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The activity of selected hydrolases of adult female *Ascaris suum*, Goeze 1782

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Summary

The activity of hydrolases was measured in the perienteric fluid, muscles and three sections of the intestine of female *Ascaris suum*. In none of the examined samples was the activity of lipase and α-galactosidase detected. The activity of hydrolases in anterior, middle and posterior sections of the *A. suum* intestine was similar. The high activity of the following glucosidases: β-galactosidase (lactase), N-acetyl-β glucosaminidase and α-fucosidase, was detected in the contents and extracts from the intestinal wall, apart from the activity of those found in earlier studies. In perienteric fluid and in extracts from muscles, a high activity of β-glucuronidase and β-glucosidase was detected; such activity was not found in the intestine.

Key words: *Ascaris suum*; nematodes; hydrolases

Introduction

Members of the phylum Nematoda belonging to the order Ascaridida absorb macromolecular compounds per os, whereas certain small molecules are absorbed by the body walls (Leštan *et al*., 1974). According to Pappas (1988) the most important role in the nutrition of mature nematoda is played by the intestine. Excretion processes are concentrated in the anterior part of the intestine, whereas nutrients are absorbed in the middle and posterior parts (von Brand, 1973, 1979). Beames and King (1972) claimed that the presence of enzymes in the intestine of parasites is affected by their hosts. According to these researchers, nematodes which inhabit the intestines avail themselves to partially digested food, therefore they do not have to produce many digestive enzymes of their own. According to Maki *et al*. (1982) and Oue *et al*. (2000) the activity of many enzymes in parasites' intestines depends on the composition of the absorbed food. The ability to digest numerous carbohydrates was observed in the intestine of *A. suum* (Gentner *et al*., 1972; Van den Bossche & Borgers, 1973; Żółtowska, 1991). The activity of endoproteases and lipase in the intestine of *A. suum* was determined by many authors, e.g. Juhasz (1979 a,b); Żółtowska and Łopieńska (1999); however, no record has been found in the available literature of isolating and determining the properties of the enzymes. According to Hogan (1980), no enzymes able to digest proteins are synthesised in the intestine of *A. suum*; the proteases there are absorbed from the host and are part of an inactive enzyme-inhibitor complex. Rupova *et al*. (1984) claim that such enzymes as trypsin, chymotrypsin and enterokinase are absent from the intestine of *A. suum*; only cathepsins are present there. According to Maki and Yanagisawa (1986), aminopeptidases are the dominant enzymes connected with the process of protein digestion in the intestine of *A. suum*.

Most of the available literature reporting the presence of carbohydrates digesting enzymes (Zenka & Prokopic, 1984; Żółtowska, 1991, 1995, 2001; Żółtowska & Dmitryjuk, 1998; Dmitryjuk & Żółtowska, 2004) but it is few information regarding the presence of other hydrolases in the intestine, perienteric fluid and muscles of *A. suum*. Therefore, it is the aim of this study to determine the activity of hydrolases found in pseudocelomatic fluid and muscles of *A. suum* and compare it to such activity in the intestine contents and in the intestine wall.

Material and Methods

Mature female *A. suum* were obtained from the intestines of swine slaughtered in the meat processing plant in Morliny. Before dissection, the parasites were washed several times in physiological saline. During the dissection, perienteric fluid was taken from the pseudocelom, muscles and the intestines were isolated. The intestine was divided into three equal parts. These parts were cleft longitudinally and the contents were washed away with 2 ml physiological saline. Muscles and each part of intestinal tissue were weighed and homogenised in a Potter glass homogeniser with physiological saline, in the ratio of 1:3. All the samples were then centrifuged at 3000 x g for 10 minutes. The supernatants were used for enzymatic determinations. The

protein contents in all samples tested were determined using Bradford's (1976) method. All the operations were carried out in a cool room and the solutions were stored in ice before being used for analysis.

