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Purification and characterization of α-amylases from the intestine and muscle of *Ascaris suum* (Nematoda)

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α-Amylase (EC 3.2.1.1) was purified from the muscle and intestine of the parasitic helminth of pigs *Ascaris suum*. The enzymes from the two sources differed in their properties. Isoelectric focusing revealed one form of α-amylase from muscles with pI of 5.0, and two forms of amylase from intestine with pI of 4.7 and 4.5. SDS/PAGE suggested a molecular mass of 83 kDa and 73 kDa for isoenzymes of α-amylases from intestine and 59 kDa for the muscle enzyme. α-Amylase from intestine showed maximum activity at pH 7.4, and the enzyme from muscle at pH 8.2. The muscle enzyme was more thermostabile than the intestinal α-amylase. Both the muscle and intestine amylase lost half of its activity after 15 min at 70°C and 50°C, respectively. The K_m values were: for muscle amylase 0.22 μg/ml glycogen and 3.33 μg/ml starch, and for intestine amylase 1.77 μg/ml glycogen and 0.48 μg/ml starch. Both amylases were activated by Ca^{2+} and inhibited by EDTA, iodoacetic acid, *p*-chloromercuribenzoate and the inhibitor of α-amylase from wheat. No significant differences were found between the properties of α-amylases from parasites and from their hosts.

The activity of amylolytic enzymes has been observed in many species of parasites, however, the available literature offers little detailed information going beyond the simple statement confirming the presence of the enzyme (Von Brand, 1979). Only the α-amylases of the protozoa *Entamoeba histolytica* (Nebinger, 1986) and *Toxoplasma gondii* (Ferrer *at al.*, 1999) have been isolated and characterized. In *Ascarididae* family the enzyme from *Ascaridia galli*, a parasite of chicken, and α-amylase from perienteric fluid of *Ascaris suum,* a parasite of humans and pig, have been partly studied (Ženka & Prokopič, 1984; Żółtowska, 1990). It is known that α-amylases are also present in *A. suum* intes-

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Abbreviations: PCMB, *p*-chloromercuribenzoate; pNGlc, *p*-nitrophenyl-α-D-glucopyranoside; $pNGlc_7$, *p*-nitrophenylhexa[α-D-glucopyranosyl(1→4)]- α-D-glucopyranoside; PPA, porcine pancreatic amylase.

tine and muscles (Żółtowska, 1991; 1995[1]). This report describes the purification of α-amylases from these tissues of *A. suum* and some of the physicochemical and catalytic properties of the enzyme. Gaining the knowledge on the parasite's enzymes and comparing them to the corresponding enzymes present in the host may offer important information, facilitating development of new chemotherapeutic compounds.

MATERIALS AND METHODS

Adult females of *A. suum* were cut along the lateral line. The alimentary tract was isolated; the layer of muscles was separated from the cuticle. The material was homogenised in a glass Potter homogenizer with 0.9% NaCl (1:4 w/v). The supernatant (crude extract) was obtained by centrifugation of the homogenate at 1000× ***g*** for 15 min.

Purification of α-amylases from the muscles of* A. suum**. The crude extract was salted out at 0.3–0.5 ammonium sulphate saturation. Following dialysis, the solution of proteins was heated for 15 min at 50°C and cooled. Then the precipitated protein was removed by centrifugation at 1500 × ***g for 15 min. The supernatant was applied to a column (28.5 × 1.6 cm) of P-cellulose equilibrated with 0.15 M veronal/Na acetate buffer, pH 3.7. The proteins were eluted from the column with a linear 0.1–0.6 M NaCl gradient. The active fractions were pooled and lyophilized after dialysis. Next, the enzyme was resolved using preparative electrophoresis on 7.5% polyacrylamide gel containing 1% starch. The activity bands were stained by iodine–starch reaction following incubation for 15 min in veronal/Na acetate buffer at pH 8.4, at 37°C. Then the enzyme was eluted from the gel with 0.9% NaCl.

Purification of α-amylase from the intestine of* A. suum.** The crude extract from the intestine of the parasite was adjusted to pH 5.75 by 0.1 M HCl. Next, it was incubated at 50°C for 10 min and centrifuged for 10 min at 1500 × ***g. The supernatant was placed in an ice bath and supplemented (1:10, v/v) with 20% suspension of DEAE-cellulose in veronal/Na acetate buffer (pH 7.4). The obtained mixture was stirred for 15 min and centrifuged for 10 min at 600 × ***g***. The supernatant was applied to P-cellulose column. The chromatography procedure was the same as in the case of the enzyme from the muscles.

