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Digestive Enzymes Complement of Freshwater Prawn *Macrobrachium idella* (Heller)

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Abstract

Hepatopancreas of the freshwater prawn, *Macrobrachium idella* was analysed for activities of different digestive enzymes. The carbohydrases were detected by employing substrates with α and β glucosidics, and α and β galactosidic linkages. Polymeric substrates and complex polymeric substrates with β glucosidic linkage were also employed. Proteases such as trypsin, chymotrypsin, pepsin, carboxypeptidase A, carboxypeptidase B and leucine aminopeptidase showed high levels of activity and were assayed using specific synthetic substrates. High level of esterase and trace of lipase activity were detected. Based on this enzyme profile, *M. idella* can be classified as an omnivore. Further, it implies that digestive enzymes are not limiting factor in utilization of the ingested feed. No relationship between enzyme and sex or size was noticed.

Introduction

The prawn, *Macrobrachium idella* (Heller) is a candidate species for aquaculture. Analysis of digestive enzymes serves as an efficient tool in the assessment of digestive ability and design of optimal diet for aquacultural practices.

Earlier reports on the digestive enzymes of crustaceans were mainly qualitative and comparative (van Weel, 1970; Dall and Moriarty, 1983). Since crustaceans gained considerable importance in commerical culture practices in recent times, the changes in enzyme activities during the life cycle, adaptation to new diets and quantitative studies of digestive enzymes have become the focal point of interest (Fair *et al.*, 1980; Galgani *et al.*, 1984). The present investigation attempts to assess the specific digestive capabilities of *M. idella* by quantitative analysis of digestive enzymes in the hepatopancreas.

Materials and Methods

M. idella (♀, ♂ 70-100 mm) obtained from cast net catches in Cochin were maintained with continuous aeration at room temperature (27-30°C) upto two weeks. They were fed with formulated feed NPCL/225 daily. The hepatopancreas of each prawn was homogenized, centrifuged and used for assay. Carbohydrases were analysed according to Chiu and Benitez (1981). Trypsin and Chymotrypsin were assayed according to Hummel (1959). Peptic activity was determined as described by Ric and Fritsch (1974). Carboxypeptidase-A was measured by the method of Folk and Schrimer (1963), carboxypeptidase B by (Folk *et al.*, 1960), and leucine amino-peptidase according to Tuppy *et al.*, (1962). Esterase and lipase activities were determined by the method of Nachlas and Seligmen (1949) and the protein content by the method of Lowry *et al.*, (1951). All enzyme activites are reported as specific activities (amount of substrate hydrolysed or end product formed/min/mg protein) or enzyme units (unit/mg protein/min).

Results and Discussion

The results indicated the presence of hydrolytic enzymes. Specific activities of the various carbohydrases are presented in (Table 1). Hepatopancreas extract hydrolysed substrates with α glucosidic linkages rapidly than with β glucosidic linkages. The hydrolysis of substrates with α glucosidic linkages of different structural types and the synthetic substrate p-nitrophenyl- αD-glucoside indicates the presence of a general α glucosidase. In addition, the rapid hydrolysis of dextrin, maltose, sucrose and trehalose indicates the presence of specific enzymes dextrinase, maltase, sucrose and trehalase. The hepatopancreatic extract of *M. lamarrei* exhibited high activity of trehalase (Murthy, 1978). High amount of hydrolytic activity towards dextrin, maltose, sucrose and trehalose was also detected in the hepatopancreatic extract of *Penaeus indicus* (unpublished data). The weak hydrolysis of cellobiose and salicin and minimal hydrolysis of P- nitrophenyl β D-glucoside indicate the presence of β glucosidase with limited substrate specificity.

An insignificant activity on raffinose and melibiose indicated the presence of a galactosidase with limited substrate specificity. Similar activity was recorded in *Carcinus maenas* and *Crangon crangon* (Kristensen, 1972). However, specific raffinase was detected in *Marinogammarus* (van Weel, 1970). *Astacus fluviatilis* exhibited a general α glactosidase activity (van Weel, 1970). Weak activity on lactose and strong activity on p-nitrophenyl β D-galactopyranoside indicated the presence of a general β glactosidase. Though it is believed that lactase is not secreted by the midgut gland of crustaceans (van Weel, 1970) there are reports on its presence in *Carcinus maenas*, *Crangon crangon* (Kristensen, 1972), *Corphium volutator* and *Astacus fluviatilis* (van Weel, 1970).

Table 1. The specific activities of the digestive carbohydrases in the hepatopancreas of *M. idella*.

