Acetylcholinesterase from *Schistosoma mansoni*: interaction of globular species with heparin

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In the cercarial and schistosomal stages of the life cycle of the trematode *Schistosoma mansoni*, acetylcholinesterase occurs as two principal molecular forms (both globular), present in approximately equal amounts, with sedimentation coefficients of 6.5 S and 8 S. The 6.5 S form is solubilized by bacterial phosphatidylinositol-specific phospholipase C from intact schistosomula. It is thus located on the outer surface of the schistosomal tegument and is most probably analogous to the glycosylphosphatidylinositol-anchored G_2 form of acetylcholinesterase found in the electric organ of *Torpedo*, on the surface of mammalian erythrocytes, and elsewhere. Both forms are fully solubilized by the non-ionic detergent Triton X-100. Upon passing such a detergent extract over a heparin–Sepharose column, only the 8 S form was retained on the column. The bound acetylcholinesterase could be progressively eluted by increasing the salt concentration, with approx. 0.5–0.6 M NaCl being needed for complete elution. Selective inhibition experiments carried out on live parasites using the covalent acetylcholinesterase inhibitor echothiophate (phospholine), which does not penetrate the tegument, selectively inhibited the 6.5 S form, but not the 8 S form, suggesting an internal location for the latter. Monoclonal antibodies raised against *S. mansoni* acetylcholinesterase also distinguished between the two forms. Thus monoclonal antibody SA7 bound the 6.5 S form selectively, whereas SA57 recognized the 8 S form. The selective binding of the 8 S form to heparin suggests that, within the parasite, this form may be associated with the extracellular matrix of the musculature.

Key words: cercaria, glycosylphosphatidylinositol anchor, monoclonal antibodies, tegument.

INTRODUCTION

The principal physiological role of acetylcholinesterase (AChE; EC 3.1.1.7) is believed to be termination of transmission at cholinergic synapses by rapid hydrolysis of the neurotransmitter acetylcholine (ACh) [1]. Other, non-cholinergic, roles for AChE have also been suggested [2,3], including a possible role as an adhesion protein [4].

ACh has long been considered a probable neurotransmitter in the nervous systems of parasitic flatworms [5]. ACh and AChE were shown to be involved in the motor activity of *Schistosoma mansoni* [6–8], and phosphonium salts, cholinomimetic agents and cholinesterase inhibitors result in paralysis of the worms [9,10]. However, the physiological role(s) of AChE in this trematode remain(s) to be clarified. Its presence has been established at several developmental stages of the parasite, i.e. in cercaria, schistosomula and adult worms, both in the internal organs and on the surface of the organism [6,11]. The surface AChE of the parasite was first described and characterized by Levi-Schaffer et al. [12]. More recent studies, using immunoelectron microscopy, on cercaria and schistosomula revealed that the enzyme is localized mainly on the surface of the tegument and, in the form of patches, in the muscles [13].

AChE occurs in a repertoire of molecular forms in both vertebrates and invertebrates [1,14]. These include the globular (G) forms, which contain one, two or four catalytic subunits (G_1, G_2 and G_4 respectively), and the asymmetric (A) forms, which contain four, eight or twelve subunits (A_4, A_8 and A_{12} respectively) attached to a collagenous tail [1]. The G forms are either water-soluble or membrane-bound, the latter being anchored to the surface membrane by hydrophobic moieties that are inserted post-translationally. The A forms are believed to be attached to the basal lamina within the synaptic cleft by interaction with heparan sulphate [15]. The reason for this elaborate polymorphism is, as yet, unknown, but has been ascribed to the functional requirements of different types of synapses [1,16,17]. It appears to arise, at least in part, from differential targeting to either the presynaptic or postsynaptic surface membranes or to secretion [18]. Thus, for example, the A forms are localized to the endplate regions in skeletal muscle [19,20], and hydrophobic G_4 forms are associated with cholinergic synapses in the central nervous system [20]. As mentioned, the A forms interact specifically with heparin or heparin-like glycosaminoglycans, and motifs on the collagen tail have been identified that are involved in this interaction [21]. The G forms of AChE lack the collagen tail, and are usually considered to lack the capacity to interact with heparin. However, Ramirez et al. [22] showed that the G_4 form from chick muscle binds to heparin with low affinity. Furthermore, Sine et al. [23] reported an interaction with heparin by an amphiphilic G_2 form of butyrylcholinesterase extracted from mucosal cells of rat intestine.

We reported previously the presence of several molecular forms of AChE in *S. mansoni*, and described some of their biochemical properties [24]. In cercaria and schistosomula, part of the enzyme is soluble at high ionic strength and part is solubilized only in the presence of detergent. In these larval life-cycle stages, both the high-salt-soluble and the detergent-soluble fractions migrate as broad peaks at approx. 8 S on sucrose

Abbreviations used: ACh, acetylcholine; AChE, acetylcholinesterase; A form, asymmetric form; DSM, defined synthetic medium; G form, globular form; GPI, glycosylphosphatidylinositol; MoAb, monoclonal antibody; PI-PLC, phosphatidylinositol-specific phospholipase C.