Determination of α-amylase activity. The activity of the enzyme was measured by Caraway (1959) method with starch or by Bernfeld (1955) method with other α-1,4-glucopolysaccharides as the substrates. Amylase activity was determined also with *p*-nitrophenyl-α-D-maltoheptaoside ($pNPGlc_7$) as a substrate (Alpha Diagnostic kit), and expressed in international units (U). Enzymatic units were converted to mg protein estimated by the method of Lowry *et al.* (1951).

Isolation of cell fractions. Subcellular distribution of the enzyme in the intestines and muscles was established according to Van den Bossche & Borgers (1973). The tissue was homogenized in 4 vol. of 0.25 M saccharose (pH 7.4). The homogenate was centrifuged for 5 min at 800 × ***g***. The sediment was rehomogenized and centrifuged as before. The supernatants were combined. The pellet was resuspended in 0.9% NaCl yielding the nuclear fraction. The mitochondrial, lysosomal and microsomal fractions were successively isolated by centrifugation at 10 000 × ***g*** for 10 min, 15 000 × ***g*** for 15 min and 100 000 × ***g*** for 2 h. The final supernatant was the cytosol fraction.

Characterization of the enzymes. The effect of pH on enzyme activity was studied in 0.07 M veronal/Na acetate buffer at pH 2.65–9.65. The optimum temperature for the enzyme was established by incubation of samples of α-amylase from the intestine and mus-

[1] *7th International Helminthological Symposium,* September 19–22, 1995. Košice, Slovak Republic, Abstract P-4.

cle for 15 or 30 min, respectively, in the temperature range of 20–80°C in 0.07 M veronal/Na acetate buffer at pH 7.4 and 8.2. The thermal stability of the enzymes was tested in the following way: the samples were incubated for 15 min at temperatures from 40°C to 90°C, and after, being cooled in an ice bath, they were transferred to a bath of 37°C. Then the substrate was added to measure the enzyme activity. The dependence between the reaction velocity and the concentration of substrate was determined with rabbit liver glycogen (Sigma no. G 8876), *A. suum* muscle glycogen (own preparation, Żółtowska, 1989), amylopectin (Koch Light m.w. >1 000 000 no. 40 000), amylose (Koch Light m.w. 150 000 no. 042100), soluble starch (POCh), *p*-nitrophenyl-α-D-maltoheptaoside (Boehringer Mannheim no. 720470). The ability of the obtained enzymes to catalyse the transglycosylation reaction was measured after Hutny & Kossobudzki (1968). The effect of $CaCl_2$, EDTA, 2-phospho- and 3-phosphoglyceric acid, glycerol, mannitol, iodoacetic acid and *p*-chloromercuribenzoate (PCMB) at the concentrations of 0.5, 5.0 and 50 μM was tested. The influence of 0.01, 0.1 and 1 mg of α-amylase inhibitor from wheat, obtained using the O'Donnel & McGeeny method (1976), was also studied. All the above compounds were preincubated with the enzyme for 15 min at 37°C, prior to initiation of the reaction by addition of substrate.

Electrophoretic methods. Following each stage of enzyme purification, polyacrylamide gel electrophoresis (PAGE) according to Davis (1964) was performed. The protein was stained using Coomassie Brilliant Blue R-250. The molecular mass of α-amylase was established using SDS/PAGE according to Weber & Osborn (1969), using Sigma Dalton Mark VI standards. The isoelectric point was identified according to Andrews (1988) on 1% agarose with the ampholine pH 3.5–10, using the IEF-Mix 3.5–9.3 set of Sigma as a standard.

RESULTS

The total amylolytic activity of the body of adult *A. suum*, as calculated per 1 g of fresh mass, was 0.37 ± 0.12 U. In the intestines it was 14.3 ± 1.7 U, in the reproductive system 0.46 ± 0.17 U, in muscles 0.14 ± 0.03 U, in cuticle 0.02 ± 0.01 U and in pseudocoelomic liquid 0.61 ± 0.12 U/ml.

