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Hepatopancreatic Amylase of *Macrobrachium lamarrei* (Crustacea: Decapoda)

PADMA SAXENA and RAMESH CHANDRA MURTHY
Department of Zoology, University of Lucknow, Lucknow 226007

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The effect of nine factors was tested on hepatopancreatic α-amylase of *Macrobrachium lamarrei*. Its optimum *p*H was 6.5 and optimum temperature 50°C. Maltose, the end product, was ineffective up to 0.2% but slightly inhibitory above it. The amylase activity was constant up to 20 min. declined thereafter up to zero at 50 min. Increase of enzyme concentration produced a similar effect. Its *Km* value is 9.0×10^{-2}%. Dialysis suppressed its activity by 15%. Six chlorides (of Li, Mg, K, Mn, Ba, Cd) were activatory at all six strengths and ten (Ca, Rb, Sr, Hg, Co, Sn, Zn, Na, Pt, Cu) were ineffective or activatory, depending upon the concentration. $AlCl_3$ alone produced an inhibitory effect at 1×10^{-2}M, besides being ineffective at 5×10^{-5}M and activatory at other strengths. Three amino acids (asparagine, β-alanine, glycine) were ineffective or activatory, three (glutamine, norleucine, phenylalanine) were both activatory and inhibitory, one (leucine) produced all three effects, depending upon the concentration, and the remaining 12 were activatory at all six strengths.

Key Words: Crustacea, Decapoda, *Macrobrachium lamarrei*, **Hepatopancreatic α-amylase**

Introduction

It is a well-known fact that the enzyme amylase (E.C. 3.2.1.1) occurs in the hepatopancreatic gland of a wide variety of crustaceans (Lockwood 1968, van Weel 1970, Vonk 1960). Yet information on the kinetic properties of hepatopancreatic amylase is scanty, being confined to its response to *p*H and temperature (Agarwal 1963, 1964, Ammal 1966, Bernice 1971, Brun & Wojtowicz 1976, Chinnayya 1968, Gopalakrishnan 1957, Hoyle 1973, Martin 1966, Nagabhushanam & Sarojini 1968, Newcomer 1956, Takahashi et al. 1964, Wojtowicz & Brockerhoff 1972). To obtain a deeper knowledge of the properties of crustacean amylase, effect of nine factors was studied on this carbohydrase from the hepatopancreas of *Macrobrachium lamarrei*, a fresh water shrimp, reported by Murthy (1978) to be rich in α-amylase.

Material and Methods

Hepatopancreatic gland from 100 *Macrobrachium lamarrei* Milne Edward was pooled in ice-cold distilled water, dried between filter paper, weighed and homogenised. The

homogenate was centrifuged at 3000×g for 15 min at 4°C and the supernatant diluted with water to a concentration of 0.1 mg/ml (wet weight) (or 0.1 ml≡0.1 mg) of hepatopancreas. The assay system for seven factors (*p*H, temperature, end product, incubation period, substrate and enzyme concentration and dialysis) consisted of: appropriate buffer, 0.2 ml; 0.5 M NaCl solution, 0.1 ml; 1% starch solution, 0.2 ml (all pre-incubated at 37°C for 10 min); and enzyme extract 0.1 ml. For two factors (chlorides and amino acids), 0.1 ml of their solution was substituted for 0.5M NaCl in the assay system. After incubating at 37°C for 45 min, 0.2 ml of N HCl was added to stop the reaction. To controls, enzyme extract was added after HCl stage. Thereafter, following the colorimetric method of Smith and Roe (1949), 0.1 ml of iodine reagent was added to the mixtures, the volumes raised to 6 ml with distilled water, and readings taken at 620 nm.

