

SHORT COMMUNICATION

SMALL INTESTINAL AND HEPATIC AMYLASE ACTIVITY OF DIFFERENT  
VERTEBRATES AND INVERTEBRATES

M.M. PALIT\* AND A.K. SHARMA

Department of Biochemistry,  
University College of Medicine, Calcutta University,  
244-B, Acharya J.C. Bose Road, Calcutta-700020

**Summary:** Studies have been done on amylase activity of the small intestine and liver of toad, calotes, pigeon, rat, guineapig and pilaglobosa. It has been found that amylase activity was maximum in pigeon, rat, guineapig and then in calotes and pilaglobosa and least activity was found in toad, and seem to be related to the phylogeny and the dietary habit.

**Key words:** intestinal and hepatic amylase                      vertebrates                      invertebrates

INTRODUCTION

Role of intestinal oligosaccharidase activity in different vertebrates and invertebrates was first observed by Palit *et al.* (6).

The role of intestinal carbohydrase activity was observed previously in human being but the intestinal carbohydrases activities in invertebrates like mollusca and vertebrates from fish upto mammals are relatively meagre in published literature. In some micro organism the lactase is an adaptive enzyme (1). The presence of trehalase, lactase and cellobiase activity in the proximal region of small intestine and sucrase, maltase and isomaltase in ileum have also been reported (2). It is claimed that  $\beta$ -glucocidase activity could only be demonstrated in the intestine of rats and toads. The intestine of pigeons, finches, turtles and frogs does not react (4). This problem was undertaken in order to explore the possibilities of presence of amylase activities in the small intestine and liver of different vertebrates and invertebrates.

MATERIALS AND METHODS

3'5 Dinitro salicylate reagent, standard starch solution and standard maltose solution was prepared as described by Hawk (5) with some modification. 3'5 Dinitro salicylate reagent was

---

\*Present address: Department of Zoology, Narasinha Dutta College, Howrah.

prepared by 1 gm of 3'5 Dinitro salicylic Acid, 30 gms of Rochelle Salt and 1.6 gm of NaOH in CO<sub>2</sub> free water and diluted to 100 ml. It was stored in a brown flask protected from CO<sub>2</sub>. Standard starch solution was prepared by dissolving 200 mgs of NaCl, 1.515 gms of KH<sub>2</sub>PO<sub>4</sub> and 1.98 gms of Na<sub>2</sub>HPO<sub>4</sub>, 2H<sub>2</sub>O in distilled water and was diluted to 500 ml. The pH was measured and was adjusted to 6.9, 2 gms of chemically pure rice starch was suspended with 100 ml of the cold buffer and was boiled on a water bath for 15 min under careful stirring. Standard maltose solution was prepared by dissolving 200 mg of maltose monohydrate in distilled water and was preserved by adding a pinch of Benzoic Acid (5). The young adult animals of different species of both sexes were selected at random from commercial sources. The body weight of each species has been discussed in the table for comparison. The amylase activity was measured as described by Hawk with some modification. A portion of the small intestine was taken from the vertebrate species like rat, guineapig, toad, calotes, bird (Pigeon) and the invertebrate species like *pila globosa* was taken out and immediately thrown into a freezing mixture of ice and Ammonium Chloride. The lumen of the gut was then thoroughly washed with normal saline to flush out the intestinal content and a horizontal incision was made along the length of the gut and was split on a sterile glass slab. The mucosa was then scraped out by means of the sharp edge of a glass slide and the volume was measured in a graduated centrifuge tube. It was then homogenised with 9 parts of cold normal saline in a bucket containing freezing mixture by means of a Potter-Elvehjem glass homogeniser, and centrifuged at 3000 rpm for 10 minutes. The supernatant was used as an aliquot for the enzyme source (8). 0.2 ml of homogenate was treated with 0.2 ml of standard starch solution and incubated for 1 hr at 37°C; keeping suitable control and to it 2 ml of Dinitro-salicylate reagent was added and boiled for 10 min. It was then cooled and the volume was made 4 ml by adding 1.6 ml of distilled water and the reading was taken in a Spectrophotometer at 530  $\mu$ m and the reading obtained was calculated from standard maltose curve. If R be the reading, then total starch converted into maltose per hour per ml of original mucosal tissue is

$$R \times D \times 1000 = \text{mg of maltose converted from starch/1 hr/1 ml of original mucosal.}$$

*The liver amylase activity* : A piece of liver tissue was excised out and was thrown into a freezing mixture of ice and Ammonium Chloride. It was then homogenised with equal part of cold Acetone and Ether in a chilled condition for 10 min. The supernatant was thrown out and the residual cake was then homogenised with equal part of cold Acetone and Ether. The residual cake was then evaporated to dryness and the dry protein obtained was the source of the enzyme. The pulverised tissue was preserved in a sterile glass container. 10 mg of pulverised tissue was homogenised with 1 ml of phosphate buffer at pH7 (5), in a chilled condition and centrifuged at 3000 r.p.m. for 10 min. The supernatant was used as an aliquot for the enzyme source. 0.1 ml of homogenate was treated with 0.2 ml of standard starch solution and 0.1 ml of phosphate buffer at pH7 and was incubated for 1 hr (9). The rest of the procedure was followed similarly as in case of small intestine. The results which were obtained have been discussed in Table I for comparison.

