HELMINTHOLOGIA, 41, 2: 63 – 66, 2004

Trehalose catabolism enzymes in tissues of *Ascaris suum* (Nematoda)

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Summary

The activity of trehalose catabolism enzymes – trehalase and trehalose phosphorylase was identified in the cuticle, muscles, intestine, reproductive system and haemolymph of *Ascaris suum.* It was shown that hydrolysis is the main path of trehalose breakdown in the tissues of this nematode with the exception of the haemolymph where the activity of trehalase was not observed. The highest activity of trehalase was recorded in the muscles (900 ± 98 ((μ) u/mg), slightly lower in the cuticle (850 ± 35 μ/mg) and intestines (809 ± 27 μ/mg), while the activity in the reproductive system was lower by ca. 40 %. Trehalose phosphorylase was present in all tissues examined. Its activity was lower by an order of magnitude than that of trehalase. In the muscles and cuticle the activity of trehalose phosphorylase was the highest (94.5 ± 14 μ/mg and 89.8 ± 9.6 μ/mg) while in the intestines, reproductive system and haemolymph it was lower by almost a half.

Key words: trehalase; trehalose phosphorylase; trehalose; *Ascaris suum*; Nematoda

Introduction

Trehalose (1-α-D-glucopyranosyl-α-D-glucopyranoside) is a common, non-reducing disaccharide built of two molecules of glucose (Elbein, 1974). In the case of nematodes, trehalose has three basic functions: 1) as a low molecular weight energy source, 2) as a protectant for tissues during stress of desiccation and freezing, and 3) similar to insects, to aid glucose uptake, by converting glucose to trehalose this maintains a favourable glucose gradient into the tissues (Behm, 1997). As a consequence, it is not only an important source of energy but it also facilitates survival and completion of the life cycle of numerous nematodes, including *Ascaris suum* (Wharton, 1994). Consequently, knowledge of the pathways and enzymes of trehalose metabolism is important for understanding the biology of nematodes.

Trehalose is broken-down along two metabolic paths: hydrolytic and phosphorolytic. Hydrolysis has been considered the main, and until recently the only path of trehalose catabolism in animals. The trehalose hydrolysis is catalyzed by a common enzyme – trehalase (Elbein, 1974; Behm, 1997).

Trehalase (EC 3.2.1.28) hydrolyzes the α-1,1-glycoside link of trehalose releasing two molecules of glucose. This enzyme has been found in a number of nematodes such as *A. suum*, *Haemonchus contortus*, *Bunostomum trigonocephalum*, *Onchocerca gibsoni*, *Trichinella spiralis*, *Caenorhabditis elegans*, *Steinernema affinis*, *Anisakis simplex, Hysterothylacium aduncum* and *Cystidicola farionis* (Gentner and Castro, 1974; Castro and Roy, 1974; Lapp and Mason, 1978; Gupta and Trivedi, 1986; Behm, 1997; Łopieńska *et al.*, 2001; Żółtowska *et al.*, 2001). *A. suum* is the only nematode in which the distribution of trehalase in the body has been studied. The presence of trehalase was confirmed in all tissues of *Ascaris*, except the haemolymph (Fukushima, 1967; Lapp and Mason, 1978). Żółtowska and Dmitryjuk (1998) have analyzed the distribution of trehalase activity in the muscles, which are the major source of the enzyme in *A. suum*, and shown that it correlates positively with the locomotor activity of individual parts of the nematode's body. Additionally, two isoenzymes of trehalase - neutral and acid were isolated from the muscles of *Ascaris suum* and characterized (Lapp and Mason, 1978; Dmitryjuk and Żółtowska, 2003).

The second enzyme that catabolizes trehalose – trehalose phosphorylase (EC 2.4.1.64) – is a much less common and less frequently studied enzyme. It is a glycosyltransferase catalyzing phosphorolysis and synthesis of trehalose according to the reaction (Inoue *et al.*, 2002):

Trehalose + orthophosphate ⇔ β(α)-D-glucose 1-phos-

phate + D-glucose.
So far presence of the trehalose phosphorylase has been reported in *Euglena gracilis* (Belecopitow and Marechal, 1970), *Plesiomonas* sp. (Yoshida *et al.*, 1995), *Pichia fermentans* (Schick *et al.*, 1995), *Micrococcus varians* (Kizawa *et al.*, 1995), *Catellatospora ferruginea* (Aisaka and Masuda, 1995), *Agaricus bisporus* (Wannet *et al.*, 1998), *Schizophyllum commune* (Eis *et al.*, 1998), *Flammulina velutipes* (Kitamoto *et al.*, 1998), *Thermoanaerobium brockii* (Chaen *et al.*, 1999), *Scytonema* sp. (Page-Sharp *et al.*, 1999) and *Bacillus stearothermophilus* (Inoue *et al.*, 2002). On the basis of the earlier status of studies it was assumed that the presence of trehalose phosphorylase is limited to microorganisms, fungi and lower plants only. There were no studies confirming the presence of this enzyme in animals.
The first information concerning the activity of trehalose phosphorylase in *A. suum* presented Dmitryjuk and Żółtowska (2000). This paper is the continuation of that study. It aims at defining the distribution of both trehalose catabolism enzymes and comparison of activity of both paths – hydrolysis and phosphorolysis in individual tissues of *A. suum*.

