Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *86* (1): 31-36 jan./mar. 1991

IDENTIFICATION AND CHARACTERIZATION OF SEX-LINKED PROTEINS OF *SCHISTOSOMA MANSONI*

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The proteins of adults worms (male and female) of two isolates (BH and RJ) of Schistosoma mansoni *were extracted using Triton X-114 phase separation. The SDS-polyacrilamide gel electrophoresis profiles of the three phases (detergent, aqueous and insoluble proteins) obtained were compared after Coomassie blue and silver staining, surface radioiodination and Western blotting.*

No major differences were detected between the 2 isolates. Of the 25 or more proteins which partitioned into the detergent phase, only about 8 proteins could be surface radiodinated on live adult worms.

A comparison was also made between the profiles of male and females worms, isolated from bisexually infected mice. Two major female-specific and one male-specific band were detected by silver and/or Coomassie staining. The female bands, 32 KDa and 18 KDa, partitioned into the detergent and aqueous phase, respectively. The male-specific band of 42 KDa remained in the insoluble phase. Antigenic differences between male and females proteins were detected by Western blotting using a sera from infected Nectomys squamipes.

Key words: *Schistosoma mansoni* – BH and RJ isolates – amphiphilic proteins – tegumental proteins – Triton X-114 – sex-linked proteins

The tegumental membrane of adult worms of *Schistosoma mansoni* play a very important biological role (Philip & Rumjanek, 1984) and is a site of nutrient uptake (Barret, 1981; Conford, 1986). The proteins and glycolipids which compose the tegument represent important antigens which elicit both humoral and cell-mediated immune responses (Smithers & Terry, 1976; Butterworth et al., 1982).

In recent years, a large amount of work has been done aiming at the identification of antigens that are expressed on the surface of the parasite's tegumental membrane, with the premise that they are candidate vaccine molecules (Shah & Ramsamy, 1982; Philip & Rumjanek, 1984; Payares et al., 1985a, b). In this context, several techniques have been widely employed to identify surface antigens of several developmental stages of *S. mansoni*. The recent advent of a new technique which relies on the phyical properties of the non-ionic detergent TX-114 provided an appropriate mean of recovering integral membrane proteins of schistosomes. In this paper we compare and characterize the integral membrane proteins of male and female worms of two isolates of *S. mansoni*.

MATERIALS AND METHODS

Parasites – The BH and RJ isolates of *S. mansoni* were harvested respectively from a patient with chronic infection (Belo Horizonte, MG, Brazil) and from a Rodent cricetidae *N. squamipes* naturally infected in an endemic region (Sumidouro, RJ, Brazil). The BH isolate was mantained in laboratory by cyclical passage throught Swiss mice and *Biomphalaria glabrata*. Five *N. squamipes* were inoculated transcutaneously with about 500 cercariae of BH isolate and sacrified after 26 week. Worms were recovered by perfusion of the hepatic portal system with at least 200 ml of citrate saline delivered from a rotatory peristaltic pump

Supported by CNPq.

Received 11 October, 1990.
Accepted 17 December, 1990.

operating at 100 ml min^{-1} (Smithers & Terry, 1965). After washing in PBS, the worms were sex separated and imediately used.

Iodination procedures and phase separation in TX-114 – Protein A from *Staphylococcus aureus* (Pharmacia Fine Chemicals, Uppsala, Sweden) was iodinated with ^{125}I (NaI, Amersham Corporation, Arlington Heights, IL) to give specific activities of 10-12 Ci/g with the use of Iodogen (Pierce Chemical, Co, USA) as oxidizing agent (Fraker & Speck, 1978).

Male and female parasites of both isolates were separately labelled with 300 μCi of ^{125}I as described previously (Fraker & Speck, 1978). After washing (three times) with cold PBS the worms were extracted for 30 min at 0 ^{o}C in 10 mM of Tris pH 7.4 containing 1% precycled Triton X-114 (TX-114, Bordier, 1981). 150 mM NaCl, 1 mM PMSF and 50 mM EDTA. The isoluble material was separated by centrifugation (15,000 g, 20 min, 4 ^{o}C) and, after washing (10 mM Tris-NaCl), was extracted with 2% SDS. The TX-114-soluble supernatant was incubated for 10 min at 37 ^{o}C, to permit phase separation, and centrifuged (10,600 g, 37 ^{o}C) to separate the TX-114 mixed micelle phase from the aqueous phase. The aqueous phase was transferred to another tube, brought to2% TX-114, equilibrated for 10 min on ice and the phases separated as described above.

