Biochem. J. (1991) 279, 837–842 (Printed in Great Britain)

Primary structure and origin of schistosomin, an anti-gonadotropic neuropeptide of the pond snail *Lymnaea stagnalis*

Peter L. HORDIJK,* Henk D. F. H. SCHALLIG,* Rob H. M. EBBERINK,† Marijke DE JONG-BRINK*‡ and Joos JOOSSE*

*Biological Laboratory, Vrije Universiteit, de Boelelaan 1087, 1081 HV Amsterdam, The Netherlands, and †Applied Biosystems, Industrieweg 66–68, 3606 AS Maarssen, The Netherlands

In the pond snail *Lymnaea stagnalis* infected with the schistosome parasite *Trichobilharzia ocellata*, a peptide called schistosomin is released from the central nervous system, which counteracts the bioactivity of a number of gonadotropic hormones. This leads to inhibition of the reproductive activities of the infected snail. In order to determine the structure of schistosomin, the neuropeptide was purified from the central nervous system using gel-permeation chromatography and reverse-phase h.p.l.c. The complete primary structure of the peptide was determined by *N*-terminal sequencing and peptide mapping. Schistosomin is a single-chain molecule of 79 amino acids with a molecular mass of 8738 Da. The peptide contains eight cysteine residues which may give rise to four intramolecular disulphide bridges that fold the peptide into a stable globular structure. A database search did not reveal any known peptides that show significant sequence similarity to schistosomin. By means of immunocytochemistry, the peptide was shown to be localized in the growth-controlling neurosecretory light green cells, which are located in the cerebral ganglia of the central nervous system of *Lymnaea*. In addition to schistosomin, these neurons are known to produce various insulin-related peptides.

INTRODUCTION

Parasites affect the growth, metabolism and reproductive activity of their hosts in order to make nutrients available for their own maintenance and reproduction. It has been shown that some parasites achieve this goal by interfering with the hormonal actions of their hosts [1–3]. We have demonstrated an endocrine component in the host–parasite inter-relationship between the snail *Lymnaea stagnalis* and the avian schistosome *Trichobilharzia ocellata*. Schistosome parasites need two types of host for their cycle. The intermediate hosts are freshwater snails, whereas the definitive hosts are vertebrates (e.g. cattle and humans). These parasites cause schistosomiasis, a tropical disease affecting at least 200 million people worldwide. Insight into the host–parasite relationship at the level of the freshwater snails may lead to a method for interrupting the parasitic cycle at this stage. Knowledge of the molecular basis of the interaction is a prerequisite for this type of approach. Since the physiology and endocrinology of *Lymnaea* have been extensively studied [4,5], the *Lymnaea–Trichobilharzia* interaction may serve as a model for related snail–schistosome combinations. Our studies focus on the mechanisms underlying the inhibition of reproductive activity in *Lymnaea* which occurs as a result of infection with *Trichobilharzia*.

In *Lymnaea*, egg laying and egg-laying behaviour are controlled by a set of gonadotropic neuropeptides which are derived from a common precursor and are synthesized and released by the neuroendocrine caudodorsal cells (CDCs) [4,6]. Among these peptides is the egg-laying hormone (caudodorsal cell hormone; CDCH). This is a peptide hormone of 36 amino acids which, upon injection, induces ovulation and egg laying [7,8]. Calfluxin (CaFl) is a peptide of 17 amino acids which is also derived from the precursor synthesized by the CDCs. CaFl stimulates the albumen gland, a female accessory sex gland which adds perivitellin fluid to freshly ovulated egg cells during egg formation [9].

Schistosomin is a recently characterized neuropeptide which inhibits the bioactivity of CDCH [10] and decreases the binding capacity of CaFl to membrane-bound receptors of the albumen gland [11]. Schistosomin was originally isolated from the haemolymph of *Trichobilharzia*-infected snails and appeared not to be present in the haemolymph of non-infected snails [11,12]. However, the observation that schistosomin could be isolated from extracts of the central nervous system (CNS) of non-infected snails [11] demonstrates not only that the peptide is produced by the CNS, but also that its release and/or synthesis is stimulated during parasitic infection. The parasite apparently makes use of an endogenous reproduction-inhibiting system of the host to lower host fecundity.

The property of schistosomin of inhibiting the receptor-binding activity of CaFl has been used to purify the peptide from the haemolymph of parasitized snails as well as from extracts of the CNS of non-infected animals by ion-exchange and reverse-phase column chromatography. Initial characterization using plasma desorption mass spectrometry and amino acid analysis indicated that schistosomin is a peptide consisting of about 75 amino acids [11].