In the intestines the enzyme was located mainly in the lysosomal fraction, which showed about 75% of the total activity. In the muscle the activity was divided between two fractions: microsomal and lysosomal, with 50% and 40% of the total enzyme activity, respectively (Fig. 1). The distribution of marker

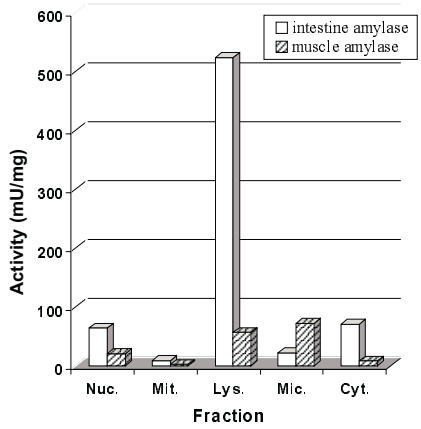

Figure 1. Intracellular distribution of α-amylases from *A. suum*.

Fractions: Nuc., nuclear, Mit., mitochondrial, Lys., lysosomal, Mic., microsomal, Cyt., cytosolic.

enzymes was similar to the results presented by Van den Bossche & Borgers (1973).

The protocol for isolation of the enzyme from the muscles and intestines and the effects of purification at each step are presented in Table 1. The enzyme obtained from the muscle was purified almost 295-fold with the yield of 9.6%. The preparation was not homogeneous; after repeated electrophoresis it showed 2 bands of which only one was active (Fig. 2). α-Amylase from the intestine, after

chromatography on P-cellulose was purified 37-fold with the yield of 30.2%. The preparation was not homogeneous, as shown by PAGE: four protein fractions were observed, including two active ones. They were called isoenzymes α-I_1 and α-I_2 (Fig. 2).

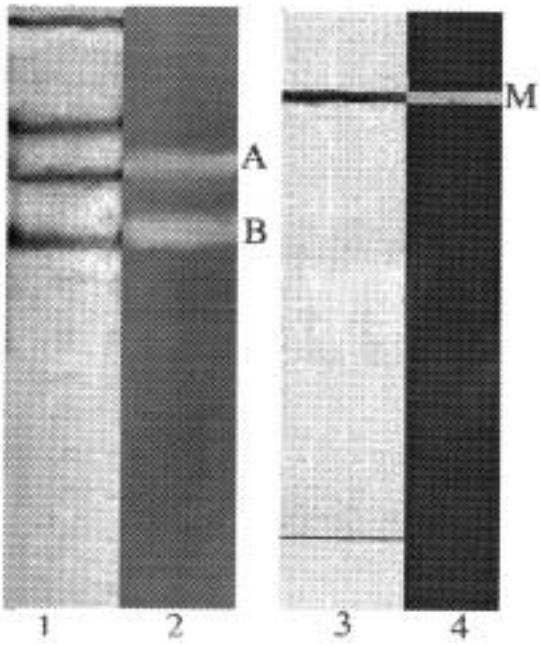

Figure 2. Separation of *A. suum* amylases by gel electrophoresis.

Lane 1, proteins in the preparation of intestinal α-amylases; lane 2, the activity of intestinal α-amylases; lane 3, proteins in the preparation of muscle α-amylase; lane 4, the activity of muscle α-amylase. A, isoenzyme α-I_2, B, isoenzyme α-I_1, M, muscle α-amylase. Protein was detected by staining with Coomassie Brilliant Blue R 250, the activity was located by iodine staining.

The molecular mass of intestinal α-amylases, as established by SDS/PAGE, was 83 kDa for α-I_2 and 74 kDa for α-I_1. The muscle amylase had a lower mass i.e. 53 kDa. The isoelectric point (pI) for intestinal isoenzymes was at pH 4.7 and 4.5, respectively, and for the muscle α-amylase at pH 5.0.

The intestinal enzyme was active over a rather wide range of pH 5.5–9.2 reaching the maximum at pH 7.4. The muscle α-amylase showed the highest activity at pH 8.4. At pH 6.5 its activity was lowered to 10% of its maximal activity (Fig. 3). The two amylases from

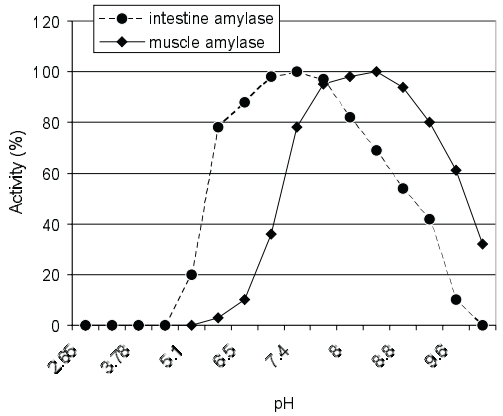

Figure 3. The pH profiles of α-amylases from *A. suum*.