Polyacrilamide gel electrophoresis – The Laemmli (1970) discontinuous sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) system was used. Before the electrophoresis the proteins were isolated from the detergent using cold acetone (Giovanni De Simone et al., 1987). The proteins resolved by SDS-PAGE were Coomassie blue or silver stained (Niellsen & Brown, 1984). The autoradiography of the dried gels was processed at –70 ^{o}C for 48-72 h using Kodak XAR-5X films and intensifying screens. Molecular weights were estimated by comparison with gels loaded with proteins of known molecular weights (BRL, Bethesda, USA).

Immunoblotting of parasite material – Western blotting was performed as previously described (Towbin et al., 1979). Briefly, the separated proteins were blotted onto nitrocellulose (NC) filters and the remaining protein binding sites blocked with defated milk (3%). The strips were washed three times with PBS-tween 20 (0.05% v/v) and probed with 1:100 dilution *N. squamipes* sera. After incubation with ^{125}I-protein A (60,000 cpm ml^{-1}) bound antibodies were detected by autoradiography.

RESULTS

SDS-profiles of TX-114 phase-separated proteins from NS S. mansoni – The protein pattern obtained following TX-114 phase separation of male adult worms of *S. mansoni* RJ is shown in the Fig. 1. Lane A shows the silver-stained profile of aqueous phase, and lane B and C shows the pattern of material contained in the detergent and insoluble material in TX-114. The protein profiles of the aqueous phase (lane A) and the TX-114

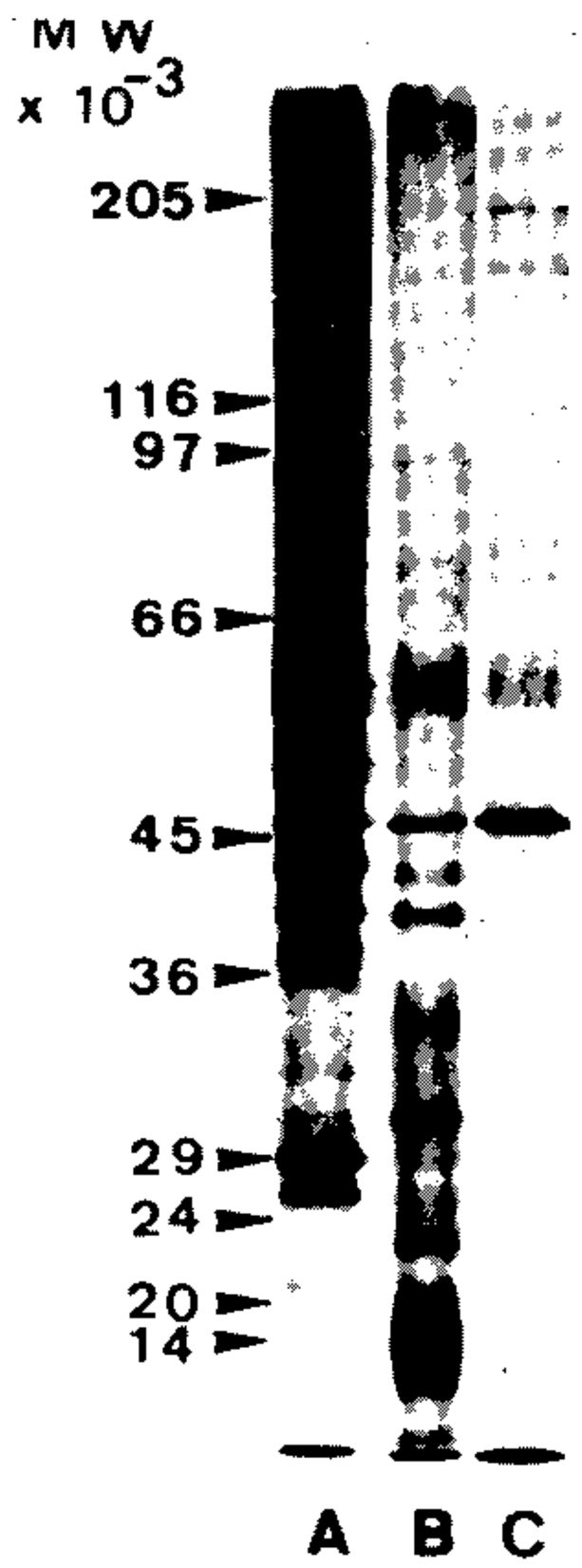

Fig. 1: polypeptide composition of TX-114 phase-partitioned RJ isolate of *Schistosoma mansoni*. Male worms were phase-partitioned as described in Materials and Methods, and samples prepared under reducing conditions were subjected to SDS-PAGE (10%).

