DOI: 10.2478/s11686-012-0007-8

Acta Parasitologica, 2012, 57(1), 46–52; ISSN 1230-2821

VERSITA

Fasciola hepatica miracidia: Lectin binding and stimulation of *in vitro* miracidium-to-sporocyst transformation

Katya Georgieva[1]*, Simona Georgieva[1], Yana Mizinska[1] and Stoyanka R. Stoitsova[2]

[1]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Street, 1113 Sofia, Bulgaria;
[2]Institute of Microbiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Street, Bl. 26, 1113 Sofia, Bulgaria

Abstract

The lectin binding properties of *Fasciola hepatica* miracidia were studied by a panel of fluorescein- and gold-conjugated lectins (ConA, LCA, WGA, LEA, SBA, HPA and UEA-I). The presence of mannose and/or glucose residues was demonstrated with ConA and LCA as weak diffuse fluorescence of the miracidial surface, which was more intense at the anterior part of the larva. The N-acetylglucosamine-binding lectins WGA and LEA reacted intensely with the whole miracidial surface. No labelling with N-acetylgalactosamine and/or galactose-specific (SBA and HPA) and fucose-specific UEA-I lectins was observed. The possibility that the specific recognition of the miracidial surface carbohydrates by lectins may initiate the process of transformation of the miracidia into sporocysts was examined *in vitro* in physiological saline for *Galba truncatula*. Incubation in the presence of ConA and WGA resulted in facilitation of the transformation process. Facilitation was absent in the presence of inhibitor sugars. Incubation in the presence of SBA or UEA-I had no effect. The results suggested a possible impact of carbohydrate-lectin interactions in transformation of miracidia of *F. hepatica* to sporocysts *in vivo*.

Keywords

Fasciola hepatica, miracidia, lectin binding, lectin-carbohydrate interactions, *in vitro* miracidium-to-sporocyst transformation

Introduction

The surface carbohydrates of trematode larvae have long attracted attention due to the important role of glycan-binding proteins (lectins) among the non-self recognition mechanisms of the intermediate gastropod host (Horák and van der Knaap 1997, Lockyer *et al*. 2004, Loker 2010). One traditional way to characterize carbohydrate surface epitopes is using panels of commercially available lectins with different sugar specificities. Extensive studies on larval stages of representatives of the family Schistosomatidae have drawn the attention to the occurrence of stage- and species-specific differences in the lectin-binding capacity. This also concerns miracidia (Coles *et al*. 1988). Notably, several species (*Schistosoma mansoni*, *S. margrebowiei*, *Trichobilharzia ocellata* and *T. szidati*) were shown to undergo drastic changes in their surface carbohydrates and lose a number of their surface epitopes while sloughing their ciliated epithelia during miracidium-to-sporocyst transformation (Yoshino *et al*. 1977, Gerhardus *et al*. 1991, Daniel *et al*. 1992, Horák 1995, Peterson *et al*. 2009).

Fasciola hepatica, a helminth of unquestionable economic importance, has attracted little attention in this respect. This motivated our interest, and we have previously shown stage-specific lectin reactivities in sporocysts and rediae (Georgieva *et al*. 2005, 2007). The present study is directed to the characterization of the lectin-binding capacity of miracidia.

While sporocysts and rediae throughout their life span are in contact with snail effector mechanisms, including hemolymph lectins, the possible role of species specificity of miracidial surface carbohydrates and the related specific lectin binding capacity is less clear. One possibility is that this might be responsible for the recognition of the specific host by the miracidia prior to the penetration of miracidia into the snail. Specificity at this level of the host-parasite interaction is however quite questionable. For instance, using a set of four digenean (echinostomatid and schistosomatid) and five snail species, Sapp and Loker (2000) failed to confirm host selectivity at the stage of miracidium-snail attachment or penetration. Rather, over half of the miracidia of each parasite species attached and attempted to penetrate into both compatible and incompatible snails.

Another possibility is that the species-specific miracidial carbohydrate disguise may be related with triggers of their developmental into a next intramolluscan larval stage upon con-

*Corresponding author: katyageor@gmail.com

tacts with the specific snail host's lectins. Lectins are a component of snail hemolymph protective arsenal but they are also present in the mucus secreted by the molluscans (Iguchi *et al*. 1985, Pales Espinosa *et al*. 2009, Ito *et al*. 2011). To check the possibility of lectin binding as a trigger for miracidial transformation, we examined the interference of commercially available lectins with *in vitro* miracidium-to-sporocyst transformation.