TABLE I: Amylase activity of the small intestine and liver of some vertebrates and invertebrates with  $\pm$ S.D.

No. of expt.	Species	Range of body weight in gms,	Enzyme	Site of enzyme activity and the values of enzyme activity expressed in mg of maltose converted from starch/hr/1 ml of original man intestinal mucosa and 1 gm of pulverised liver tissue.	
				Small Intestine	Liver
<i>Vertebrates</i>					
6	Toad	60-70	Amylase	40.8 $\pm$ 2.38	38.5 $\pm$ 1.9
6	Calotes	30-40	Amylase	131.6 $\pm$ 8.87	104.2 $\pm$ 0.56
6	Pigeon	200-250	Amylase	232.6 $\pm$ 3.74	218 $\pm$ 10.62
6	Rat	150-200	Amylase	205.8 $\pm$ 4.465	176 $\pm$ 4.45
6	Guineapig	200-250	Amylase	201.3 $\pm$ 2.64	160.3 $\pm$ 1.17
<i>Invertebrate</i>					
6	Pilaglobosa	70-80 (with shell)	Amylase	130.5 $\pm$ 8.85	102.5 $\pm$ 1.85

S.D. = Standard Deviation.

### DISCUSSION

From our present observations it is evident that amylase activity is present in measurable quantity in the alimentary tract and liver of both vertebrates and invertebrates, used in the series of study and the degree of the amylase activity showed a wide species variation. Maximum activity was found in pigeon and then in rat, guineapig, calotes and pilaglobosa, whereas least activity was found in toad. In our previous study we demonstrated the presence of enzymes, lactase, sucrase and maltase in the alimentary tract of all the above species of vertebrates and invertebrates and the presence of lactase activity in non-mamalian species was a very interesting phenomenon. The presence of amylase activity of liver in the present series of study is also another interesting observation (6). Gossarou observed the presence of  $\beta$ -glucocidase and  $\beta$ -galactocidase activities could only be demonstrated in the intestine of Rat and Toad only, while these were absent in pigeon, finches, turtles and frogs (4). Alvariz also observed the presence of  $\beta$ -galactocidase activity in developing rat. Dhalquist noticed the presence of trehalase, lactase, cellobiase in the small intestine of pig (1). Parson and Prichard reported the hydrolysis of disaccharides during absorption by the perfused small intestine of amphibia (7). So far the literature have been studied, the comparative analysis of the presence of amylase activity in different vertebrates and invertebrates are relatively meagre. However, from our present observations the presence of amylase activity in the small intestine and liver in the above species is due to their food habit which they acquire from the vegetable kingdom of land and water which contain starch or polysaccharides, and is essential for the hydrolysis of their dietary starch into simpler molecules like maltose. The maltose is subsequently hydrolysed by the enzyme maltase into glucose was proved earlier. The rate of variation of amylase activity in different vertebrates and invertebrates is due to the systemic requirement of the individual species. Further work is going on in this laboratory regarding the source and utility of

polysaccharides in relation to their habitat and the amylase activity as well as the activities of other carbohydrases like maltase, sucrase and lactase, as described earlier in some species during hibernation.

### ACKNOWLEDGEMENTS

Thanks are due to Miss Dipali Dutta, Research Scholar, for her kind help during the course of the study. Our heartiest thanks are also due to Dr. A. K. Maiti, Head of the Department of Biochemistry, University College of Medicine, Calcutta University, for his kind help during the course of the study.

### REFERENCES

1. Alvariz, A. and J. Sas.  $\beta$ -galactosidase changes in the developing intestinal tract of the rat. *Nature*, **190** : 826-827, 1961.
2. Dhalqvist, A. The location of carbohydrases in the digestive tract of the pig. *Biochem. J.*, **78** : 280-288, 1961.
3. Dhalqvist, A. Methods for assay of intestinal disaccharidase. *Anal. Biochem.*, **7** : 18-25, 1964.
4. Gossarou, R.  $\beta$ -glucosidase and lactase in the intestine of vertebrates. *Anal. Inst. Univ. Warburg, Warburg, W. Germany*, **35(2)** : 143-151, 1973.
5. Hawk. *Physiological Chemistry*. Oser 14th Ed., P. 518, 464.
6. Palit, M.M., S.R. Dasgupta, Dipali Dutta, A.K. Sharma and Sucheta Sircar. Comparative Studies on different small intestinal oligosaccharidase activities in some vertebrates and invertebrates like molluscs. *Ind. J. Physiol. Pharmac.*, **20(3)**, 156-159, 1976.
7. Parsons, D.S. and J.S. Prichard. Hydrolysis of disaccharides during absorption by the perfused small intestinal of amphibia. *Nature*, **208** : 1097, 1965.
8. Sharma, A.K. and A.C. Majumdar. Studies on the effect of some broad spectrum antibiotics on small intestinal oligosaccharidase activities and absorption of lactose. *The Jap. J. Exptl. Med.*, **40 (4)** : 221-225, 1970.
9. Sharma, Ajoy Kumar. Effect of Alloxan diabetes on small Intestinal and Pancreatic carbohydrase activities. *Calcutta Medical Journal (C.M.J.)*, **73** : (11 and 12), 124-127(Nov.-Dec.), 1976.