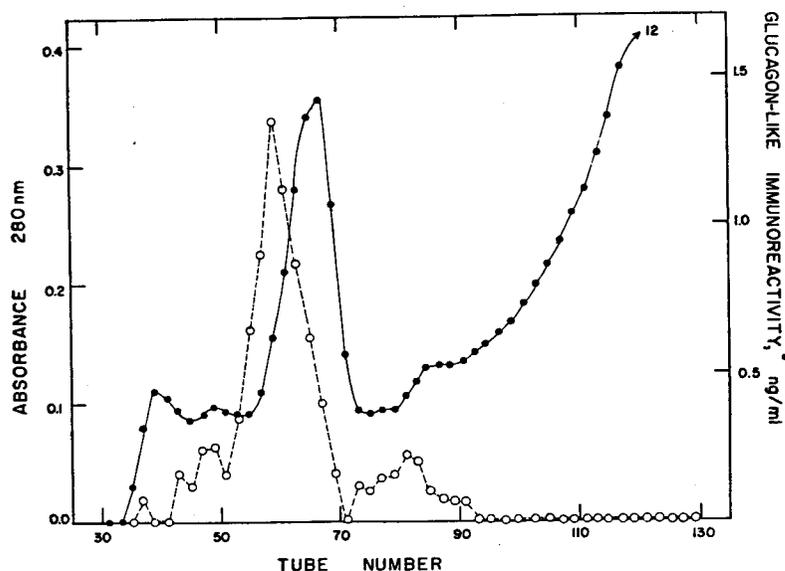


Fig. 1. Gel filtration of an acid-ethanol extract of midgut from *M. sexta* on BioGel P-30. The figure shows optical absorbance at 280 nm as a measure of protein concentration (●) and glucagon-like immunoreactivity using an antiserum specific for the central portion of glucagon-related peptides (○). The column was calibrated by the application of standard proteins including bovine serum albumin (Research Products, Elk Grove Village, IL) soybean trypsin inhibitor (Sigma Chemical, St. Louis, MO), hen lysozyme (Worthington Biochemical, Freehold, NJ) and bovine insulin (Sigma). Details for the preparation of the sample and for the radioimmunoassay are provided in Materials and Methods.

like peptides in the intestinal tract (FALKMER and MARQUES, 1972; FALKMER *et al.*, 1975) antedates the divergence of insects from other animals in the phylogenetic scheme.

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