Materials and methods

Miracidia

Fasciola hepatica life cycle was maintained in our laboratory using *Galba truncatula* as intermediate and male Wistar rats as definitive hosts. Metacercariae of *F. hepatica* were initially purchased from Baldwin Aquatics, Inc. (USA). Rats were fed with 15 metacercariae each. The adult flukes were isolated from rats 10 weeks post infections and washed in physiological saline. Parasite mature eggs were collected from the upper uterine coils of adult flukes, washed several times with distilled water and incubated in distilled water at 25°C for 14 days in the dark. Hatching of the miracidia was induced by light stimulation.

The snails originally came from the natural population from Razlog, South-West Bulgaria. They were grown in Petri dishes on mixed culture of algae. The field-collected algae *Oscillatoria* spp. originated from the same place as snails were reared on hand-smoothed mud surface, covered with boiled tap water, at room temperature and a diurnal photoperiod of 12 hours with a 3,000–4,000 l × light intensity. Snails measuring 4 mm were infected by placing them individually in small pots with boiled tap water, containing 10 miracidia by pot, for 4 hours at room temperature.

Fifty days post infection the snails were placed in glass bowls with transparent cellophane and boiled tap water so that they would release *F. hepatica* cercariae. The snails were stimulated by strong light and upon ventilator up to 2 hours. The emerged cercariae quickly spontaneously encysted to metacercariae. They were washed with distilled water and stored in distilled water at 4°C before infection of rats.

Lectins

The lectins applied in this study and their carbohydrate specificities are listed in Table I. Unlabelled lectins, lectin-FITC and lectin-gold conjugates were purchased from Sigma-Aldrich. They were dissolved in PBS (0.1 M Na_2HPO, 0.1 M $NaH_2PO_4.2H_2O$, 0.15 M NaCl, pH 7.2) with the addition of 0.1 mM $CaCl_2$ and $MnCl_2$ for ConA. For inhibitory controls, the buffers were supplemented with 0.2 M methyl-α-D-mannopyranoside for ConA and LCA, N-acetyl-D-glucosamine for LEA, N-acetyl-D-galactosamine for SBA and HPA, and α-L-fucose for UEA-I. For WGA, the concentration of N-acetyl-D-glucosamine was 0.5 M.

Lectin-FITC and lectin-gold labelling

Miracidia were fixed in 4% paraformaldehyde and 0.1% glutaraldehyde in PBS for 2 hours at 4°C. After a buffer rinse, the miracidia were incubated with 5% bovine serum albumin (Sigma-Aldrich) in PBS (BSA-PBS) for 1 hour at room temperature to block unspecific binding. Blocked larvae were incubated for 1 hour with lectins-FITC at concentration 20 µg/ml in PBS. Following lectin labelling, larvae were washed five times with 1% BSA-PBS and buffer only (10 min/wash). The treated parasites were transferred onto microscopic slides, covered with cover slips and observed using a Leica DM 5000B fluorescence microscope.

In vitro *miracidium-to-sporocyst transformation*

In vitro transformation of miracidia into sporocysts was performed in physiological saline for *Galba truncatula* (PSGtr) (Pullin 1971) consisting of: 1.182 g NaCl, 1.5 g $NaHCO_3$, 0.243 g $KHCO_3$, 0.041 g $(NH_4)_2SO_4$, 0.030 g $Na_3PO_4.12H_2O$, 0.605 g $CaCl_2.6H_2O$ and 0.418 g $MgCl_2.6H_2O$ per litre of distilled water, pH 7.8. Incubation was done at 25°C in glass vials, each containing 1.5 ml of PSGtr and 50 miracidia. The vitality and morphology of the larvae was checked under inverted microscope. To trace the dynamics of miracidia-to-sporocyst transformation, at different time intervals the parasites were fixed in 1% glutaraldehyde in PBS and examined microscopically.