Polypeptides were visualized by staining the gel with silver. Lanes: A, Aqueous phase; B, TX-114 phase; C, proteins not solubilized with 1% of Triton X-114 but solubilized with 2% of SDS.

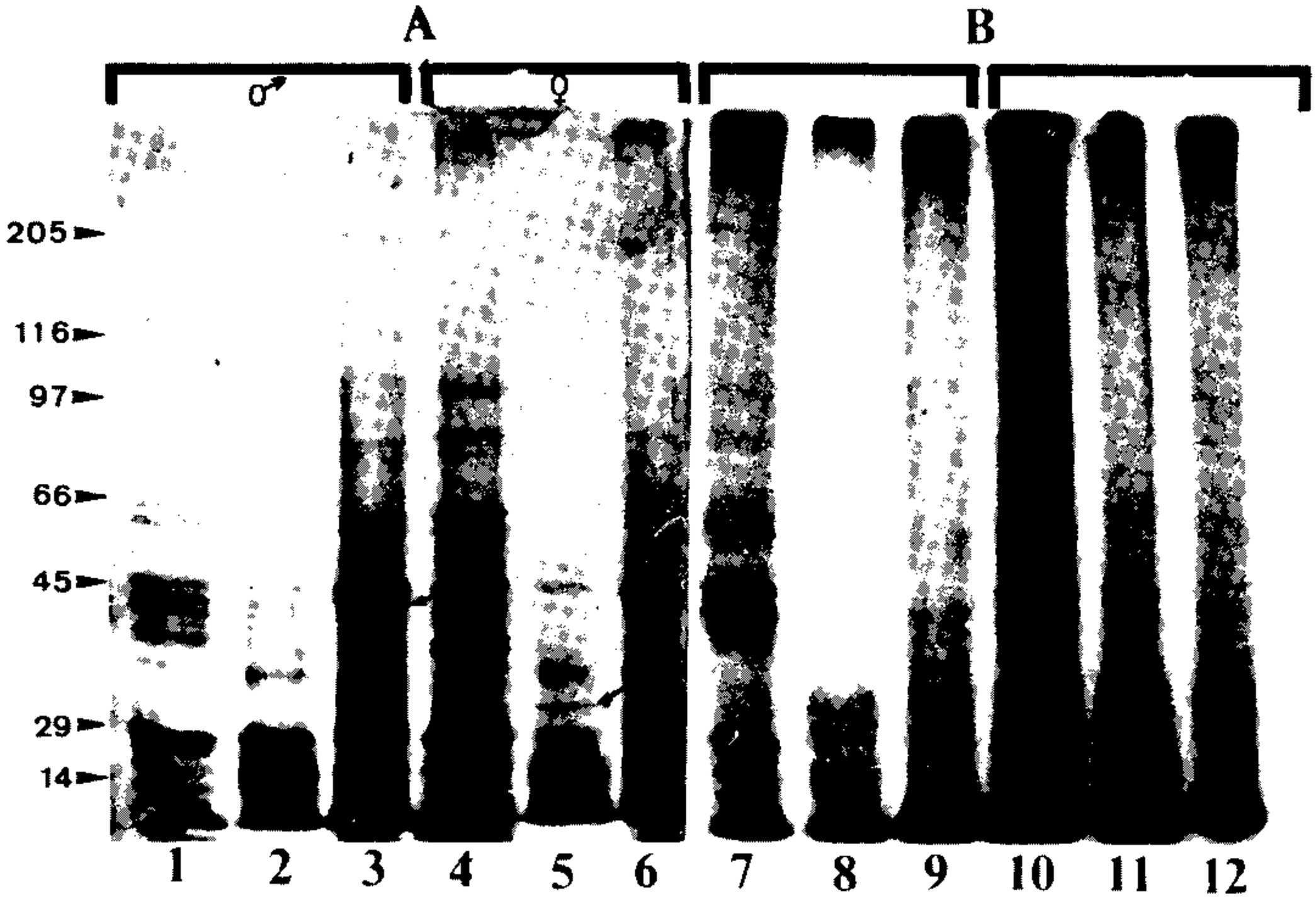

Fig. 2: comparison by SDS-PAGE (8-15%) of detergent (2,5), aqueous (1,4) and insoluble (3,6) Triton X-114 proteins silver stained (A) and surface labelled (B) *in vitro* males (1,2,3,7,8,9) and females (4,5,6,10,11,12) of *Schistosoma mansoni* worms of BH isolate. Arrows in 3 and 5 indicate sex-specific bands.

phase (lane B) show clear differences. The TX-114 phase, which includes most integral membrane proteins, shows a clear enrichment for some proteins which are depleted from the aqueous phase. This TX-114 phase includes at least 25 bands some weakly stained with silver.

