Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *84* (1): 13-18, jan./mar. 1989

CHARACTERIZATION OF PLASMA MEMBRANE POLYPEPTIDES OF TRYPANOSOMA FROM BATS

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Cell surface proteins of Trypanosoma dionisii, Trypanosoma vespertilionis *and* Trypanosoma sp. *(M238) were radiodinated and their distribution both in the detergent-poor (DPP) and detergent-enriched phase (DRP) was studied using a phase separation technique in Triton X-114, as well as polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS-PAGE). Significant differences were observed in the proteins present in the DRP when the three species of trypanosoma were compared. Two major bands with 88 and 70 KDa were observed in* T. sp. *(M238) but were not detectable in* T. dionisii *and* T. vespertilionis. *Three polypeptides with 96, 77 and 60 KDa were identified in the DRP of* T. vespertilionis. *Three major bands with 84, 72 and 60 KDa were observed in the DRP of* T. dionisii. *Two polypeptides with 34-36 KDa present in the DPP, were observed in the three* Trypanosome *species analyzed. Our observations show that* T. sp. *(M238) has characteristic surface polypeptides not found in* T. dionisii *and* T. vespertilionis.

Key words: Triton X-114 fractionation – *Trypanosoma* from bats – amphiphilic proteins

Models of membrane structure reveal that the membrane matrix is made up of a bilayer of phospholipids into which proteins are intercalated (Singer & Nicolson, 1972; Nicolson, 1976). Some of these proteins play an important structural role and are involved in different processes such as adsorption and transportation of nutrients, cell recognization, etc. In the case of parasites, many of the surface proteins represent important antigens which elicit both humoral and cell-mediated immune response when they enter in contact with the vertebrate host.

In recent years a large number of studies has been done aiming at the identification of surface proteins in members of the Trypanosomatidae family, especially in those which belong to the genera *Trypanosoma* and *Leishmania* (Mancini et al., 1982; Gardiner & Dwyer, 1983; Lanar & Manning, 1984). Few data are available on surface proteins of members of bat trypanosomes. The difficulty of distinguishing trypanosome species and subspecies of the subgenus *Schizotrypanum,* is a major problem in epidemiological studies on Chagas' disease. In this paper we analyzed the surface proteins of *Trypanosoma sp.* (M238), a species of bat *Trypanosoma* recently isolated in Venezuela and characterized. For this study we used labelling with ^{125}I followed by solubilization with Triton X-114, separation of proteins into an aqueous and a detergent phase (Bordier, 1981) and their characterization by polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS-PAGE). Many of the bat trypanosomes make up a group of parasites belonging the subgenus *Schizotrypanum* together with the etiological agent of Chagas' disease, *Trypanosoma cruzi.* Although, some of the bat trypanosomes show a host restriction some stocks isolated in America were found to be infective to laboratory animals (Marinkelle 1976, 1982). These parasites can be differentiated by patterns of isoenzymes (Baker et al., 1978) isoelectrofocusing (Ebert, 1983) and lectins (Schottelius & Uhlenbruck, 1983). However, not much is known about the amphiphilic nature of surface cell proteins. In this paper, we use this parameter to compare *T. sp.* (M238) with *T. dionisii* and *T. vespertilionis*, two other species of bat (Microchiroptera) trypanosomes isolated in Europe.

Work supported by a grant from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), Brazil.

Received August 18, 1988.
Accepted November 2, 1988.

MATERIAL AND METHODS

Parasites – *T. dionisii, T. sp.* (M238) (Hernandez, 1979) and *T. vespertilionis* were maintained in Liver Infusion-Tryptose medium (Difco Laboratories, Detroit, USA) at 28 °C, with serial passages at 4-5 day intervals.

Radiodination of parasite membranes – Cells were pelleted by centrifugation (1.5 min at 9,650 g, using a Microfuge B miniature centrifuge, Beckman Instruments Inc, California, USA) and washed three times in cold PBS, pH 7.2. Surface proteins were radiolabelled by incubating 1 to 2 x 10^8 washed viable parasites with 11.1 MBq of iodine ($Na^{125}I$, Amersham, England, UK) and 125 μg of iodogen for 10 min at 0 °C (Markwell & Fox, 1978).

