**Schistosoma mansoni:** Surface Membrane Isolation with Lectin-Coated Beads

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**Abstract** Lectins from Lens culinaris and Arachis hypogaea immobilized on polyacrylamide beads were used for selective isolation of glycosylated surface membrane domains of adult Schistosoma mansoni worms, and the method was compared with the membrane isolation procedure developed with polycationic (Affi-Gel) beads. The lentil lectin proved to be suitable for interaction with surface membrane components: an increment in the specific activities of tegumental phosphohydrolases was observed in the bound fraction with respect to that observed in a total worm homogenate. A characteristic polypeptide pattern on gel electrophoresis was also seen, more restricted than that obtained with the bound Affi-Gel fraction. Immobilized peanut lectin was not successful as a method for isolating membrane material from the tegument of adult worms. Solubilization and dissociation of the lentil lectin-bound enzyme markers was achieved after addition of detergent and competing sugars. Glycosylation of the solubilized enzymes was further confirmed by affinity chromatography with fresh lentil lectin-coated beads. These results, together with histochemical evidences, suggest that the active sites of some of these enzymes are locted within or close to the cytoplasmic leaflet of the surface tegumental membranes, and allow us to propose a model for the double surface membrane complex where some proteins may be crossing the two bilayers.

**Keywords** *Schistosoma mansoni*, lectin-coated beads, lentil lectin, peanut lectin, alkaline phosphatase, Ca$^{2+}$-ATPase, type I phosphodiesterase, membrane isolation.

**Introduction**

*Schistosoma mansoni* adult worms are surrounded by a syncytial tegument which is covered by a double membrane of great importance in the host–parasite relationship (16). A detergent-free procedure for isolating this surface membrane has been proposed, based upon the high negative charge density present in the surface of the parasite and its interaction with polycationic beads, in a short 30-min period (4).

The surface of adult *S. mansoni* is rich in concanavalin A (Con A) lectin receptors and there are also components that interact with ricin, peanut and wheat germ lectins (1, 13, 17, 25). Thus, the use of lectin-coated beads could be a more specific method for...
the isolation of the surface membrane. Lectin-coated beads have been previously used for the isolation of erythrocyte membranes (10).

Two immobilized lectins were assayed: *Lens culinaris* lectin (LcH), with a sugar specificity similar to Con A but with a lower affinity and it is easier to desorb (9), and *Arachis hypogaea* lectin (PNA). The interaction of these lectins with the parasite surface was evaluated by determination of three membrane-bound tegumental phosphohydrolase activities, alkaline phosphatase, type I phosphodiesterase, and Ca$^{2+}$-stimulated ATPase, and by analysing the polypeptide pattern of the isolated membrane fragments. The possible topographical localization of these enzymes in the double bilayer was explored by using non-ionic detergents, and this is discussed below.

**Materials and Methods**

**Materials**

Except as otherwise mentioned, all reagents were from Sigma Chemical Co. (St Louis, MO). LcH and PNA were isolated according to previously described methodologies (20, 24) from lentil grains and peanut seeds purchased in the local market, and immobilized on aminoethyl-P2 beads (21). Affi-Gel 731 was from Bio-Rad (Richmond, CA).

**Parasites**

Adult worms from a Venezuelan strain (JL patient) of *S. mansoni* were obtained by perfusion of mouse infected 7 weeks previously with 400 cercariae.

**Membrane Fraction Isolation**

Surface membrane isolation with Affi-Gel 731 was performed according to a previously described methodology (4) in acetate-buffered saline (ABS, 150 mM NaCl, 20 mM acetate buffer, pH 5) with some modifications; membrane-containing Affi-Gel 731 beads were separated from the worms by a brief vortexing and sieving through a 50-mesh grid, washed three times with ABS and resuspended finally v/v in 150 mM NaCl. When LcH-coated P2 beads (P2-LcH) or PNA-coated beads (P2-PNA) were used, the worms were first fixed as above by washing in ABS but the incubation with the beads was achieved in 150 mM NaCl, 20 mM Tris/maleate buffer pH 7 (TMBS). Bovine serum albumin (BSA)-coated-P2 beads (P2-BSA) were used as control.