SDS-profiles of BH isolates of male and female proteins comparation – The protein pattern obtained with the male worms of BH isolates is shown in the Fig. 2 for comparison to the profile of NS males (Fig. 1). No major differences could be observed between the profiles from either aqueous or detergent proteins from RJ and BH isolates. On the other hand, comparing respectively male and female TX-114 phases, the silver stained profiles show the presence of at least two specific proteins with relatively low molecular weight. One band with 42,000 in the TX-114 insoluble proteins of male preparations (Fig. 2A, lane 3) and one with aproximately 32,000 in the detergent phase of female preparations (Fig. 2A, lane 5). To obtain a best resolution of the low molecular weight bands and determinate their proteic nature, one more cross-linked gel (20%) was prepared and Coomassie blue stained. The Fig. 3A shows for comparasion the profiles of male and female extractions using three times more protein than applied in the gel of the Fig. 2A. The aqueous protein profile shows a stricking increase in the staining intensity of a group of polypeptide bands in female preparations with an approximate Mr of 18,000 to 40,000. In addition, there were differences in the lower-molecular-weight sex specific polypeptide detectable by Coomassie blue with approximately Mr 18,000 (Fig. 3, lane 2). These differences were not due to different protein concentration applied in the gel, since the hightest m.w. proteins of both sexes were similary stained.

On the other hand, contrasting with the silver stained gel (Fig. 2), using Coomassie blue no apparent difference was detected in the detergent and insoluble proteins (Fig. 3) showing that some of the bands detected in the silver stained gel are not well stained by Coomassie blue.