The enzymatic activity was determined by the semi-quantitative test API ZYM, manufactured by Bio Mèrieux SA (France, Lyon). The test contains substrates which may be used to determine the activity of 19 hydrolases (Table 1). 65 µl of the tested solution, containing 100 µg of protein, was put in each well containing appropriate substrates, and incubated for 4 hours at 37ºC. Under illumination, the reagents produce a colour reaction with compounds formed by the enzymes. Activity of the hydrolases was expressed in volumetric units (nmol) of the hydrolysed substrate with respect to colour reaction intensity in 5 stage scale: 1-five, 2-ten, 3-twenty, 4-thirty, 5-forty and more nanomoles.

Results

The activities of 13 hydrolases were determined in extracts from muscles (Table 1). The highest activity was found for leucine arylamidase, β-galactosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-fucosidase (40 nmol) and acid phosphatase (30 nmol). No activity of lipase (C14), cystine arylamidase, trypsin, α-galactosidase, β-glucuronidase and α-mannosidase was found.

In the liquid from the body cavity activity of 16 hydrolases was found (Table 1). The highest activity was determined for leucine arylamidase, β-galactosidase, β-glucuronidase, β-glucosidase and N-acetyl-β-glucosaminidase (40 nmol). No activity of lipase (C14), naphthol-AS-BI-phosphohydrolase or α-galactosidase was found.

In the contents of the anterior intestine activity of 13 hy-

Table 1. Activity of hydrolases in selected tissues of *Ascaris suum*

Enzyme	Classification	Substrate	pH	Activity in nmoles of substrate hydrolysed per 4 hr							
				Muscle	Perienteric fluid	Intestine contents			Extract from the intestine wall		
						a	m	p	a	m	p
Alkaline phosphatase	3.1.3.1	2-naphtyl phosphate	8.5	20	30	40	40	30	40	40	40
Esterase (C 4)	3.1.1.6	2-naphthyl butyrate	6.5	10	30	30	20	30	30	30	20
Esterase Lipase (C 8)	3.1.1.3	2 - naphthyl caprylate	7.5	20	30	30	40	30	40	30	20
Lipase (C 14)	3.1.1.3	2- naphthyl myristate	"	0	0	0	0	0	0	0	0
Leucine arylamidase	3.4.11.14	L-leucyl-2-naphthylamide	"	40	40	40	40	30	40	40	40
Valine arylamidase	3.4.11.14	L-valyl-2-naphthylamide	"	20	5	40	40	40	40	40	40
Cystine arylamidase	3.4.11.14	L-cystyl-2-naphthylamide	"	0	5	5	20	0	20	5	20
Trypsin	3.4.4.4	N-benzoyl-DL-arginine-2-naphthylamide	8.5	0	5	0	0	0	0	0	0
α-chymotrypsin	3.4.4.5	N-glutaryl-phenylalanine-2-naphthylamide	7.5	5	5	5	0	0	40	40	20
Acid phosphatase	3.1.3.2	2- naphthyl phosphate	5.4	30	10	40	40	40	40	40	40
Naphthol-AS-BI-phosphohydrolase	3.1.3.31	Naphthol-AS-BI-phosphate	"	5	0	40	40	40	40	40	40
α-galactosidase	3.2.1.22	6-Br –2- naphtyl-αD-galactopyranoside	"	0	0	0	0	0	0	0	0
β-galactosidase	3.2.1.23	2-naphthyl-βD- galactopyranoside	"	40	40	10	5	20	40	40	30
β-glucuronidase	3.2.1.31	Naphthol-AS-BI-βD-glucoronide	"	0	40	0	0	0	0	0	0
α-glucosidase	3.2.1.20	2-naphthyl-αD-glucopyranoside	"	5	10	40	40	40	40	40	40
β-glucosidase	3.2.1.21	6-Br-2-naphthyl-βD-glucopyranoside	"	40	40	0	5	5	0	0	0
N-acetyl-β-glucosaminidase	3.2.1.50	1-naphthyl-N-acetyl-βD-glucosaminide	"	40	40	40	40	40	40	40	40
α-mannosidase	3.2.1.24	6-Br-2-naphthyl-αD-mannopyranoside	"	0	5	0	0	0	5	0	0
α-fucosidase	3.2.1.51	2-naphthyl-αL-fucopyranoside	"	40	10	40	40	40	40	40	40