**Protein and Enzyme Assays**

Protein determinations were performed as previously described (4). An alkaline Ca$^{2+}$-stimulated adenosine triphosphatase (Ca$^{2+}$-ATPase), alkaline phosphatase (AP, EC 3.1.3.1) and type I phosphodiesterase (PD, EC 3.1.4.1) activities were determined using, respectively, ATP, p-nitrophenyl phosphate, and thymidine-5'-monophospho-p-nitrophenyl ester as substrates, according to previously described procedures (3, 4).

**SDS Polyacrylamide-Gel Electrophoresis (SDS PAGE)**

Membrane preparations isolated either with Affi-Gel or P2-LcH from 3000 worms were solubilized in 2.5% SDS for 10 min at 100°C and subjected to electrophoresis on 10% polyacrylamide gels which were then stained with Coomassie Brilliant Blue (12).
Non-ionic Detergent Solubilization

P2-LcH beads bearing membrane fragments were incubated for 30 min with either 0.1% saponin, 1% Triton X-100 (w/v) or 150 mM NaCl (as control) and then centrifuged at 900 g for 1 min. The enzymatic activities were determined in the supernatant and then were compared with the total activity in the fraction.

**Affinity Chromatography on P2-LcH**

The membrane fraction obtained on P2-LcH beads was solubilized with 1% Triton X-100, D-glucose and D-mannose 0.1 M each in TMBS. After dialysis against TMBS, the preparation was applied to a P2-LcH column; bound protein was eluted with D-glucose and D-mannose as above. Phosphohydrolase activities were monitored in the fractions.

Results

**Surface Membrane Isolation**

Ca\(^{2+}\)-ATPase, AP and PD were detected in the P2-LcH isolated fraction, with specific activities significantly higher than in worm homogenates (Table 1). The use of P2-BSA or P2-LcH in the presence of competing sugars showed that there was a specific adsorption of membrane material onto the beads (Table 1). None of the above enzymatic activities could be detected when using P2-PNA, even if the worms were incubated previously with 0.01 U/ml neuraminidase (Table 1); this enzyme concentration was the same used for removing sialic acid from erythrocytes to test the hemagglutinating activity of this lectin (21). The specific enzyme activities and the total amount of protein obtained in the P2-LcH fraction (70–130 μg/ml suspension v/v) were lower than those in the Affi-Gel fraction (170–180 μg/ml suspension v/v) (Table 1).

The polypeptide patterns of the membrane fractions obtained with P2-LcH or Affi-Gel are shown in Figure 1. Nearly 36 neat bands were observed in the total worm homogenate, 31 in the Affi-Gel, and 21 in the P2-LcH fraction (Figure 1). A polypeptide band of Mr 19 000 appeared to be present exclusively in the P2-LcH fraction; this component seemed to correspond with the LcH half unit (8) as the purified lectin migrated at the same position (data not shown).

### Table 1

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Specific Activity: Mean ± SEM (^a)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Ca(^{2+})-ATPase</td>
</tr>
<tr>
<td>Affi-Gel</td>
<td>2346 ± 238</td>
</tr>
<tr>
<td>P2-LcH</td>
<td>942 ± 27</td>
</tr>
<tr>
<td>Homogenate</td>
<td>56 ± 9</td>
</tr>
<tr>
<td>P2-BSA</td>
<td>0</td>
</tr>
<tr>
<td>P2-LcH*</td>
<td>0</td>
</tr>
<tr>
<td>P2-PNA</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\) Specific activity expressed as nmol product/min/mg protein, mean ± SEM. \(^b\) Number of experiments. \(^*\) P2-LcH incubated with the worms in the presence of 0.1 M mannose and 0.1 M glucose.
To test if the enzyme markers were intrinsically associated to either one or both schistosome surface lipid bilayers, the membrane fragments which are primarily bound by their outermost surface to the P2-LcH beads were subjected to solubilization with either Triton X-100 or saponin. No activity was solubilized with the former detergent, and only a small solubilization percentage was achieved with saponin (Figure 2).

On the other hand, enzyme solubilization and dissociation occurred when the P2-LcH membrane-bound fraction was treated with Triton X-100 in the presence of the competing sugars. The enzymes released after this treatment were totally readsorbed on a P2-LcH column and subsequently eluted again with the inhibitory sugars for confirmation (data not shown).