Labelled parasites were washed four times in cold PBS as described above and maintained on ice until extraction with detergent. Phase contrast microscopic observation of the radiolabelled cells showed that they remained motile.

Solubilization of radiolabeleld proteins and phase separation in Triton X-114 – Surface-labelled parasites were solubilized in 10 mM Tris-saline buffer pH 7.4 containing 2% Triton X-114 and cleared by centrifugation at 15.000 g for 15 min at 4 °C. The detergent enriched phase (DRP) and the detergent poor-phase (DPP) were obtained as described by Bordier (1981).

SDS-PAGE – Samples of the proteins (DRP and DPP) were previously precipitated with cold acetone (three times) and solubilized in the same volume of sample buffer (30 mM Tris-HCl, pH 6.8 1 mM EDTA, 1% SDS, 5% glycerin and 0,5% B-mercaptoethanol) and further boiled for 3 min. Samples corresponding to 7.5 x 10^5 cells were loaded into each well. Electrophoresis was performed on 10-20% polyacrylamide gels according to Laemmli (1970), under 20 mA constant current for approximately 4h.

Staining and autoradiography – The proteins resolved by SDS-PAGE were silver stained according to the procedure described by Niellsen & Brow (1984). Low and high-molecular weight standards were obtained from Sigma Chemical Co, USA.

Autoradiograms of the dried gels were made using Sakura Medical Imaging films (Sakura, Japan) in Kodak X-0-Matic regular intensifying screens (Eastman Kodak Co., Rochester, N.Y., USA), at −70 °C for 72-96h.

RESULTS

Phase-separation of proteins – Epimastigote stage surface of *T. dionisii, T. vespertilionis* and *T. sp.* (M238) were iodinated, Triton X-114 extracted and SDS-PAGE compared. The two phases (DRP and DPP) obtained were analyzed by SDS-PAGE after silver staining (Fig. 1) and autoradiography (Fig. 2). A large number of bands were observed in the silver stained gels, among the three bat trypanosomes analyzed. A very similar pattern of bands was observed in gel with *T. dionisii* and *T. sp.* (M238) silver stained in DPP and DRP (Fig. 1, lanes A, B, D, E). However, significant differences in the pattern of proteins between *T. vespertilionis* (Fig. 1, lane C) and *T. dionisii* or *T. sp.* (M238) was observed (Fig. 1, lanes A, B).

The major radiolabelled membrane proteins were recovered in the DRP of the three trypanosomes analyzed (Fig. 2). In the case of *T. vespertilionis* there were three major bands with MW of 96,000, 77,000 and 60,000 (Fig. 2, lane D). Other minor bands with MW of 160,000 and 27,000 were also seen. With *T. sp.* (M238) (Fig. 2, lane E) two major bands with MW of 88,000 and 70,000 were evident. Three major bands with 84, 72 and 60 KDa were observed, with *T. dionisii* (Fig. 2, lane F).

Few radiolabelled membrane proteins were recovered in the DPP (Fig. 2, lanes A-C). Two minor common polypeptides with MW of 34 and 36,000 were seen in the three trypanosome species. In the case of *T. dionisii* (Fig. 2, lane C) one additional major band with MW of 25,000 was also seen.

Triton X-114 insoluble proteins – To characterize the proteins not solubilized in 2% of Triton X-114, the material precipitated before the phase separation was treated with 4% of SDS and, after centrifugation, analyzed by SDS-PAGE. The silver stained gels are shown in Fig. 1. Although, several proteins can be solubilized from the three trypanosomes, minor proteins radiolabelled with similar molecular weights were detected by auto-