Materials and Methods

The material for the study consisted of adult female *A. suum*. The enzymatic extracts were prepared from muscles, cuticle, intestines and reproductive system isolated from the nematodes. Haemolymph was also collected for tests. The tissues were homogenized with 0.9 % NaCl at 1 : 4 w/v in a glass homogenizer. The homogenates and haemolymph were centrifuged at 1500 × g for 15 min at 4°C. The supernatants were dialyzed for 18 hours against 0.9 % NaCl at 4°C. The products of dialysis were centrifuged at 1500 × g for 10 min. Activity of trehalase and trehalose phosphorylase as well as proteins content were identified in above supernatants.
Trehalose activity in enzymatic extracts was identified by Dahlqvist's method (1968). The reaction mixture contained: 0.1 ml of the test extract, 0.1 ml 50 mM trehalose and 0.8 ml 70 mM sodium veronal – sodium acetate buffer at optimal pH values: pH 7.4 for the reproductive system, pH 4.3 for muscles, cuticle and haemolymph and pH 4.9 for the alimentary system. Controls contained 0.1 ml distilled water instead of the substrate. The reaction was carried out at 37°C for 1 hour. Glucose released by the enzyme from trehalose during the reaction was measured using glucose oxidase (LTS-120 by Cormay, Lublin, Poland).
Activity of trehalose phosphorylase was measured in the direction of decomposition of sugar by a modified method of Wannet *et al.* (1998). That identification took place in two stages. The first stage (I) covered the reaction of trehalose phosphorolysis to glucose and glucose-1-phosphate. During the second stage (II) the volume of glucose-1-phosphate was measured according to Michal (1981) using phosphoglucomutase (EC 5.4.2.2) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49).
The reaction mixture for stage I contained: 0.2 ml of the test extract, 0.6 ml 70 mM Sörensen's buffer at pH 7.0 and 0.2 ml 50 mM of trehalose solution. Test samples were incubated for 15 min at 37°C. Next they were placed on an ice bath, protein was precipitated using 3 ml 1 M perchloric acid and immediately alkalized to pH 7.6 with 5 M $CaCO_3$ solution. The sediment was removed by centrifuging at 1500 × g for 10 min. The glucose-1-phosphate was identified in the supernatant (stage II). For that purpose 0.2 ml of supernatant was added to a spectrophotometric cuvete containing: 1.8 ml 0.1 M. Tris – HCl buffer at pH 7.6, 50 µl 24 mM NADP and 10 µl glucose-6-phosphate dehydrogenase (Sigma, G-8404). After 5 minutes absorbance was read at wavelength of 365 nm. Next 10 µl of phosphoglucomutase was added (Sigma, P-3397) and after 5 minutes another reading was done. The procedure for controls was the same with the only exception that they were not incubated during stage I.
The enzymatic unit [u] represents the quantity of enzyme that within 1 hour, at 37°C, produces 1 nM of glucose from trehalose in case of trehalase or 1 nM glucose-1-phosphate in case of trehalose phosphorylase. The enzymatic activity was converted to 1 mg protein identified by Bradford's method (1976). The results are average of five replicates.

Results

The activity of both trehalose catabolism enzymes was recorded in all tissues studied of *A. suum*. Haemolymph was exceptional as only trehalose phosphorylase was present in it (Table 1). Comparing the activity of both enzymes in individual tissues of the nematode it was established that the activity of trehalase was generally higher by one order than that of trehalose phosphorylase. The highest activity of both enzymes was recorded in muscles extracts and slightly lower in cuticle. Activity of trehalase in intestines was high and did not differ significantly from that measured in the cuticle while in the reproductive system of the nematode it was lower by ca. 40 % than in the muscles. On the other hand, the activity of trehalose phosphorylase reached similar levels (ca. 52–57 µ/mg) in the intestines, reproductive system and haemolymph. In those tissues it was lower by almost a half than in the muscles and cuticle of the nematode.