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gradients [24]. Camacho et al. [25] reported that, in adult worms, the AChE activity could be partially resolved into two peaks of approx. 6 S and 8 S, presumably both representing G forms of the enzyme. Exposure of intact schistosomula to phosphatidylinositol-specific phospholipase C (PI-PLC) released AChE from the surface of the parasite. This indicates that, like many other parasite surface proteins [26], this surface AChE is a glycosylphosphatidylinositol (GPI)-anchored protein [27], analogous to the GPI-anchored G_2 form of AChE present in, for example, the *Torpedo* electric organ [28]. The removal of the surface AChE by PI-PLC *in situ* activates *de novo* biosynthesis of AChE to replenish the surface enzyme [29]. This phenomenon is specific for AChE, and is not observed for another GPI-anchored enzyme of the parasite, alkaline phosphatase. These data suggest an important physiological role for AChE on the surface of the parasite, although this role remains to be established [30,31].

In the present paper, we describe the heparin-binding characteristics of schistosomal AChE. We show that the internal (8 S) form of AChE interacts with heparin, whereas the external (6.5 S) GPI-anchored form does not.

MATERIALS AND METHODS

Parasite

An Egyptian strain of *S. mansoni* is routinely maintained in our laboratory by passage through *Biomphalaria glabrata* snails and outbred CD-1 mice. Cercaria were collected after a shedding period of 90–100 min. Schistosomula were prepared by mechanical transformation [13].

Reagents

[^{3}H]ACh iodide (90 mCi/mmol) was purchased from New England Nuclear (Boston, MA, U.S.A.). Echothiophate iodide was from Wyeth-Ayers (Montreal, Canada). The AChE inhibitors BW284c51 and eserine sulphate, the protease inhibitors leupeptin, pepstastin, bacitracin and aprotinin, and *Clostridium histolyticum* collagenase (type III) were all from Sigma (St. Louis, MO, U.S.A.). Recombinant PI-PLC from *Bacillus thuringiensis* was from Oxford Glycosystems (Oxford, U.K.). Heparin, as the sodium salt, was from Calbiochem (La Jolla, CA, U.S.A.), and Triton X-100 was from Packard (Meriden, CT, U.S.A.). Heparin–Sepharose CL-6B was from Pharmacia Fine Chemicals AB (Uppsala, Sweden), and the Fab' fragment from rabbit anti-mouse Ig was purchased from The Jackson Laboratory (Bar Harbor, MN, U.S.A.). Sucrose, NaCl and other reagents were of analytical grade.

Fractionation of parasite tissue

Fresh cercaria (1×10^6) or schistosomula (5×10^5) were cool-concentrated by centrifugation at 4 °C for 2 min at 800 ***g***. Then 50 mM Tris/HCl, pH 7.5, containing a cocktail of protease inhibitors (10 mM EGTA, 40 μg/ml leupeptin, 20 μg/ml pepstatin, 1 mg/ml bacitracin, 20 units/ml aprotinin and 1 mM benzamidine [32]) was added (10 ml), followed by sonication at 50 % duty cycle/output control 4 in a bath sonicator (Heat Systems–Ultrasonics, Plainview, NY, U.S.A.). Sonication was performed for 3×3 min at 4 °C. Each homogenate was divided into two equal portions before solubilization. One portion was solubilized in the presence of detergent (in 50 mM Tris/HCl, pH 7.5, containing 0.5–1 % Triton X-100), and the other in the same buffer with Triton X-100 being replaced by 3 mg/ml heparin. In both cases the buffers contained the anti-protease cocktail. Following incubation for 30 min at 22 °C, the homogenates were centrifuged for 5 min at 10000 ***g*** and 4 °C, and the supernatants were re-centrifuged for 2 h at 100000 ***g*** and 4 °C. Each of the pellets was resuspended in a third of the total initial volume using various buffers.

Assay methods

AChE was routinely assayed by the radiometric method of Johnson and Russell [33], using [^{3}H]ACh as substrate. The assay was performed at 25 °C in a final concentration of 50 mM Tris/HCl, pH 7.5, containing 0.1 M NaCl. An aliquot of 100 μl of reaction mixture contained 50 μl of the sample and 100000 c.p.m. of [^{3}H]ACh, to which unlabelled ACh was added to a final concentration of 3 mM. The reaction was terminated by addition of 100 μl of a solution consisting of 1 M chloroacetic acid, 0.5 M NaOH and 2 M NaCl. The radiolabelled product was extracted in 4 ml of 10 % (v/v) 3-methylbutan-1-ol in toluene-based scintillation fluid, and radioactivity was counted in a Packard counter. The Ellman assay for AChE [34], using acetylthiocholine as substrate, was used in the gel filtration experiments.