a – anterior section of the intestine; m – middle section of the intestine; p – posterior section of the intestine

drolases was determined. Only the activity of the same hydrolases as in the contents was determined in the extracts from this part of intestine; additionally, a certain activity of α-mannosidase (5 nmol) was determined. In most cases, the activity of enzymes in the intestine contents and in the wall of this part of the intestine was at the same or similar level. Only in the case of cystine arylamidase and α-chymotrypsin was the activity in the tissue much higher than in the contents.

In the contents and extracts from the middle part of intestinal wall 13 hydrolases exhibited activity. The activity of most hydrolases was similar to those determined in the tissue. The activity of cystine arylamidase found in the contents was 4 times higher than in the tissue, whereas the activity of β-galactosidase was 8 times higher in the tissue from this part of intestine. The activity of α-chymotrypsin was found in the tissue, but it was not found in the intestine contents.

Twelve (12) hydrolases exhibited activity in the contents taken from the terminal part of the intestine of *A. suum*; whereas the activity of 13 enzymes was found in the respective intestinal tissue. The activity of most hydrolases was found to be of a similar level in both samples. No activity of cystine arylamidase and α-chymotrypsin was found in the intestine contents, and in the extract from the tissue the activity of these enzymes was found to be 20 nmol. The activity of β-glucosidase was found in the intestine contents; this enzyme was not found in the extract from this part of the intestine.

Discussion

The results obtained in this experiment concerning the activity of hydrolases in particular sections of the intestine of *A. suum* are similar in most cases. This is attributed to the rapid flow of contents along the intestine of *A. suum*. According to Bird and Bird (1991) the time of contact between food and digesting enzymes in the intestine of *A. suum* is very short, because the intestine is completely emptied every 3 minutes.

The results obtained in this study are, in most cases, consistent with those obtained by other authors and they confirm that the intestine of *A. suum* is able to digest most kinds of food. The presence of phosphatases and esterases and their high activity is considered to indicate places which are responsible for secretive and metabolic activity, and for the absorption of food (von Brand, 1979). In this experiment, the very high activity of phosphatases and esterases was determined in the contents of intestines, and in the homogenates of the intestinal wall. The high activity of these enzymes was confirmed in flatworms (Niemczuk, 1993) in which food products are absorbed primarily through the tegument.

According to many authors (Beames & King, 1972; Maki *et al*., 1982; Oue *et al*., 2000), the habitat and the type of absorbed food is a limiting factor on the presence and activity of enzymes in the intestine of a parasite. According to these authors, if food partly digested by the host's enzymes is available to *A. suum*, the latter does not have to synthesise many digestive enzymes of its own, mostly those which hydrolyse macromolecular compounds. The above authors found the activity of acid protease in the alimentary tract of *A. suum* to be much lower than in the alimentary tract of those parasites which feed on blood. Dziekońska-Rynko *et al*., (2003) found activity for all 19 hydrolases in extracts from mature individuals of *Cystidicola farionis* with the use of API Zym tests. This parasite lives in the bladder of Salmonid fishes. Nisbet and Billingsley (2000) found a significant effect of diet on the activity of hydrolases in extracts obtained from saprophytes. The highest activity of these 19 hydrolases was found in the extract obtained from a free-living *Acarus siro,* whereas in extracts from saprophytes which live on blood and plants, the amount of active hydrolases was much lower. The authors did not find any trypsin activity in any sample.