Table 1. Activity of trehalose catabolism enzymes in tissues of *Ascaris suum* (µ/mg)

Tissue	Trehalase	Trehalose phosphorylase
Muscles	900 ± 98*	94.5 ± 14
Cuticle	850 ± 35	89.8 ± 9.6
Intestine	809 ± 27	57 ± 5.5
Reproductive system	536 ± 18	55.1 ± 2.6
Haemolymph	0	52.5 ± 20.7

* Mean ± SD; n = 5

Discussion

As mentioned in the introduction, the literature contained no information on the phosphorolytic path of trehalose breakdown in animals. *A. suum* is the animal species in which we have shown, for the first time, the presence of a phosphorolytic path of trehalose breakdown (Dmitryjuk and Żółtowska, 2000). Later, the activity of trehalose phosphorylase was confirmed by us in three other parasitic nematodes: *Cystidicola farionis*, *Hysterothylacium aduncum* and *Anisakis simplex* (Żółtowska *et al.*, 2001, 2002; Łopieńska *et al.*, 2002). In the case of *C. farionis*, a fish bladder parasite, the activity of trehalose phosphorylase was similar to that of *Ascaris* (Żółtowska *et al.*, 2001). On the other hand, in the case of the intestinal parasite of fish - *H. aduncum,* the activity of this enzyme was exceptionally high. In case of adult forms of this nematode the activity of trehalose phosphorylase was ca. 10 times higher (1.194 μM/mg) than that recorded in the tissues of *A. suum* (Żółtowska *et al.*, 2002).

As indicated by the above examples, the intensity of trehalose breakdown along the phosphorylase path differs not only among the individual representatives of Nematodes type but also among species belonging to the same superfamily - Ascaridoidea. However, the role of trehalose phosphorylase in nematodes is not as important as in *Euglena gracilis*, where it is the only enzyme degrading trehalose (Belocopitow and Marechal, 1970). Similarly in the case of yeast *Pichia fermentas* and in fruiterers of numerous other fungi phosphorolysis is the main path for degrading trehalose (Schick *et al.*, 1995; Kitamoto *et al.*, 1998; Wannet *et al.*, 1998).

It is surprising that trehalose phosphorylase is present in haemolymph of *A. suum*, where there is no trehalase. According to Behm (1997), lack of trehalase activity in haemolymph, combined with the high level of trehalose and low concentration of free glucose, indicates that trehalose, in case of *Ascaris* functions as a circulation sugar. Then the role of trehalose phosphorylase in that body liquid, where presence of mechanisms guaranteeing a high level of trehalose seems justified, is interesting. Possibly the enzyme in nematodes, similar to fungi and bacteria, is able to maintain a reverse reaction – synthesis of trehalose from glucose and glucose-1-phosphate (Schick *et al.*, 1995; Kizawa *et al.*, 1995; Kitamoto *et al.*, 1998; Wannet *et al.*, 1998). Explaining that interesting issue would require further focused studies.

A comparison of activity between trehalose phosphorylase and trehalase in the tissues of *A. suum* allows the conclusion that the hydrolytic path is the main path for breakdown of trehalose. The meaning of the phosphorolytic trehalose catabolism path in *Ascaris* seems lower than that of the hydrolytic one as the activity of trehalose phosphorylase in tissues of the nematode is 10 times lower than that of trehalase.

The results of this study concerning the activity of trehalase confirmed the earlier results by Fukushima (1967) and Lapp and Mason (1978). These authors described presence of trehalase in all tissues of *A. suum* except haemolymph. The distribution of trehalase activity in the body of the nematode is identical to that described by Lapp and Mason (1978). For the first time, during that study, the activity of trehalase in the cuticle of the parasite was studied. It should be highlighted that the activity of the enzyme in that tissue was high, only slightly lower than in the muscles. A high level of trehalase activity in the cuticle of the parasite may suggest the possibility of direct intake and hydrolysis of trehalose present in the host's chyme through the nematode's body wall. It is known that enzyme fulfilled a similar role in the intestine. According to Gentner and Castro (1974), activity of trehalase in the intestine of *A. suum* is limited mainly to the brush border. It allows hydrolysis of trehalose present in the gut of the nematode. Similarly, digestion of trehalose contained in the diet is the main function of intestinal trehalases of mammals (Oestereicher *et al.*, 2001).

Among the studied tissues of the nematode, the lowest level of trehalase was found in the reproductive system. That contradicts the results obtained by Fukushima (1967), who recorded the highest activity of the enzyme in that tissue. These differences may result from the status of material used for studies. Our studies (not published) indicate that the distribution of trehalase in the reproductive system of the nematode is highly differentiated – activity of the enzyme depends on the part of the system and the contents of gametes in it.

Concluding, it should be emphasized that trehalose hydrolysis, similar to the majority of living organisms, is the main path of trehalose breakdown in *A. suum*. In the case of this parasite, the path of phosphorolytic trehalose breakdown seems to be of lesser importance. Nevertheless the fact that such a path of trehalose catabolism exists in nematodes deserves notice and it inspires further studies.

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RECEIVED APRIL 16, 2003

ACCEPTED SEPTEMBER 10, 2003