In the present paper the primary structure of schistosomin, isolated from the CNS, is presented. The inhibitory activity of the purified peptide was tested in the *in vitro* bioassay for CaFl. Finally, immunocytochemistry was used to determine the cellular origin of schistosomin within the CNS of *Lymnaea*.

MATERIALS AND METHODS

Animals

Adult specimens of *Lymnaea stagnalis* (shell height > 23 mm) were used for all experiments, unless stated otherwise. The animals were bred under standard conditions [13].

Isolation of schistosomin

For the isolation of schistosomin, the CNS was dissected out

Abbreviations used: CaFl, calfluxin; CNS, central nervous system; CDC, caudodorsal cell; CDCH, caudodorsal cell hormone; LGC, light green cell; PBS, phosphate-buffered saline (50 mM-Na_2PO_4,$7H_2O$, 140 mM-NaCl, pH 7.2), Ac, acetate.

‡ To whom correspondence and reprint requests should be sent.

and collected in tubes on solid CO_2. All reverse-phase h.p.l.c. procedures were performed using a flow rate of 1 ml/min and spectrophotometric detection at 214 nm. Fractions of 1 min were collected and concentrated by lyophilization in a Speedvac (Savant).

In a preliminary experiment, schistosomin was purified from the CNS of *Lymnaea* as described previously [11] and this material (500 pmol) was *N*-terminally sequenced, resulting in the identification of 26 amino acids. In the present study, a simplified purification method was developed, resulting in a higher recovery of the purified peptide. This protocol consists of a five-step procedure starting with the homogenization of the tissue in 0.1 M-HAc (Ac = acetate) using an all-glass Elvehjem–Potter homogenizer at 0 °C, followed by centrifugation (10000 *g*, 10 min). This was done batchwise, processing 100 CNS in 2 ml of extraction solvent at a time. Next, the supernatant was pre-purified using Supelclean LC-18 disposable cartridges (Supelco). After application of an extract of 100 CNS in 2 ml of 0.1 M-HAc, the cartridge was washed with 5 ml of water and eluted with 5 ml of a solvent consisting of 7 mM-trifluoroacetic acid and 60 % (v/v) acetonitrile. The material was lyophilized and applied in batches of 250 animal equivalents to high-performance gel-permeation chromatography using two columns in tandem arrangement (one I-125 and one I-300 Protein Pak, 7.8 mm × 300 mm and 7.5 mm × 300 mm respectively; Waters Associates). The columns were equilibrated and run in an isocratic mode using 7.5 mM-trifluoroacetic acid containing 30 % acetonitrile [14]. The void volume of these columns was 11 ml, and salt was eluted after 30 ml. After chromatography, schistosomin-containing material was concentrated by lyophilization and separated with two reverse-phase h.p.l.c. steps using a Nucleosil Wide Pore C-18 column (4.6 mm × 250 mm).

In the first reverse-phase h.p.l.c. step, 7.5 mM-trifluoroacetic acid (solvent A) was used for column equilibration. A gradient was developed in 45 min from 0 to 100 % of a solution containing 7 mM-trifluoroacetic acid and 60 % acetonitrile (solvent B). After concentration by lyophilization, fractions containing schistosomin were rechromatographed on the same column equilibrated with 25 mM-NH_4Ac, pH 6.7 (buffer A). A gradient was developed in 60 min from 0–100 % of 25 mM-NH_4Ac containing 60 % acetonitrile (buffer B). The purified peptide was concentrated by lyophilization and either subjected to direct sequencing or, after reduction and modification of the cysteine residues, cleaved with the different proteases (see below). Sequencing reactions were performed using an Applied Biosystems model 473A protein sequencer.

Bioactivity of schistosomin

In order to test the presence of schistosomin, the *in vitro* ultracytochemical CaFl bioassay was used. This assay is based on the influx of Ca^{2+} into the mitochondria of secretory cells of the albumen gland. This influx is the result of an increase in the cytosolic Ca^{2+} concentration which is caused by the neuropeptide CaFl. Following incubation of the glands in the presence of CaFl and in the presence or the absence of the test samples, the tissue is fixed and calcium is deposited using potassium antimonate. The tissue is further processed for electron microscopy and the percentage of mitochondria containing calcium deposits is determined by counting (see [15] for further details). A crude extract of cerebral commissures of the CNS of *Lymnaea* (three animal equivalents/incubation) was used as source of CaFl [9]. As a control, lyophilized eluate from the final purification step, containing no protein, was used. The shell height of the snails used for the assay was 21 mm. Statistical analysis of the results was performed as described [15].

