LECTINS OF *Trichobilharzia szidati* CERCARIAE

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Summary :

Cercariae of *Trichobilharzia szidati* were examined for the presence of endogenous lectins. The haemagglutination caused by the cercarial homogenate was inhibited by glycoconjugates (heparin, hyaluronic acid, lipopolysaccharide, bovine submaxillar mucin, thyroglobulin) and saccharides (lactulose, laminarin, D-galacturonic acid). Ligand blotting with laminarin-conjugate revealed the existence of one laminarin-binding protein in the sample. This protein migrates mostly as a double-band under non-reducing conditions (48-52 kDa), and as a single-band under reducing conditions (54-56 kDa). Identical bands were recognized by specific mouse antibodies raised against agglutinins bound on mouse erythrocytes. Labeled laminarin and/or heparin reacted with postacetabular glands on histological sections. Similarly, the binding of *Lotus tetragonolobus* lectin as a glycoprotein ligand supported the finding that the cercarial lectin is localized in postacetabular glands. Moreover, there is an indication that a lectin is present on the cercarial surface. In agreement with affinity fluorescence, mouse antibodies to the cercarial haemagglutinins recognized the postacetabular penetration glands and the surface of cercariae.

KEY WORDS : *Trichobilharzia szidati*, schistosomes, cercaria, lectin, agglutinin.

Résumé : LECTINES DE CERCAIRES DE *TRICHOBILHARZIA SZIDATI*

Des lectines endogènes ont été recherchées dans des extraits de cercaires de Trichobilharzia szidati. *L'hémagglutination provoquée par l'homogénat de cercaires est inhibée par des substances glycoconjuguées (héparine, acide hyaluronique, lipopolysaccharide, mucine de sous-maxillaire bovine, thyroglobuline) et des saccharides (lactulose, laminarine, acide D-galacturonique). La réaction de floculation avec un conjugué de laminarine révèle l'existence d'une protéine de liaison à la laminarine dans l'échantillon. Cette protéine migre principalement : en deux bandes en conditions non réduites (48-52 kDa), et en une seule bande SDS-PAGE en conditions réduites (54-56 kDa). Des bandes identiques sont reconnues par des anticorps de souris spécifiques dirigés contre des agglutinines liées à des érythrocytes de souris. La laminarine et/ou l'héparine réagit avec les glandes post-acétabulaires sur des coupes histologiques. De même la liaison de la lectine de* Lotus tetragonolobus *conforte la localisation de la lectine de cercaire dans les glandes post-acétabulaires. De plus, une lectine pourrait être située à la surface des cercaires. En immunofluorescence, les anticorps de souris dirigés contre les hémagglutinines de cercaire reconnaissent*

MOTS CLÉS : Trichobilharzia szidati, *schistosomes, cercaire, lectine, agglutinine.*

INTRODUCTION

Lectins are considered to possess many functions in living systems, especially in animals (Drickamer, 1995). As lectins specifically recognize and reversibly bind glycans, they may be involved in host-parasite recognition processes (see e.g., Adler *et al.*, 1995 for *Entamoeba histolytica* lectin).

In helminths (plathelminths, nemathelminths and acanthocephalans) data on parasite lectins are scarce. The cyst fluid of the cestode *Taenia multiceps* contains a mitogen with carbohydrate-binding properties (Judson *et al.*, 1987). Lectins, haemagglutinins and/or surface receptors for carbohydrates were found in nematodes: *Ascaris suum* (Čuperlovič *et al.*, 1987, 1988; Kato, 1995), *Ostertagia ostertagi* (Klesius, 1993), *Onchocerca volvulus* (Klion & Donelson, 1994), phytophagous nematodes (Spiegel *et al.*, 1995) and the free-living nematode *Caenorhabditis elegans* (Hirabayashi *et al.*, 1992*a*, 1992*b*, 1996).

In trematodes, only one report exists on the topic. The surface of *in vitro* transformed mother sporocysts of *Schistosoma mansoni* contains binding sites which are specific for α-D-mannose, D-mannose-6-phosphate, α–D-glucose, β-D-galactose, N-acetyl-D-galactosamine and N-acetyl-D-glucosamine (Zelck, 1993). It could be expected that other trematode developmental stages also possess lectins. Such lectins could bring advantage to the parasites being involved in parasite escape reactions, orientation in the (host) environment or in invasion mechanisms towards the next host. Of course, trematode-derived lectins could cover some internal needs of parasites (growth regulation, cell-cell recognition, etc.).