**Discussion**

The use of P2-LcH proved to be successful for a specific interaction with external glycosylated membrane components of the *S. mansoni* tegument, as inferred from the increment in specific activities of traditional tegumental phosphohydrolase enzyme markers (Table 1). These enzymes, however, do not seem to be restricted to the double surface membrane: Ca$^{2+}$-ATPase has been histochemically shown to be located in the cytoplasmic leaflet of both the surface and the basal tegumental membranes (6). AP, although certainly restricted to the tegument, as confirmed by the immunoreactivity of a monoclonal antibody against this enzyme (22), seems to be present in the double surface membrane and also in small intrategumental cytoplasmic inclusions called discoid bodies (5, 16, 23). Thus,
it is possible that in the case of the Affi-Gel fractionation procedure other non-surface, enzyme-containing tegumental membrane components could have interacted non-specifically with the polycationic charge of the beads and contributed to the higher increment in specific phosphohydrolase activities seen in the respective fraction as compared with the P2-LcH one. On the other hand, it cannot be ruled out that the lectins used could inhibit these phosphohydrolases activities; however, soluble lectins did not produce any detectable inhibition on them (data not shown).

The specific activities obtained with Affi-Gel (Table 1) were higher than those reported previously using the same solid matrix (4). There is some evidence of variation in the specific activities of phosphohydrolases between different S. mansoni strains (14). Small modifications introduced to the original technique (4), like sieving through a grid, may have also contributed to these differences and may also explain the somewhat different polypeptide pattern of our Affi-Gel fraction with respect to that obtained by Payares and Evans (19).

Unlike Affi-Gel (4), no lectin-coated beads could be observed over the surface of the worms when using the microscope. The glycosylated surface groups are quite probably less uniformly distributed than the overall negative surface charge and it might be more difficult for a lectin-coated bead to stick persistently to the surface membrane. Therefore it could not be assessed whether P2-LcH was directly grasping fragments from the surface membrane through selective plucking of glycoconjugate-rich domains or indirectly interacting with membrane microvesicules shed from the surface by the parasite. However, the apparent non-interaction of immobilized peanut lectin with the surface, because of the low density or the non-uniform distribution of the corresponding receptors (13), strengthens the hypothesis that a selective interaction of P2-LcH with the surface of the parasite has occurred.

The topographical localization of the studied enzymes within the double bilayer was explored taking advantage on the fact that the lectin on the bead must interact with
glycosylated receptors on the outermost leaflet of the outer schistosome surface bilayer and that the corresponding membrane fragment remains immobilized on the surface of the bead. Thus, non-ionic detergents were used to attempt differential solubilization of components from the P2-LcH fraction. In contrast with tegumental membrane preparations in suspension, where almost 90% of the three enzyme activities tested could be solubilized with 1% Triton X-100 (3), very little enzyme solubilization was obtained from P2-LcH membranes with this detergent (Figure 2), suggesting that these enzymes are directly immobilized by their carbohydrate moieties exposed on the surface. On the other hand, 0.1% saponin, which has been claimed to remove sequentially the two bilayers (15), caused a small percentage solubilization (Figure 2); however, we cannot rule out that the latter effect could be due to a competing action of the glycosidic moieties of these detergent molecules.

The affinity chromatography of the material released from P2-LcH showed that the three markers enzymes were indeed glycosylated, as was previously shown for AP (18). These enzymes are then probably exposing their glycosylated, antigenic regions (7) on the surface of the parasite (1, 11). Ultrahistochemical evidence (5, 23) and the difficulty to surface radioiodinate the AP (18) suggest that the active site of this enzyme is probably located inner in the double bilayer. The same seems to be true for Ca^{2+}-ATPase (6). Consequently, it is conceivable that these proteins might be crossing and spanning in some degree the double membrane complex and may explain the high degree of immobilization observed for some receptors upon lectin interaction (11). On the other hand, this does not exclude the presence of more fluid domains (2, 26, 27).

The possible position of these enzymes within the surface membrane allows us to propose a model for the double surface membrane of S. mansoni. The surface bilayers are not simply two juxtaposed, digitonin-separable bilayers (15), they may be strongly interconnected by some spanning intrinsic enzyme protein whose detailed intramembranous array we still do not know.

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References