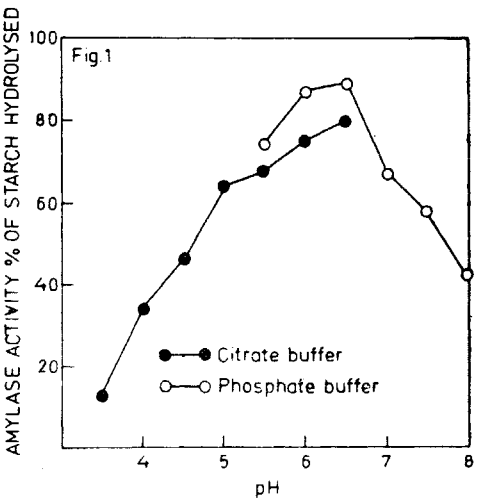

Figure 1 Effect of *p*H on the hepatopancreatic amylase

Observations

Determination of optimum *p*H: The results using two buffer series 0.1M sodium citrate-HCl buffer (*p*H 3.5-6.5) and 0.1M Sorenson's phosphate buffer (*p*H 5.5-8.0) show that amylase remained quite active at *p*H 5.0-7.5 —being optimum at *p*H 6.5 (figure 1). While the optimum *p*H with both the buffers coincided, its activity with citrate buffer was 10.82% less compared to phosphate buffer.

Effect of temperature: Amylase activity was tested at 10 temperatures ranging from 10 to 80°C (figure 2A). Although it remained fairly active from 30 to 60°C, optimum activity occurred at 50°C.

Effect of end-product: To the assay system, 0.2 ml of maltose solution of ten strengths, increasing by 0.05% was added. The concentrations of 0.05 to 0.2% were ineffective but above these strengths a slight inhibition in the enzyme activity was evident (figure 2B).

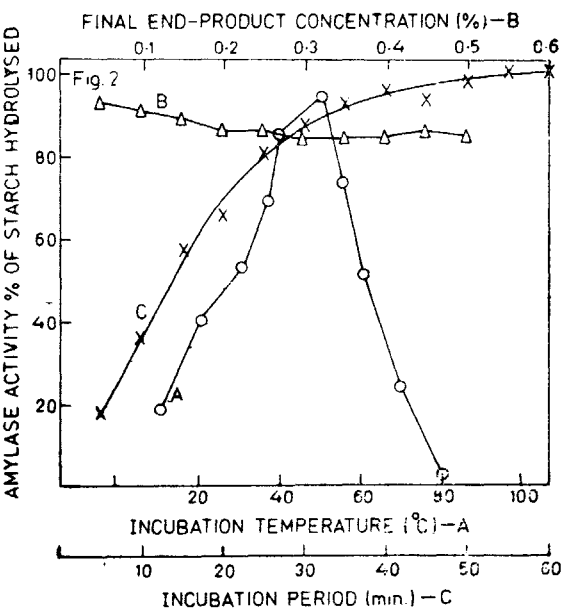

Figure 2 A–C Effect of temperature (A), end-product (maltose) (B), and incubation period (C) on the hepatopancreatic amylase

Effect of incubation period: Mixtures were incubated for progressively longer duration by 5 min. Within first 15–20 min almost 60% of starch was hydrolysed at a constant rate (figure 2C). Thereafter, the rate of hydrolysis declined, ultimately reaching near zero at and after 50 min of incubation.

Effect of substrate concentration: Starch solutions of 12 concentrations, from 0.25 to 3%, were tested. Starch hydrolysis increased with increasing substrate concentration up to 1%; thereafter it slowed down, becoming constant at 1.75% (figure 3). It thus seems that at 1.75% virtually all available enzyme is converted into ES-complex. (*Km* value $=9.0\times10^{-2}$%) (figure 4).

Effect of enzyme concentration: Enzyme extracts of 12 strengths, increasing by 0.1 mg hepatopancreas/ml, were tested. The starch hydrolysis increased in a somewhat linear manner up to 0.4 mg, hepatopancreas/ml and thereafter declined up to 0.9 mg hepatopancreas/ml and above this concentration totally ceased (figure 5).

Effect of dialysis: The enzyme activity was lowered by 15% following dialysis against double distilled water for 24 hr at 4°C.

In the study of the effects of metallic chlorides and amino acids, dialysed enzyme preparation was used. As to the assay system 0.4 ml of water was also added, raising its volume to 1 ml, the concentration of the added chloride or amino acid solution was reduced to one-tenth of its original strength.