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The aim of our study was to determine, characterize and localize lectins of *Trichobilharzia szidati* cercariae. This free-living stage of the avian schistosome enters the skin of a duck and, incidentally, of humans (Kolářová *et al.*, 1992). The origin and possible importance of the lectins are discussed in the parasite-definitive host relationship.

MATERIALS AND METHODS

Cercariae of *Trichobilharzia szidati* (originally isolated in South Bohemia, Czech Republic) were obtained from the laboratory reared infected snails of the species *Lymnaea stagnalis*. In order to quickly and quantitatively collect cercariae, the emerged cercariae were killed in 0.1 % neutral formaldehyde. After 30 min in refrigerator, cercariae were washed three times in 0.1 M glycine in order to block reactive aldehyde groups and consequently three times in TRIS-buffered saline (TBS; 0.02 M Tris(hydroxymethyl)aminomethan (TRIS) and 0.15 M NaCl; pH 7.8). Protease inhibitors were added to cercariae to a final concentration of 2 µg/ml aprotinin, 1 µg/ml leupeptin and 5 µg/ml tosyl-lysine-chloromethyl ketone. Cercariae were stored at – 20° C until use but no longer than for three weeks. Just before use, cercariae were homogenized by sonification for 3 x 30 sec, centrifuged by 8,000 g for 15 min and the supernatant was taken as a sample for further experiments.

In order to determine the presence of agglutinins in the sample, haemagglutination activity test using native erythrocytes of laboratory mice (inbred strain BALB/c) was performed. Erythrocytes were prepared as a 2 % suspension in TBS. The wells of microtitration plates (U-type) were filled with 50 µl of TBS and 50 µl of the sample that had been serially diluted; 50 µl of the erythrocyte suspension were added to each well. Haemagglutination activity was tested at room temperature and expressed as a titre, *i.e.*, the reciprocal of the dilution in the last well of the row in which agglutination can be recognized (Rüdiger, 1993). Inhibitors (see Table I) were serially diluted in TBS in microtitration plates. pH of acidic inhibitors was adjusted to 7.8 prior use. Then, 50 µl of sample containing 1 haemagglutination unit (dilution of the sample according to the highest titre giving agglutination) and 50 µl of erythrocyte suspension were added into each well. Minimal inhibitory concentration (MIC) was expressed as the lowest inhibitor concentration blocking 1 haemagglutination unit.

Polyclonal antibodies to the agglutinins bound on erythrocytes were raised in laboratory mice (inbred strain BALB/c) according to Yeaton (1986). Agglutinated erythrocytes were collected, centrifuged at 1,000 g/5 min and washed twice in TBS. The resulting suspension was injected into mice in order to generate antihaemagglutinin(s) antibodies. Immunization was repeated weekly for four weeks by intraperitoneal and intravenous injections on the fifth week. Four days after the last injection, serum from immunized mice was acquired. Control serum was taken from the same mice which were subsequently immunized.

Inhibitor	MIC	Inhibitor	MIC
Saccharides		Other sugar compounds	
D-arabinose	40 mM	D-galacturonic acid	1 mM
D-mannose	40 mM	D-glucuronic acid	40 mM
L-fucose	20 mM	hyaluronic acid	0.1 mg/ml
L-sorbose	20 mM	heparin	0.004 IU/ml
lactulose	0.5 mM	LPS *E.coli* 055 B5	3.2 µg/ml
melezitose	40 mM	bovine submax. mucin	0.01 mg/ml
laminarin	0.1 µg/ml	thyroglobulin	0.05 mg/ml

Slight inhibitors (MIC 170-80 mM): D-ribose, D-lyxose, D-fructose, D-mannoheptulose, maltose, cellobiose, sucrose, turanose, α-lactose, β-lactose, melibiose, D-raffinose, inulin (1.7 mg/ml),
No reaction: D-xylose, D-glucose, D-galactose, D-tagatose, D-sedoheptulose, trehalose, gentibiose, palatinose, fetuin, chondroitin sulphate C.
MIC: minimal inhibitory concentration; LPS: lipopolysaccharide

Table I. — Inhibitors of haemagglutination caused by the *T. szidati* cercarial homogenate.

The sodium dodecyl sulphate — polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli (1970) under reducing (2-mercaptoethanol) and non-reducing conditions. The sample was mixed with the sample buffer in ratio 1:1 and boiled for 3 min. Protein concentrations were determined with the Bradford method (Bradford, 1976) using bovine serum albumin as a standard. The amount of protein per line was 20 µg. Electrophoretic separation was performed in 4 % stacking and 15 % separation gels at a constant voltage 150 V. After electrophoresis one part of the gel was stained with Coomassie Blue R-250 and the other was used for transfer of proteins to nitrocellulose membrane.