The incubation mixture contained: 0.1 ml of enzyme (23 μg intestinal or 17 μg muscle α-amylase), 0.9 ml of 0.07 M veronal/Na acetate buffer pH 2.64–9.64, and 1.0 ml of 1% starch. The maximal activity (100%) was 6.43 U/mg and 0.86 U/mg for intestine and muscle amylase, respectively.

A. suum showed each two temperature dependent optima of activity (Fig. 4). For intestinal α-amylase they were found at 40°C and 50°C. The peaks of activity of muscle α-amylase were identified at 50°C and 30°C (Fig. 4).

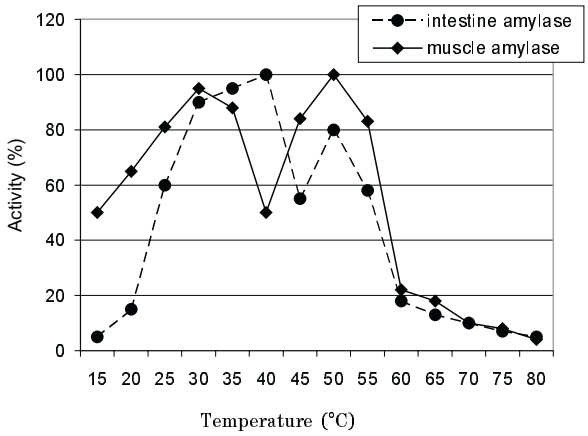

Figure 4. Dependence of activity of α-amylases from *A. suum* on temperature.

The incubation mixture contained: 0.1 ml of enzyme solution (23 μg intestinal or 17 μg muscle α-amylase), 0.9 ml of 0.07 M veronal/Na acetate buffer pH 7.4 or 8.4 for intestine and muscle amylase respectively, and 1.0 ml of 1% starch. Time of incubation was 15 min or 30 min at 37°C. The relative activities were expressed as percentages of maximum activity.

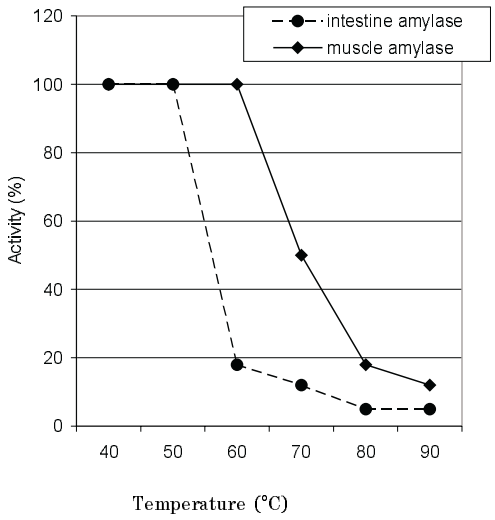

Figure 5. Thermal stability of α-amylases from *Ascaris suum*.

The incubation mixture contained: 0.1 ml of enzymes (23 μg intestinal or 17 μg muscle α-amylase), 0.9 ml of 0.07 M veronal/Na acetate buffer pH 7.4 or 8.4 for intestine and muscle amylase, respectively. After 15 min of preincubation at appropriate temperature, the probes were cooled to 37°C and then 1.0 ml of 1% starch was added. Then the incubation lasted 15 min or 30 min. The relative activities were based on the activity at 37°C taken as 100%.

Intestinal α-amylase was more sensitive to higher temperatures than the muscle one (Fig. 5). After 15 min incubation at 70°C the muscle enzyme maintained a half of its activity while the intestinal one only around 10%.

For studies on the dependence of the enzyme activity on the type and concentration of the substrate five polysaccharides were used; two of them amylose and Ascaris glycogen have been partly characterized (Żółtowska, 1989). The values of Michaelis constant (K_m) with all the tested substrates for the intestinal α-amylase differed from those for the muscle enzyme (Table 2). The latter showed a higher affinity to glycogen than the intestinal one, which, in turn, hydrolysed more intensely starch, amylopectin and amylose. The two enzymes differed not only in the value of K_m in the presence of $pNPGlc_7$ but also in the pattern of products obtained after hydrolysis of this substrate (Tables 2 and 3). The major products of the intestinal enzyme were $pNPGlc_2$ and $pNPGlc_4$, which represented around 45% of the total products while in the case of muscle α-amylase the main product was $pNPGlc_{5-6}$. Muscle α-amylase, in contrast to the intestinal form, did not release *p*-nitrophenol. Both the enzyme from the intestine and the muscle showed transglycosylation abilities as in addition to product of $pNPGlc_2$ hydrolysis (pNGlc), the product of transglycosylation ($pNPGlc_3$) was

