Properties of trehalase from the muscles of fresh water prawn Macrobrachium lamarrei

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Summary. The trehalase from the extramitochondrial fraction of abdominal and thoracic muscles of Macrobrachium lamarrei was purified to give a 12.85fold increase in specific activity. Its optimum pH was 6.0, K_m value was 2.85 mM and had a mol.wt around 120,000. Tris HCl buffer inhibited the enzyme activity by 21.7%.

Trehalase (E.C.3.2.1.28., α-α'-glucoside 1-glucohydrolase) was first discovered in insects by Frerejacque[2] even before the recognition of trehalose in them. Since then, it has been found in many insects[3]. Recently it has been confirmed that both soluble and particle-bound trahalase are present in the muscle tissues of Hyalophora cecropia[4], Blaberus discoidalis[5], Phormia regina[6] and Schistocerca gregaria[7]. Both the trehalases have been reported to show similar enzymatic properties. However, no reports are available on the presence of trehalase in crustacean muscle which might play an important role as in insects. During the study of enzymes in the tissues of fresh water prawn Macrobrachium lamarrei, an appreciable trehalase activity was found and the present communication reports the isolation and properties of the enzyme.

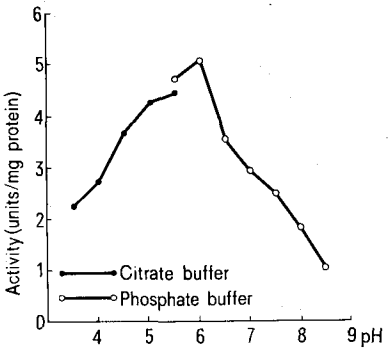

Fig. 1. Effect of pH on the activity of tissue trehalase of M. lamarrei.

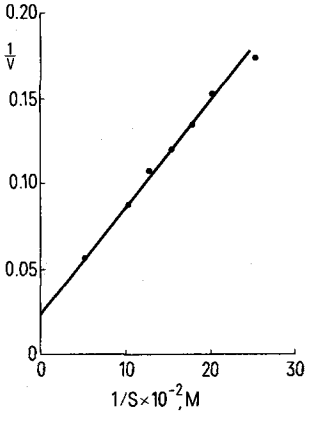

Fig. 2. Lineweaver-Burk plot for Michaelis constant (K_m) of tissue trehalase of *M. lamarrei*. V, enzyme units/mg protein; S, substrate concentration.

Material and methods. Animals were procured from the local river Gomati. After a thorough washing, homogenate was prepared from the thoracic and abdominal muscles in 0.1 M phosphate buffer (pH 6.0) using all-glass homogenizer and the homogenate centrifuged at 10,000 × g for 30 min at 0 °C to separate the mitochondrial and extramitochondrial fractions. Since extramitochondrial fraction contained most of the enzyme activity it was subjected to repeated freezing and thawing and then to ammonium sulphate precipitation. The resulting fraction was applied on a DEAE Cellulose column as described previously[8]. Mol.wt of the enzyme was determined by a sephadex Gel-100 column previously caliberated with mol.wt markers[9].

For enzyme assay reaction mixture containing 0.2 ml each of the enzyme, 0.1 M phosphate buffer and trehalose dihydrate (0.2 M) was incubated at 37 °C for 1 h. The reaction was stopped by placing the tubes in boiling water bath for 5 min and the amount of glucose liberated was determined by Glucostat Reagent. Protein content was estimated by the method of Lowry et al.[10] and all colour measurements were made on a Bausch & Lomb Spectronic 20.

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Purification of tissue trehalase from Macrobrachium lamarrei

Stage of purification	Volume (ml)	Total protein (mg)	Total units*	Specific activity**	Purification factor	Recovery (%)
Crude homogenate	150.00	472.5	190.50	0.403	1×	100
10,000 × g supernatent	116.00	324.8	165.88	0.510	1.26×	87.07
Freezing and thawing (8 times)	116.00	324.8	186.76	0.575	1.42×	98.03
Ammonium sulphate precipitation	24.00	38.8	51.60	1.328	3.30×	27.1
DEAE cellulose fraction	17.5	4.9	25.37	5.178	12.85×	13.3

*1 enzyme unit is the amount of enzyme required to release 1 mole glucose from the substrate in 1 h at 37 °C. **Specific activity = units of enzyme/mg protein.