Reduction of disulphide bonds and modification of cysteine residues

Reduction of disulphide bonds was performed by adding 15 μl of 14.3 M-2-mercaptoethanol to the peptide dissolved in 200 μl of 6 M-guanidinium hydrochloride/0.1 M-Tris, pH 8.5 (2 h at 50 °C). Modification of the cysteine residues was subsequently performed with 15 μl of distilled 4-vinylpyridine (1.5 h, room temperature, in the dark). After this procedure, the reduced peptide was desalted using reverse-phase h.p.l.c. After 30 min of washing with solvent A, a gradient of 0–100 % solvent B was developed in 30 min. Schistosomin-containing fractions were partly lyophilized and subjected either to direct sequencing or to enzymic cleavage.

Digestion with *Staphylococcus aureus* V8 protease

Reduced and *S*-pyridylethylated schistosomin (900 pmol in 200 μl of 0.1 M-ammonium acetate, pH 4) was subjected to cleavage with 0.3 μg of *Staphylococcus aureus* V8 protease (Sigma) for 18 h at 37 °C. The peptide fragments were purified by application of the mixture to a Nucleosil Wide Pore C-18 column in combination with the trifluoroacetic acid solvent system described above. A linear gradient of 0–100 % solvent B was generated in 60 min. The fractions were lyophilized and the peptide fragments were sequenced.

Digestion with endoproteinase Asp-N

Purified schistosomin was subjected to reduction and alkylation as described above. Fragments were generated by incubation of 1.4 nmol of the reduced peptide with 0.5 μg of endoprotease Asp-N from *Pseudomonas fragi* (Boehringer) in 50 mM-sodium phosphate buffer, pH 8.0 (18 h, 37 °C). Fragments were resolved on a Nucleosil C-18 column as described above and then sequenced.

Mass determination

The molecular mass of schistosomin was determined using a plasma desorption mass spectrometer (Bio-ion, Applied Biosystems). Lyophilized schistosomin (1.2 μg) was analysed for 3×10^5 fission events, corresponding to 30 min.

Predictions of secondary structure

Hydropathy was calculated according to Kyte & Doolittle [16], and prediction of turns was performed according to Chou & Fasman [17].

Production and specificity of an anti-schistosomin serum

H.p.l.c.-purified lyophilized schistosomin (600 animal equivalents) was dissolved in 0.5 ml of phosphate-buffered saline (PBS, pH 7.3). An equal volume of Freund's complete adjuvant was added and the solution was mixed. A female Balb/c mouse was injected with the immunogen, and 3 weeks after the first injection the mouse received a booster injection with the same amount of immunogen, this time using Freund's incomplete adjuvant. The mouse was killed 1 week after the booster injection and the serum was collected. The specificity of the antiserum was tested by means of a dotting immunobinding assay. Serial dilutions of h.p.l.c.-purified schistosomin (30–1 pmol) were dotted (1 μl per dot) on strips of nitrocellulose paper. As a control, two non-related peptides from *Lymnaea* were used, i.e. CDCH and the C-peptide of the molluscan insulin-related peptide, which share no sequence similarity with schistosomin [8,18]. After heating for 60 min at 108 °C, the strips were wetted in a mixture of PBS and 0.05 % Tween-20 (PBS/Tween) for 20 min and then for 2 h with the anti-schistosomin serum or, as a control, with a polyclonal antiserum against the C-peptide [19].