Table 1. Purification of α-amylases from muscle and intestine of *Ascaris suum*

No.	Step	Specific activity mU/mg	Total protein mg	Purification	Yield %
		muscle α-amylase			
1	Crude extract	3.2	2760	–	100
2	Ammonium sulfate (0.3–0.5)	6.9	670	2.16	52.34
3	50°C denaturation	14.3	286	46.7	46.71
4	P-cellulose	864	1.8	270	17.65
5	PAGE	943	0.9	294.7	9.61
		intestine α-amylase			
1	Crude extract	208	1287	–	100
2	50°C denaturation	324	648	1.57	78.4
3	DEAE-cellulose	716	284	3.44	76.0
4	P-cellulose	7 710	10.5	37.07	30.2

Amylase activity was determined as described in Materials and Methods with $pNPGlc_7$ as substrate.

Table 2. Kinetics parameters of α-amylases from *A. suum*

No.	Substrate	Enzyme from			
		muscle		intestine	
		K_m μg/ml	V_{max} mol/min per mg	K_m μg/ml	V_{max} mol/min per mg
1	Glycogen from rabbit	0.537	0.041	1.449	1.089
2	Glycogen from *A. suum*	0.222	0.062	1.773	0.6861
3	Amylopectin	2.032	0.124	1.243	0.4873
4	Amylose	3.762	0.104	2.381	0.7408
5	Starch	3.333	0.139	0.476	0.5811
6	$pNPGlc_7$	0.345*	0.0816	0.112*	0.3175

The concentrations of substrates were varied from 2.5 - 15 mg/ml for glycogen and starch, 1.6 - 6 mg/ml for amylopectin, 0.5 - 3 mg/ml for amylose, and 0.35 - 2.1 mM for $pNPGlc_7$. Volume was 5 ml, the content of enzyme was 115 μg for intestial and 85 μg for muscle α-amylase. Values are average of at least 4 replicates. * mM

Table 3. Hydrolysis of *p*-nitrophenyl α-maltooligosaccharides by amylases from *A. suum*

Substrate	Enzyme from	Percentage in the hydrolysate						
		pNP	pNPGlc	$pNPGlc_2$	$pNPGlc_3$	$pNPGlc_4$	$pNPGlc_{5-6}$	$pNPGlc_7$
$pNPGlc_7$	intestine	16.5	15.9	23.4	13.8	22.2	2.9	5.3
	muscle	ND	16.4	14.4	18.2	16.8	29.5	4.7
$pNPGlc_2$	intestine	18.7	49.2	19.8	12.3			
	muscle	ND	14.8	55.5	29.7			

The mixture of 0.1 ml solution of enzyme (23 μg intestinal or 17 mg muscle α-amylase), 0.5 ml of 21.5 mM $pNPGlc_2$ or $pNPGlc_7$ and 0.4 ml of 0.07 M veronal/Na acetate buffer was incubated at 37°C for 30 min. The reaction was stopped by immersion of the probe in boiling water for 5 min, and the probes were cooled and filtered. Then 0.1 ml of probe was applied on Whatman No. 3 paper and chromatographed in n-propanol/nitromethane/water (5:4:3, by vol.). The spots of sugars were identified in UV, cut out and eluted with water.

found. The transferase activity of muscle amylase is stronger than that of the intestinal enzyme (Table 3).

The influence of some compounds on the activity of amylases is presented in Table 4. $CaCl_2$, 2-phospho- and 3-phosphoglycerate activated both enzymes. These latter two compounds raised several fold the activity of muscle α-amylase. EDTA, PCMB and iodoacetic acid, as well as α-amylase inhibitor from wheat, acted as inhibitors of both enzymes.

DISCUSSION

α-Amylases are present in representatives of such systematic groups as Archea, Bacteria and Eucarya. They form the most numerous family of glycosyl hydrolases. Their structure has been reviewed in numerous works concerning evolutionary relations (Svensson, 1988; Robins & Samuelson, 1992; Qian *et al.*, 1993; Janeček, 1994; Henrissat & Bairoch, 1996; Henrissat & Davies, 1997; D'Amico *et al.*, 2000). Their structure, as well as the mechanism of catalysis and properties are interesting also for biotechnology because of the practical use of α-amylases in storage, in starch processing (baking, brewing industries) and also in diagnostics and treatment of diseases of the alimentary tract and glands which cooperate in the digestion process (Skude, 1977; Ishizuka *et al.*, 1996; Matsui *et al.*, 1992).

