WIADOMOŚCI PARAZYTOLOGICZNE
T. 50 (2) 2004: 269–272

# ACTIVITY OF HYDROLASES IN THE REPRODUCTIVE SYSTEM AND IN CONSECUTIVE STAGES OF *ASCARIS SUUM* DEVELOPMENT

**JANINA DZIEKOŃSKA-RYNKO**

Chair of Zoology, Faculty of Biology, University of Warmia and Mazury in Olsztyn, 10-957 Olsztyn, Oczapowskiego 5; E-mail:jdr@matman.uwm.edu.pl

**ABSTRACT.** The activity of hydrolases in the eggs isolated from the uterus of *A. suum* is in most cases similar to that in the reproductive system. The activity of hydrolases was low in the stages of cleavage and gastrulation, and it grew until the larva stage.

**Key words:** *Ascaris suum*, eggs, hydrolase, reproductive system.

## INTRODUCTION

Hydrolases are found not only in the digestive system of *A. suum* but also in other tissues (Lee 1962 a, b; Rhodes et al. 1969; Wimmer et al. 1998; Żółtowska and Łopieńska 1999). Their role is particularly significant in the processes associated with the hatching and moulting of larvae. The activity of proteases, esterases, lipase, chitinase, α and ß-glukosidases, hyaluronidase and leucine aminopeptidase were found in the hatching liquid of the parasites (Hinck and Ivey 1976, Jabłonowski et al. 1993, Rhoads et al. 1997, Young et al. 1999, Geng et al. 2002). The available literature does not contain any papers concerning the activity of hydrolases in the reproductive system and in the consecutive stages of *A. suum* development.

## MATERIALS AND METHODS

An ovary, oviduct and uterus were isolated from mature female *A. suum*. Eggs were squeezed out of the terminal sections of uterus. Some eggs were used directly for enzymatic determinations, while the others were divided into three parts and, after being suspended in 0.1 N solution of HCl, they were placed in a thermostat at 28°C. Every two days the liquid was replaced and the development was checked. After the stage of cleavage, gastrulation or the larval stage had been achieved by 60% of the eggs, incubation was discontinued. Parts of the reproductive system and eggs were homogenised in a glass Potter's homogeniser with the addition of phy-

siological saline. Following this, all samples were centrifuged at 3000 x g for 10 minutes. The supernatants were used for enzymatic determinations with API ZYM tests manufactured by bio Merieux.

## RESULTS AND DISCUSSION

The activity of 16 out of the determined 19 hydrolases was found in the ovary, 14 in the oviduct and 15 in the uterus (Table 1). The activity of such hydrolases as esterase, aminopeptidase and acidic protease in the reproductive system of *A. suum* has been ascertained by numerous authors: Lee (1962 a, b), Rhodes et al. (1969), Oue et al. (2000). Żółtowska and Łopieńska (1999) found a high activity of lipase in ovaries and eggs of *A. suum*, whereas the activity of this enzyme in the uterus was notably lower. No activity of lipase, trypsin, α-galactosidase or α-mannosidase was found in any of the samples in this experiment. The activity of 10 hydrolases was found in eggs immediately after they were obtained from the uterus. The highest activity was determined for esterase, leucine and valine arylamidase, ß-galactosidase, ß-glucosidase. The activity of 6 hydrolases was found in eggs homogenates in the cleavage stage. The highest activity was determined for alkaline and acid phosphatase, naphthol-AS-BI-phosphohydrolase and ß-galactosidase. Similar results were obtained in the stage of gastrulation. The activity of 10 hydrolases was determined in the homogenates of eggs in the larval stage. The highest activity was determined for acid and alkaline phosphatase, naphthol-AS-BI-phosphohydrolase, ß-galactosidase, ß-glucuronidase, N-acetyl-ß-glucosaminidase and α-fucosidase. The results suggest that there is a correlation between the stage of embryonic development and the activity of hydrolases. Skotarczak (1987) observed changes in acid and alkaline phosphatase activity during *A. suum* embryogenesis. According to the author, in this initial stages the enzymes are associated with utilising carbohydrates and later with their resynthesis from reserve fat. No activity of alkaline phosphatase was found in the eggs obtained from the uterus in this experiment, and the activity of the acid phosphatase was very low. However, starting with the period of cleavage, a gradual increase in the activity of both enzymes was observed. It may be explained by a higher demand for energy by the developing embryo. No activity of such enzymes as esterase, esterase lipase, leucine arylamidase, N-acetyl-ß-glucosaminidase were found in the stages of cleavage or gastrulation, but it was found in the larval stage, which may indicate the preparation of larvae for the process of hatching and moulting. In the hatching liquid from *A. suum* eggs, the following enzymes were found: esterase, protease, chitinase (Ward and Fairbairn 1972, Hinck and Ivey 1976, Rogers 1982, Rhoads et al. 1997, Young et al. 1999, Geng et al. 2002). Nisbet and Billingsley (2000) found a high activity of N-acetyl-ß-glucosaminidase during the moulting process of parasitic saprophytes. The above authors claim that it is an enzyme which is commonly found in invertebrates during the moulting process.