Table I. Lectins used in this study and their carbohydrate specificities

Lectin from:	Carbohydrate specificity
Canavalia ensiformis (ConA)	Mannose, glucose
Lens culinaris (LCA)	Mannose, glucose
Triticum vulgaris (WGA)	N-acetylglucosamine, N-acetylneuraminic acid
Lycopersicon esculentum (LEA)	N-acetylglucosamine
Glycine max (SBA)	N-acetylgalactosamine, galactose
Helix pomatia (HPA)	N-acetylgalactosamine, galactose
Ulex europaeus (UEA-I)	Fucose

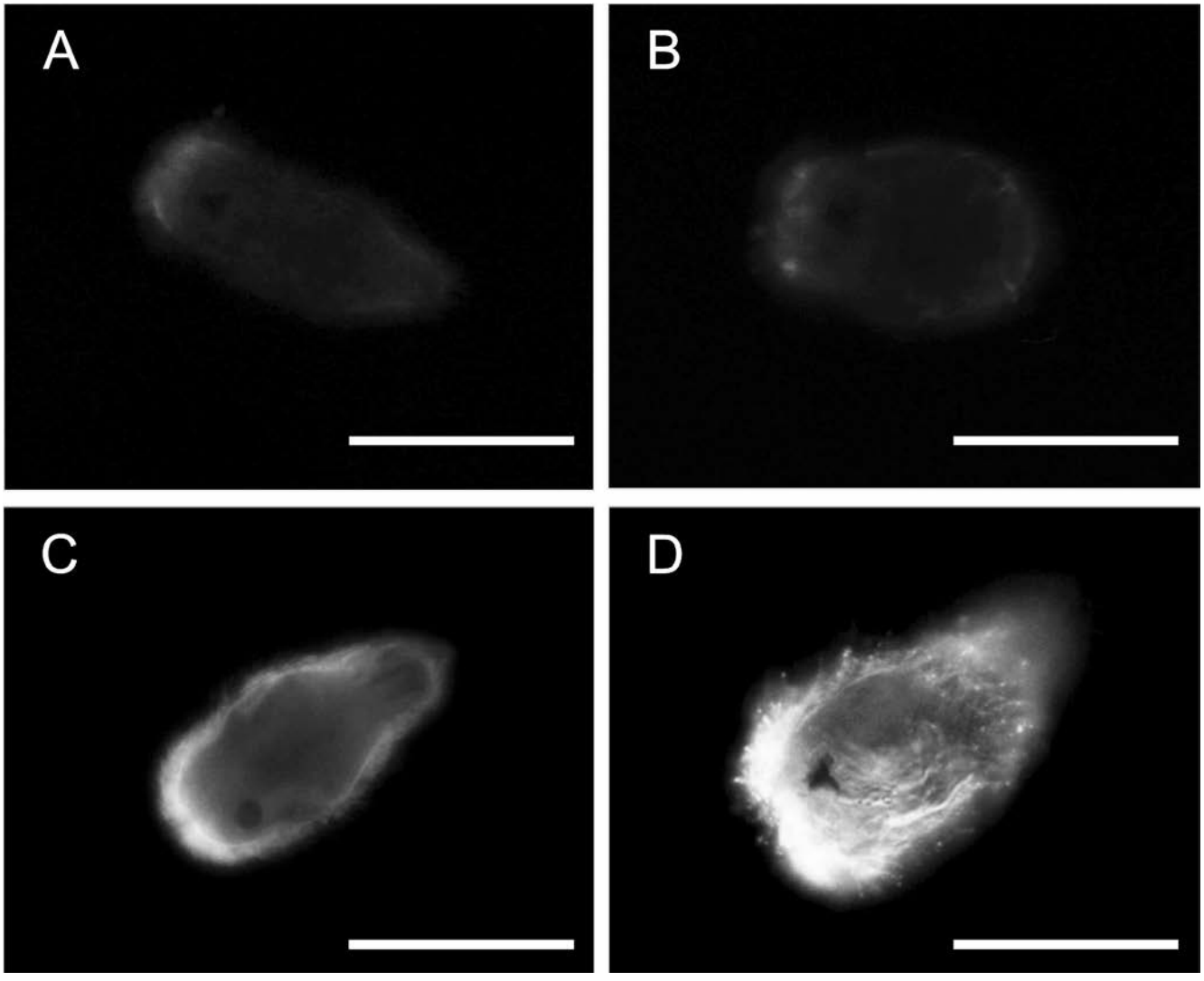

Fig. 1. Lectin-FITC labelling of the *F. hepatica* miracidia. ConA (A) and LCA (B). Staining located in the anterior end of the miracidia. The N-acetylglucosamine binding lectins WGA (C) and LEA (D) reacted intensely with the whole miracidial surface. Scale bars = 80 μm

The impact of lectins to *in vitro* miracidial transformation was estimated by the addition of 10 μg/ml of ConA, WGA, SBA or UEA-I. Controls comprised incubation in PSGtr alone, or in the presence of lectins plus 0.1 M of their specific inhibitory sugars. After 18 hours of incubation, the parasites were fixed and examined as above. The transformation was estimated by counting the miracidia, transforming miracidia and sporocysts. The experiment was repeated 10 times.

