Glycoconjugate Journal (1998) **15:** 147–153

Expression, purification, and biochemical characterization of a recombinant lectin of *Sarcocystis muris* (Apicomplexa) cyst merozoites

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The mature major microneme protein of *Sarcocystis muris* cyst merozoites, which is known as a dimeric lectin with high affinity to galactose and some of its derivatives, was expressed in *Escherichia coli* as a histidine-tagged fusion protein. The recombinant polypeptide, which was recognized by a monoclonal antibody directed against the native lectin, was purified from inclusion bodies after solubilization and refolding, using a combination of metal chelate and lactose affinity chromatography. The apparent molecular mass of the refolded polypeptide as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoreses was 16 kDa, whereas gel filtration chromatography clearly demonstrated that the recombinant protein, like its native counterpart, exists as a homodimer of two non-covalently associated subunits. Inhibition of haemagglutination suggests that the combining site of the recombinant lectin recognizes *N*-acetyl-galactosamine as the dominant sugar, thus confirming the correct folding of the monosaccharide combining site in the renatured lectin. To the best of our knowledge, this work represents the first reported detailed characterization of a recombinant lectin from apicomplexan parasites, and may contribute to a better understanding of the process of host cell recognition and invasion by these obligate intracellular protozoa.

***Keywords*: *Sarcocystis muris*, micronemes, recombinant protein, refolding, lectin**

Introduction

Invasive stages of apicomplexan parasites (*eg Plasmodium*, *Toxoplasma*, *Sarcocystis*) are characterized by rhoptries, micronemes and dense granules, specialized secretory organelles at the anterior end of the cell [1, 2]. Several proteins which have been proposed to interact with receptors on the surface of host cells are initially located within the micronemes, *eg* the thrombospondin related anonymous protein (TRAP/SSP-2) [3, 4] and the erythrocyte binding proteins of malaria parasites [5–7]. It could therefore be hypothesized that the microneme antigens contribute to the specific recognition of the corresponding host cell. Consistant with this theory is the observation that the microneme contents are exocytosed during an early phase of invasion [8, 9].

The cyst-forming coccidian parasite *Sarcocystis muris* propagates in mice as intermediate and in cats as final hosts. Entzeroth *et al.* [9] utilized a monoclonal antibody (mAb 3A8; [10]) directed against the major microneme antigen of *S. muris* cyst merozoites to localize this protein before and during invasion of embryonic feline cells. Cyst merozoites occur in the muscle tissue of the intermediate host (mouse). They become motile and penetrate the intestinal cells of cats after being ingested with raw meat [11]. Results of confocal laser scanning microscopy of invading cyst merozoites revealed the secretion of this polypeptide at the apical tip of the parasite. Furthermore, an increased amount of microneme antigen could be localized at the moving junction, an electron-dense contact zone, which forms between the apical cone of the merozoite and the host cell membrane after reorientation of the invading parasite.

Using the mAb 3A8 (see above), we have isolated a full-length cDNA clone encoding the major microneme antigen of *S. muris* cyst merozoites from an expression library [12]. The hypothetical polypeptide precursor consists of a N-terminal secretory signal sequence followed by a hydrophilic propeptide of unknown function and the mature protein with a theoretical molecular mass of 15.1 kDa.

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Additional evidence of the *S. muris* microneme antigen function came from further studies by our group (unpublished results) showing that the protein isolated from cyst merozoites is a lectin with high affinity to *N*-acetyl-galactosamine and galactose. As determined by gel filtration chromatography, the *S. muris* lectin (SML) exists as a homodimer of two non-covalently associated subunits, each with an apparent molecular mass of 15 kDa. Due to the lack of an *in vitro* culture system for *S. muris* cyst merozoites, it is difficult to obtain sufficient quantities of the native protein from infected animals. As an initial step towards obtaining more information on the structure and function of this polypeptide, we have used an *Escherichia coli* expression system for the production of a recombinant form of the *S. muris* lectin [rSML(His)$_6$] suitable for further analysis. We report here the expression of the mature microneme antigen in *E. coli*. In addition, we describe an efficient method for *in vitro* renaturation and purification of the *E. coli* material, and a biochemical and immunological characterization of the recombinant protein.