α-Amylases are proteins generally of low molecular mass, which for animal α-amylases is

Table 4. Influence of different effectors and inhibitors on the activity of α-amylases

	The agent concentration (μM)	Activity of enzyme (%)* from intestine			Activity of enzyme (%)* from muscle		
		50	5	0.5	50	5	0.5
I	**Inhibitors**						
	EDTA	36	38	40	0	63	86
	Iodoacetic acid	0	10	55	0	14	92
	PCMB	48	62	82	32	60	98
	Inhibitor of α-amylase from wheat**	24[a]	39[b]	69[c]	54[a]	54[b]	76[c]
II	**Effectors**						
	$CaCl_2$	151	132	128	168	132	124
	2-Phosphoglyceric acid	136	116	104	744	444	226
	3-Phosphoglyceric acid	146	123	98	526	416	196
	Glycerol	42	75	82	105	100	100
	Mannitol	95	98	98	61	82	88

The enzymes were preincubated in 1.0 ml of veronal/Na acetate buffer, pH 7.4 or 8.4 for 15 min at 37°C with each of the effectors before the addition of substrate. Then 1 ml of 1% starch was added, and the activity was measured after 15 or 30 min, respectively, for intestine and muscle amylase. * The activities of control samples were taken as 100%. ** The concentration of inhibitor of α-amylase from wheat were in the samples; a, 1.0 mg, b, 0.1 mg, and c, 0.01 mg.

within the range of 45–67 kDa. The molecular mass of muscle α-amylase of *A. suum* (59 kDa) is well within this range. However, molecular mass of the intestinal isoenzymes of *A. suum* is higher i.e. 74 kDa α-I_1 and 83 kDa α-I_2. It seems that the molecular mass of the enzyme of the free-living nematode *Caenorhabditis elegans* estimated on the basis of nucleotide sequence of the amylase gene seems to be equally high (D'Amico *et al.*, 2000). The *C. elegans* enzyme has an unusually long C-terminal amino-acid sequence. As compared to the porcine pancreatic amylase (PPA), at the C-terminus it has additional 197 amino acids, their total number being 693 (D'Amico *et al.*, 2000). These data, however, might be inaccurate as mature amylase protein of that nematode has not so far been isolated and we do not know its true characteristics. Muscle and intestinal enzymes from *A. suum* as established in this study, have a higher molecular mass than α-amylases of the host of that parasite i.e. the porcine and human enzymes (Ishikawa *et al.*, 1993; Fereyroux *et al.*, 1998).

The intestinal α-amylase of *A. suum* was present mainly in the lysosomal fraction of cells, similarly as the enzyme of the intestine of the larvae and imago of the housefly (*Musca domestica)* and *E. histolytica* (Nebinger, 1986; Terra *et al.*, 1988). The microsomal fraction of the muscle enzyme of *A. suum* had higher activity than the lysosomal one (Fig. 1). In the case of the parasitic protozoon *T. gondii*, the membrane-bound α-amylase was present along with the cytoplasmic enzyme (Ferrer *et al.*, 1999). The activity of *A. suum* enzyme in the cytosolic fraction of cells both from muscle and intestine was generally low; in neither case did it exceed 10% of total enzyme activity (Fig. 1).

The so far completed studies have proven that α-amylases of vertebrates, as well as those of the invertebrates are encoded by two genes: amy-1 and amy-2. In mammals, these genes code for salivary and pancreatic α-amylases (Robins & Samuelson, 1992). As a consequence of the gene size and multitude of alleles, polymorphism of α-amylases is observed even among individuals belonging to the same species (Nebinger, 1986; Strumeyer *et al.*, 1988; McMahon *et al.*, 1999; Terzič *et al.*, 1999). From two to six isoenzymatic forms dif-

fering more or less in their physical, chemical and immunological properties can be simultaneously present (Strumeyer *et al.*, 1988; Anindyawati *et al.*, 1998; Fereyroux *et al.*, 1998; Ferrer *et al.*, 1999; Matsuo *et al.*, 1999). Similarly, in *A. suum* at least three α-amylases differing in their properties – pseudocoelomic (Ženka & Prokopič, 1984), muscle and intestinal – were found. The last one, in turn, was found in two isoforms.