Statistics

Averages and standard deviations were calculated by Microsoft Excel. Comparisons of the average values between test and control samples were done by the Student's t-test using the Statistika software. In comparisons between experimental data sets, probability values of $P \leq 0.05$ were accepted as indication of statistically significant differences.

Results

Lectin-binding characteristics of the miracidia of F. hepatica

The results of the lectin labelling experiments are shown in Fig. 1. ConA and LCA binding resulted in very weak diffuse fluorescence of the miracidial surface, more intense at the anterior part of the larva (Fig. 1A, B). The N-acetylglucosamine-binding lectins (WGA and LEA) reacted intensely with the miracidial surface (Fig. 1C, D). No labelling of N-acetylgalactosamine and/or galactose-specific (SBA and HPA) and fucose-specific UEA-I lectins was observed. Specificity of the labelling reactions was confirmed by the

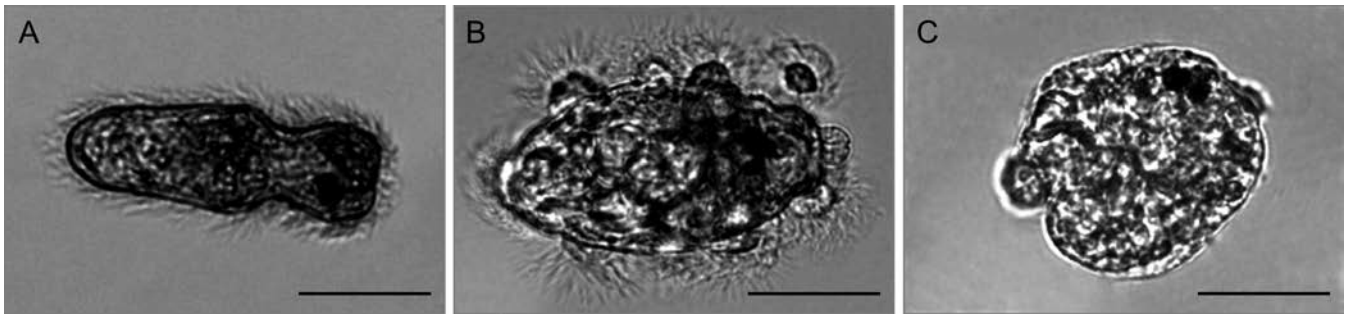

Fig. 2. *In vitro* transformation stages: (A) control – ciliated miracidium, (B) transforming miracidium with ciliary plates blebbing or in a state of detachment, (C) *in vitro* formed sporocyst. Scale bars = 50 μm

lack of fluorescence in the presence of the respective specific sugars.

Impact of lectin binding on in vitro *miracidium-to-sporocyst transformation*

Microscopic examinations on unfixed miracidia during the *in vitro* incubation in PSGtr showed a series of events. Initially, cilia stopped beating and miracidia became immobile. Subsequently, the ciliated plates were swelled out and started their spontaneous detachment from the surface, leading to the formation of young sporocyst. Thus, in fixed materials, the presence of three morphologically distinct forms was recorded: ciliated miracidia maintaining the typical miracidial shape and swimming freely (Fig. 2A), transforming miracidia with blebbed surface in the process of losing their ciliated plates (Fig. 2B) or individuals fully-transformed into sporocysts when all of ciliated plates were lost (Fig. 2C). Initial quantitative experiments aimed to trace the behaviour of the miracidia in PSGtr only (Fig. 3). The transformation process was quantified at 6-hour intervals. On 6 hour, the samples contained almost equal amounts miracidia and transforming miracidia, with only single sporocysts. After 18 hours, the transforming miracidia predominated. By 24 hours, almost 90% of miracidia have started or completed their transformation into sporocysts. No miracidia were present after 48 hours. Based on the above dynamics obtained in PSGtr only, quantitative estimation of the effect of lectins (10 μg/ml) was performed after 18 hours of incubation. Addition of ConA and WGA resulted in facilitation of the miracidium-to-sporocyst transformation.