Results and discussion. Nearly 13fold purification was obtained by the method used and the results of purification are summarized in the table. The tissue trehalase from M. lamarrei showed optimum activity at pH 6.0 in 0.1 M phosphate buffer (figure 1). The pH optimum of whole body trehalase of insects ranged from 5.2 to 6.5; as for example Bombyx mori 5.2[11], Galleria mellonella 5.5[12], P. regina 5.8[13], B. discoidalis 6.0[5], Leucophaea maderae 6.0[14], H. cecropia 6.5[4], S. gregaria 6.0–6.5[7]. This value also resembles the pH optimum obtained for digestive gland trehalase of M. lamarrei (5.5)[15]. The purified preparation is highly specific for trahalose, as revealed by the substrate specificity tests using other α-linked substrates (sucrose, maltose, melezitose and p-nitrophenyl-α-D-glucoside).

A value for the Michaelis constant (K_m) of 2.85 mM was calculated from a Lineweaver-Burk plot (figure 2). K_m values obtained for the tissue trehalase from Blaberus 3.3 mM[5], Hyalophora 3.6 mM[4], Phormia 1.3 mM[13] and Schistocerca 3.8 mM[7]. The mol.wt of the enzyme was estimated to be around 120,000. This value is far less than the value obtained for the enzyme of Mandusa (250,000)[8] and Drosophila (200,000)[16] but it is higher than that reported for Blaberus (80,000)[5]. About 21.7% of the enzyme activity was inhibited in Tris-HCl buffer supporting the previous observations[7,17]. Repeated freezing and thawing of the 10,000 × g supernatent considerably increased the specific activity of the enzyme. The same results were found by earlier workers[4,5,7,14]. The enzyme was stable at all temperatures below 45 °C for at least 3 h, but at 65 °C it was completely destroyed. The enzyme could remain in deep freeze without any loss of activity for several weeks.

No reports are available so far on the properties of trehalase from a crustacean source. The present study reveals that the tissue trehalase of M. lamarrei has many features in common with the tissue trehalase of insects. The pH optimum falls within the same range, its activity is inhibited by Tris-HCl buffer and it has a K_m value of 2.85 mM. Hence it may be concluded that the trehalase of M. lamarrei is more or less similar in nature to that obtained from insect sources.

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Electrically-induced mechanical activity of the isolated guinea-pig sciatic nerve. Influences of calcium and anoxia[1]

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Summary. The electrically-induced motility of isolated segments of guinea-pig sciatic nerves is reported. This motility was characterized by waves of tensional variations. The removal of Ca^{2+} from the suspending solution, the addition of EDTA as well as anoxia, resulted in a decrement of tension accompanied by a prolongued duration of the cycles of mechanical activity.

One of the first reports of the existence of an axonal flow dates back to approximately 30 years ago[4,5]. Nevertheless, the mechanism by which axoplasmic components migrate along nerves are not presently known. The 2 theories which are more widely accepted[6] postulate: a) Periaxonal elements generate peristalic pressure waves which, by squeezing the axoplasm, can move its components distally. b) There are structural changes in axoplasmic macromolecules able to propel inner materials. Öchs has proposed the 'transport filament hypothesis' as an explanation for the axoplasmic transport. According to this theory, crossbridges existing between transport filament and neurofilaments or microtubules were considered to be involved in axonal movements, similarly to the situation described for the sliding filament model of muscle contraction[7].

Borisy and Taylor suggested a specific colchicine binding protein as the mayor component of many axonal microtubules[8]. Puskin and Berl have shown that the microtubular protein has actin-like properties[9] and lately an actomyosin-like protein has been isolated from the synaptosomal fraction of bovine and rat brain[10]. On the other hand, Weiss was able to show continuous peristaltic motions in isolated nerves of young mice using microcinematographic methods[11]. – Based on all these data, we considered it of interest to explore the possibility of recording distinct signs of mechanical activity in isolated nerve preparations electrically stimulated.

Methods. Sciatic nerves isolated from adult (500–700 g) guinea-pigs were used. After the animals were killed by decapitation, the distal part of the principal trunk of the sciatic nerve was dissected out. Segments of approximately 2 cm were removed, placed in a Petri dish con-

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