



## CHYMOTRYPSIN I FROM *PENAEUS CALIFORNIENSIS*

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**Introduction.** Besides being useful as food and delicatessens, crustaceans like crabs, lobsters, prawns, crayfish and shrimps are models to understand basic biology as how digestive enzymes work and as potential biological reagents in biotechnologies (1-2).

A chymotrypsin from shrimp *Penaeus californiensis* was purified to homogeneity as demonstrated by mass spectrometry, NH<sub>2</sub>-terminal amino acid analysis, and electrophoresis-silver staining, characterized and compared to the prototype bovine chymotrypsin. The enzyme peculiar characteristics called for a detailed structure-function analysis.

**Methods.** The enzyme was taken from the digestive gland and purified by affinity chromatography. It was characterized for catalytic and molecule properties, and the effect of environmental and processing variables on activity assessed. The deduced sequence of the protein was obtained from mRNA. All properties were compared to the prototype bovine chymotrypsin.

**Results.** Shrimp chymotrypsin was purified to homogeneity. During storage, the pure chymotrypsin autohydrolyzed, yielding a lower molecular mass molecule that keeps catalytic activity. Table 1. shows some properties of the bovine chymotrypsin and shrimp chymotrypsin. The enzyme, in spite of being a serine peptidase and hence sharing the scaffold of two  $\beta$ -barrels, hydrolyze collagen, while the bovine one didn't.

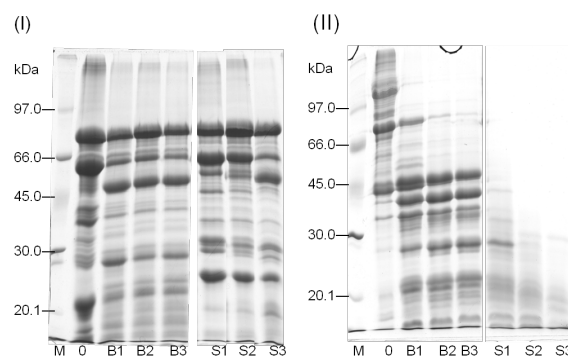
Unlike bovine, shrimp chymotrypsin doesn't retain their activation propeptide and mature protein begins at residue Ile<sup>16</sup>. Shrimp chymotrypsinogen has a putative activation peptide of 30 amino acid residues, including a trypsin Arg cleavage. Instead of the second step of bovine chymotrypsinogen self-activation where Tyr<sup>146</sup> and Asn<sup>148</sup> are released, a single and continuous polypeptide forms the mature shrimp chymotrypsin in contrast with the three polypeptides interconnected by disulphide bridges in bovine chymotrypsin.

The molecular mass of the shrimp chymotrypsin by electrophoresis was of 27 kDa. When the molecular mass was derived from the deduced primary it was found to be of 23.72 kDa. The theoretical pI of the deduced protein was 4.87 due to high content of Asp and Glu amino acid residues. Catalytic variables of the shrimp chymotrypsin were compared to those of bovine one. Shrimp chymotrypsin cDNAs encode for Ser<sup>189</sup> at the bottom of the S1 pocket. This structural composition at the binding site confirms the shrimp chymotrypsin nature of the purified enzyme. However, loops interactions important to substrate recognition are highly homologous with other crustacean serine proteases, making them with broader substrate specificity (Fig. 1).

Table 1. Kinetic parameters for the hydrolysis of SAAPFNA at pH 8 for bovine (*Bos taurus*) and shrimp (*P. californiensis*) chymotrypsin.

Species	pI	Molecular weight (kDa)	K <sub>M</sub> ( $\mu$ M)	K <sub>cat</sub> (seg <sup>-1</sup> )	K <sub>cat</sub> /K <sub>M</sub> ( $\mu$ M <sup>-1</sup> seg <sup>-1</sup> )	Reference
<i>P. californiensis</i>	3.6	27.0	0.12	21.5	176	Present work
<i>Bos taurus</i>	8.7	25.0	0.08	60.0	750	Present work
<i>P. vannamei</i>	3.1	33.2	1.60	15.5	10	3
<i>P. monodon</i> II	3.0	25.0	0.56	78.0	139	4

**Table 1.** Properties of bovine chymotrypsin and shrimp chymotrypsin I. Substrate is succinyl-Ala-Ala-Pro-Phe-p-nitroanilide.



**Fig.1** Hydrolysis of proteinaceous substrates. I. BSA, B1-B3 bovine chymotrypsin; S1-S3 shrimp chymotrypsin. II. Collagen, B1-B3 and S1-S3 same as above

**Conclusions.** Shrimp chymotrypsin differs in several molecular and catalytic properties, making it suitable to investigate phylogeny and the structure-function relationship. Due to its capacity to hydrolyze all sort of protein, including collagen, it is suited for further analysis for biotechnologies, mostly in food technology.

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