Effect of metallic chlorides: Seventeen metallic chlorides were tested, each in six concentrations ranging from 5×10^{-5}M to 1×10^{-2}M. Chlorides of Li, Mg, K, Mn, Ba and Cd activated amylase at all six concentrations. Chlorides of Na, Ca, Rb, Sr, Pt, Hg, Co, Zn and Sn exerted no effect at 1×10^{-4}M and/or 5×10^{-5}M; at the remaining strengths amylase was activated. $CuCl_2$ was ineffective from 5×10^{-5}M to 5×10^{-3}M but caused 4% activation at the strongest (1×10^{-2}M)

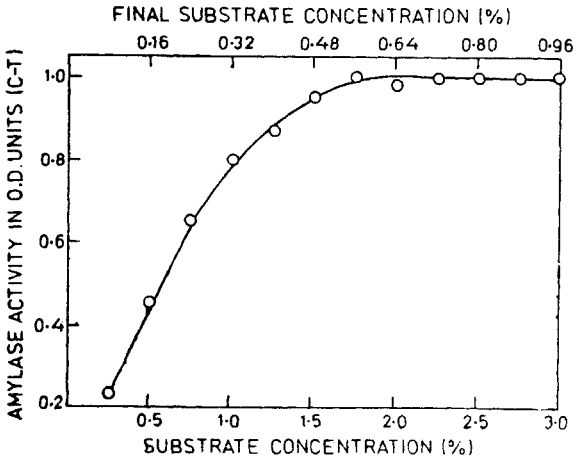

Figure 3 Effect of substrate concentration on the hepatopancreatic amylase

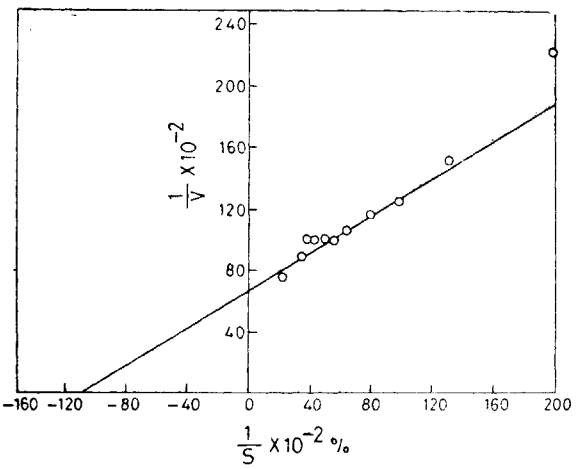

Figure 4 Line Weaver-Burk plot for Michaelis constant (*Km* value) of the hepatopancreatic amylase

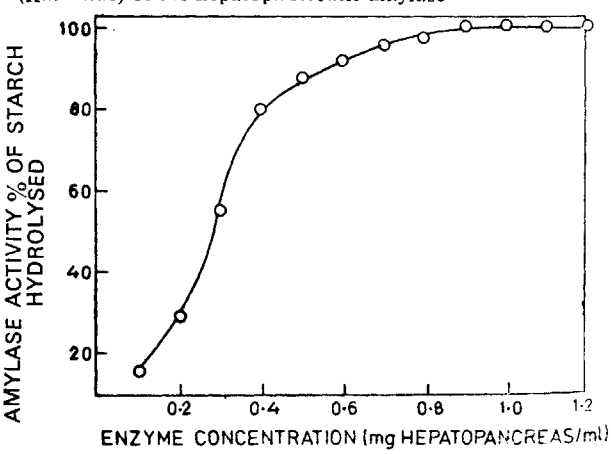

Figure 5 Effect of enzyme concentration on its activity

concentration. $AlCl_3$ was ineffective at 5×10^{-5}M, caused 73% inhibition at 1×10^{-2}M and activated amylase from 6 to 171% at the four intermediate strenghths (table 1).