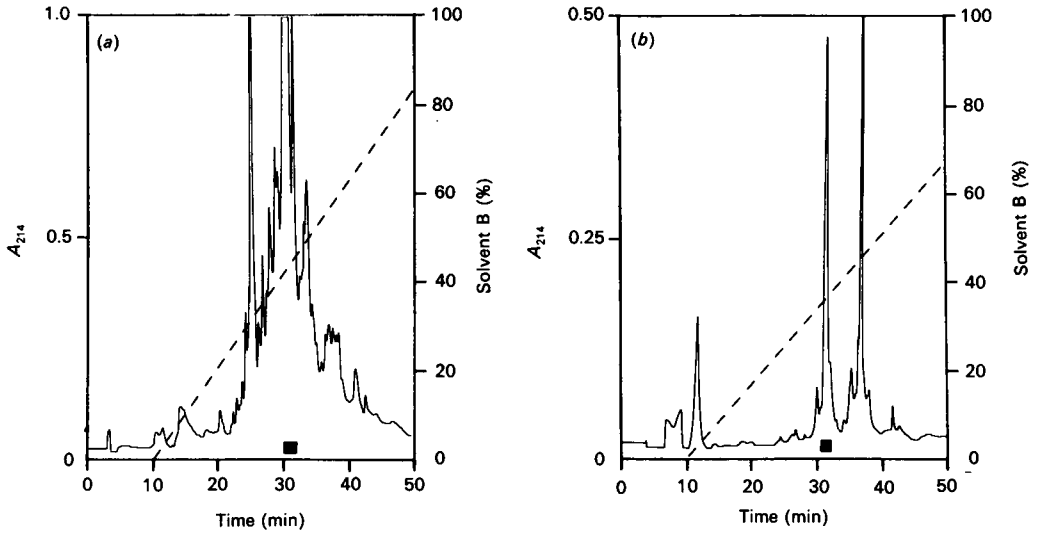

Fig. 1. Purification of schistosomin from the CNS of *Lymnaea stagnalis* by reverse-phase h.p.l.c.

CNS were extracted in 0.1 M-HAc, after which the material was pre-purified using LC C-18 disposable columns and gel-permeation chromatography. Schistosomin-containing fractions were subsequently applied to reverse-phase h.p.l.c. using a trifluoroacetic acid solvent system at pH 2 in combination with a wide-pore C-18 column (*a*). Schistosomin was then purified on the same column using an NH_4Ac buffer system at pH 6.8 (*b*). See the Materials and methods section for details. The gradient of solvent B is indicated (----). Schistosomin-containing fractions are indicated by ■.

The antisera were diluted 1:500 in PBS/Tween. After washing in PBS/Tween, the strips were incubated for 1 h with the appropriate second antiserum, rabbit anti-mouse IgG conjugated with horseradish peroxidase or pig anti-rabbit IgG conjugated with horseradish peroxidase, both diluted 1:1000 in PBS/Tween. Next, the strips were washed with PBS and the horseradish peroxidase was visualized using 0.05% 3,3 diaminobenzidine tetrahydrochloride (Sigma) in PBS containing 0.01% H_2O_2. The anti-schistosomin antibody reacted with h.p.l.c.-purified schistosomin; the detection limit was 2 pmol. The antiserum did not react with the C-peptide and showed a slight cross-reaction with CDCH (only with the 30 pmol dot).

Immunocytochemistry

CNS of *Lymnaea* were dissected out and transferred to Bouin's fixative (saturated aqueous picric acid/40% formaldehyde/acetic acid, 15:5:1, by vol.) and fixed overnight at 4 °C. Next, the tissue was dehydrated according to conventional methods with ethanol and amyl acetate and embedded in paraplast. Paraplast was removed from 7 μm thick sections by two changes of xylene and the sections were rehydrated. Endogenous peroxidase activity was inhibited by incubating the sections in 100% methanol containing 1% acetic acid and 0.01% H_2O_2 (30 min, 20 °C). The sections were incubated overnight at 4 °C or 1 h at 20 °C with the anti-schistosomin serum diluted 1:250 in PBS/Tween. After washing in PBS/Tween, the sections were incubated for 1 h at 20 °C with the second antiserum diluted 1:100, and, after washing with PBS, the horseradish peroxidase was visualized as described above. The following controls were included: sections were incubated with non-immune mouse serum or with anti-schistosomin serum (diluted 1:250) which had been preincubated with 50 pmol of purified schistosomin to block the antigen-binding site.

RESULTS

Purification of schistosomin

The protocol for the purification of schistosomin described here resulted in a higher recovery of bioactive schistosomin (1.5 pmol/CNS equivalent) as compared with the method applied previously (0.5–1 pmol/CNS equivalent [11]). For all peptide mapping experiments this method was used and the identity of schistosomin was confirmed after the final purification step using the CaFl bioassay. After pre-purification using the LC-18 cartridges, about 250 CNS equivalents were applied to high-performance gel-permeation chromatography. Schistosomin-containing fractions, which were eluted in the molecular mass range 6–10 kDa, were concentrated by lyophilization and applied to reverse-phase h.p.l.c. in batches of about 250 CNS equivalents. In this first reverse-phase h.p.l.c. step at pH 2, schistosomin is eluted at 45% solvent B (Fig. 1*a*). The schistosomin-containing fractions were combined to give 1000 CNS equivalents and further resolved using a second reverse-phase h.p.l.c. step at pH 6.7. In this system schistosomin is eluted as a sharp peak at 34% buffer B (Fig. 1*b*).