Catalase was monitored by measuring absorbance at 407 nm, β-galactosidase was assayed as described by Craven et al. [35], and protein concentrations were determined as described by Bradford [36].

RIA for AChE

Solid-phase RIA was performed essentially as described by Pierce and Klinman [37]. Briefly, microtitre plates were coated for 2 h, at room temperature, with 10 μg of solubilized cercarial fractions per well. The wells were first treated with various dilutions of anti-AChE hybridoma Ig [38] for 2 h at 24 °C or overnight at 4 °C, and then incubated for 2 h at room temperature with affinity-purified ^{125}I-labelled anti-mouse Fab' (10^5 c.p.m./well). The washed wells were dried and cut out, and radioactivity was counted in an autogamma counter.

Sucrose-gradient centrifugation

Apparent sedimentation coefficients ($s_{20,w}$) were determined as described by Lyles et al. [39] by sedimentation in 5–20 % (w/v) sucrose gradients. Sedimentation was performed at 100000 ***g*** (SW 41 rotor) for 16–18 h at 4 °C in a Beckman L8-M ultracentrifuge. Fractions of 200 μl were collected. *Escherichia coli* β-galactosidase (16 S) and bovine liver catalase (11.4 S) served as markers.

Tissue culture of schistosomula

Schistosomula, after mechanical transformation [40], were maintained in culture at a concentration of 20000 organisms per ml in defined synthetic medium (DSM) consisting of a 1:1 (v/v) mixture of F12 and RPMI media (Gibco, Grand Island, NY, U.S.A.), supplemented with 200 units/ml penicillin, 200 μg/ml streptomycin, 2 mM L-glutamine and 20 mM Hepes, pH 7.2. They were cultured for 3 or 24 h at 37 °C in a humidified atmosphere containing 7 % CO_2 [12].

For inhibition of surface AChE of the schistosomula, 1 mM echothiophate iodide (phospholine) was added to cultures. Incubation for 30 min was followed by extensive washing with DSM and one wash with PBS before processing.

Heparin–Sepharose affinity chromatography

A column containing 6 ml of heparin–Sepharose was equilibrated with the appropriate buffer before use. Usually, equilibration was with 50 mM Tris/HCl, pH 7.5, containing 0.2% Triton X-100. Samples of 2 or 5 ml (protein concentration 3 mg/ml) were applied, and 1.5 ml fractions of the unbound material were collected. Elution from the column involved successive application of 5–10 ml aliquots of the same buffer containing 0.1, 0.2, 0.5, 1.0 and 2 M NaCl; again, 1.5 ml fractions were collected. Alternatively, elution was performed with heparin (3 mg/ml) in 50 mM Tris/HCl, pH 7.5, without Triton X-100.

Gel filtration

An aliquot of the 100000 *g* supernatant from the cercarial extract (40 mg of protein in 10 ml) was subjected to gel filtration on a column (100 cm × 15 cm) of superfine Sephacryl S-300 equilibrated with extraction buffer. Fractions of 2 ml were collected. The column was calibrated with the following protein markers: ferritin (420 kDa), catalase (220 kDa) and aldolase (156 kDa).

RESULTS

Binding of AChE to heparin–Sepharose

Figure 1 shows chromatography of extracts from cercaria on a heparin–Sepharose column in 0.5% Triton X-100/50 mM Tris/HCl, pH 7.5; 40–50% of the activity bound to the column. This result was consistently observed in 10 different experiments, and similar results (not shown) were obtained with extracts of schistosomula. The bound enzyme was eluted at a salt concentration of 0.5–0.6 M NaCl for both life-cycle stages. Increasing the gel bed volume 3-fold did not increase the percentage of AChE activity bound; nor did re-application of unbound material to a fresh column result in additional binding. In most cases, all the bound AChE could be recovered from the column. However, in some experiments, part of the bound AChE (20–30%) could not be recovered. This appeared to be related to the batch of heparin–Sepharose employed.

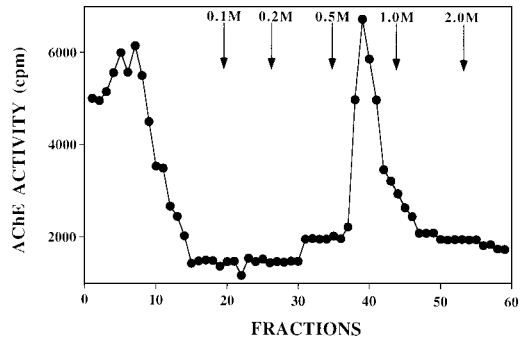

Figure 1 Affinity chromatography of AChE from *S. mansoni* on a heparin–Sepharose column

A total extract of cercaria in 0.5% Triton X-100/50 mM Tris/HCl, pH 7.5, was applied to a heparin–Sepharose column. Bound AChE was eluted from the column using a step gradient from 0 to 2 M NaCl in the same buffer containing 0.1% Triton X-100.