Using a semidry blotting unit, the transfer of proteins to nitrocellulose membrane (Serva, pore size 0.2 µm) was performed at 1 mA/cm^2 constant current density for 2 h. Then, the membrane was rinsed in Tween-20-TRIS-buffered saline (T-TBS; TBS supplemented with 0.05 % Tween-20, pH 7.8) and incubated for 3 h in 5 % delipidized milk powder in T-TBS. After washing in T-TBS the membrane was incubated for 2 h with specific mouse antibodies (diluted 1:500) or control serum (1:500). As far as the β(1 → 3)-glucan-binding protein determination is concerned, laminarin (a β(1 → 3)-linked D-glucose polymer) was used here as a ligand. Laminarin-poly(acrylamide-allylamine)-copolymer-biotin conjugate (LBC) was prepared in laboratory according to Novotná *et al.* (1996). Membranes were incubated

with LBC (250 µg/ml), LBC (250 µg/ml) with free laminarin (2.5 mg/ml) or laminarin-free copolymer (250 µg/ml) for 2 h. The blocking solution in the first step was enriched with 2.5 mg/ml of free laminarin in order to sufficiently block the strip exposed in the second step to LBC together with free laminarin. Membranes were then rinsed in T-TBS and incubated for 1 h either with peroxidase-conjugated swine anti-mouse immunoglobulins (SwAM-Px; Sevac, Prague, Czech Republic) diluted 1:500 in T-TBS in the case of antigen analysis, or with 2.5 µg/ml peroxidase-conjugated avidin in T-TBS in the case of LBC binding. The product of peroxidase reaction was developed by incubation of blot membrane in the substrate solution (TBS supplemented with 0.01 % hydrogen peroxide, 0.6 mM 3,3′-diaminobenzidine tetrahydrochloride, pH 7.8). Controls for binding of SwAM-Px and peroxidase-conjugated avidin were also done.

Using mouse antibodies against cercarial haemagglutinins, indirect immunofluorescence was performed. Histological sections of both the emerged cercariae and the infected snail hepatopancreases fixed in Bouin's fluid (Danguy & Gabius, 1993) and embedded in JB4-resin (Polysciences, Inc., Warrington, USA) were cut with the thickness of 3 µm and further processed. Sections were blocked with 1 % bovine serum albumin in TBS for 1 h. Then, mouse antihaemagglutinin(s) antibodies diluted 1:100 in TBS were applied. After 30 min, slides were rinsed twice in TBS and overlaid for 30 min with fluorescein-conjugated swine anti-mouse immunoglobulins (SwAM-FITC) (Sevac, Prague, Czech Republic) diluted 1:100 in TBS. Slides were washed twice in TBS and immediately examined by epifluorescence microscope.

As ligands for the detection of lectins on sections, LBC (25 µg/ml), heparin-albumin-biotin conjugate (HBC; 25 µg/ml) and fluorescein-conjugated *Lotus tetragonolobus* lectin (LTA-FITC; 50 µg/ml) were used. Sections were blocked as described above excluding the control sections where appropriate inhibitor (laminarin 2.5 mg/ml, heparin 2,500 IU/ml, saccharides 250 mM or 1 M and glycosaminoglycans 2.5 mg/ml — see Table III) in blocking solution (1 % bovine serum albumin in TBS) was used. Then, labeled probes with or without inhibitor in the same blocking concentration were applied for 30 min. After washing in TBS, slides were either directly examined (direct fluorescence of LTA-FITC) or incubated with fluorescein-conjugated avidin (5 µg/ml) for 30 min (indirect fluorescence using LBC or HBC). After washing the slides, immediate examination was provided.

All experiments mentioned in the methods were repeated four times except for the immunization experiment. All chemicals listed above were purchased from Sigma Chemical Company unless stated otherwise.

RESULTS

Using supernatant of the *T. szidati* cercarial homogenate, haemagglutination of mouse native erythrocytes was observed. The haemagglutination titre was 2,048 with an original sample protein concentration of 1.0 mg/ml. Testing of 37 potential inhibitors of haemagglutination (Table I), the highest degree of inhibition was observed with laminarin (MIC 0.1 µg/ml), heparin (MIC 0.004 IU/ml) and bacterial lipopolysaccharide (MIC 3.2 µg/ml) followed by lactulose (= D-galactopyranosyl-β(1 → 4)-D-fructose; MIC 0.5 mM), D-galacturonic acid (MIC 1 mM) and glycoproteins — bovine submaxillar mucin (MIC 0.01 mg/ml) as well as thyroglobulin (MIC 0.05 mg/ml).