Effect of amino acids: Nineteen amino acids were tested, each in six strengths ranging from 5×10^{-5}M to 1×10^{-2}M. Twelve caused activation of amylase at all six strengths which, in relation to increasing concentration, showed a linear decrease (threonine and α-alanine) or increase (α- and γ- aminobutyric acid, tryptophan, isoleucine, valine, serine and methionine) or was hyperbolic (lysine, proline and hydroxyproline). Asparagine, β-alanine and glycine were ineffective at 1×10^{-4}M and/or 5×10^{-5}M but they activated amylase at the remaining higher strengths. Glutamine, norleucine and phenylalanine were inhibitory at 5×10^{-3}M and 1×10^{-2}M but accelerated amylase at the lower ones. Leucine was ineffective at 5×10^{-5}M, inhibitory at 1×10^{-2}M; at all other strengths it caused activation. Maximum activation and inhibition was caused respectively by tryptophan (125%) and norleucine (70%) (table 2).

Table 1 *Concentrations of metallic chloride solutions causing maximum activatory or inhibitory effect on hepatopancreatic amylase of* M. lamarrei

Chloride	Final molar concentration (s)	Relative amylase activity	% Activation	% Inhibition
Nil	—	100	Nil	Nil
Lithium	1×10^{-2}	281	181	—
Magnesium	1×10^{-2}	280	180	—
Potassium	1×10^{-2}	281	181	—
Manganese	5×10^{-3}	276	176	—
Barium	5×10^{-3}	281	181	—
Cadmium	1×10^{-3}	266	166	—
Sodium	1×10^{-2}	271	171	—
Calcium	5×10^{-3}	282	182	—
Rubidium	1×10^{-2}	280	180	—
Strontium	5×10^{-3}	277	177	—
Platinum	1×10^{-2}	272	172	—
Mercuric	1×10^{-2}	280	180	—
Cobalt	1×10^{-3}	280	180	—
Zinc	1×10^{-3}	272	172	—
Stannous	1×10^{-3}	269	169	—
Cupric	1×10^{-3}	103	3	—
Aluminium	5×10^{-3}	271	171	—
	1×10^{-2}	27	—	73

Discussion

The optimum *p*H of crustacean amylase ranges from 4.5 to 7.5 and is either a sharp peak or extends over a narrow range. In *Astacus fluviatilis* it has been reported as 6.09 (Krüger & Graetz 1928) and as 5.5–5.7 (Wiersma & van der Ween 1928). Being 6.5, the optimum *p*H of hepatopancreatic amylase of *M. lamarrei* is higher than that of *Panulirus japonicus* (5.0 to 5.6, Takahashi et al. 1964) but is lower than that of other decapods —*Paratelphusa hydrodromus* (6.75 to 7.25, Ammal 1966), *Cancer borealis* and *C. irroratus* (7.0, Brun & Wojtowicz 1976) and *Diogenes bicristimanus* (7.2, Nagabhushanam & Sarojini 1968). The optimum *p*H (6.5) of amylase being within the *p*H range of the stomach contents of *M. lamarrei* (6.4 to 6.7, Murthy 1978), amylase would be fully active in vivo.

The optimum temperature of hepatopancreatic amylase (50°C) of *M. lamarrei* is lower than that of *Caridina weberi* (52°C, Chinnayya 1968), *Penaeus indicus* (52°C, Gopalakrishnan 1957), but is higher than that of *P. hydrodromus* (40°C, Ammal 1966), *D. bicristimanus* (49°C, Nagabhushanam & Sarojini 1968) and *P. japonicus* (45°C, Takahashi et al. 1964).

The effect of added end-product and of prolonged incubation period on crustacean amylase remains uninvestigated; the effect of substrate and enzyme concentrations on

Table 2 *Concentrations of amino acid solutions causing maximum activatory or inhibitory effect on hepatopancreatic amylase of* M. lamarrei