Statistically significant differences from control values were obtained for ConA, i.e. reduction of the number of miracidia ($t = 5.32$, $n = 18$, $P = 0.001$) and increase of the number of sporocysts ($t = 11.76$, $n = 18$, $P = 0.001$) as well as for WGA, i.e. reduction of miracidia ($t = 5.32$, $n = 18$, $P = 0.001$) and increase of sporocysts ($t = 11.87$, $n = 18$, $P = 0.001$). All other values were within the range of the control samples (Fig. 4). No statistically significant difference was found between data obtained for ConA and WGA. Specificity of the interac-

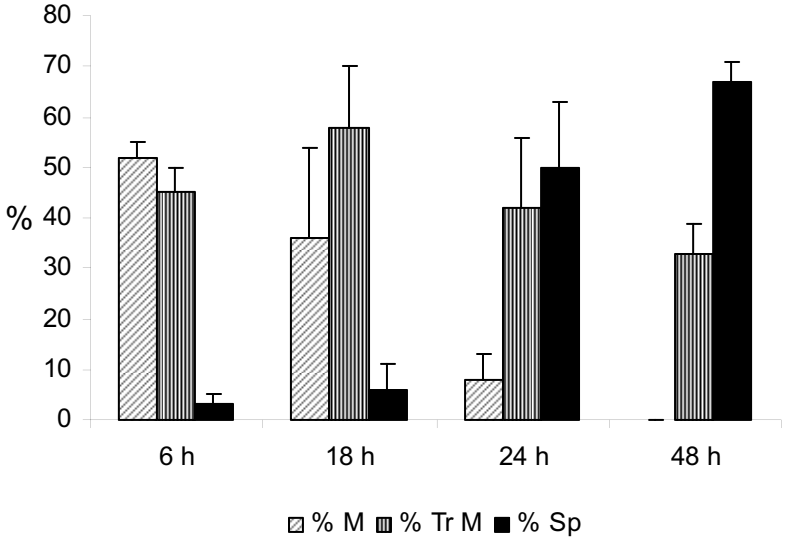

Fig. 3. Dynamics of the *in vitro* transformation of *F. hepatica* in PSGtr M – miracidia, Tr M – transforming miracidia, Sp – sporocysts

