ACID PROTEINASE OF HYPOTHALAMUS
Purification, Some Properties, and Action on
Somatostatin and Substance P

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In a continuing study of the physiological role of protein breakdown in the
hypothalamus, acid proteinase from bovine hypothalamus was purified about
1000-fold. The molecular weight of the enzyme was approximately 50,000.
Maximal activity against hemoglobin was obtained at pH 3.2-3.5; serum albumin
was split much more slowly. Hypothalamus acid proteinase was partially
inhibited by β-phenyl pyruvate, or benzethonium Cl, and was completely
inhibited by low concentrations of pepstatin. This proteinase splits somatostatin,
substance P, and analogs of substance P. The probable sites of enzyme action on
these peptides were determined by the end group dansyl technique. The enzyme,
most likely cathepsin D, may play an important role in the formation and
breakdown of peptide hormones in the hypothalamus.

INTRODUCTION

The metabolism of peptide hormones (synthesis, storage, degradation)
plays an important part in the control of numerous physiological
mechanisms. In recent years, many studies showed that possibly most
peptide hormones derive from larger-molecular-weight protein precur-
sors. In turn, the hydrolysis of some peptide hormones results in the
formation of new physiologically active hormones. For example, oxyto-
cin serves as a precursor for the factor inhibiting the release of
melanotropin (MSH-RF-IF) and for leucylglycinamide, a dipeptide with an effect on dopaminergic transmission (1). Endo- and exopeptidases influence hormone action also by degrading the carrier proteins of hormones, for example, neurophysins I and II (2). The importance of peptide metabolism in the hypothalamus is further underscored by the finding that several peptides act as hormone-releasing factors (3,4), by the finding of the formation from specific hypothalamic proteins by pepsin or trypsin of a factor causing dilation of coronary arteries (5), and by the finding that extracts of hypothalamus inactivate peptides such as substance P, bradykinin, and vasopressin (6).

We are studying the possibility that specific peptide hydrolases in the hypothalamus regulate various hormone levels and activities. We have previously described the distribution of proteases, peptidases, and arylamidases in the hypothalamus (7).

In the present study, we report our observations on the properties of purified acid proteinase (E.C. 3.4.2.3.) from bovine hypothalamus, including its action on somatostatin, substance P, and substance P analogs.

**EXPERIMENTAL PROCEDURE**

Hypothalamus tissue was separated from bovine brain and frozen at −20°C within a few hours of death. SP-Sephadex C-50 (fine grade), Sephadex G-100 (fine grade), and DEAE-Sephadex (fine grade) were obtained from Pharmacia (Uppsala, Sweden). The inhibitors, phenylmethyl sulfonyl fluoride (PMSF), t-1-tosyl-amino-2-phenylethyl chloromethyl ketone (TPCK), β-phenylpyruvate, and benzethonium chloride were from Sigma (St. Louis, Missouri). Pepstatin (isovaleryl-L-valyl-L-valyl-4-amino-3-hydroxy-6-methylheptanoyl-L-alanyl-4-amino-3-hydroxy-6-methylheptanoic acid) was kindly supplied by Dr. H. Umezawa (Institute of Microbial Chemistry, Tokyo, Japan). Somatostatin was a gift from Dr. R. Guillemin (Salk Institute, San Diego, California). Analog of substance P were supplied by Dr. P. Oehme (Akademie der Wissenschaften der DDR, Berlin). Other materials used were denatured hemoglobin (Worthington, Freehold, New Jersey), albumin (Biomed, Krakow), and Triton X-100 (Ferak, Berlin).

**Enzyme Assay**

A typical assay of acid proteinase was performed in 2 ml of a reaction mixture containing 100 μmol of sodium citrate buffer (pH 3.2), 10 mg of denatured hemoglobin, and a sufficient amount of enzyme. In some experiments acid-denatured bovine serum albumin (8) was used as substrate. Incubation was carried out at 37°C for 1 h in a shaking bath. The reaction was stopped by adding 0.5 ml of 30% (w/v) trichloroacetic acid. Acid-soluble products were measured after centrifugation by three methods: (1) absorption at 280 nm; (2) the modified Folin procedure (9); (3) the ninhydrin procedure (10). Controls were the identical reaction mixture at zero time. The incubation mixture contained 50–200 millunits