Bioactivity of schistosomin

Purified schistosomin (5 pmol) was tested in the *in vitro* assay for CaFl (Table 1). The activity of CaFl in the presence of schistosomin was decreased to a similar extent as was observed in the presence of haemolymph from schistosome-infected snails. A buffer control from the final purification step of schistosomin had no effect on CaFl activity.

Reduction of schistosomin and modification of cysteine residues

After reduction of schistosomin and modification of the cysteine residues with 4-vinylpyridine, the peptide was desalted by reverse-phase h.p.l.c. This resulted in elution of a number of contaminants, followed by the reduced schistosomin eluting at 60% solution B. Reduction was always complete and the yield of the peptide was > 90% (based on u.v. absorption).

Sequencing of schistosomin and of its proteolytic fragments

During all sequencing experiments, cysteine residues could be identified as a result of reduction and *S*-pyridylethylation. *N*-Terminal sequencing of native schistosomin (500 pmol) purified according to Hordijk *et al.* [11] resulted in the elucidation of the

Table 1. Inhibition of the bioactivity of the gonadotropic hormone CaFl by purified schistosomin

The accumulation of calcium into the mitochondria of cells of the albumen gland of *Lymnaea stagnalis* (expressed as the percentage of mitochondria containing calcium deposits) was studied in the absence and the presence of CaFl (an extract of the cerebral commissure of the CNS) and in the presence of CaFl in combination with different test samples. Values represent the means ± S.E.M. of five determinations. *Significantly different from control ($P < 0.05$).

Treatment	Ca^{2+}-positive mitochondria (%)
Control	24.4±2.1
CaFl (three animal equivalents)	66.0±1.9*
CaFl + haemolymph of parasitized snails	29.6±2.2
CaFl + reverse-phase h.p.l.c. buffer control	65.0±2.1*
CaFl + 5 pmol of purified schistosomin	36.4±2.6*

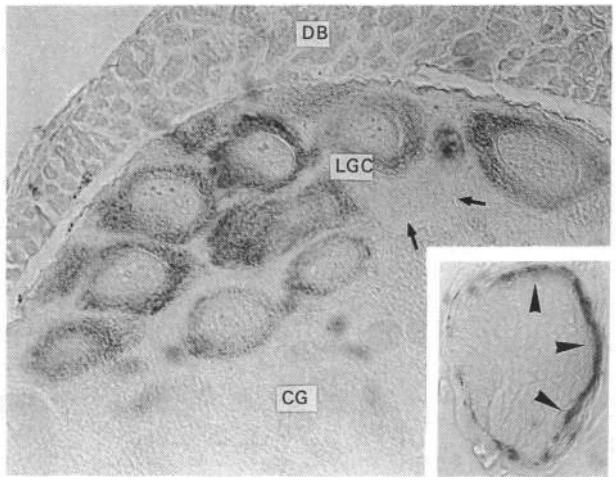

Fig. 3. Localization of schistosomin in the CNS of *Lymnaea stagnalis*

Light micrograph of a section through a cerebral ganglion (CG) of the CNS after immunostaining with a polyclonal antiserum directed against purified schistosomin. The neurosecretory LGCs, which show a positive reaction, and the immunonegative endocrine dorsal bodies (DB) are indicated. A LGC which does not show a positive reaction with the antiserum is also indicated (arrows). Magnification ×225. Inset: cross-section of the median lip nerve showing immunoreactive material (arrowheads) at the periphery. Magnification ×300.

sequence of the first 26 amino acids (fragment N1, Fig. 2). This sequence was confirmed in a second experiment in which the peptide (1.5 nmol), purified using the newly developed protocol, was sequenced again up to Gly-48 (fragment N2, Fig. 2). As a result of a proteolytic digest of 900 pmol of purified schistosomin using the *S. aureus* V8 protease, four fragments were isolated and sequenced (fragment E1–E4, Fig. 2). These fragments represented almost the compete schistosomin sequence. Based on the information from mass spectrometry [11], it appeared that the sequence information was incomplete. Therefore we used the Asp-N-specific protease in order to obtain *C*-terminal sequence information. A total of three fragments (D1–D3, Fig. 2) could be resolved using reverse-phase h.p.l.c. Fragment D3 represented the *C*-terminal part of the peptide (residues 71–79). There was no phenylhydantoin-amino acid detectable after Phe-79, indicating that this is the *C*-terminal residue. The peptide fragment corresponding to residues 11–57 was not found, for unknown reasons. Schistosomin contains eight cysteine residues, and the calculated isoelectric point of schistosomin is 6.25. The amino acid sequence of schistosomin was compared with all proteins in the NBRF-Protein (release 26.0) and Swiss-Prot. (release 15.0) databases at EMBL [20], but there was no substantial sequence similarity observed between schistosomin and any other protein.