The optimum temperature and thermal stability of α-amylases are usually related to their origin. For example, the optimum temperature of α-amylase from the Antarctic psychrotroph *Alteromonas haloplancits* A23 is at 5°C, and this enzyme is particularly thermolabile (Feller *et al.*, 1992). On the other hand, the enzymes from thermophile microorganisms *Bacillus licheniformis* and *Streptomyces sp.* IMD 2679 were unusually thermostable (McMahon *et al.*, 1999; Declerck *et al.*, 2000). The optimum activity of both studied *A. suum* isoenzymes found at 40°C and 50°C is in line with the temperature of pig intestine where the worms live. The curves of thermal dependence of the activity of these enzymes have two peaks (Fig. 4). It is supposed this is due to different thermal sensitivity of amylase isoenzymes observed in the case of isoamylases from *A. suum* intestine (unpublished).

The muscle enzyme is more resistant to thermal denaturation than the intestinal one; its activity decreased by a half after 15 min incubation without substrate at 70°C, while the intestine amylase retained only 10% of its initial activity (Fig. 5). Thus it is evident that, in *A. suum* both the muscle and intestinal α-amylases are less sensitive to higher temperatures than those from pseudocoelomic liquid (Ženka & Prokopič, 1984).

Stability of α-amylases is a consequence not only of the specific sequence of amino acids but also of the presence of Cl^- and Ca^{2+} ions in the molecule (Buisson *et al.*, 1987; Yamashita *et al.*, 1991; Lecker & Khan, 1998; D'Amico *et al.*, 2000). The α-amylase of *C. elegans* is considered a chloride dependent amylase (D'Amico *et al.*, 2000). That relation was not studied for the two α-amylases investigated during this study, as chloride ions were present at all stages of enzyme isolation. Probably the α-amylases of *A. suum* also are chloride-dependent, like the α-amylase from the pseudocoelomic liquid of the same parasite (Ženka & Prokopič, 1984). The effect of calcium on the activity of α-amylases from Ascaris was typical for many amylases; Ca^{2+} seems to be essential for both the muscle and intestinal *A. suum* enzymes (Table 4). Their activity was strongly inhibited by the bivalent ion chelator EDTA. This result is consistent with the role of Ca^{2+} as an enhancer of the activity of amylases from Ascaris.

The effect of disulfide bonds and free residues of cysteine on α-amylase was analysed by D'Amico *et al.* (2000). Porcine pancreatic amylase (PPA) has 5 disulfide bonds and 2 free cysteines (Pasero *et al.*, 1986). Cysteines are important for stabilisation of α-amylase structure (Sakano *et al.*, 1986). Their modification results in a decrease in the enzyme activity by 80% (Pasero *et al.*, 1986). Similarly, the two α-amylases of *A. suum* were inhibited by compounds reacting with –SH groups (Table 4). Iodoacetic acid was a particularly strong inhibitor of both investigated α-amylases. As shown by earlier studies, this inhibitor did not influence the activity of α-amylases from another intestinal nematode, *A. galli* (Żółtowska, 1990). This is one of the features differentiating amylases of those two species belonging to the same family of *Ascarididea*. Karpiak & Sobiech (1982) tried to differentiate α-amylases of bacteria, invertebrates and mammals using small-molecular organic compounds. The muscle α-amylase of *A. suum* reacted with 50 μM mannitol and glycerol similarly as did PPA and the muscle enzyme of *Celerio euphorbiae,* while the reaction of intestinal α-amylase was similar to that of the corresponding enzyme from *B. subtilis.* The role of polyalcohols on amylase activity was discussed by De Cordt *et al.* (1994a; 1994b). Gen-

erally, polyols act as protein stabilizers. The mechanism of this stabilization at higher concentration of polyalcohols is indirect and non-specific. The variation in hydrophilicity/hydrophobicity among proteins, particularly in their surface patterns, can lead to opposite effects of a given polyol on different proteins (De Cordt *et al.*, 1994a), as its has also been observed during the present studies.

2-Phospho- and 3-phosphoglyceric acids strongly activated α-amylases from *A. suum,* particularly the muscle enzyme (Table 4). The mechanism and physiological significance of this activation are not quite clear. Vegetable inhibitors of α-amylases are used for inhibition of human and animal α-amylases and their differentiation for diagnostic purposes (O'Donnel & McGeeny, 1976; Kutty & Pattabiraman, 1986). The inhibitor from wheat inhibited by 90% the activity of human salivary amylase and only by 20% that of pancreatic amylase (O'Donnel & McGeeny, 1976). Its influence on the *A. suum* intestinal α-amylase appeared to be a little stronger than that on the muscle enzyme (Table 4).