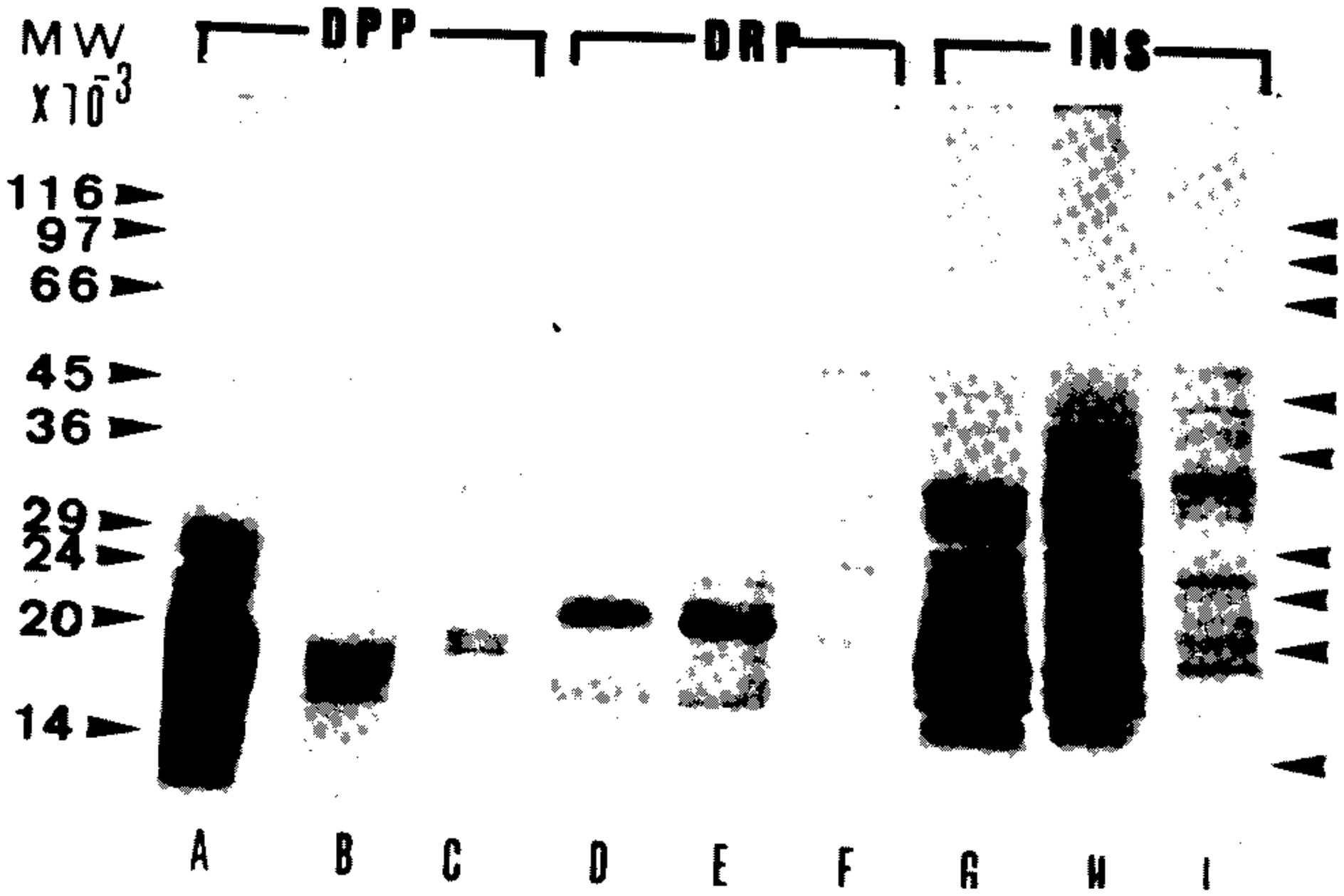

Fig. 1: SDS-PAGE of proteins from radiolabelled parasites after phase separation and silver staining. A, D, G: *Trypanosoma dionisii;* B, E, H: *Trypanosoma sp.* (M238); C, F, I: *Trypanosoma vespertilionis.* DPP, detergent-poor phase; DRP, detergent-rich phase. INS, insolube proteins in 2% Triton X-114 but which were solubilized in 4% SDS.

radiography. Two major radiolabelled polypeptides with MW of 50,000 and 15,000 were observed in the three trypanosomes analyzed. One additional band with MW of 60,000 was seen in the *T. vespertilionis* preparation (data not shown).