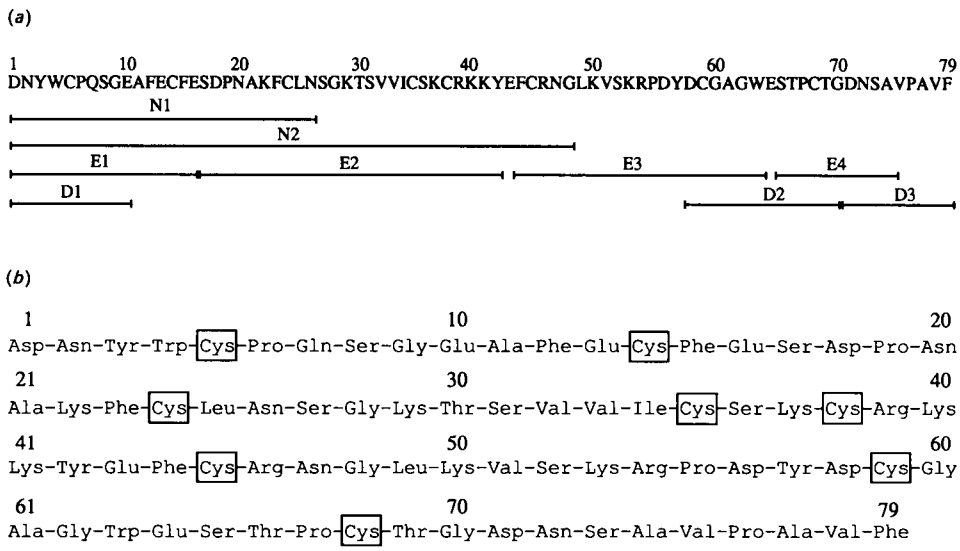

Fig. 2. Peptide mapping and primary structure of schistosomin isolated from the CNS of *Lymnaea stagnalis*

Purified schistosomin was reduced, after which the cysteine residues were modified with 4-vinylpyridine. (*a*) *N*-Terminal sequencing was performed twice (fragments N1 and N2). Cleavage with *Staphylococcus aureus* V8 protease resulted in the isolation of four fragments representing residues 1–16 (E1), 17–42 (E2), 44–63 (E3) and 65–75 (E4) respectively. Cleavage with endoproteinase Asp-N resulted in the isolation of three fragments representing residues 1–10 (D1), 58–70 (D2) and 71–79 (D3) respectively. The sequence of schistosomin is depicted in the one-letter code to visualize the exact positions of the proteolytic fragments and the specificity of the enzymes used. (*b*) Primary structure of schistosomin as deduced from the peptide mapping experiments. Cysteine residues are boxed.

Mass determination

Based on the primary structure, the molecular mass of schistosomin was calculated to be 8738 Da. Mass determination by plasma desorption mass spectrometry using freshly purified schistosomin gave a measured molecular mass of 8729 Da.

Immunocytochemistry

The anti-schistosomin serum reacted with neurons within both clusters of light green cells (LGCs) in each cerebral ganglion (Fig. 3). Remarkably, only approx. 90% of the LGCs reacted with the antiserum. The immunoreactive LGC-axon tracts run towards the median lip nerve and terminate at the periphery of this nerve (Fig. 3 inset). In addition, immunoreactivity was found in the canopy cells in the lateral lobes of the cerebral ganglia and in five to eight neurons located in the medio-caudal part of each pedal ganglion. Immunopositive fibres were found in the dorsal pedal commissure and in the median pedal nerves (results not shown). No staining was observed either with non-immune mouse serum or with the anti-schistosomin serum after preincubation with 50 pmol of the purified peptide.

DISCUSSION

In the present study we present the primary structure of an anti-gonadotropic neuropeptide, schistosomin, from the pond snail *Lymnaea stagnalis*. The peptide was purified from the CNS of the snail using pre-purification of LC-18 columns followed by gel-permeation chromatography and reverse-phase h.p.l.c. The identity of the purified material was tested using the *in vitro* CaFl assay. Automated Edman degradation of the purified intact peptide and subsequent peptide mapping resulted in the elucidation of the complete primary structure of this 79-residue neuropeptide.