Materials and methods

Construction of the *E. coli* expression vector

The cDNA clone pSM/1.6, which was known to carry the complete open reading frame encoding the major microneme antigen of *S. muris* cyst merozoites [12] was used as a template for polymerase chain reaction (PCR) amplification of the region in the open reading frame encoding the mature microneme antigen (Ala_{104}–Ala_{241}) of *S. muris* cyst merozoites. PCR amplification was performed using 1 ng template DNA, 0.5 μM of the appropriate primers (5′-CGGGATCCGCAGACGGACAACTCGAC-3′ [5′-primer] and 5′-TCCAAGCTTTTAGGCACGTTCTACAGA-3′ [3′-primer]) and 2 U Vent DNA polymerase (New England Biolabs, USA) in a final reaction volume of 100 μl, according to the manufacturer's protocol. The reaction mix was subjected to amplification for 35 cycles (95 °C, 1 min; 50 °C, 1 min; 75 °C, 1 min) using a Techne PHC-3 thermal cycler (Techne, UK). After purification, the PCR products were cleaved with *Bam*HI and *Hin*dIII (Gibco/BRL, USA) and ligated between the corresponding restriction sites of the vector p*Bluescript* for DNA sequence analysis. *E. coli*-XL-1 cells were transformed with the ligation mixture and recombinant plasmids were isolated after selection in the presence of ampicillin. Automated sequencing [13] of both strands of double-stranded plasmid DNA was performed on an A.L.F. DNA sequencer (Pharmacia, LKB, Sweden). Subcloning of the appropriate DNA insert between the unique *Bam*HI and *Hin*dIII restriction sites of the expression vector pQE30 (Qiagen, Germany) gave rise to the recombinant plasmid pQE30-*r*SML. The *E. coli* strain M15[pREP4] was transformed with the corresponding expression construct and the resultant transformants were used for fusion protein expression.

Overexpression and nickel-nitrilotriacetic acid (Ni-NTA) affinity purification of the recombinant protein

E. coli cells transformed with pQE30-rSML were grown overnight with shaking at 37 °C in 2 × YT medium (20 ml) containing ampicillin (100 μg ml^{-1}). The inoculant culture was added to growth medium (1 litre 2 × YT supplemented with 100 μg ml^{-1} ampicillin), incubated at 37 °C until optical density $(OD)_{590}$ had reached a value of 0.8, and then induced for 5 h with 2 mM isopropyl thiogalactoside (IPTG). Bacteria were harvested by centrifugation (4000 × **g**, 20 min), and lysed in 8 M urea, 10 mM Tris/HCl, pH 8.0, 100 mM Na-phosphate at 5 ml g^{-1} wet weight for 1 h at room temperature. After centrifugation at 10 000 × **g** for 30 min, the supernatant was collected and loaded, at a flow rate of 10–15 ml h^{-1}, onto a 4 ml Ni-NTA column (Qiagen, Germany) pre-equilibrated in lysis buffer. The resin was washed with 10 column volumes of lysis buffer until the OD_{280} of the flow-through was below 0.01. After washing with the same buffer at pH 6.0 (OD_{280} of the flow-through below 0.01) bound proteins were eluted with buffer containing 250 mM imidazole [14].

Refolding of rSML(His)$_6$ from *E. coli*

Ni-NTA affinity purified rSML(His)$_6$ was diluted in nine volumes (v/v) of folding buffer [10 mM Tris/HCl, 0.01% (v/v) Tween-80, 0.25 M NaCl, 1 mM reduced glutathione (GSH), 0.1 mM oxidized glutathione (GSSG), pH 8.5] to a final protein concentration of 50 μg ml^{-1} by gently mixing at 30 °C. The total volume in the refolding assay was 20 ml. The solution was held at the same temperature for an additional two hours to allow refolding of the denaturated rSML(His)$_6$. After the refolding process, the pH was adjusted to 7.4. To remove residual denaturant, the suspension was dialysed extensively at 4 °C overnight against 200–300 volumes of the folding buffer. Subsequently, the protein solution was concentrated by vacuum ultrafiltration using collodion bags (Sartorius, Germany).