Electrophoretical separation of cercarial proteins is shown on Figure 1. There is one dominant protein band which migrates under non-reducing conditions mostly as a double-band in the range between 48-52 kDa and under reducing conditions as a single band of molecular mass between 54-56 kDa.

Affinity blot (Fig. 2) shows that LBC binds exclusively to the dominant double- and single-bands under non-reducing and reducing conditions, respectively, *i.e.*, LBC-binding bands are identical with major bands on separation gels. No specific reaction was observed under presence of free laminarin as a blocking agent. No activity was revealed in incubations of blots in laminarin-free copolymer and in peroxidase-conjugated avidin as well.

Immunoblot of the sample (Fig. 3) showed, that mouse antibodies raised against cercarial haemagglutinins in the non-reduced sample were bound to the dominant double-band appearing on gels and affinity blots (Fig. 3, lines 1-2). Under reducing conditions they bound to three bands : one of them again corresponds to the dominant band on gels and affinity blots (Fig. 3, lines 5-6), the other two, recognized by specific antibodies, are of molecular masses of 59-61 kDa and 46-49 kDa (Fig. 3, line 6). No reaction was observed with control sera and SwAM-Px.

Immunofluorescence results (Table II) brought the evidence that the haemagglutinin(s) is localized on the cercarial surface (body and tail) and in the postacetabular penetration glands. The same reaction was achieved both in free-dwelling cercariae (Fig. 4*a*) and in cercariae still preserved within daughter sporocysts (Fig. 4*b*). SwAM-FITC and/or control antibodies reacted to a certain degree with glands and this reaction was fully blocked with laminarin. Titration of specific antibodies shows that they bind to the sections in titres up to 640.

Using affinity fluorescence (Table III), it was found that LBC and HBC bound exclusively to the postacetabular penetration glands and their ducts (Fig. 4*c*). This find-

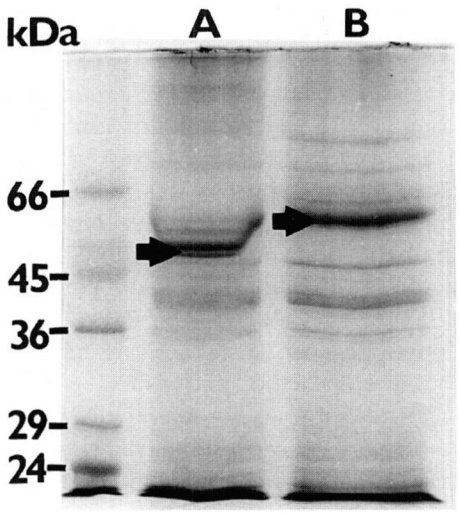

Fig. 1. — Cercarial protein pattern on SDS-PAGE under non-reducing (A) and reducing (B) conditions. Dominant bands of gland origin are clearly visible (arrows).

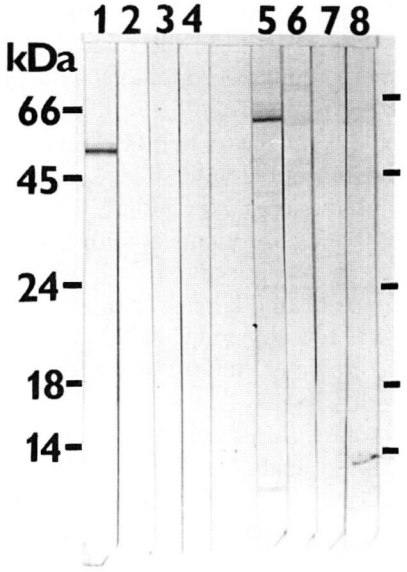

Fig. 2. — Affinity blot of cercarial proteins under non-reducing (lines 1-4) and reducing (lines 5-8) conditions. The β(1 → 3)-glucan binding lectin is demonstrated in lines 1 and 5. No signals were obtained after treatment with laminarin-conjugate + free laminarin (lines 2 and 6), laminarin-free copolymer (lines 3 and 7) and avidin-peroxidase alone (lines 4 and 8).