Amino acid	Effective molar concentration (s)	Relative amylase activity	% Activation	% Inhibition
Nil	—	100	Nil	Nil
α-Aminobutyric acid	1×10^{-2}	157	57	—
γ-Aminobutyric acid	1×10^{-2}	157	57	—
L-Tryptophan	1×10^{-2}	225	125	—
DL-Isoleucine	1×10^{-2}	175	75	—
DL-Valine	1×10^{-2}	145	45	—
DL-Serine	1×10^{-2}	132	32	—
DL-Methionine	1×10^{-2}	175	75	—
L-Threonine	5×10^{-5}	138	38	—
DL-α-Alanine	5×10^{-5}	143	43	—
L-Lysine	5×10^{-4}	196	96	—
L-Proline	1×10^{-3}	132	32	—
L-Hydroxyproline	1×10^{-3}	123	23	—
L-Asparagine	1×10^{-3}	107	7	—
DL-β-Alanine	5×10^{-3}	118	18	—
Glycine	1×10^{-2}	159	59	—
L-Glutamine	5×10^{-5}	150	50	—
	1×10^{-2}	93	—	7
Norleucine	5×10^{-4}	135	35	—
	1×10^{-2}	30	—	70
DL-Phenylalanine	1×10^{-3}	139	39	—
	1×10^{-2}	36	—	64
L-Leucine	5×10^{-3}	143	43	—
	1×10^{-2}	54	—	46

hepatopancreatic amylase of *M. lamarrei* and on caecal amylase of *Orchestia gammarella* (Agarwal 1962) are greatly similar.

Whilst prolonged incubation and increasing enzyme and substrate concentrations produced an identical effect (an initial acceleration followed by retardation) on the activity of hepatopancreatic amylase of *M. lamarrei*, the explanation for the retardation for the three factors is not the same. That caused by prolonged incubation and stronger enzyme concentrations cannot be attributed to accumulation of maltose as stronger concentrations of the end-product have proved only slightly inhibitory (figure 2B); it, therefore, most likely, is due to exhaustion of the substrate. That caused by stronger strengths of the substrate is due possibly to conversion of the total enzyme into ES-complex (Karlson 1969). The K_m value indicates the affinity for starch of hepatopancreatic amylase (9×10^{-2}%) to be slightly lesser than that of mammalian amylase (6×10^{-2}%, Barman 1969).

Dialysis caused total loss of activity of amylase from the salivary glands of *Aedes aegypti* (McGeachin et al. 1972), with 75% recovery thereof by NaCl solution. In comparison, the 15% loss in activity in dialysed hepatopancreatic amylase of *M. lamarrei* is strikingly low and the 6-171% accelaratory effect produced by NaCl solutions is strikingly high.

Over 65% enhancement of activity of hepatopancreatic amylase of *M. lamarrei* by all metallic chlorides, with the exception of $CuCl_2$, is noteworthy. Being common to all, Cl^- can be inferred to be responsible for the activation. Since, Cu^{++} inhibited the activity of the midgut amylase of *Tenebrio molitor* (Applebaum et al. 1961), the slight activation of hepatopancreatic amylase of *M. lamarrei* by $CuCl_2$ can be explained by assuming that its cation neutralised almost completely the accelaratory effect of its anion (table 1).

Whilst the effect of amino acids on crustacean amylase remains uninvestigated, Hori (1969) seems to be the only worker to have studied the effect of 16 amino acids on amylase from the salivary glands of the bug, *Lygus disponsi*. On comparison with his results, the following comments have been considered desirable: (i) Threonine and isoleucine activated and inhibited the amylase

of *M. lamarrei*, but only activated that of *L. disponsi*; (ii) Serine and methionine activated amylase of *M. lamarrei* but were ineffective on that of *L. disponsi*; (iii) Whereas 10^{-1} M solution of glutamine produced maximum activation (448%) of amylase of *L. disponsi*, the maximum activation (50%) of *M. lamarrei* amylase was caused by a 5×10^{-5} M solution. Since higher strengths progressively inhibited the activity of hepatopancreatic amylase, 1×10^{-1} M solution, as used by Hori (1969), would be expected to cause even greater inhibition.

The majority of amino acids caused, at one or more strenghts activation of hepatopancreatic amylase of *M. lamarrei*, albeit to different extents. The –COOH and $-NH_2$ groups being common to all may be inferred to be responsible for the activation. The third acyl (R–) group which is specific for each amino acid could likewise be held responsible for the degree of activation or inhibition. On this basis, the strong activation by tryptophan can be ascribed to the activatory effect of its R– or α–amino-β-indole propionyl group in conjunction with the activatory effect of –COOH and $-NH_2$ groups. Likewise the moderate inhibition by norleucine may be due to inhibition by its R– or caproic group which being excessive overrode the activation due to –COOH and $-NH_2$ groups.

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