Immunoblot analysis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed under reducing and non-reducing conditions according to Laemmli [15] using 15% gels (mini gel system; Biorad, Germany). For immunoblot analysis, proteins were transferred onto nitrocellulose membranes (Sartorius, Germany) using a semi-dry blotting apparatus (Biometra, Germany). Transfer was allowed to proceed for 30 min at 2.5 A cm^{-2}. Immunodetection followed standard procedures [16]. Culture supernatant of hybridomas producing the microneme specific monoclonal antibody 3A8 [10] was diluted 1 : 10. Alkaline phosphatase-conjugated goat anti-mouse IgG (BioRad, Germany) was used as secondary antibody (dilution 1 : 3000). Prestained SDS-PAGE standards (BioRad, Germany) were myosin

(203 kDa), β-galactosidase (118 kDa), bovine serum albumin (86 kDa), ovalbumin (51.6 kDa), carbonic anhydrase (34.1 kDa), soybean trypsin inhibitor (29 kDa), lysozyme (19.2 kDa), and aprotinin (7.5 kDa).

Lactose affinity chromatography

Sugar affinity chromatography was performed using the SmartSystem (Pharmacia, LKB, Sweden) under conditions previously optimized for native SML. 2.5 ml of preswollen alpha-lactose gel (EY Laboratories, Inc., USA) were packaged in a HR 10/2 column (Pharmacia, LKB, Sweden) and equilibrated with 50 mM Tris/HCl, pH 8.4, 150 mM NaCl. 1 ml of the refolded protein solution (1 mg ml^{-1}) was loaded onto the column (flow rate 300 µl min^{-1}), in order to allow specific interaction of the recombinant lectin with the gel. The flow-through was collected in 600 µl fractions and the protein concentration was monitored continuously by measuring the absorbance at 280 nm. After unbound material had passed through, the column was washed with three column volumes of equilibration buffer. Sugar binding material was eluted using the same buffer, containing a linear galactose gradient (0 M to 0.3 M, 7 min, 2.1 ml). Again, the eluate was collected (600 µl fractions) and its protein content determined as described above.

Reducing and non-reducing SDS-PAGE analysis of the peak fractions was carried out using the PhastSystem (8–25% gradient gel; Pharmacia, LKB, Sweden) according to the manufacturer's recommendations. Protein bands were visualized by silver-staining. Molecular size standards (Pharmacia, LKB, Sweden) were phosphorylase B (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa). In preparation for haemagglutination assays and gel filtration, the eluting carbohydrate was removed from the rSML$(His)_6$ containing fraction by gel filtration (Sephadex-G25 superfine matrix packaged in a HR 16/10 column; Pharmacia, LKB, Sweden). Finally, the protein solution was passed through membrane filters (pore size 0.2 µm; Sartorius, Germany) to eliminate contaminating bacteria and stored at $-20\,^{\circ}C$.

Gel filtration chromatography

In order to determine the molecular mass of rSML$(His)_6$, an aliquot (50 µl) of sugar affinity purified rSML$(His)_6$ (0.65 mg ml^{-1}) was loaded onto a calibrated Superdex 75 PC 3.2/30 column (Pharmacia, LKB, Sweden) previously equilibrated with 50 mM Tris/HCl, pH 8.4, 150 mM NaCl at 20 °C. The protein was eluted at a flow rate of 30 µl min^{-1} and protein concentration of the eluate was monitored by absorbance at 280 nm.

Haemagglutination (HA) assay and inhibition of HA

Haemagglutination (HA) tests were performed in 96-well microtitre plates with 0.3% human blood group A2 erythrocytes suspension, previously washed in phosphate-buffered saline (PBS). Two-fold dilutions of rSML$(His)_6$ (0.65 mg ml^{-1}) were placed in microwells (100 µl per well) and HA was initiated by adding 100 µl of the above erythrocyte suspension. Native SML served as a positive control. HA titres (expressed in units) were defined as the reciprocal of the highest protein dilution giving visible haemagglutination after 24 h incubation at room temperature. HA inhibition was carried out by incubating equal volumes of rSML$(His)_6$ (HA titre 8 U ml^{-1} rSML$(His)_6$ solution) with two-fold dilutions of different sugar solutions followed by the standard HA assay.