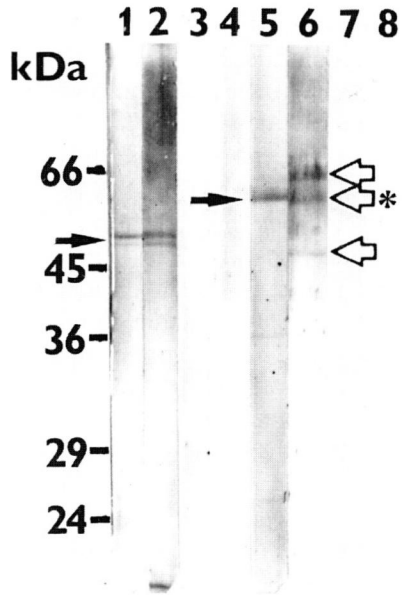

Fig. 3. — Immunoblot of cercarial proteins under non-reducing (lines 1-4) and reducing (lines 5-8) conditions. Laminarin-conjugate binding is shown for comparison in lines 1 and 5 (full arrows); binding of specific antibodies to the gland lectin is documented in lines 2 and 6. No reaction was found with control antibodies (lines 3 and 7) or SwAM-Px alone (lines 4 and 8). Three bands in line 6 are indicated by empty arrows (one of them, marked by asterisk, is identical with the laminarin-binding protein).
SwAM-Px — Peroxidase-conjugated swine anti-mouse immunoglobulins.

Tissue	Postacetabular glands	Surface
Specific Ab	++	++
Control Ab	+*	– –
Specific Ab + laminarin	++	++
Control Ab + laminarin	– –	– –

* SwAM-FITC and/or control antibodies were bound by the gland lectin (+); this binding was fully inhibited by laminarin. SwAM-FITC and/or control antibodies did not react with cercarial glands on sections of the infected hepatopancreases.
++: strong fluorescence, +: weak fluorescence, – –: no fluorescence. Ab: antibodies; SwAM-FITC: fluorescein-conjugated swine anti-mouse immunoglobulins.

Table II. — Reaction of specific antibodies with *T. szidati* cercarial structures.

ing corresponds to antibody reaction with glands (see above) (Figs. 4*a, b*). The LBC- and HBC-bindings were successfully blocked with free laminarin and heparin, respectively. LTA-FITC binds to both, the cercarial surface and the penetration glands (Figs. 4*a, b*). Blocking by a specific inhibitor (L-fucose; Fig. 3*c*) showed that L-fucose-based binding of LTA-FITC appeared on the surface, not in glands (*i.e.,* glands remained positive). Moreover, the use of non-specific saccharide inhibitors showed that there were three inhibitor groups of *Lotus tetragonolobus* lectin (LTA) binding. The first group (heparin, de-N-sulphated-N-acetylated heparin, chondroitin sulphate C, laminarin and D-galacturonic acid) inhibited the reaction of LTA-FITC with penetration