The presence of eight cysteine residues in the schistosomin sequence probably leads to the formation of a total of four disulphide bonds. The disulphide bonds will probably fold the molecule into a rigid structure, which explains the stability of the peptide during, for example, heat treatment [11,15,21]. Another intriguing feature of the sequence of schistosomin is the distribution of the positively and negatively charged amino acid residues. With the exception of Glu-43, there is a strict separation of three domains of charge, starting with the region Asp-1–Asp-18, containing five acidic residues. Next, there is the region Lys-22–Arg-54, containing 10 basic residues. The *C*-terminal part of the molecule (Asp-56–Asp-71) contains four acidic residues [16]. These areas are separated from each other by proline residues (Pro-19 and Pro-55 respectively), which probably give rise to β-turns in the molecule [17]. The high number of turns predicted for the overall sequence suggests that schistosomin is a highly folded globular peptide. The most hydrophilic areas of the peptide are predicted to be the sequence Arg-Lys-Lys-Tyr (residues 39–42) and the Lys and Arg residues at positions 53 and 54. Both parts might be exposed at the surface of the molecule. These positively charged areas are probably important for the biological activity of schistosomin.

The molecular mass of schistosomin was determined twice by plasma desorption mass spectrometry. The value measured previously was 8780 Da [11]. Based on the sequence information, the calculated molecular mass is 8738 Da. The discrepancy was suggested to result from oxidation of tryptophan residues in the first sample due to prolonged storage. Therefore a second experiment was performed in which freshly isolated schistosomin was used. This resulted in a measured molecular mass of 8729 Da, in close agreement with the calculated molecular mass of 8738 Da (the resolution of the technique is 0.2% [22]).

Based on the observed inhibition of CaFl activity by purified schistosomin (Table 1), it can be concluded that the inhibitory effects of the haemolymph of parasitized snails [12,15] must be ascribed to the presence of schistosomin in the haemolymph of these animals. The inhibition by purified schistosomin was slightly lower than that observed in the presence of infected snail haemolymph. The reason for this might be that additional unknown inhibitory factors are present in the haemolymph. This inhibitory effect of schistosomin is known to be exerted at the level of the hormone receptor [11,12], although the exact mechanism of action remains to be elucidated. Purified schistosomin is also capable of inhibiting the ovulation response to the egg-laying hormone (CDCH) of *Lymnaea* [10]. Since the primary structures of schistosomin, CaFl and CDCH [6] do not share any similarity, a direct interaction of schistosomin with the binding sites of these peptides is unlikely. It seems plausible to suggest that schistosomin interferes with the signal transduction pathways of the different gonadotropic hormones by acting via its own receptor.

The immunocytochemical investigations demonstrate that schistosomin immunoreactivity is present in the LGCs and that the peptide is transported to the neurohaemal area of the LGCs, the periphery of the median lip nerve. The fact that not all LGCs react with the anti-schistosomin serum suggests that the LGC cluster is heterogeneous. The positive reaction with the antiserum of the canopy cells in the lateral lobes of the CNS (results not shown) corresponds with the notion that these cells are in fact ectopic LGCs [5]. The LGCs and the canopy cells have been shown to express different genes coding for insulin-related peptides and are known to be involved in the regulation of growth and metabolism in *Lymnaea* [5]. Parasitic infection results in gigantism, indicating that the co-ordination of growth by the LGCs and the canopy cells is disturbed. Gigantism can also be induced in non-infected snails by removal of the lateral lobes, which contain the canopy cells [23]. Since schistosomin is present in the LGCs and the canopy cells and inhibits reproduction, the peptide apparently plays an important role in the antagonism between growth and reproduction.

A parasite-induced decrease in fecundity by either mechanical or chemical means is found in numerous parasite–host combinations [3]. Schistosome parasites do not harm host tissues mechanically. In the combination studied here, the schistosome manipulates its host by chemical means to increase the synthesis and/or release of schistosomin, which results in inhibition of reproduction of the host. It has been suggested that parasite-derived ecdysteroids are involved in this process [24].

Reproduction-inhibiting systems have also been identified in other invertebrates. However, only a limited number of the (neuro)hormones involved have been structurally characterized. These are the locust neuroparsins A and B, which have been shown to delay oocyte growth [25,26], and a vitellogenesis-inhibiting hormone from the lobster *Homarus americanus*, which has only been partially characterized [27]. These hormones have molecular masses of 7–8 kDa. Although the molecular masses and the biological effects of these molecules are similar to those of schistosomin, their amino acid sequences do not show any similarity. The vertebrate hormones of the inhibin family, which inhibit the release of follicle-stimulating hormone, also do not show a structural relationship with schistosomin [28].