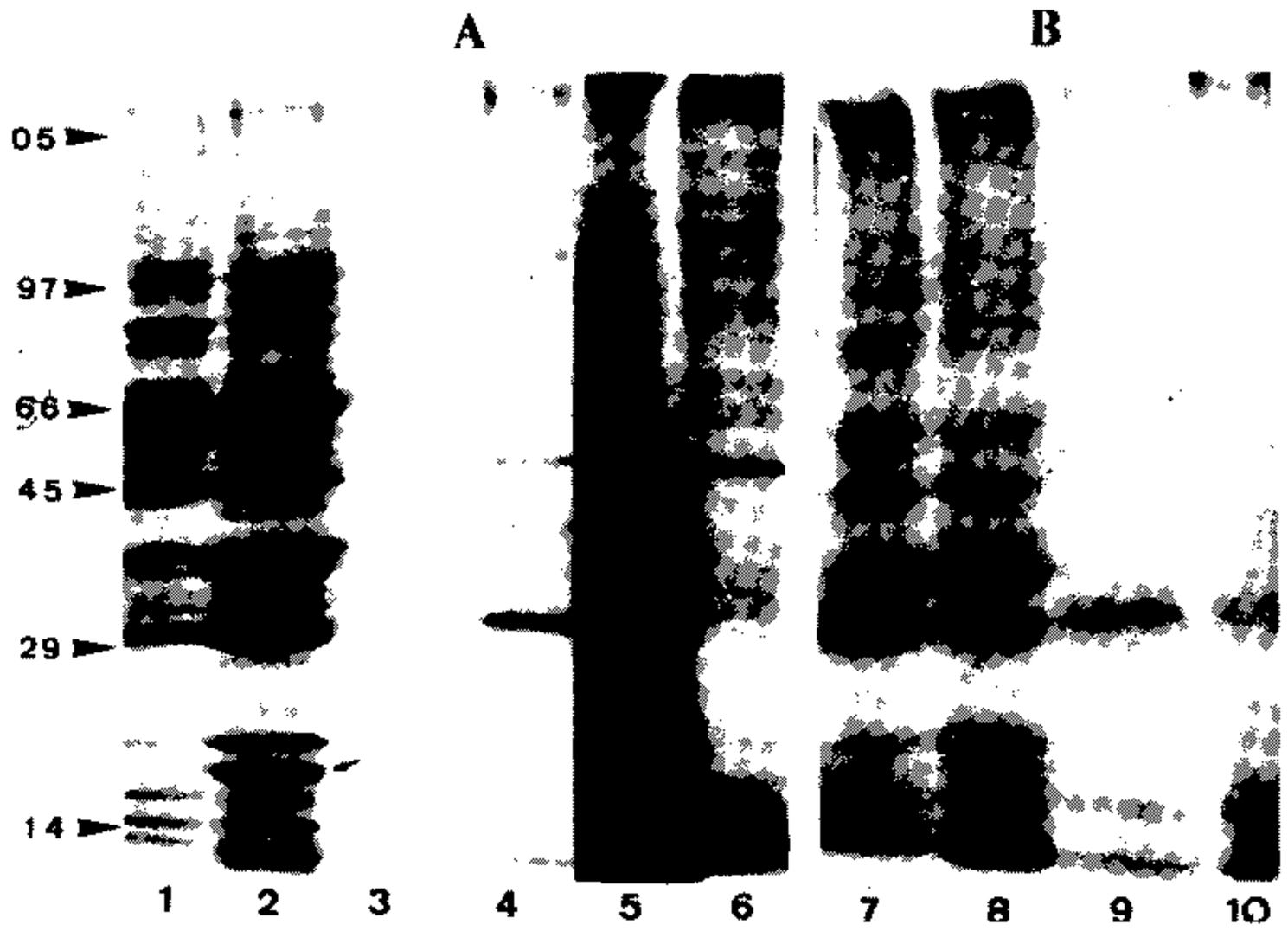

Fig. 3: comparison by SDS-PAGE (20%) of male (1,3,5,7,9) and female (2,4,6,8,10) TX-114 phases Coomassie blue stained (A). Aqueous phases (1,2,7,8); detergent phases (3,4,9,10); insoluble TX-114 proteins solubilized with SDS (5,6). The panel B shows the autoradiography of the same gel. The material loaded in the slots correspond to a material obtained from 20 worms. Arrow in 2 indicate the 18 KDa sex-specific band.

To determine if any of the TX-114 phase proteins seen was externally oriented on the parasite surface, iodogen iodination of males and females surface proteins were performed, followed by TX-114 phase separation. As shown in the Fig. 2B and 3B the direct iodination of intact parasites was less resolutive, however at least 8 different polypeptides were detected in the detergent phase (Fig. 2B lanes 8 and 11; Fig. 3B, lanes 9 and 10). The major bands in this phase are two peptides with Mr 29,000 and 14,000 which are dominant surface-labeled proteins in adult worms of *S. mansoni* (Payares et al., 1985 a, b). Several minor different polypeptide bands were apparent in the TX-114 insoluble fraction (Fig. 2B lanes 9 and 12). The major bands in this fraction correspond to proteins detected in the aqueous or detergent fractions and probably represent incomplete separation of these molecules into the TX-114 phase during the extraction procedure. The several minor polypeptides partitioning in the aqueous phase may represent the watersoluble forms of the integral membrane proteins released during the lengthy sample preparation or may correspond to hydrophilic proteins.

The Coomassie blue stained gel and its autoradiography revealed the presence of at least 25 integral membrane and 8 surface oriented proteins. The iodination pattern of surface proteins of male worms of *S. mansoni* was similar to that shown for female worms, however, intensity differences could be demonstrated. For example, comparing the 36 KDa proteins, it was more weakly detected by Coomassie blue in male preparations (Fig. 3A, lane 3) them that the female preparations (Fig. 3A, lane 4) but this band was more intensively detected in male preparations after autoradiography (Fig. 3B, lane 9). This difference of intensity could be also detected in several aqueous proteins (Fig. 3A, lanes 1, 2; Fig. 3B, lanes 7, 8).

Western immunoblotting – Lysates of male and female adult worms were obtained by SDS extraction and western blotted onto nitrocellulose filters. The filters were then probed with chronically infected *N. squamipes* sera (26 weaks of infection). Two major high molecular weight bands were detected principally in male preparations, contrasting this same antisera showed no reactivity against molecules with 21, 45 and 50 KDa present in female preparations (Fig. 4). In addition, other male and female common bands were also detected by this sera.