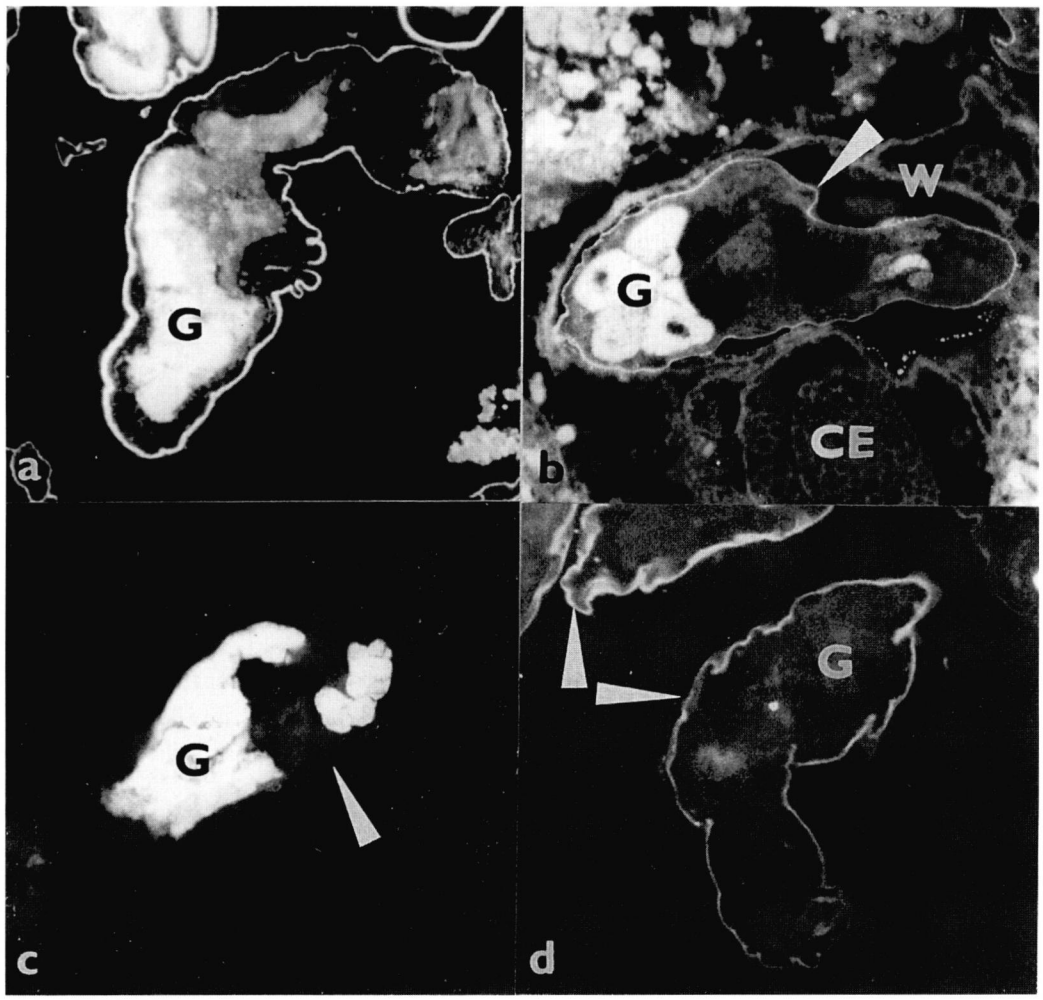

Fig. 4. —
a) Binding of LTA-FITC to cercarial sections. Surface positivity and reaction of postacetabular glands (G) is shown. The same fluorescence reaction was obtained with specific antibodies directed against the cercarial haemagglutinins.
b) Positive reaction of surface (arrow) and postacetabular penetration glands (G) of intrasporocystic cercaria labeled with specific antibodies. Similar binding pattern was seen with LTA-FITC. CE: cercarial embryo, W: sporocyst wall (unstained).
c) Positive reaction of cercarial glands (G) with laminarin-conjugate. Cercarial surface (arrow) appears to be totally negative. The same situation was observed using labeled heparin. Similar fluorescence results were obtained after LTA treatment together with L-fucose or with non-specific inhibitors of the group No. 2 (D-fructose, maltose, turanose, D-glucose, D-mannitol, gentibiose).
d) Positive surface fluorescence (arrows) and negative gland (G) fluorescence reactions of cercariae treated by LTA-FITC together with laminarin. Similar result was obtained with remaining non-specific inhibitors of the group No. 1 (heparin, de-N-sulphated-N-acetylated heparin, chondroitin sulphate C, D-galacturonic acid).
LTA-FITC: Fluorescein-labeled *Lotus tetragonolobus* lectin.

Ligand	laminarin-biotin (25 µg/ml)		heparin-biotin (25 µg/ml)		LTA-fluorescein (50 µg/ml)	
Inhibitor	glands	surface	glands	surface	glands	surface
None	++	– –	++	– –	++	++
Specific inhibitors*	– –	– –	– –	– –	++	– –
Non-specific inhibitors No. 1:						
laminarin (2.5 mg/ml)	– –	– –	ND	ND	– –	++
heparin (2500 IU/ml)	ND	ND	– –	– –	– –	++
de-N-sulphated-N-acetylated heparin (2.5 mg/ml)	– –	– –	++	– –	– –	++
remaining inhibitors	ND	ND	ND	ND	– –	++
Non-specific inhibitors No. 2:						
D-mannitol (1 M)	++	– –	++	– –	++**	– –**
gentibiose (1 M)	++	– –	++	– –	++**	– –**
remaining inhibitors	ND	ND	ND	ND	++	– –
Non-specific inhibitors No. 3:						
lactulose (1 M)	– –	– –	+	– –	– –	– –

*Respective specific inhibitors: laminarin (2.5 mg/ml); heparin (2,500 IU/ml); L-fucose (250 mM); **Tested also at concentration of 250 mM.
++: strong fluorescence; +: weak fluorescence; --: no fluorescence; ND: not tested; LTA: *Lotus tetragonolobus* lectin.
Remaining non-specific inhibitors No. 1: chondroitin sulphate C (2.5 mg/ml), D-galacturonic acid (500 mM); Remaining non-specific inhibitors No. 2: D-fructose (250 mM), D-glucose (250 mM), maltose (250 mM), turanose (250 mM), lactulose (250 mM).