In the present study, no lipase activity was found in any part of the *A. suum* organism, but esterase or esterase lipase were found in all samples, which is consistent with the results obtained by Lee (1962a), who found esterase activity in all the parts of the *A. suum* organism. Żółtowska and Łopieńska (1999) found the activity of lipase in all examined parts of *A. suum*; the highest activity was found in the intestine. In the present study activity of trypsin was indication only in pseudocelomic fluid. Juhasz (1979a) found activity of both endopeptidases, trypsin and chymotrypsin, in the contents and tissue of the intestine, with trypsin activity 10 times lower than that of chymotrypsin. The high activity of chymotrypsin found in the present study suggests that this enzyme is associated with the brush border of the ascarid intestine, as its highest activity was found in the homogenates from the intestinal wall. Rupova *et al*. (1984) claim that in the intestine of *A. suum* there are no enzymes similar to trypsin, chymotrypsin and enterokinase, but cathepsins are there. According to Hogan (1980) the intestine of *A. suum* is not able to digest proteins to a significant extent, and the proteases which are found there are assimilated from the host. According to Maki and Yanagisawa (1986) aminopeptidases are the dominant protein digesting enzymes in the *A. suum* intestine, although aspartyl and cysteine proteases were also found. The results obtained in the present study are similar to those obtained by these authors and demonstrate that the *Ascaris* intestine is more able to digest small-molecular compounds, which is indicated by a high activity of aminopeptidases. A high activity of aminopeptidases has also found in the intestines of other parasites like *Haemonchus contortus* (Newton, 1995) and *Fasciola hepatica* (Acosta *et al*., 1998). In these parasites, the enzymes are integrally bound to intestine microvilli and are never found in the culture medium. However, according to Rhoads and Fetterer (1998), in *A. suum* aminopeptidases are found in various parts and may be secreted to the environment. The high activity of aminopeptidases determined in muscles and pseudocelomic fluid in this experiment, is similar to the results obtained by the above authors. Leucine aminopeptidase is a very important enzyme which is found in various parts of most parasites.

Its presence was detected in the ovaries, uterus, intestine and perienteric fluid of *A. suum* (Lee, 1962b; Rhodes *et al*., 1966; Rhodes *et al.,* 1969; Rhoads & Fetterer, 1998). In our experiments, apart from this aminopeptidase, high activities of valine aminopeptidase and cystine aminopeptidase were seen. In the body of a parasite, aminopeptidases may play various roles, including the hatching and moulting of larvae, degrading host proteins during the process of infiltration and migration, destroying antibodies and modulating the entire host immunological system (McKerrow, 1989; Sajid & McKerrow, 2002). According to Sajid *et al*., (1997) the aminopeptidases from the muscles of *A. suum* may play an important role in intracellular metabolism and inactivate neuropeptides.

The presence of carbohydrates digesting enzymes in the intestine and in other parts of the body of *A. suum* has been detected by many authors (Palma *et al*., 1970; Gentner *et al.,* 1972; Zenka & Prokopic 1984; Żółtowska, 1991, 1995, 2001; Żółtowska & Dmitryjuk, 1998; Dmitryjuk & Żółtowska 2004). These authors determined the activity of α-amylase, maltase, invertase, palatinase and trehalase, but they did not find any activity of β-galactosidase (lactase) and β-glucosidase (celobiase). In the present study, high activity of β-galactosidase (lactase) was found in all the examined samples. The activity of β-glucosidase (celobiase) in perienteric fluid and muscles was very high, but was very low in the intestine and was confined to the contents of the middle and posterior sections of the intestine. β-galactosidase (lactase) activity was found in all parts of the intestine in the present study, but was much lower than in intestine tissue, which may indicate that, like other disaccharides, this enzyme is associated with the brush border of the intestine of *A. suum*, which is consistent with the results obtained by the above authors. In all the examined samples, there was a high activity of α-fucosidase and N-acetyl-β-glucosaminidase. In the muscles and, perienteric fluid high activity was found for β-glucuronidase and β-glucosidase, whose activity was not found in the intestine. These are enzymes commonly found in tissues and body fluids of many animals but their functions are not well-known. It is notable that the very high activity of N-acetyl-β-glucosaminidase was found in all cases. This enzyme plays an important role in the hydrolysis of digestion products of hyaluronic acid, one of the main components of connective tissue. It is broken down when parasites penetrate host tissues (Hotez *et al*., 1994). Nisbet and Billingsley (2000) found a particularly high activity of N-acetyl-β-glucosaminidase in excretes obtained from parasitic saprophytes (*Dermanyssus gallinae* and *Psoroptes ovis).* According to these authors, the enzyme plays an important role in the development processes of all invertebrates.