Results

Expression of recombinant *S. muris* lectin in *E. coli* and *in vitro* renaturation

Recombinant *S. muris* lectin was expressed as a fusion protein in the cytoplasm of *E. coli*. The expressed protein consisted of an N-terminal $(His)_6$ sequence that permits purification of the recombinant protein from crude cell lysates by nickel-affinity chromatography. Four additional amino acids (Met, Arg, Gly, Ser) were located in front of the poly-histidine sequence. The affinity tag was followed by residues 104–241 of the deduced amino acid sequence of the cDNA clone pSM/1.6, known to represe nt the mature portion of the microneme antigen. Because we utilized the *Bam*HI restriction site of the expression vector pQE30 for ligation of the appropriate DNA insert at the 5′-end, the recombinant construct contained only two amino acids (Gly, Ser) between the affinity tag and the N-terminus of the reading frame described above. SDS-PAGE analysis of cell lysates obtained after IPTG induction revealed an additional major protein band migrating at 16 kDa (Figure 1, lane 2) that was not evident in a crude extract preparation from uninduced bacteria (Figure 1, lane 1). The overexpressed polypeptide could be detected with the mAb 3A8, which is specific for the major microneme antigen of *S. muris* cyst merozoites (Figure 2, lane 2), thus confirming antigenicity of the *E. coli* derived material. The fusion protein was produced completely as insoluble aggregates that could be solubilized in denaturating buffer and partially purified by Ni-NTA affinity chromatography (initial purification; Figure 1). As determined by SDS-PAGE analysis, the 16 kDa polypeptide was localized exclusively in the eluate (Figure 1, lane 4), whereas virtually none could be detected in the flow-through (Figure 1, lane 3), indicating the efficiency of the purification procedure. The total yield of imidazole-eluted protein was approximately 60 mg per litre bacterial cell suspension. Two minor proteins with apparent molecular masses of 32 kDa and 8 kDa were also eluted from the Ni-NTA column (Figure 1, lane 4), both reacting with the mAb 3A8 (Figure 2, lane 4).

Because of the high cysteine content (12 cysteine residues) of the deduced amino acid sequence of the mature

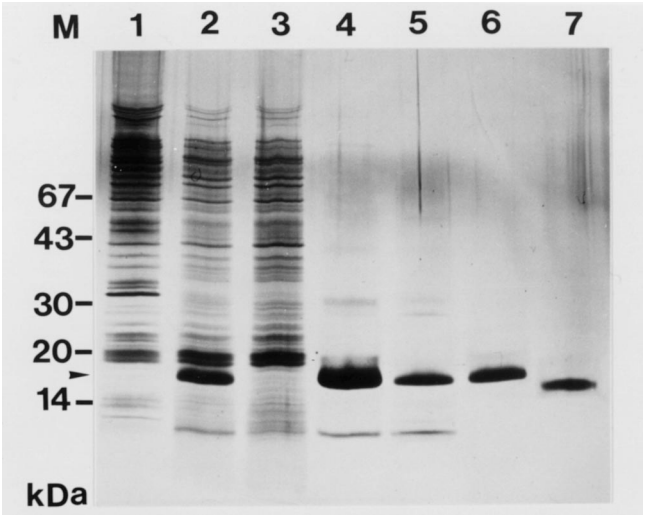

Figure 1. Expression of rSML(His)$_6$ and successive purification by Ni-NTA and sugar affinity chromatography (silver-stained SDS-PAGE gel, 8–25%, reducing conditions). Lane 1: crude extract from non-induced bacteria harbouring the plasmid pQE-30-rSML. Lane 2: proteins obtained from the same bacteria after induction with IPTG. Lane 3: flow-through of the Ni-NTA column. Lane 4: eluate of the Ni-NTA column. After refolding, the protein solution was applied to a lactose affinity column. Lane 5: flow-through of the lactose column. Lane 6: recombinant lectin after elution with a galactose gradient. Lane 7: native SML purified from *S. muris* cyst merozoites. The position of the recombinant lectin is indicated by an arrow. Molecular size standards are given on the left side (M).