Table III. — Inhibitors of the ligand binding by postacetabular glands and surface of *T. szidati* cercariae.

glands; surface LTA-positivity was not influenced (Fig. 4*d*). The second group (D-fructose, maltose = D-glucopyranosyl-α(1 → 4)-D-glucose, turanose = D-glucopyranosyl-α(1 → 3)-D-fructose, D-glucose, D-mannitol and gentibiose = D-glucopyranosyl-β (1 → 6)-D-glucose) caused the same inhibiton as observed with the specific inhibitor (L-fucose), *i.e.,* LTA-reaction on the surface was fully blocked and glands remained positive (Fig. 4*c*). The third group is comprised of lactulose. Whereas 250 mM concentration of lactulose inhibited LTA binding to the cercarial surface only, higher concentration (1 M) of this disaccharide blocked the reaction of glands with LTA as well as LBC and HBC. All these reactions were observed in the case of penetration glands with lowered intensity in intrasporocystic cercariae as well.

It is concluded, therefore, that LTA as a glycoprotein ligand is bound by two cercarial lectins which differ in their location and specificity. The first one fills the postacetabular glands and is identical with the lectin detected on immunoblot, affinity blot, immunofluorescence and affinity fluorescence using LBC, HBC and specific antibodies; the second lectin is deposited on the cercarial surface and its occurrence correlates with surface positivity detected by immunofluorescence.

DISCUSSION

Lower agglutination titers and limited number of inhibitors in our initial experiments (unpublished results) suggested that the addition of protease inhibitors was essential for full expression of lectin activity in the sample. The spectrum of haemagglutination inhibitors indicated the presence of more lectins in the sample (Table I).

As certain lectins (proteins) are able to restore their carbohydrate-binding activities after SDS-PAGE and blotting (Soutar & Wade, 1990) or after histological fixation and embedding procedures (Danguy & Gabius, 1993), the presence of cercarial lectins has been examined on nitrocellulose membranes and JB4-resin sections. High correlation between immunological and affinity results on both, the immuno- and affinity blots and the histological sections of cercariae was obtained. Specific antibodies raised against cercarial haemagglutinins recognized the same band on the blot as that which was detected using LBC. Moreover, there are two additional bands on immunoblot under reducing conditions which failed to react with LBC. These might represent some other agglutinin(s) or their subunits which are located on the surface of cercariae (see

results of LTA binding and immunofluorescence). The LBC-binding protein migrates, interestingly, slower under reducing conditions compared to non-reducing ones. This might be caused by high content of intramolecular S-S bonds as was already demonstrated for several proteins (Jonáková *et al.*, 1989). The dominance of the LBC-binding protein on the electrophoretical gel is most probably explained by the fact that the protein represents the cercarial gland content, *i.e.*, the content of the largest organ within cercariae.

Histological examination of cercariae demonstrated that LBC binds exclusively to postacetabular penetration glands (Fig. 4*c*). The same result was obtained using HBC. It is concluded that the LBC-binding protein on blots which is recognized by specific antibodies and thus being present on agglutinated erythrocytes, is localized in penetration glands of cercariae.

In our previous experiments on *T. szidati* (Horák, unpublished results), non-specific binding of LTA to the penetration glands was observed. We tried, therefore, to use known inhibitors of haemagglutination for blocking of this binding. The reaction has been blocked by laminarin, heparin, de-N-sulphated-N-acetylated heparin, chondroitin sulphate C, D-galacturonic acid and lactulose. This implies that LTA as a glycoprotein is bound by the gland lectin and this binding is inhibited by the formerly recognized ligands of the gland lectin.

Tests with other saccharide inhibitors produced unpredictable results. It was repeatedly shown (Horák, 1995; Horák & Mikeš, 1995) that LTA binds to the surface of cercariae and this binding is inhibited by L-fucose. This fulfills criteria for consideration that L-fucose residues are present in surface layers. Although LTA has affinity exclusively for L-fucose and to some lower extent for N-acetyl-D-glucosamine (Lima *et al.*, 1994), a complete inhibition of its binding to the cercarial surface by D-fructose, D-glucose, D-mannitol, maltose, turanose, gentibiose and lactulose, *i.e.*, by non-related neutral saccharides was also observed. This probably means that LTA as a glycoprotein ligand is bound *via* its carbohydrate moieties by a cercarial surface lectin and that the own L-fucose-binding sites of LTA are not involved in the reaction. From this view, the inhibition of the LTA binding by L-fucose could be caused by the L-fucose specificity of the cercarial lectin in the case when LTA contains L-fucose residues, too. Unfortunately, the carbohydrate composition of LTA is not fully known (Goldstein & Poretz, 1986). The inhibition of LTA binding by D-glucose and gentibiose shows, moreover, the limitation of haemagglutination experiments, i.e., that ligands (inhibitors) of haemagglutinins can be found only in those cases when they may compete with saccharide moieties on erythrocytes.