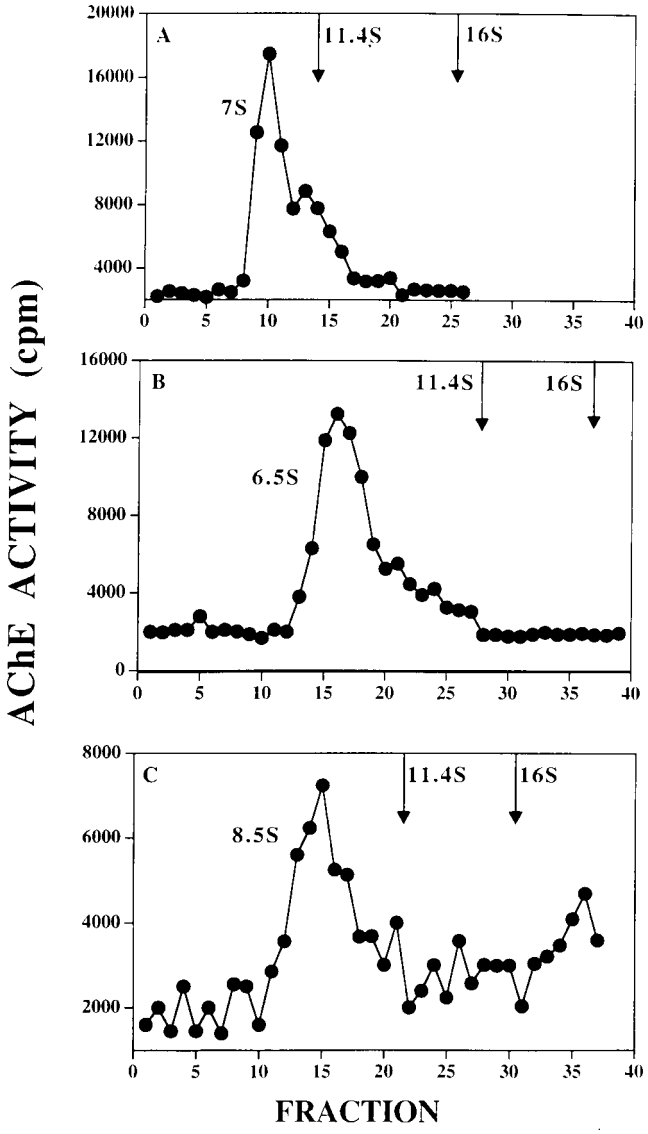

Figure 2 Differential binding of molecular forms of AChE from cercaria to a heparin–Sepharose column

A total extract of cercaria in 1% Triton X-100/50 mM Tris/HCl, pH 7.5, was applied to a heparin–Sepharose column as described in the legend to Figure 1. Bound AChE activity was eluted with a 0–2 M NaCl step gradient in the same buffer containing 0.1% Triton X-100, and the fractions of activity were pooled. Samples were analysed by sucrose-gradient centrifugation on a 5–20% (w/v) sucrose gradient in 0.1% Triton X-100/50 mM Tris/HCl, pH 7.5. (**A**) Total extract; (**B**) unbound fraction; (**C**) heparin-bound fraction, eluted with NaCl. The arrows denote the positions of the markers catalase (11.4 S) and β-galactosidase (16 S).

Characterization of molecular forms of bound and non-bound AChE

The molecular forms present in the total soluble extracts of cercaria, and in the fractions that either were not bound to the heparin–Sepharose column or were eluted from it by high salt or heparin, were all analysed by sucrose-gradient centrifugation. The AChE activity in the total cercarial extract appeared primarily in a peak at 6.5–7 S, with a shoulder at 8–8.5 S (Figure 2A). The non-bound material, representing 40–50% of total activity, displayed a major peak at 6.5 S, with a small shoulder at 8 S (Figure 2B). For the heparin-bound fraction eluted by