We thank Dr. Ka Wan Li, Mr. Roel van der Schors and Mrs. Marion Bergamin-Sassen for advice and technical assistance. The first *N*-terminal sequencing was kindly performed by Dr. Rudolf van den Broek. This investigation received financial support from the UNDP/World

Bank/WHO Special Programme for Research and Training in Tropical Diseases.

REFERENCES

1. Ramaley, J. A. & Phares, C. K. (1980) Endocrinology (Baltimore) **106**, 1989–1993
2. Beckage, N. E. (1985) Annu. Rev. Entomol. **30**, 371–413
3. Hurd, H. (1990) Adv. Parasitol. **29**, 271–318
4. Geraerts, W. P. M., ter Maat, A. & Vreugdenhil, E. (1988) in Invertebrate Endocrinology: Endocrinology of Selected Invertebrate Types (Laufer, H. & Downer, G. H., eds.), vol 2, pp. 141–231, Alan R. Liss, New York
5. Geraerts, W. P. M., Smit, A. B., Li, K. W., Vreugdenhil, E. & van Heerikhuizen, H. (1990) in Current Aspects of the Neurosciences (Osborne, N. N., ed.), vol. 3, pp. 255–304, MacMillan Press, London
6. Vreugdenhil, E., Jackson, J. F., Bouwmeester, T., Smit, A. B., Van Minnen, J., van Heerikhuizen, H., Klootwijk, J. & Joosse, J. (1988) J. Neurosci. **81**, 4184–4191
7. Dogterom, G. E., Bohlken, S. & Geraerts, W. P. M. (1983) Gen. Comp. Endocrinol. **60**, 476–482
8. Ebberink, R. H. M., van Loenhout, H., Geraerts, W. P. M. & Joosse, J. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 7767–7771
9. Dictus, W. J. A. G., de Jong-Brink, M. & Boer, H. H. (1987) Gen. Comp. Endocrinol. **65**, 439–450
10. Hordijk, P. L., van Loenhout, H., Ebberink, R. H. M., de Jong-Brink, M. & Joosse, J. (1991) J. Exp. Zool., in the press
11. Hordijk, P. L., Ebberink, R. H. M., de Jong-Brink, M. & Joosse, J. (1991) Eur. J. Biochem. **195**, 131–136
12. de Jong-Brink, M., Elsaadany, M. M. & Boer, H. H. (1988) Exp. Parasitol. **65**, 109–118
13. van der Steen, W. J., van den Hoven, N. P. & Jager, J. C. (1969) Neth. J. Zool. **19**, 131–139
14. Li, K. W., Geraerts, W. P. M., van Elk, R. & Joosse, J. (1989) J. Chromatogr., **472**, 445–446
15. de Jong-Brink, M., Elsaadany, M. M. & Boer, H. H. (1988) Exp. Parasitol. **65**, 91–100
16. Kyte, J. & Doolittle, R. F. (1982) J. Mol. Biol. **157**, 105–132
17. Chou, P. Y. & Fasman, G. D. (1987) Annu. Rev. Biochem. **47**, 251–276
18. Smit, A. B., Vreugdenhil, E., Ebberink, R. H. M., Geraerts, W. P. M., Klootwijk, J. & Joosse, J. (1988) Nature (London) **331**, 535–538
19. Van Minnen, J. & Schallig, H. D. F. H. (1990) Cell Tissue Res. **260**, 381–386
20. Devereux, J., Haeberli, P. & Smithies, O. (1984) Nucleic Acids Res. **12**, 387–395
21. Joosse, J., van Elk, R., Mosselman, S., Wortelboer, H. & van Diepen, J. C. E. (1988) Parasitol. Res. **74**, 228–234
22. Tsarbopoulos, A. (1989) Pept. Res. **2**, 258–266
23. Geraerts, W. P. M. (1976) Gen. Comp. Endocrinol. **29**, 97–108
24. de Jong-Brink, M., Schallig, H. D. F. H., Charlet, M. & Zonneveld, C. (1989) Int. J. Invertebr. Reprod. Dev. **15**, 201–209
25. Couillard, F., Girardie, A. & Girardie, J. (1989) Int. J. Invertebr. Reprod. Dev. **16**, 17–22
26. Girardie, J., Girardie, A., Huet, J. & Pernollet, J. (1989) FEBS Lett. **245**, 4–8
27. Soyez, D., van Deijnen, J. E. & Martin, M. (1987) J. Exp. Zool. **244**, 479–484
28. Ying, S. Y. (1988) Endocr. Rev. **9**, 267–293

Received 22 April 1991/10 June 1991; accepted 14 June 1991