We have no explanation for the high affinity to laminarin, a $\beta(1 \rightarrow 3)$-glucan. This glucose polysaccharide (> 99 % pure polysaccharide according to the supplier information) may contain small amounts of $\beta(1 \rightarrow 6)$ linkages and 2-3 % of D-mannitol. As both D-mannitol and gentibiose (= D-glucopyranosyl-$\beta(1 \rightarrow 6)$-D-glucose) block exclusively the LTA binding to the cercarial surface and have no influence on LBC, HBC and LTA reaction with the postacetabular glands (even at 1 M saccharide concentration), we can deduce that the $\beta(1 \rightarrow 3)$-glucan chain is the putative ligand of the gland lectin when laminarin is used. Experiments using lactulose show that at low concentration (250 mM), this disaccharide prevents the surface binding of LTA whereas at high concentration (1 M), the gland lectin is blocked, too. The latter concentration of lactulose is inhibitory for LBC- and HBC-bindings as well. Taken together, the gland lectin seems to recognize more complex (clustered) carbohydrate ligands in contrast to that on the cercarial surface.

It is possible that the gland lectin is a protein being involved in parasite invasion into the host, e.g., in recognition of proteoglycans (glycosaminoglycans) of skin or extracellular matrix of host tissues. This can be supported by the fact that some haemagglutination inhibitors in our experiments belong to skin or connective tissue components (hyaluronic acid, D-glucuronic acid, glycoproteins). Moreover, glycosaminoglycans are high-affinity ligands for cercarial glands on histological sections (heparin, chondroitin sulphate C) even in their desulphated form (de-N-sulphated-N-acetylated heparin). Non-specific reactions due to charged groups can be excluded by the fact that laminarin and lactulose served as efficient ligands, too. Maybe the specificity to $\beta(1 \rightarrow 3)$-glucan and lactulose reflects certain degree of similarity between these saccharides and glycosaminoglycan chains which are known to possess very often $\beta(1 \rightarrow 3)$ or $\beta(1 \rightarrow 4)$ linkages of glucose/galactose-derived carbohydrates. In concert with this view, some glycoconjugates (including glycoproteins and glycosaminoglycans) have been found to play a role in invasion processes of trematode cercariae (see Haas, 1994 for review). Of course the detected gland lectin may play some other roles (*e.g.*, in regulation of the host immunity, parasite protection, etc.).

It is necessary to note that SwAM-FITC and/or control antibodies were to certain degree bound by the glands. This is probably caused by the ability of the gland lectin to bind saccharide moieties of immunoglobulins, a phenomenon known for e.g., snail plasma lectins (Hahn *et al.*, 1996). This reaction was totally blocked by laminarin and thus, exclusively the specific reaction of antibodies has been evaluated.

It has been argued that cercariae of *Trichobilharzia ocellata* possess either surface snail-like antigens or

antigens of snail origin adsorbed onto the surface (Roder *et al.*, 1977; van der Knaap *et al.*, 1985) including probably snail lectins, too. There is, however, no doubt that the lectins detected in our study are of cercarial origin. This view is supported by observing the same surface fluorescence reaction on free-swimming cercariae and those within daughter sporocysts (i.e., preserved from snail components).

In conclusion, two lectin activities have been demonstrated. The first one is restricted to postacetabular penetration glands and was confirmed by many methods, and the second activity appears on the surface of the cercariae as indicated by immunofluorescence and indirectly using non-specific inhibitors of LTA. Both activities are most probably of endogenous origin, because mature cercariae preserved within schistosome daughter sporocysts show identical binding patterns.

ACKNOWLEDGEMENTS

We wish to thank Lubomír Kovář, BSc. for his cooperation in non-specific blocking of LTA. Experiments in this contribution were partially supported by the grant of the Charles University Grant Agency No. 20163/93, grant of the Grant Agency of the Czech Republic No. 204/96/0248 and the grant of the Czech Ministry of Health No. IGA 2797-3/95.

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Reçu le 19 août 1996
Accepté le 25 novembre 1996