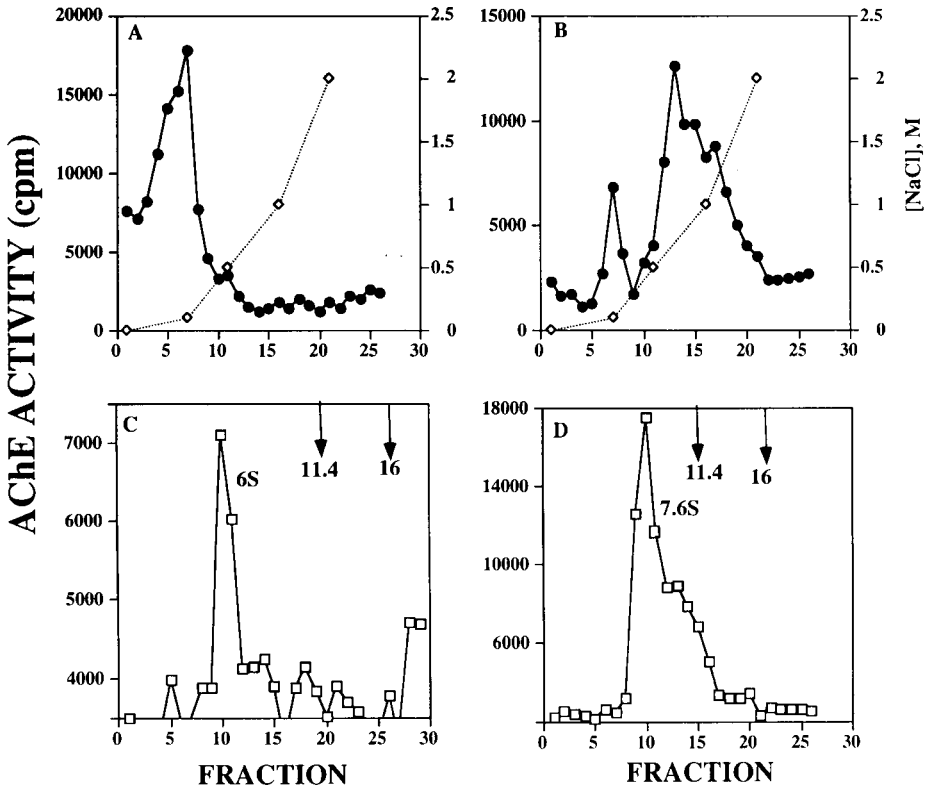

Figure 3 Heparin-binding capacity and characterization on sucrose gradients of AChE from schistosomula

Schistosomula obtained after 24 h in culture were incubated in fresh DSM with PI-PLC (8 μg/ml) for 2 h at 37 °C. The parasites were separated from the incubation medium by centrifugation (80 ***g***, 1 min). The supernatant, representing the PI-PLC-solubilized fraction, was retained. The parasite pellet, containing residual AChE activity, was solubilized with 1% Triton X-100 in 50 mM Tris/HCl, pH 7.5, as described in the Materials and methods section. Both the PI-PLC supernatant (**A**) and the extract (**B**) were subjected to affinity chromatography on heparin–Sepharose, as described in the legend to Figure 1. The AChE activity passing through the heparin–Sepharose column in the case of the PI-PLC supernatant was analysed by sucrose-gradient centrifugation on a 5–20% (w/v) sucrose gradient in 0.1% Triton X-100/0.05 M Tris/HCl, pH 7.5 (**C**), and the material eluted by NaCl from the heparin–Sepharose column to which the extract of the pellet had been applied was similarly analysed (**D**). The arrows denote the positions of the markers catalase (11.4 S) and β-galactosidase (16 S).

NaCl, the major peak was at 8–8.5 S (Figure 2C), and this accounted for 30–50% of the total AChE activity. Elution with heparin similarly yielded a single peak at approx. 8 S (results not shown). Treatment of cercarial extracts with collagenase prior to their passage through the heparin–Sepharose column did not reduce the percentage of AChE activity bound, nor did it affect the sedimentation coefficient of the eluted material (results not shown).

Binding of surface and internal AChE forms to heparin–Sepharose

In order to study separately the heparin-binding properties of the surface and internal AChE forms, schistosomula, cultured for 24 h, were treated with PI-PLC as described previously [27,29]. The AChE activity released from the surface of the intact schistosomula by PI-PLC treatment, and the residual enzyme extracted from the whole organism with detergent, were each fractionated on the heparin–Sepharose column. Figure 3(A) clearly shows that the surface-localized AChE released by PI-PLC did not bind to the column. Between 70 and 100% of the PI-PLC-solubilized AChE was recovered in the effluent, and no significant activity was eluted by high salt. Sucrose-gradient centrifugation revealed, as expected for PI-PLC-released material [25], a major peak at 6.5 S (Figure 3C). The elution pattern on the heparin–Sepharose column for the detergent-solubilized material obtained from the schistosomula after PI-PLC treatment is shown in Figure 3(B). Part of the AChE activity (10–30%) was not bound to the column, and may represent replenished surface enzyme synthesized subsequent to exposure to PI-PLC [29]. However, most of the AChE activity was bound to the column, and was only eluted at approx. 0.6 M NaCl. The sedimentation coefficient of the eluted material showed a major peak at approx. 8 S, with a shoulder at 10–12 S (Figure 3D).

