CRUSTACEAN ERYTHROPHORE-CONCENTRATING HORMONE AND LOCUST ADIPOKINETIC HORMONE IN CRUSTACEA AND INSECTS: DETECTION, ISOLATION AND BIOASSAY[1/]

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MINI REVIEW

Reverse phase-high performance liquid chromatography (rp-hplc) has been applied to the analyses of synthetic crustacean erythrophore-concentrating hormone (CECH, p-Glu-Leu-Asn-Phe-Ser-Pro-Gly-Trp-NH_2) and locust adipokinetic hormone (LAKH, p-Glu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2) (Jaffe and Hayes 1972a). Both CECH and LAKH were analyzed on several different reverse phase columns by means of gradient elution with the following buffers: 0.01M KH_2PO_4, 0.1% H_3PO_4, 0.1% trifluoroacetic acid (TFA), or 0.25N triethylammonium phosphate (TEAP) at pH 2.20, versus acetonitrile. Ultraviolet absorbance was monitored at two wavelengths 254 and 195 nm or 254 and 210 nm in the case of TFA buffer, permitting the determination of absorbance peak ratios for positive confirmation of peptides. Application of a computerized background correction was sometimes employed to correct excessive baseline drift during the course of the gradient run.

By use of the Supelcosil and LC-18DB Column and the TEAP buffer system 4 ng of LAKH or 2 ng of CECH were detectable at 195 nm. Approximately 400 pg of CECH and LAKH could be detected by use of fluorescence detection (excitation 280 nm, emission 340 nm) of the endogenous tryptophan present in these peptides.

A rp-hplc method was employed to detect and isolate CECH from tissue homogenates of the shrimp, Paleomenetes pugio (Jaffe et al. 1982b), and the lobster, Homarus americanus (Jaffe et al. 1982c). Tissue homogenates were subjected to preliminary clean-up followed by sequential gradient elution on two different reverse phase systems. CECH was identified by its retention times and absorbance peak ratio. CECH, which remained after removal of volatile TFA buffer, gave a positive response in the bioassay of erythrophore-concentrating activity in the shrimp, Paleomenetes pugio.

Current work involves detection and isolation of LAKH and similar hormones from central nervous tissue of the face fly, Musca autumnalis, by rp-hplc methods.

LITERATURE CITED

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1/ Mention of a commercial product in this paper does not constitute an endorsement of this product by the USDA.