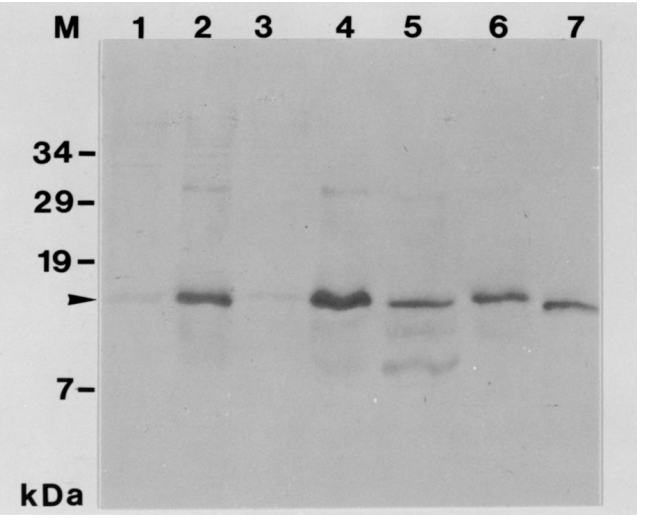

Figure 2. Western blot analysis of rSML(His)$_6$, probed with mAb 3A8 directed against the major microneme antigen of *S. muris* cyst merozoites (15% SDS-PAGE gel, reducing conditions). Lanes 1–7 contain the same material described in Figure 1. The position of the recombinant lectin is indicated by an arrow. Molecular size standards are given on the left side (M).

microneme antigen, renaturation was expected to be limited by the rate of formation of the correct disulfide bonds. Thus folding of the Ni-NTA affinity purified recombinant protein was performed at pH 8.5 in the presence of a redox exchange buffer containing a mixture of reduced and oxidized glutathione, with conditions under which disulfide exchange is expected to be rapid.

Lactose affinity chromatography (final purification)

Because refolding did not result in a 100% yield, it was necessary to separate active rSML(His)$_6$ molecules from incorrectly folded polypeptides. Since the microneme antigen of *S. muris* cyst merozoites appears as a galactose lectin and can be purified by lactose affinity chromatography, the same method was employed to further purify biologically active rSML(His)$_6$. The result of a typical separation is depicted in Figure 3. Peak 1 (P1) represents the flow-through, whereas P2 contains material which has been eluted at a galactose concentration of approximately 0.2 M. A silver-stained SDS-PAGE analysis of aliquots from the two peak fractions is shown in Figure 1 (lanes 5 and 6, respectively). In addition to wrongly folded rSML(His)$_6$, the flow-through (Figure 1, lane 5) contains primarily the two contaminating polypeptides mentioned above. Correctly folded recombinant lectin was localized in the second peak fraction. Sugar affinity purified rSML(His)$_6$ eluted as a single band with an apparent molecular mass of 16 kDa under reducing conditions (Figure 1, lane 6), whereas the native lectin migrates at 15 kDa (Figure 1, lane 7). When the reducing agent was omitted from the SDS-PAGE sample buffer, the recombinant protein migrated slightly more slowly than the reduced one (approximately 17 kDa; data not shown). The similarity of highly purified native and recombinant SML was further established by their reaction with the mAb 3A8 (Figure 2, lanes 6 and 7). The total yield of highly purified and active rSML(His)$_6$ was approximately 400 μg per 1 mg refolded protein solution applied onto the sugar column.

Gel filtration chromatography

Sugar affinity purified rSML(His)$_6$ was run on a Superdex 75 size exclusion column that had been calibrated with gel filtration molecular size standards. The protein eluted as a single species with an apparent molecular mass of approximately 32 kDa (Figure 4) indicating that the recombinant lectin, like its native counterpart, is dimeric.

Haemagglutination and inhibition of HA

Recombinant *S. muris* lectin produced a haemagglutination titre of 32 U ml^{-1} rSML(His)$_6$ solution when tested against human erythrocytes (Figure 5). The specific haemagglutination activity was 50 U mg^{-1} protein. Among the sugars tested, *N*-acetyl-D-galactosamine was found to be the best inhibitor of haemagglutination (inhibitory concentration < 1 mM), whereas D-glucose and α-methyl-D-mannoside did