Characterization of surface-associated and internal AChE activity

To distinguish further between the surface and internal forms of AChE, we utilized the quaternary AChE inhibitor echothiophate (phospholine), which inhibits AChE irreversibly and, due to its charge, penetrates the surface membrane very slowly. Consequently, under appropriate experimental conditions, it can be used to inhibit surface AChE selectively [41]. Schistosomula (100000) were incubated with 0.1 mM echothiophate for 30 min. The parasites were then washed four times with 10 ml of DSM and once with PBS, sonicated and extracted with 50 mM Tris/HCl, pH 7.5, containing 1% Triton X-100. The activity in this fraction should correspond exclusively to internal AChE. The 100000 ***g*** supernatant was diluted 2-fold and then fractionated on the heparin–Sepharose column. Figure 4(A) shows the profile for control schistosomula, and Figure 4(B) that

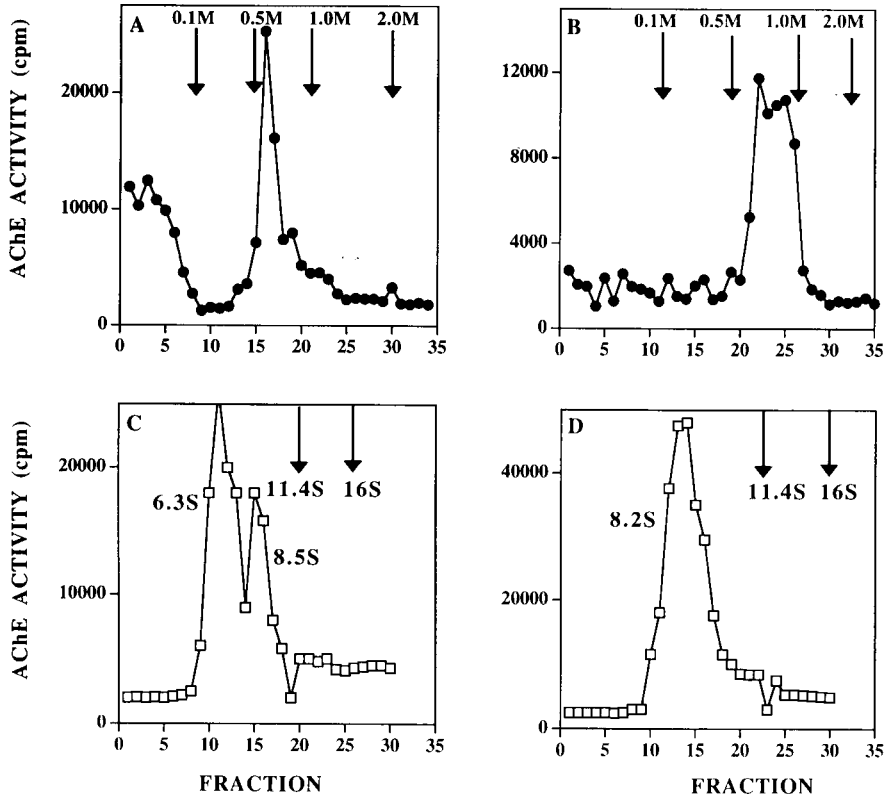

Figure 4 Heparin-binding capacity and analysis on sucrose gradients of the echothiophate-resistant pool of AChE from schistosomula

Schistosomula obtained after 24 h in culture were incubated with fresh DSM containing 1 mM echothiophate for 20 min at 37 °C, as described in the Materials and methods section. The total detergent extract was then applied to a heparin–Sepharose column, and bound AChE activity was eluted with a 0–2 M NaCl step gradient. (**A**) Chromatography on heparin–Sepharose of an extract of control schistosomula, not exposed to echothiophate; (**B**) chromatography on heparin–Sepharose of an extract of echothiophate-treated schistosomula; (**C**) sucrose-gradient centrifugation of the eluate from the heparin–Sepharose column for control schistosomula; (**D**) sucrose-gradient centrifugation of the eluate from the heparin–Sepharose column for the echothiophate-treated schistosomula. The arrows in (**C**) and (**D**) denote the positions of the markers catalase (11.4 S) and β-galactosidase (16 S).

for the echothiophate-treated parasites. With the control, approx. 30–40 % of the AChE activity was not bound, and the remainder was eluted by NaCl. In the sample that had been exposed to echothiophate, no unbound material was observed. As expected, sucrose-gradient centrifugation showed two peaks (at approx. 6 and 8 S) in the control sample (Figure 4C), but only a single peak (approx. 8 S) in the echothiophate-treated sample (Figure 4D). These data clearly show that it is the internal pool of AChE, with a sedimentation coefficient of approx. 8 S, that has the capacity to bind to heparin.