Table 1. Activity of hydrolases in the reproductive system and development stage of *Ascaris suum*

| Enzyme | * | Substrate | pH | Activity in nmoles of hydrolysed substrate | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ovary | oviduct | uterus | eggs | | | |
| | | | | | | | Z | C | G | L |
| Alkaline phosphatase | 3.1.3.1 | 2-naphtyl phosphate | 8.5 | 30 | 30 | 40 | 0 | 30 | 30 | 40 |
| Esterase (C 4) | 3.1.1.6 | 2-naphthyl butyrate | 6.5 | 30 | 30 | 30 | 30 | 0 | 0 | 5 |
| Esterase Lipase (C 8) | 3.1.1.3. | 2 – naphthyl caprylate | 7.5 | 30 | 30 | 30 | 5 | 0 | 0 | 5 |
| Lipase (C 14) | 3.1.1.3 | 2- naphthyl myristate | 7.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Leucine arylamidase | 3.4.11.14 | L-Leucyl-2 -naphthylamide | 7.5 | 40 | 40 | 40 | 40 | 0 | 0 | 5 |
| Valine arylamidase | 3.4.11.14 | L-valyl-2 -naphthylamide | 7.5 | 40 | 40 | 40 | 40 | 0 | 0 | 0 |
| Cystine arylamidase | 3.4.11.14 | L-cystyl-2 -naphthylamide | 7.5 | 5 | 5 | 20 | 10 | 5 | 0 | 0 |
| Trypsin | 3.4.4.4 | N-benzoyl-DL-argini-ne-2-naphthylamide | 8.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| α-chymotrypsin | 3.4.4.5 | N-glutaryl-phenylala-nine-2-naphthylamide | 7.5 | 5 | 5 | 5 | 0 | 0 | 0 | 0 |
| Acid phosphatase | 3.1.3.2 | 2- naphthyl phosphate | 5.4 | 40 | 40 | 40 | 10 | 40 | 40 | 40 |
| Naphthol-AS-BI -phosphohydrolase | 3.1.3.31 | Naphthol-AS-BI -phosphate | 5.4 | 10 | 10 | 40 | 10 | 30 | 40 | 30 |
| α-galactosidase | 3.2.1.22 | 6-Br -2- naphtyl- αD -galactopyranoside | 5.4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ß- galactosidase | 3.2.1.23 | 2-naphthyl- ßD -galactopyranoside | 5.4 | 40 | 40 | 40 | 20 | 40 | 40 | 40 |
| ß-glucuronidase | 3.2.1.31 | Naphthol-AS-BI ßD-glucoronide | 5.4 | 40 | 30 | 10 | 0 | 0 | 5 | 30 |
| α- glucosidase | 3.2.1.20 | 2-naphthyl-αD -glucopyranoside | 5.4 | 20 | 0 | 10 | 0 | 0 | 0 | 0 |
| ß- glucosidase | 3.2.1.21 | 6-Br-2-naphthyl-ßD -glucopyranoside | 5.4 | 5 | 40 | 40 | 30 | 0 | 0 | 0 |
| N- acetyl-ß -glucosaminidase | 3.2.1.50 | 1-naphthyl-N-acetyl-ßD -glucosaminide | 5.4 | 40 | 40 | 40 | 0 | 0 | 0 | 30 |
| α– mannosidase | 3.2.1.24 | 6-Br-2-naphthyl- αD mannopyranoside | 5.4 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| α- fucosidase | 3.2.1.51 | 2-naphthyl- αL -fucopyranoside | 5.4 | 40 | 10 | 40 | 5 | 5 | 10 | 30 |

*clasiffication; Z – zygote; C – cleavage; G – gastrulation; L – larval stage

## Literature

Geng J., Plenefisch J., Kumieniecki P.R., Kumieniecki R. 2002. Secretion of a novel developmentally regulated chitinase (family 19 glycosyl hydrolase) into the perivitelline fluid of the parasitic namatode, *Ascaris suum*. *Molecular & Biochemical Parasitology* 124: 11-21.

Hinck L.W., Ivey M.H. 1976. Proteinase activity in *Ascaris suum* eggs, hatching fluid and excretions-secretions. *Journal of Parasitology* 62: 771-774.

Jabłonowski Z., Łukaszewicz-Babecka J., Piechocki D. 1993. The processes connected with hatching of larvae from eggs of parasitic nematodes. *Wiadomości* Parazytologiczne 39: 3-12.

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.

Nisbet A.J., Billingsley P.F. 2000. A comparative survey of the hydrolytic enzymes of ectoparasitic and free-living mites. *International Journal for Parasitology* 30: 19-27.

Oue M., Yamaguchi H., Sato K., Suzuki M. 2000. Immunohistochemical localization of acid proteinase in *Ascaris suum*. *Experimental Parasitology* 95: 144-147.

Rhoads M.L., Fetterer R.H., Urban J.F. 1997. Secretion of an aminopeptidase during transition of third- to fourth- stage larvae of *Ascaris suum*. *Journal of Parasitology* 83: 780-784.

Rhodes M.B., Marsh C.L., Ferguson D.L. 1969. Studies in helminth enzymology. VI. Aminopeptidases from uterine extracts of *Ascaris suum*. *Experimental Parasitology* 26: 140-149.

Rogers W.P. 1982. Enzymes in the exsheathing fluid of Nematodes and their biological significance. *International Journal for Parasitology* 12: 495-502.

Skotarczak B. 1987. Histochemical studies on eggs of *Ascaris suum* during embryogenesis. I. Phosphatases. *Wiadomości Parazytologiczne* 33: 681-685.

Ward K.A., Fairbairn D. 1972. Chitinase in developing eggs of *Ascaris suum* (Nematoda). *Journal of Parasitology* 58:546-549.

Wimmer M., Schmid B., Tag C., Hofer H.W. 1998. *Ascaris suum*: Protein phosphotyrosine phosphatases in oocytes and developing stages. *Experimental Parasitology* 88:139-145.

Young A.R., Macuso N., Bowles V.M. 1999. Biochemical aspects of egg hatch in endo- and ectoparasites: potential for rational drug design. *International Journal for Parasitology* 29:861-867.

Żółtowska K., Łopieńska E. 1999. Distribution of the activity of triacyloglycerol lipase in the tissues of female *Ascaris suum* (Nematoda). *Helminthologia* 36: 75-78.

*Zaakceptowano do druku 30 maja 2004*