References

ACOSTA, D., GONI, F., CARMONA, C. (1998): Characterization and partial purification of a leucine aminopeptidase from *Fasciola hepatica*. *J. Parasitol*., 84: 1 – 7

BEAMES, C. G. JR., KING, G. A. (1972): Factors influencing the movement of materials across the intestines of *Ascaris*. In VAN DEN BOSSCHE, H. (Ed): Comparative Biochemistry of Parasites. New York, Academic Press: 275 – 282

BIRD, A. F., BIRD, J. (1991): The structure of nematodes. New York, Academic Press

BRADFORD, M. M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt. Biochem*., 44: 653 – 662

BRAND, VON T. (1973): Biochemistry of parasites. Acad. Press, New York-London

BRAND, VON T. (1979): Biochemistry and physiology of endoparasites. Elsevier/North-Holand Biomedical Press, Amsterdam

DMITRYJUK, M., ŻÓŁTOWSKA, K. (2004): Trehalose catabolism enzymes in tissues of *Ascaris suum* (Nematoda). *Helminthologia*, 41: 63 – 66

DZIEKOŃSKA-RYNKO, J., ROKICKI, J., JABŁONOWSKI, Z. (2003): The activity of selected hydrolases in excretion-secretion products and extracts from larvae and mature specimens of *Cystidicola farionis*. *Oceanol. and Hydrobiol. Studies*, 4: 117 – 129

GENTNER, H., SAVAGE, W. R., CASTRO, G. A. (1972): Disaccharidase activity in isolated brush border from the gut of *Ascaris suum*. *J. Parasitol*., 58: 247 – 251

HOGAN, B. J. (1980): Function of the gut in the parasitic roundworm *Ascaris lumbricoides* var. *suum*. *Proceedings of the South Dakota Academy of Sciences*. 59: 283

HOTEZ, P., CAPPELLO, M., HAWDON, J. (1994): Hyaluronidases of Gastrointestinal Invasive Nematodes *Ancylostoma caninum* and *Anisakis simplex*: Possible functions in the Pathogenesis of Human Zoonoses. *J. Inf. Dis.*, 170: 918 – 926

JUHASZ, S. (1979a): Proteolytic enzymes and enzyme inhibitors in *Ascaris suum*. I. Proteolytic enzymes and enzyme inhibitors in the gut. *Acta Vet. Acad. Sci. Hung*., 27: 83 – 87

JUHASZ, S. (1979b): Proteolytic enzymes and enzyme inhibitors in *Ascaris suum*. IV. Estimation of molecular weights of chymotrypsin inhibitors and an intestinal protease by gel chromatography. *Acta Vet. Acad. Sci. Hung*., 27: 217 – 224

LEE, D. L. (1962a): The distribution of esterase enzymes in *Ascaris lumbricoides*. *Parasitology,* 52: 241 – 260

LEE, D. L. (1962b): The histochemical localization of leucine aminopeptidase in *Ascaris lumbricoides*. *Parasitology,* 52: 533 – 538

LEŠTAN, P., DUBINSKÝ, P., RYBOŠ, M. (1974): The nutrition of Nematodes. *Helminthologia,* 15: 763 – 772

MAKI, J., FURUHASHI, A., YANAGISAWA, T. (1982): The activity of acid proteases hydrolysing haemoglobin in parasitic helminths with special reference to interspecific and intraspecific distribution. *Parasitology,* 84: 137 – 147

MAKI, J., YANAGISAWA, T. (1986): Demonstration of carboxyl and thiol protease activities in adult schistosoma mansoni, *Dirofilaria immitis*, *Angiostrongylus cantonensis* and *Ascaris suum*. *J. Helminthol*., 60: 31 – 37