Differential recognition of heparin-bound and non-bound AChE forms by monoclonal antibodies (MoAbs)

In previous studies in our laboratory, a series of MoAbs against *S. mansoni* AChE were obtained that displayed cross-reactivity with *Electrophorus electricus* AChE [38]. These MoAbs differ from each other in various properties, such as their capacity to recognize different life-cycle stages of the parasite, to cross-react with mammalian AChE and to inhibit AChE activity on the surface of intact schistosomula. Thus MoAb SA7 inhibits almost 50 % of the surface AChE activity of schistosomula, whereas MoAb SA57 produces almost no inhibition. These two MoAbs were evaluated for their capacity to differentiate between the fractions of schistosomal AChE that bind to heparin and those that do not. Pooled fractions of the high-salt eluate from the heparin–Sepharose column and of the non-retained material were adsorbed on to polystyrene plates at protein concentrations of 10 μg/well for RIA. MoAb SA7 interacted preferentially with the fraction that did not bind to the heparin column (5083 ± 375 c.p.m., compared with 1360 ± 65 c.p.m. for the fraction that did bind to the column). In contrast, MoAb SA57 preferentially recognized the fraction that did bind to the heparin column (5895 ± 244 c.p.m., compared with 1290 ± 34 c.p.m. for the fraction that did not bind to the column). These data are in agreement with our earlier observations which suggested that the former MoAb (SA7) recognizes the surface enzyme preferentially, whereas the latter (SA57) recognizes the internal enzyme [38]. The epitopes recognized by these MoAbs are not known, but we have established that they are protein (rather than carbohydrate) epitopes [38].

Analysis of the soluble extract by gel filtration

Total soluble extracts of cercaria were analysed by gel filtration. In addition to a fraction that was excluded from the column, two principal peaks of AChE activity were resolved (Figure 5A). When the peaks of these two fractions (tubes 106 and 116) were analysed on sucrose gradients, they each displayed a single major peak, at approx. 6.5 S (Figure 5B) and approx. 8 S (Figure 5C) respectively. Thus we were able to cleanly resolve these two species, yet their close migration on the gel-filtration column supports the notion that they are both G forms of the enzyme.

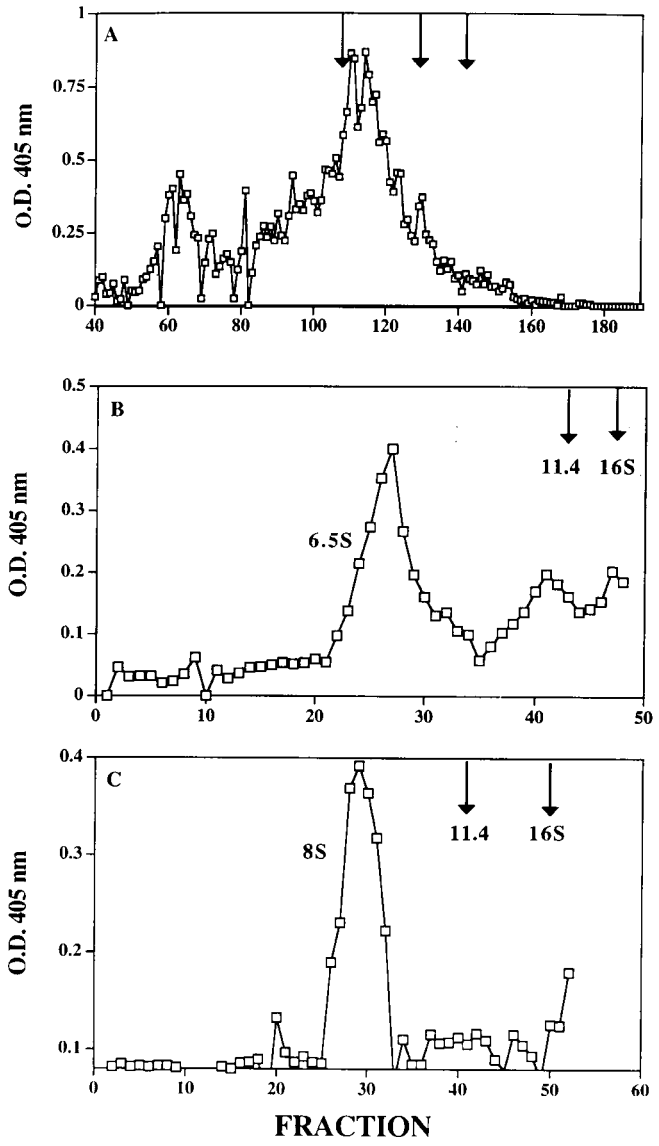

Figure 5 Analysis by gel filtration and sucrose-gradient centrifugation of AChE activity in a total extract of cercaria