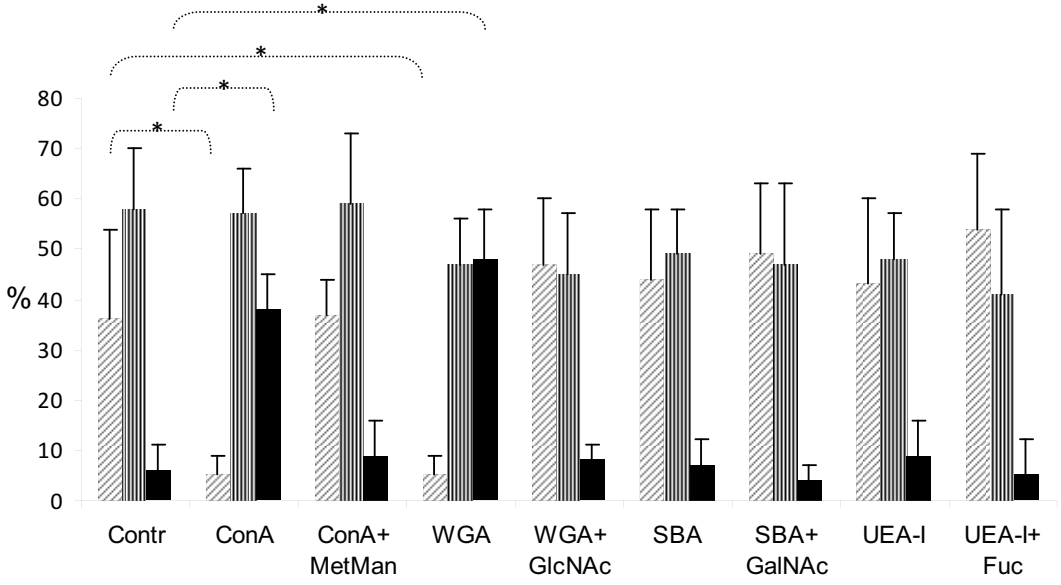

Fig. 4. Effects of the incubation in the presence of 10 μg/ml lectins on the occurrence of miracidia (M), transforming miracidia (Tr M) and sporocysts (Sp). (i) Control: incubation in physiological saline for *Galba truncatula* (PSGtr) (Contr); (ii) incubation in PSGtr plus ConA, WGA, SBA or UEA-I; (iii) inhibitory trial incubations: ConA + 0.1 M methylmannopyranoside; WGA + 0.1 M N-acetylglucosamine; SBA + 0.1 M N-acetylgalactosamine; UEA-I + 0.1 M fucose. Values are given as % of the total number of vital larvae recovered after 18 hours of incubation. The results are the means of 10 independent experiments, each starting with 50 miracidia. Error bars stand for standard deviations between the experiments. Statistically significant differences are marked by asterisks, $P < 0.001$

tion was confirmed by the lack of effect of the two lectins in the presence of their specific inhibitory sugar. The amounts of miracidia, transforming miracidia and sporocysts in the presence of SBA or UEA-I were similar to those for the controls.

Discussion

In this study, the lectin-binding characteristics of *F. hepatica* miracidia are described and specific recognition of miracidial surface carbohydrates by lectins is implied among the possible signals for miracidium-to-sporocyst transformation.

The experiments with FITC- and gold-labelled lectins have indicated that on the miracidia of *F. hepatica* there are mannose and/or glucose and N-acetylglucosamine carbohydrate termini. WGA and LEA label the entire larval surface but ConA and LCA recognize predominantly epitopes at the anterior miracidial surface. This lectin staining pattern of the miracidia of *F. hepatica* is different from that of sporocyst stage (Georgieva *et al*. 2005). It appears that the surfaces of miracidia and sporocysts of *F. hepatica* display stage-specific carbohydrates. While the mannose and/or glucose-binding lectins ConA and LCA label the anterior part of the miracidium of *F. hepatica*, they are the only positive labels for the sporocyst where they interact with its entire surface (Georgieva *et al*. 2005). Similarly, markers that bind only locally in miracidia of other trematode species have been shown to be overall labels in sporocyst surfaces. Thus, ConA and WGA have been demonstrated as located over intercellular ridges in *S. margrebowiei* miracidium but bound all over the sporocyst (Daniel *et al*. 1992). A monoclonal antibody against carbohydrate epitopes on the whole tegument of the primary sporocyst of *S. mansoni* has interacted only with intercellular ridges of the miracidium (Dunn and Yoshino 1988). These examples are in support of that the sporocyst tegumental glycocalyx might have started to differentiate under the ciliary epithelium.

Together with the commented above stage specificity, the comparison of our data with those on other miracidia implies species-specific characteristics of the carbohydrate disguise. Thus, the miracidia of *S. mansoni* are recognized by lectins with specificity to terminal mannose and/or glucose as well as N-acetylglucosamine but, unlike *F. hepatica*, also by lectins specific for N-acetylgalactosamine, galactose and fucose (Yoshino *et al*. 1977, Coles *et al*. 1988). Miracidia of *S. margrebowiei* reacted only with lectins recognizing N-acetylgalactosamine and galactose carbohydrates (Daniel *et al*. 1992). Avian schistosomes *T. ocellata* (see Gerhardus *et al*. 1991) and *T. szidati* (see Horák 1995) also differed in their lectin binding properties, i.e. fucose is detected on the miracidia of *T. szidati* in addition to mannose and/or glucose-, N-acetylglucosamine, N-acetylgalactosamine and galactose.

The specific carbohydrate coating of this developmental stage may have role in the fate of miracidia upon contacts with a snail environment. One guess is that specific binding of miracidial surface epitopes by exogenous lectins may initiate surface changes and influence miracidium-to-sporocyst transformation. In another *in vitro* system, it has been shown that

soluble protein factor(s) released by *Biomphalaria glabrata* embryonic cell line can trigger miracidium-to-sporocyst transformation in *S. japonicum* and *Fascioloides magna* (Coustau *et al*. 1997, Laursen and Yoshino 1999). In the present study, a facilitation of *in vitro* miracidium-to-sporocyst transformation has been shown in relation with the recognition of specific receptor sugars by the glycan-binding proteins ConA and WGA. In the presence of the ConA- and WGA-specific inhibitor monosaccharides, no facilitation is registered in comparison with the PSGtr control. Also, the lectins SBA and UEA-I, which do not bind to the miracidium, have no influence on the *in vitro* transformation process. This implies that stimulation of the transformation process is related with specific protein-carbohydrate interaction.