DISCUSSION

Most of the work on the identification of surface proteins of trypanosomatids has been done using whole radiolabelled cells solubilized in SDS. Using this approach proteins, most of which are glycoproteins characteristic of certain species or even characteristic of development stages of certain species, have been identified. Although, the functional nature of some membrane proteins has been determined, in some trypanosomatids (Ramaley et al., 1985; Das et al., 1986; Lettelier et al., 1986; Torruella et al., 1986), the mechanism of insertion into the membrane has been a subject of study only recently (Etges et al., 1985; Ward et al., 1987). In the present study, we have first solubilized the proteins of three bat trypanosomes species using Triton X-114 and then separated the membrane proteins depending on their ability to be included or excluded from the detergent during phase separation. A comparison of proteins which remain in the DRP by SDS-PAGE did not show a degree of homology among the parasites examined.

Some radiolabelled proteins were not completely solubilized in 2% of Triton X-114, but could be solubilized when 4% SDS was used. This suggests that specific detergent conditions are required for their solubilization. It is possible that these polypeptides represent transmembrane proteins which interact with cytoskeletal components, as previously shown in other systems (Glenney et al., 1982; Jacobson, 1983; Glenney & Glenney, 1984; Tiruppathi et al., 1986).

It is important to point out that radiolabelled polypeptides with the same molecular weight were seen in the DPP of the three trypanosomes analyzed. In the case of *T. vespertilionis* one additional polypeptide was evident. As these polypeptides were found only in the DPP, the result suggests that these proteins are