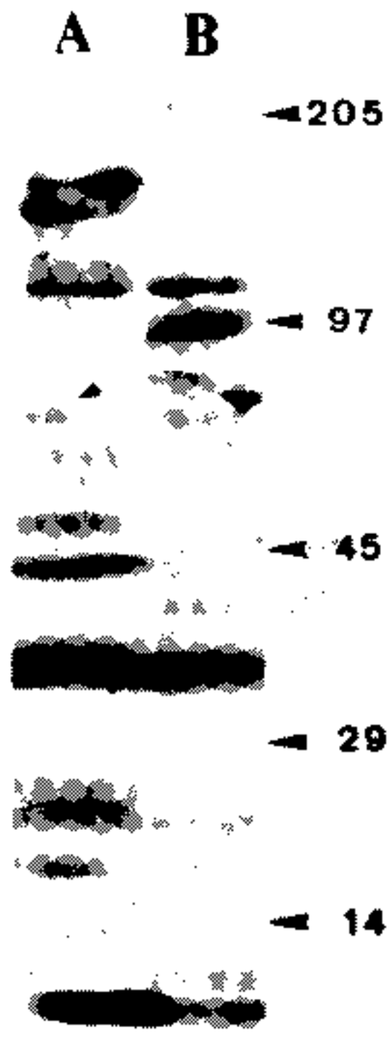

Fig. 4: Western blot analysis of sera from *Nectomys squamipes* exposed to *Schistosoma mansoni* (26 weeks) separated on SDS-PAGE (10-15% polyacrylamide gel) against male (A) and female (B) SDS extracted proteins.

DISCUSSION

In this study, we used the temperature induced phase separation of TX-114 to solubilize and separate proteins from males and females of two isolates of *S. mansoni* into 3 distinct groups: one water-soluble, one soluble in detergent and the other insoluble in TX-114 but soluble in SDS. Using this technique we have identified a minimum of 25 integral membrane polypeptides, aproximately 8 of which are surface oriented as demonstrated by surface radiodination. The TX-114 procedure used to fractionate the labelled surface proteins into three distinct groups, showed that the surface proteins of *S. mansoni* are hardly labelled by direct iodination. However, this difficulty in the identification of surface proteins of adult worms of *S. mansoni* is described in the literature (Roberts et al., 1983; Payares et al., 1985a, b).

Silver staining analysis of SDS-PAGE gels containing the detergent phase proteins isolated from female and male preparations revealed the presence of a major specific component of 32 KDa in female preparations. This specific band was detected in both BH (Fig. 2) and NS isolates (data not shown), showing the reproducibility of the results. The 32 KDa antigen does not appear to be proteinaceous, on the basis of the inability to detect a major Coomassie blue-staining band in this region of an SDS-PAGE gel (Fig. 3A). However, this antigen may not stain well with Coomassie blue. The low affinity of integral membrane proteins for Coomassie blue is described in the literature. Additional information using surface labelled parasites showed that the 32 KDa antigen is not accessible to radiodination, suggesting that this hydrophobic component is exposed to the cytoplasmic side of the parasite membrane or is a component of internal membranes.

The female-specific band identified in this work did not correspond to any major proteins present in both male and female worms, and it may therefore be a transitory or minor constituent of the parasite tegument, since it was not detected by Western blotting using sera from cronically infected *N. squamipes*. On the other hand, it is possible that this protein corresponds to the 32 KDa protein detached by poly-lysine beads (Cesari et al., 1983; Payares & Evans, 1987). This detached protein is located in underlying regions of the surface membrane of the tegument and is not accessible to radiodination.

In addition to the 32 KDa band, female- and male-specific proteins of 18 KDa and 42 KDa respectively were detected in the aqueous and insoluble fractions. The 18 KDa protein is probably a soluble protein and the 42 KDa a membrane protein since the latter remained insoluble under the condition used.

Significant differences in the total levels of calcium in the tegument of male and female *S. mansoni* worms were detected (Shaw & Erasmus, 1983, 1984) suggesting physiological differences between sexes with respect to calcium metabolism. In this context it is possible that some of the female specific proteins could be related to the degree of sexual development of the female worms, since no reactivity against these low m.w proteins was detected using cronically infected *N. squamipes* sera.

In conclusion, although the adult worms of *S. mansoni* present a complex tegumental membrane structure, the technique used in this work was useful in the identification and isolation of a sex specific group of proteins. The physiological and immunological importance of these proteins in single-sexually infected animals remains to be elucidated.

ACKNOWLEDGMENTS

To Dr L. Rey and Dr K. Lowdes for careful reading of the English version of the manuscript.

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