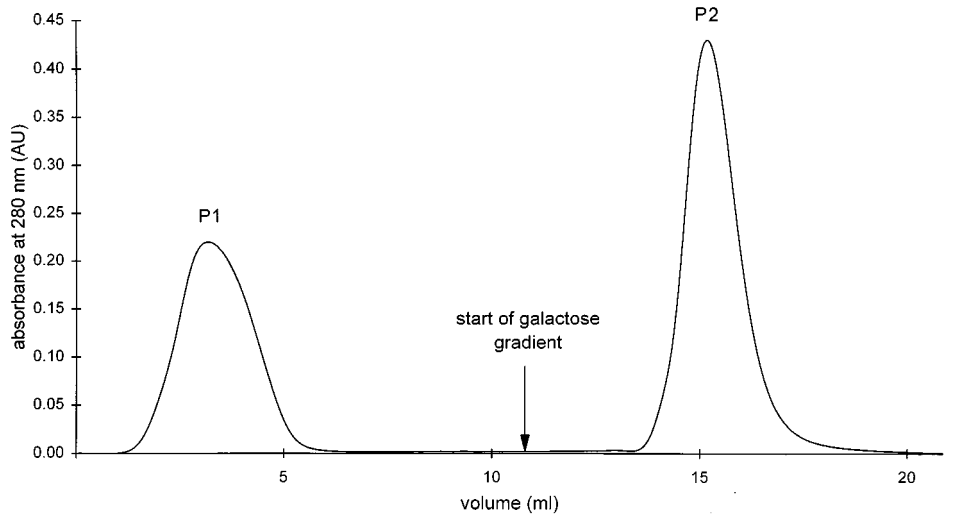

Figure 3. HPLC based lactose affinity chromatography of refolded rSML(His)$_6$. P1 and P2 represent the two peak fractions. P1 contains material which had passed through the column without interacting with the gel (flow-through). P2 was eluted with a linear galactose gradient, ranging from 0 M to 0.3 M. The time point at which the sugar gradient was applied onto the column is indicated by an arrow.

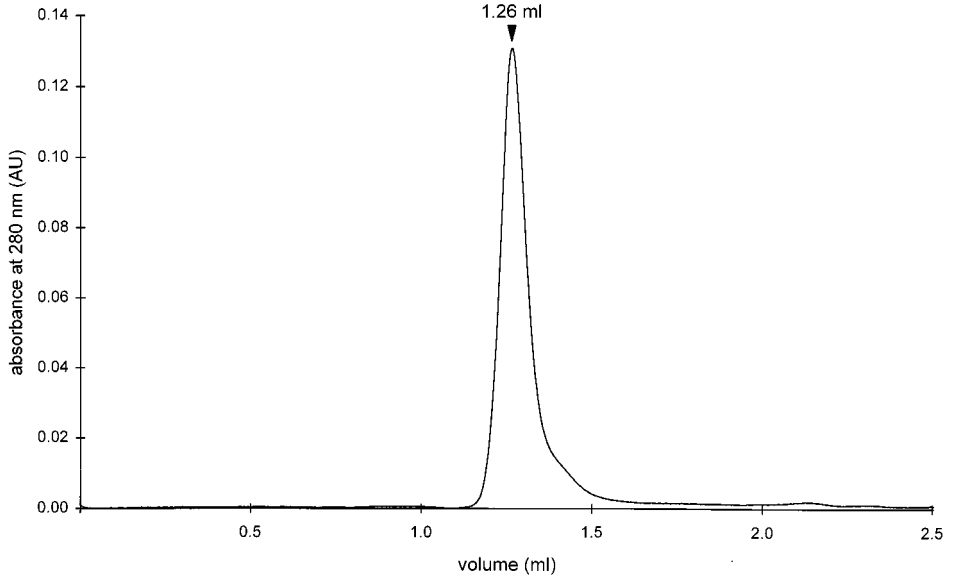

Figure 4. Gel filtration chromatography of sugar affinity purified rSML(His)$_6$. A Superdex 75 column was calibrated using molecular size standards as follows: myoglobin (17.5 kDa), chymotrypsinogen (25 kDa), and ovalbumin (43 kDa). The elution volumes were 1.46 ml, 1.33 ml, and 1.13 ml, respectively. The recombinant lectin was eluted at 1.26 ml.