MCKERROW, J. H. (1989): Parasite Proteases. *Exp. Para-*

sitol., 68: 111 – 115
NEWTON, S. E. (1995): Progress on vaccination against *Haemonchus contortus*. *Int. J. Parasitol.*, 25: 1281 – 1289
NIEMCZUK, W. (1993): Localization and activity of several enzymes in tissues of *Bothriocephalus acheiloghathi* and *Khawia sinensis* tapeworms parasitizing in carp. *Wiad. Parazytol.,* 39: 189 – 197
NISBET, A. J., BILLINGSLEY, P. F. (2000): A comparative survey of the hydrolytic enzymes of ectoparasitic and free-living mites. *Int. J. Parasitol*., 30: 19 – 27
OUE, M., YAMAGUCHI, H., SATO, K., SUZUKI, M. (2000): Immunohistochemical localization of Acid proteinase in *Ascaris suum*. *Exp.Parasitol*., 95: 144 – 147
PALMA, R., ORREGO_MATTE, H., SALINAS, A. (1970): Disaccharidase activity in the intestine of *Ascaris lumbricoides* Linne, 1758. *Comp. Biochem. Physiol*., 34: 405 – 413
PAPPAS, P.W. (1988): The relative roles of the intestines and external surfaces in the nutrition of monogeneas, digeneas and namatodes. *Parasitology,* 96: 105 – 121
RHOADS, M. L., FETTERER, R. H. (1998): Purification and characterisation of a secreted aminopeptidase from adult *Ascaris suum*. *Int. J. Parasitol.,* 28: 1681 – 1690
RHODES, M. B., MARSH, C. L., FERGUSON, D. L. (1966): Studies in helminth enzymology.V. An aminopeptidase of *Ascaris suum* which hydrolyzes L-leucyl-β-naphthylamide. *Exp. Parasitol*., 19: 42 – 51
RHODES, M. B., MARSH, C. L., FERGUSON, D. L. (1969): *Ascaris suum*: Purification and characterization of an intestinal aminopeptidase. *Exp. Parasitol*., 26: 150 – 155
RUPOVA, L., DRAGNEVA, N., BANKOV, OSSIKOVSKI, E. (1984): Investigation on a protease complex from adult *Ascaris suum*. I. Isolation of an acid protease and characteristic of its properties. *Helminthologia,* 21: 257 – 265
SAJID, M., MCKERROW, J. H. (2002) Cysteine proteases of parasitic organisms, *Mol. Biochem. Parasitol.,* 120: 1 – 21
SAJID, M., ISAAK, R. E., HARROW, A. N. D. (1997): Purification and properties of membrane aminopeptidase from *Ascaris suum* muscle that degrades neuropeptides AF1 and AF2. *Mol. Biochem. Parasitol.,* 89: 225 – 234
VAN DEN BOSSCHE, H., BORGERS, M. (1973): Subcellular distribution of digestive enzymes in *Ascaris suum* intestine. *Int. J. Parasitol*., 3: 59 – 65
ZENKA, J., PROKOPIC, J. (1984): Isolation and properties of alpha-amylase from perienteric fluid of *Ascaris suum*. *Folia Parasitol*., 31: 183 – 186
ŻÓŁTOWSKA, K. (1991): Distribution of enzymes hydrolyzing carbohydrates in the alimentary tract of *Ascaris suum*. *Acta Parasitol. Polon*., 36: 93 – 96
ŻÓŁTOWSKA, K. (1995): The arrangemement of amylase in muscles of *Ascaris suum. Acta Parasitol*. *Polon*., 40: 214 – 216
ŻÓŁTOWSKA, K., DMITRYJUK, M. (1998): Activity of trehalase in muscles of *Ascaris suum* (Nematoda). *Helminthologia,* 35: 3 – 6
ŻÓŁTOWSKA, K., ŁOPIEŃSKA, E. (1999): Distribution of the activity of triacyloglycerol lipase in the tissues of female *Ascaris suum* (Nematoda). *Helminthologia,* 36: 75 – 78
ŻÓŁTOWSKA, K. (2001): Purification and characterization of α-amylases from the intestine and muscle of *Ascaris suum* (Nematoda). *Acta Biochim Polon*., 48: 763 – 774

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