THE HYDROLYSIS OF DIPEPTIDES AND AMINOACYL NAPHTHYLAMIDES BY THE HUMAN SEMINAL PLASMA

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Summary. High dipeptidase and aminopeptidase activity is present in human seminal plasma. The two activities can be easily and clearly separated from each other by chromatographic procedures. Each of the three dipeptidase fractions has characteristic substrate specificity but is not affected by divalent metals. The seminal aminopeptidase activity is inhibited by EDTA and activated by cobalt.

Among the enzymic constituents of the human seminal plasma is a number of proteases (Mann, 1964). Some of them have been partially purified and characterized by Lundquist (1953), who described an aminopeptidase, which readily hydrolyses both di- and tripeptides to leucylpeptides, without any preference. During recent years, however, several new chromogenic substrates have been introduced to the study of proteolytic activity (Nachlas, Goldstein & Seligman, 1962), and the seminal aminopeptidase has been shown to hydrolyse some of the newly synthesized aminoacyl naphthylamides (Krampitz & Doepfmer, 1961), as well.

In the course of a study on the seminal proteases, we have been able to demonstrate that, contrary to the previous reports, the dipeptidase and aminopeptidase activities are well separated in the human seminal plasma. Pooled semen from an infertility laboratory was used. It was fractionated by DEAE-cellulose chromatography or Sephadex G-100 gel filtration. Semen samples were diluted with equal volumes of the elution buffer (20 mM-tris-HCl buffer, pH 7-6). Aminopeptidase activity was estimated using a number of l-amino acid naphthylamides as substrates and determining the amount of naphthylamine liberated with p-dimethyl-aminobenzaldehyde. The hydrolysis of the dipeptides was followed with a pH-stat (Jacobsen, Léonis, Linde-strem-Lang & Otteson, 1957).

In DEAE-chromatography, the aminopeptidase activity was clearly separate from the dipeptidase activity (Text-fig. 1). The latter was present in three different chromatographic fractions which could also be obtained by filtration of seminal plasma through a Sephadex G-100 column. The hydrolysis rate of various dipeptides (gly-gly, gly-leu, gly-phe ala, ala-gly, pro-gly) by the fractions varied considerably (Text-fig. 2), but neither EDTA nor any of the
divalent metal ions tested (Mg, Mn, Zn, Ca and Co) had any significant effect on the activity of the dipeptidase fractions.

The aminopeptidase activity showed two activity peaks in DEAE-cellulose chromatography (Text-fig. 1). When the sodium chloride gradient was made
slower, the activity maxima became clearly separated. On the other hand, when the salt gradient was made steeper in the end of the chromatography, a third separate fraction became evident. The rate of hydrolysis was different for each substrate by every fraction. However, the relative hydrolysis rates were similar in every fraction. Alanyl-NA was the most readily hydrolysed substrate followed by leucyl-NA and arginyl-NA. Valyl-NA and tyrosyl-NA were only poorly hydrolysed and gamma-glutamyl-NA did not split at all.

The aminopeptidase activity was inhibited 50% by 1 mM-EDTA, and a 10 mM concentration produced complete inhibition. Of the divalent metals, cobalt (1 mM) was the only one which brought about an activation.

When seminal peptidases were first investigated, it was thought that seminal aminopeptidase hydrolysed both dipeptides and tripeptides (Lundquist, Thorsteinsson & Buus, 1955). The earlier work was based on the ammonium sulphate precipitation of enzyme proteins, which probably could not effectively separate the enzymes. The data presented here demonstrate that the enzymes have clearly separate activities. The dipeptidase activity seems to be composed of subfractions with some degree of substrate specificity, and the aminopeptidase activity might be composed of several isoenzymic forms as suggested by the immunological investigations of Mattila (1968).

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REFERENCES