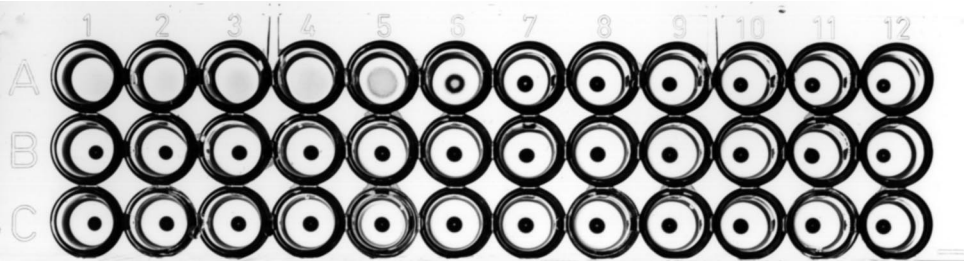

Figure 5. Haemagglutination of human type A2 erythrocytes by sugar affinity purified rSML(His)$_6$. Starting with 65 µg (A1), two-fold dilutions of rSML(His)$_6$ were incubated with human A2 erythrocytes in a final volume of 200 µl per well for 24 h. Haemagglutination, indicated by a uniform erythrocyte distribution, occurred in the first five wells (A1–A5).

not inhibit rSML(His)$_6$ at the highest concentration tested (55 mM); lactose and D-galactose inhibited HA at a concentration of 1.7 mM and 3.4 mM, respectively. These values are in good agreement with those obtained for the native lectin isolated from *S. muris* cyst merozoites. Therefore, it could be concluded that the monosaccharide specificity and affinity of the recombinantly produced lectin did not differ significantly from its native equivalent.

Discussion

The *E. coli* construct used in the work presented here was designed to produce a recombinant *S. muris* microneme antigen closely analogous to the native protein. Comparison between the N-terminal sequence of the corresponding polypeptide purified from cyst merozoites and the deduced amino acid sequence obtained from a full-length cDNA clone revealed that the protein would be synthesized *in vivo* as a precursor [12, 17]. Therefore, only the part of the reading frame encoding the mature microneme antigen (Ala_{104}–Ala_{241}) was amplified by PCR and cloned into the expression vector pQE30. This plasmid enables high-level synthesis of the protein of interest as a fusion with a six histidine residue tag, thus facilitating purification of the polypeptide by affinity chromatography on a Ni-NTA adsorbent [14]. Despite several efforts, we were unable to produce a soluble form of the recombinant lectin in *E. coli*. Instead, the desired polypeptide accumulated in the cytoplasm of the bacteria in the form of inclusion bodies, electron dense particles that consist primarily of the recombinant protein and corresponding non-reducible polymers [18].

The observed insolubility of rSML(His)$_6$ is in good agreement with the result obtained by a theoretical approach to determine the probability of *in vivo* protein insolubility/solubility [19]. Based on a statistical analysis of 81 proteins that do and do not form inclusion bodies in *E. coli* it was concluded that six parameters are correlated with inclusion body formation: charge average, turn-forming residue fraction, cysteine fraction, proline fraction, hydrophilicity, and total number of residues [19]. These findings were used to develop a model to predict the probability of inclusion body formation solely on the basis of the amino acid composition of a protein [19]. Using this model, we were able to predict accurately the insolubility of rSML(His)$_6$ in *E. coli* (probability 0.8).

In order to obtain biologically active lectin from bacteria overexpressing rSML(His)$_6$, we developed a solubilization and refolding protocol which employs three essential steps. First, the insoluble protein aggregates were effectively solubilized in denaturing buffer and partially purified by Ni-NTA-chromatography. Besides a 16 kDa protein, whose identity was established by immunoblot analysis with a monoclonal antibody directed against the major microneme antigen of *S. muris* cyst merozoites, two additional minor polypeptides with apparent molecular masses of 32 kDa and 8 kDa were eluted from the Ni-NTA column. Their reactivity with the mAb 3A8 indicates that the 32 kDa band may represent an artificial dimeric form of rSML(His)$_6$, which is resistent to reduction or denaturation by boiling in the presence of SDS. A similar phenomenon has also been observed for the major surface antigen of *Toxoplasma gondii* tachyzoites [20]. The 8 kDa fragment could be the result of protein degradation or premature chain termination during transcription or translation. In the second step of the renaturation protocol, the solubilized protein was brought into an environment which favours the native, active structure. Because of the high cysteine content of the *S. muris* lectin (12 residues; [12]), folding was performed at pH 8.5 in the presence of an oxido-shuffling system containing a mixture of GSH and GSSG, which allows the formation of disulfide bonds [21]. Although we have not yet investigated the potential involvement of these cysteines in the formation of intramolecular disulfide bridges, it seems unlikely that, especially in secretory proteins, all thiols remain in their reduced state [22]. In the last step of our refolding procedure, we took advantage of the sugar binding properties of native SML (unpublished results) to separate active recombinant molecules from incorrectly folded polypeptides and to further purify rSML(His)$_6$ from contaminating proteins. Since lectins are able to specifically bind carbohydrates and glycoconjugates but, in contrast to enzymes, do not convert them chemically, the method of choice for lectin purification is affinity chromatography with immobilized carbohydrates [23]. Biologically active rSML(His)$_6$ was eluted from the lactose carrier with a hapten monosaccharide (galactose) which resulted in a single peak (P2) containing the lectin. Desorbtion was accomplished at a galactose concentration of about 0.2 M, a value which has also been determined for SML (unpublished results). The flow-through of the sugar affinity column consisted primarily of wrongly folded rSML(His)$_6$ and the two minor polypeptides mentioned above, indicating that these proteins did not bind sugar under the conditions applied to the column. Silver-stained SDS-PAGE analysis and HPLC-based gel filtration chromatography revealed that the recombinant lectin was purified to near homogeneity, as contaminating proteins could not be detected by either method. The final yield of correctly folded and biologically active rSML(His)$_6$ was approximately 24 mg per litre of bacterial cell suspension.