The optimum pH for activity of *A. suum* muscle α-amylase with starch as substrate is at pH 8.4. At pH 7.1 the enzyme shows only 36% of its maximal activity (Fig. 3). Its sensitivity to acidity of the environment seems to contradict the physiological importance of this enzyme. It should be mentioned, however, that *A. suum* possesses an effective mechanism to remove acids generated as the final metabolites from the muscles, and eventually to secrete them through the body walls (Ho *et al.*, 1990). The optimum pH for the intestinal α-amylase of *A. suum* is 7.4. It is more alkaline than for the enzyme from the pseudocoelomic fluid of this nematode (pH 6.8) and than for α-amylases from its hosts – pigs (pH 6.9) and humans (pH 6.9–7.1) (Skude, 1977; Ženka & Prokopič, 1984; Sakano *et al.*, 1986; Yamashita *et al.*, 1991). Similarly alkaline (pH 7.8) was the optimum pH for the activity of α-amylase from the gut of the worm *A. galli* (Żółtowska, 1990).

As indicated in an earlier paper (Żółtowska, 1991), in the *A. suum* intestines, in addition to α-amylases, were present also α-glycosidases with the optimum activity at pH 5.0–6.8. It seems that the wide range of pH for the activity of intestinal α-amylases (Fig. 3) makes possible their good cooperation with α-glycosidases, the more so that the distribution of the two enzymes in the alimentary tract of *A. suum* overlaps (Żółtowska, 1991).

The affinity of α-amylases to the substrate depends on the length of the chain and the number of branching points (Seinger *et al.*, 1987; Ishikawa *et al.*, 1993). Comparing the K_m values it can be observed that the α-amylase from the muscles of the nematode has a higher affinity to the more branched substrates than the intestinal one (Table 2). The enzyme from the muscles of the worm shows a significantly higher affinity to glycogen isolated from *A. suum* than to glycogen from muscles of a rabbit. The glycogen of this nematode has a very complex branched structure (Żółtowska, 1989). Conversely, the intestinal α-amylase of *A. suum* has a 10-fold higher affinity to starch than the muscle enzyme. Also amylose, amylopectin and $pNPGlc_7$ are hydrolysed much better by the intestinal than by the muscle enzyme. This indicates the adjustment of the two α-amylases studied to the substrate type most abundant in their natural environment. The affinity to starch of intestinal α-amylase of *A. suum* is around 20-fold higher than that of the host enzyme from pancreas (Sakano *et al.*, 1986).

Many of α-amylases show stronger or weaker capacity for transglycosylation (Svensson, 1988). In addition to the bacterial enzymes, two of four isoenzymes of *E. histolytica* α-amylases and mammalian α-amylases also possess such a capacity (Nebinger, 1986; Omichi & Ikenaka, 1988). Both *A. suum* α-amylases show also the transferase activity, which is more pronounced in the muscle enzyme than in the intestinal one (Table 3).

To conclude, it can be said that, in *A. suum,* at least three forms of α-amylase differing in

their tissue origin and properties: the muscle, intestinal and pseudocoelomic forms, are present. The question whether the α-amylase present in the perienteric fluid of *A. suum* body (Ženka & Prokopič, 1984) is a separate enzyme or whether it permeates into the pseudocoelomic liquid from the other tissues cannot yet be answered. This α-amylase differs in the optimum pH from either of the two α-amylases described in this paper. However, it is supposed, on the basis of the studies in progress (unpublished), that it might be one of the isoenzymes of the intestinal α-amylase.

Analysis of the data presented in this paper did not show any substantial differences between the properties of α-amylases from *A. suum* and their host. Therefore, there is little hope for finding any potential chemotherapeutic drug specifically blocking the parasite α-amylase without simultaneous inhibition of the host's enzyme. The antiparasitic agents used in the current medical and veterinary practice have, as shown in our earlier studies, differentiated influence upon the activity of trypsin and lipase from pig and *A. suum*, while they have a similar effect on α-amylase from the host and the parasite (Żółtowska, 1993). This is one more piece of evidence confirming the similarity of the enzymes belonging to the family of α-amylases, the enzymes, which have many common features (MacGregor, 1993).

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