The specific lectin binding to live parasite specimens has earlier been shown to interfere with the surface membrane turnover. Specific binding of both ConA and WGA to living adult *F. hepatica* provoke surface membrane-related tegumental alterations like blebbing and blocked secretory vesicle discharge (Rogan and Threadgold 1984, Stoitsova *et al*. 1991). Similarly, it can be suggested WGA interacts with the total surface of the *F. hepatica* miracidium evoking blebbing of the ciliary epithelium. However, this is not likely to be the case with ConA, which binds predominantly at the anterior part of the larva but facilitates miracidial transformation to the same extent as WGA.

One peculiarity of *F. hepatica* is that the miracidium starts shading its ciliated epithelium prior to penetration into the snail host (Southgate 1970, Wilson *et al*. 1971, Køie *et al*. 1976), i.e., prior to coming in contact with host's hemolymph lectins. Another source of glycan-binding proteins however is the host's mucus. The presence of lectins in mucus of molluscs has been reported and several lectins have been identified in mucus covering the snails and bivalves (Iguchi *et al*. 1985, Pales Espinosa *et al*. 2009, Ito *et al*. 2011). While our experimental dataset supports the suggestion that lectin binding to the surface may trigger the transformation of *F. hepatica* miracidium into sporocyst, the relevance to *in vivo* situations still needs knowledge on glycan-binding proteins available in the mucus of the gastropod host *G. truncatula*.

In conclusion, the present results describe the stage-specific carbohydrates in the miracidium of *F. hepatica* and imply that specific miracidia-lectin interactions may facilitate the process of miracidium-to-sporocyst transformation. This may reflect mechanisms also active upon the contact with glycan-binding proteins of *G. truncatula* during *F. hepatica* miracidia establishment into the snail.

Acknowledgements. We are grateful to Dr A. Yoneva for her help and support during the present study. The comments of two anonymous reviewers and Dr B.B. Georgiev helped in improving the final version of the manuscript. This study was supported by the European Social Fund and Republic of Bulgaria, Operational Programme "Development of Human Resources 2007–2013", Grant BG051PO001-3.3.04/46.

References

Coles G.C., Jansson H.B., Zuckerman B.M. 1988. Lectin studies of surface carbohydrates and induction of gland secretion in the free-living stages of *Schistosoma mansoni*. *Journal of Chemical Ecology*, 14, 691–700.

Coustau C., Ataev G., Jourdane J., Yoshino T.P. 1997. *Schistosoma japonicum*: *in vitro* cultivation of miracidium to daughter sporocyst using a *Biomphalaria glabrata* embryonic cell line. *Experimental Parasitology*, 87, 77–87. DOI: 10.1006/expr.1997.4184.

Daniel B.E., Preston T.M., Southgate V.R. 1992. The *in vitro* transformation of the miracidium to the mother sporocyst of *Schistosoma margrebowiei*; changes in the parasite surface and implications for interactions with snail plasma factors. *Parasitology*, 104, 41–49. DOI: 10.1017/S0031182000060789.

Dunn T.S., Yoshino T.P. 1988. *Schistosoma mansoni*: origin and expression of a tegumental surface antigen on the miracidium and primary sporocyst. *Experimental Parasitology*, 67, 167–181. DOI: 10.1016/0014-4894(88)90064-1.

Georgieva K., Yoneva A., Popov I., Mizinska-Boevska Y., Stoitsova S. 2005. Lectin-binding properties of the surface of *Fasciola hepatica* sporocysts. *Comptes Rendus de l'Académie Bulgare des Sciences*, 58, 973–976.

Georgieva K., Yoneva A., Mizinska-Boevska Y. 2007. Lectin binding characteristics of *Fasciola hepatica* rediae. *Comptes Rendus de l'Académie Bulgare des Sciences*, 60, 315–318.

Gerhardus M.J.T., Baggen J.M.C., Van Der Knaap W.P.W., Sminia T. 1991. Analysis of surface carbohydrates of *Trichobilharzia ocellata* miracidia and sporocysts using lectin binding techniques. *Parasitology*, 103, 51–59. DOI: 10.1017/S003118200005928X.

Horák P. 1995. Developmentally regulated expression of surface carbohydrate residues on larval stages of the avian schistosome *Trichobilharzia szidati*. *Folia Parasitologica*, 42, 255–265.