Substrate	Enzyme activity[a] (µmol glucose liberated/mg protein/min)
α-glucosides	
Trehalose	108.30 ± 70.38
Maltose	399.87 ± 160.06
Sucrose	149.96 ± 80.90
Dextrin	635.92 ± 270.76
P-nitrophenyl α-D glucoside	466.68 ± 190.32[b]
β-glucosides	
Cellobiose	22.22 ± 10.36
Salicin	48.87 ± 23.68
P-nitrophenyl β-D glucose	126.68
α-galactosides	
Raffinose	2.78 ± 1.98
Melibiose	55.54 ± 26.29
β-galactosides	
Lactose	61.09 ± 27.32
P-nitrophenyl β-D galacto pyranoside	400.00 ± 186.23[b]
Polymeric substrates	
Starch	722.84 ± 316.18
Glycogen	583.16 ± 282.23
Complex polymeric substrates with β-glucosidic linkages	
Microcrystalline cellulose	0
Carboxymethyl cellulose	11.10 ± 6.80

a. the activities are reported as the mean ± standard deviation (n=6)

b. the activities are expressed as nmol p. nitrophenol released/mg/min in the assay conditions.

Activity towards starch and glycogen was high. Amylase activity was reported in crustaceans (van Weel, 1970; Dall and Moriarty, 1983). Among the carbohydrases, amylase was the major enzyme with prominent activity in *M. idella*. This suggests that probably these enzymes play an important role in the energy metabolism of this prawn as starch could be efficiently broken down to sugars. Tyagi and Prakash (1967) have observed high specific activities for several carbohydrases including amylase in the prawn *M. dayanam*. Murthy (1978) recorded many carbohydrases in the digestive system of *M. lamarrei* and the amylase was specifically characterised with reference to several factors by Saxena and Murthy (1981). The high amylase activity was also detected in *M. rosenbergii* (Lee *et al.*, 1980) and it was characterised in *Parapenaeopsis hardwickii* and *P. stylifera* (Kulkarni *et al.*, 1979) and in *Penaeus indicus* and *Metapenaeus monoceros* (Karunakaran and Dhage, 1977).

The hepatopancreas of *M. idella* contained proteinases such as trypsin, chymotrypsin, pepsin, carboxypeptidase A and B and leucineaminopeptidase (Table 2). All these enzymes were reported earlier in *M. rosenbergii* (Lee *et al.* 1980) and in other decapods (Dendinger, 1987). In *M. lamarrei*, trypsin, aminotripeptidase, leucine amino peptidase and glucyl-L-leuine dipeptidase were detected, but not chymotrypsin (Murthy, 1977). It is interesting to note that the absence of chymotrypsin in *M. lamarrei* and its presence in *M. rosenbergii* and *M. idella* suggest differences in enzyme composition within the genus *Macrobracdhium*. A similar situation was described for *Penaeus* spp. (see Gaigani *et al.*, 1984).

Peptic activity was detected in *M. idella* which is in agreement with the studies of Lee *et al.* (1980). Carboxypeptidase A and B and leucine aminopeptidase (Table 2) activity were detected in *M. idella*. These three exopeptidases were reported in *M. rosenbergii* (Lee *et al.*, 1980), *P. japonicus* (Galgani *et al.*, 1984) and *Callinectes sapidus* (Dendinger, 1987) while recording carboxypeptidase A and B in *P. setiferus* (Gates and Travis, 1973) and leucine aminopeptidase in *M. lamarrei* (Murthy, 1977). The wide range of endo and exopeptidases in *M. idella* suggest an efficient enzyme system which can hydrolyse complex protein enabling the prawn to secure all essential dietary amino acids from any diet composed of various proteins.

In *M. idella* acetate chains were more radily hydrolysed than laurate chains while hydrolysis of stearate chains was negligible (Table 2).

Table 2. The specific activities of the digestive proteases and lipases in the hepatopancreas of *M. idella*

Enzyme	Substrate	Mean±S.D.[b]	Units
Trypsin	TAME	59.8 ± 25.6	Unit/mg protein[a]
Chymotrypsin	BTEE	86.4 34.2	Unit/mg protein[a]
Pepsin	APAIT	0.02 ± 0.01	µmol/mg/min
Carboxypeptidase A	HPA	9.50 ± 3.28	µmol/mg/min
Carvoxypeptidase B	HA	4.00 ± 2.30	µmol/mg/min
Leucineaminopeptidase	LNA	$12.13 \times 10^{-3} \pm 5.18 \times 10^{-3}$	µmol/mg/min
	β-naphthyl acetate	5.4 ± 2.8	µg β-napthol/ min/mg protein
	β-naphthyl laurate	1.8 ± 0.98	
	β-naphthyl stearate	Trace	µβ-napthol/min/ mg protein

a. unit of activity is equal to the amount of enzyme that is necessary to increase the absorbance 0.001 optical density units per minute.

b. the activity are reported as mean ± standard deviation (n=6)

This observation supports the earlier reports on *M. lamarrei* (Murthy, 1977) and *M. rosenbergii* (Lee *et al*., 1980).

The present study suggests that *M. idella* is gifted with an efficient machinery to utilize complex carbohydrates. Hence carbohydrates can spare protein to a certain extent and hence the cost of prawn feed for this species can be brought down by including cheap carbohydrate sources and keeping the costlier protein level just adequate to maintain normal growth. Further it can be inferred that, since all the three groups of enzymes are well represented, the prawn *M. idella* can be considered as an omnivore capable of utilising all types of ingredients in the feed efficiently and hence offers most economic food to product conversion in aquaculture.

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