After solubilization, as described in the Materials and methods section, the 100 000 ***g*** supernatant (20 ml) was applied to a Sephacryl S-300 column (100 cm × 15 cm) in 0.1% Triton X-100/0.05 M NaCl/50 mM Tris/HCl, pH 7.5. Two activity peaks from the gel-filtration column, corresponding to fractions 106 and 116, were then analysed by sucrose-gradient centrifugation as described in the legend to Figure 3. (**A**) AChE activity profile on the Sephacryl S-300 column; the arrows show the positions of the molecular mass markers (from left to right: ferritin, 420 kDa; catalase, 232 kDa; aldolase, 158 kDa); (**B**) sucrose-gradient centrifugation profile for fraction 116; (**C**) sucrose gradient centrifugation profile for fraction 106. The arrows show the positions of the markers catalase (11.4 S) and β-galactosidase (16 S). O.D., absorbance.

DISCUSSION

The principal finding of the present study is that a globular species of AChE, present in the larval stages (i.e. cercaria and schistosomula) of the life cycle of *S. mansoni*, interacts with heparin.

Solubilization with and/or interaction with heparin have been used by Inestrosa and co-workers [42] to study interactions of AChE with the extracellular matrix. These workers provided evidence, in vertebrate skeletal muscle and *Torpedo* electric organ, that only A forms of AChE interact with heparin and/or other proteoglycans, via motifs on their collagen tails [15,21]. We showed previously that approx. 50% of the AChE of *S. mansoni* larval stages is a G_2 form, attached via a GPI anchor to the surface of the tegumental membrane, while the other 50% is internal, and associated mainly with the musculature [25,29]. Our present data show that only this internal AChE interacts with heparin; however, despite the evidence for its association with the musculature, the molecular form involved is a G form rather than an A form. This conclusion is based on the following findings: (1) The bulk of the enzyme is extracted at low ionic strength, in the presence of detergent; (2) sedimentation on sucrose gradients is not affected by collagenase; and (3) the close migration of the 6.5 S and 8 S species on gel filtration (Figure 5) argues against the 8 S species being an A_4 form, rather than a G form. Although this assignment is unusual, interaction of G forms of both AChE and butyrylcholinesterase with heparin has been reported [22,23].

Another line of evidence that corroborates the finding that it is the internally located AChE that interacts with heparin–Sepharose was provided by the accessibility of the active site of the surface AChE to the membrane-impermeable irreversible inhibitor echothiophate [41,43]. Hence, upon exposure of schistosomula to this inhibitor, all surface enzymic activity was blocked selectively, with little or no inhibition of internal AChE, and all the AChE extracted was bound to the heparin–Sepharose column (Figure 4B).

The surface and internal forms of AChE were also distinguished by use of MoAbs SA7 and SA57 [38]. Our data clearly demonstrate that the former recognizes the surface enzyme and the latter the internal enzyme. The epitopes recognized are unknown, but they must be peptide rather than sugar epitopes, since both MoAbs still bind to AChE after deglycosylation [38].

As mentioned in the Introduction section, immuno-electron microscopy has revealed an association of AChE with the musculature of schistosoma, and pharmacological evidence supports the functional role of this enzyme at motor endplates of the parasite. Since the GPI-anchored AChE is on the external surface of the tegument, it is not surprising that exposure of schistosomula to MoAb SA7 does not affect motor activity; presumably it is the heparin-binding fraction that is the functional form in the muscles. In the extensive literature on molecular forms of AChE in skeletal muscle, both A and G forms are described [1]. However, it is generally assumed that the A forms, associated with the basal lamina at the motor endplate, are the functional forms, at least in fast- and slow-twitch muscles [44]. Our present data suggest that, in striated muscles of *S. mansoni*, the functional forms of AChE are G forms, and that these are associated with the extracellular matrix. In this context, it should be noted that the major form of AChE in frog synaptic basal lamina is globular [41].

In summary, our findings clearly demonstrate the presence of two forms of AChE in *S. mansoni*: one external, associated with the tegument, and the other internal, associated with the musculature. The fact that the internal enzyme interacts with heparin supports its assignment as a functional species associated with motor function.

This study was supported in part by grant no. I-0354-080-02-94 from the German–Israeli Foundation for Scientific Research and Development. During the course of this work Ruth Arnon was a Scholar-in-Residence at the Fogarty International Center for Advanced Studies in the Health Sciences, National Institutes of Health, Bethesda, MD, U.S.A. Israel Silman is the Bernstein–Mason Professor of Neurochemistry. Ruth Arnon is the Paul Ehrlich Professor of Immunology.

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Received 10 May 1999/11 August 1999; accepted 29 September 1999