Lectin activity of the P2 fraction was further confirmed by HA of human erythrocytes. Studies on native SML suggested that the protein exists primarily as a dimer in physiological solution, and dimerization does not depend on disulfide bond formation. SDS-PAGE analysis of rSML(His)$_6$ showed that the band obtained from the reduced lectin had a higher mobility (16 kDa) than that when the reducing agent was omitted (17 kDa). A similar observation has also been made for the lectin isolated from *S. muris*

cyst merozoites (unpublished results). The different behaviour of both proteins during gel electrophoresis with regard to their redox state further indicates the presence of intramolecular disulfide bridges. These covalent intrachain bonds can hold proteins in compact configurations that are more or less retained in the presence of SDS [24]. The slight difference in the electrophoretic mobilities of SML, which migrates at 15 kDa, and rSML(His)$_6$ is very likely due to the additional amino acids attached to the N-terminus of the recombinant lectin. However, size exclusion chromatography clearly demonstrated that under non-denaturating conditions the recombinantly produced protein behaves like a dimer, thus confirming the results obtained with the native lectin.

Dimerization and oligomerization is one of the strategies most commonly employed by lectins to enhance both their affinity and specificity of interactions with more complex carbohydrate ligands [25]. Furthermore, this so called subunit multivalency is often necessary for haemagglutination of erythrocytes (see above). Among the sugar haptens investigated as possible HA inhibitors, *N*-acetyl-D-galactosamine was found to be the monosaccharide with highest affinity towards rSML(His)$_6$. Glucose had no inhibitory effect on HA at the maximum concentration tested, indicating that in the disaccharide lactose the primary counterpart was galactose. We therefore inferred that after refolding, a functional sugar combining site with the same monosaccharide specificity and affinity as that of the native lectin was reconstituted in the recombinant protein. It should be kept in mind that lectins are usually classified according to the monosaccharide which inhibits the interaction between a lectin and a glycoprotein or which allows the specific elution of a bound lectin from an immobilized sugar column. This method is convenient, but it often gives an oversimplified concept of the specificity of lectins [26].

In the last decade it has become well established that lectins act as mediators of cell recognition in a wide range of biological systems [27]. Hence it is conceivable that the microneme antigen of *S. muris* may have a role in mediating adherence of the parasite to a host cell during the invasion process (see introduction). As it is now possible to obtain a sufficient quantity of pure, correctly folded and biologically active recombinant *S. muris* microneme antigen, it should be feasible to gain further insights into the mechanisms by which apicomplexan parasites attach to and invade host cells. In addition, the availability of rSML(His)$_6$ produced in *E. coli* should faciliate X-ray crystallographic studies of a parasite derived lectin.

Acknowledgements

We gratefully acknowledge R. Entzeroth (Technische Universität, Dresden, Germany) for kindly providing the monoclonal antibody 3A8 and Stephen Norley for critically reading the manuscript.

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Received 10 February 1997, accepted 26 March 1997