Horák P., van der Knaap W.P.W. 1997. Lectins in snail-trematode immune interactions: a review. *Folia Parasitologica*, 44, 161–172.

Iguchi S.M.M., Momoi T., Egawa K., Matsumoto J.J. 1985. An N-acetylneuraminic acid-specific lectin from the body surface mucus of African giant snail. *Comparative Biochemistry and Physiology Part B*: *Comparative Biochemistry*, 85, 4, 897–900. DOI: 10.1016/0305-0491(85)90085-9.

Ito S., Shimizu M., Nagatsuka M., Kitajima S., Honda M., Tsuchiya T., Kanzawa N. 2011. High molecular weight lectin isolated from the mucus of the giant African snail *Achatina fulica*. *Bioscience*, *Biotechnology and Biochemistry*, 75, 20–25. DOI: 10.1271/bbb.100389.

Køie M., Christensen N.Ø., Nansen P. 1976. Stereoscan studies of eggs, free-swimming and penetrating miracidia and early sporocysts of *Fasciola hepatica*. *Zeitschrift für Parasitenkunde*, 51, 79–90. DOI: 10.1007/BF00380530.

Laursen J.R., Yoshino T.P. 1999. *Biomphalaria glabrata* embryonic (Bge) cell line supports *in vitro* miracidial transformation and early larval development of the deer liver fluke, *Fascioloides magna*. *Parasitology*, 118, 187–194.

Lockyer A.E., Jones C.S., Noble L.R., Rollinson D. 2004. Trematodes and snails: an intimate association. *Canadian Journal of Zoology*, 82, 251–269. DOI: 10.1139/z03-215.

Loker E.S. 2010. Gastropod immunobiology. In: (Ed. K. Söderhäll) *Invertebrate Immunity*. Landes Bioscience and Springer Science + Business Media, http://www.ncbi.nlm.nih.gov/books/NBK45994/.

Pales Espinosa E., Perrigault M., Ward J.E., Shumway S.E., Allam B. 2009. Lectins associated with the feeding organs of the oys-

ter *Crassostrea virginica* can mediate particle selection. *Biological Bulletin*, 217, 130–141.

Peterson N.A., Hokke C.H., Deelder A.M., Yoshino T.P. 2009. Glycotope analysis in miracidia and primary sporocysts of *Schistosoma mansoni*: differential expression during the miracidium-to-sporocyst transformation. *International Journal for Parasitology*, 39, 1331–1344. DOI: 10.1016/j.ijpara.2009.06.002.

Pullin R.S.V. 1971. Composition of haemolymph of *Lymnaea truncatula*, the snail host of *Fasciola hepatica*. *Comparative Biochemistry and Physiology Part A*: *Physiology*, 40, 617–626. DOI: 10.1016/0300-9629(71)90246-5.

Rogan M.T., Threadgold L.T. 1984. *Fasciola hepatica*: tegumental alterations as a consequence of lectin binding. *Experimental Parasitology*, 57, 248–260. DOI: 10.1016/0014-4894(84)90098-5.

Sapp K.K., Loker E.S. 2000. Mechanisms underlying digenean-snail specificity: role of miracidial attachment and host plasma factors. *Journal of Parasitology*, 86, 1012–1019. DOI: 10.1645/0022-3395(2000)086[1012:MUDSSR]2.0.C0;2.

Southgate V.R. 1970. Observations on the epidermis of the miracidium and on the formation of the tegument of the sporocyst of *Fasciola hepatica*. *Parasitology*, 61, 177–190. DOI: 10.1017/S0031182000040993.

Stoitsova S., Polyakova-Krusteva O., Vinarova M. 1991. *Fasciola hepatica*: a cytochemical study of tegumental blebs, apical vesicles and infolds and their possible relation to surface membrane shedding. *Helminthologia*, 28, 5–11.

Wilson R.A., Pullin R., Denison J. 1971. An investigation of the mechanism of infection by digenetic trematodes: the penetration of the miracidium of *Fasciola hepatica* into its snail host *Lymnaea truncatula*. *Parasitology*, 63, 491–506.

Yoshino T.P., Cheng T.C., Renwrantz L.R. 1977. Lectin and human blood group determinants of *Schistosoma mansoni*: alteration following *in vitro* transformation of miracidium to mother sporocyst. *Journal of Parasitology*, 63, 818–824.

(Accepted January 18, 2012)