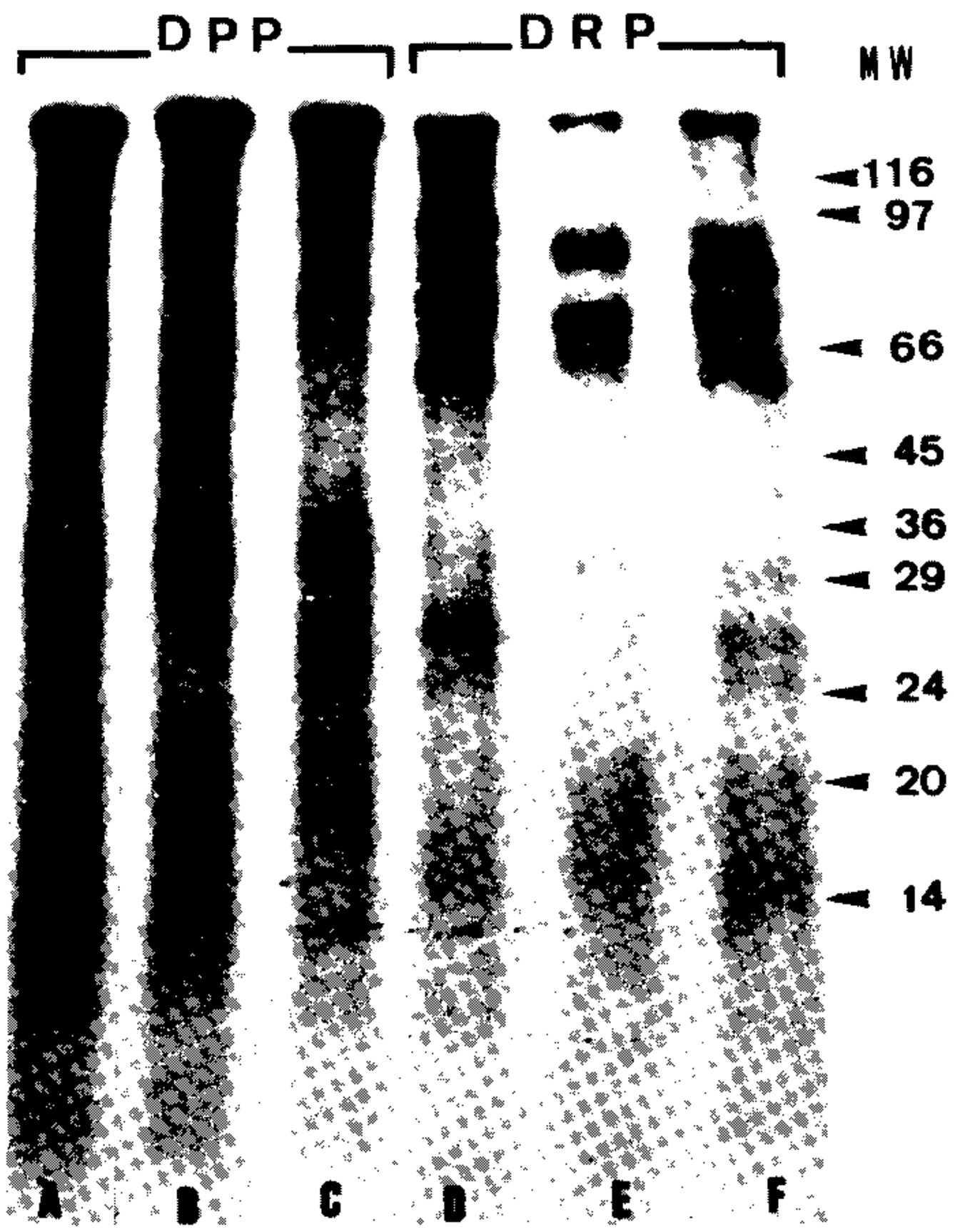

Fig. 2: autoradiography of SDS-PAGE (10-20%) of parasite radiolabelled proteins after phase separation. A, D: *Trypanosoma vespertilionis;* B, E: *Tripanosoma sp.* (M238); C, F: *Tripanosoma dionisii* DPP, detergent-poor phase; DRP, detergent-rich phase.

peripheral and exposed on the surface cell. Our observations, in association with others previously reported (Etges et al., 1985; Kumar, 1985; Bouvier et al., 1985; Bordier et al., 1986), indicate that Triton X-114 is very useful for the analysis of plasma membrane polypeptides. It is interesting to point out that in some trypanosomatids so far analyzed, proteins have been detected in the DRP (Etges, 1985; Bordier et al., 1986). However, hydrophilic polypeptides were observed in some Crithidia species (Giovanni De Simone et al., 1987). In the case of *Plasmodium gallinaceum* a polypeptide was also detected in the DPP (Kumar, 1985).

It is also important to point out that the pattern of labelled surface polypeptides seen in SDS-PAGE of *T. sp.* (M238) was different from the other two species of bat trypanosomes analyzed. This observation reinforces the suggestion, based on morphological and behavioural observations and analysis of KDNA (Pinho R. T., in preparation) that *T. sp.* (M238) is a species different from *T. vespertilionis* and *T. dionisii.*

RESUMO

Caracterização de polipeptídeos de membrana plasmática de tripanosomas de morcegos – As proteínas de superfície de *Trypanosoma dionisii, Trypanosoma vespertilionis* e *Trypanosoma sp.* (M238) foram radiodinados e sua distribuição na fase rica em detergente (DRP) e fase pobre em detergente (DPP) foram estudadas pela técnica de separação de fases com Triton X-114 e por eletroforese em gel de poliacrilamida em presença de dodecil sulfato de sódio (SDS-PAGE). Foram observadas diferenças significativas nas proteínas presentes na DRP quando as três espécies de tripanosomas

foram comparadas. Duas bandas com 88 e 70 KDa foram observadas em *T. sp.* (M238) e não foram detectadas em *T. dionisii* e *T. vespertilionis.* Três polipeptídeos com 96, 77 e 60 KDa foram identificados na fase DRP de *T. vespertilionis.* Três bandas com 84, 72 e 60 KDa foram visualizadas na fase DRP de *T. dionisii.* Dois polipeptídeos com 34-36 KDa presentes na fase DPP, foram observados nas três espécies de tripanosomas analisadas. Nossas observações mostraram que *T. sp.* (M238) possui polipeptídeos de superfície característicos, que não são encontrados em *T. dionisii* e *T. vespertilionis.*

Palavras-chave: fracionamento com Triton X-114 – polipeptídeos de trypanosomas de morcegos – proteínas anfifílicas

ACKNOWLEDGEMENTS

We are grateful to Dr L. Deane and Dr M. P. Deane for the revision and comments on the manuscript.

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