TRYPANOSOMA CRUZI CELL SURFACE PROTEINS: IDENTIFICATION OF ONE MAJOR GLYCOPROTEIN

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1. Introduction

Chagas' disease is a chronic debilitating disease which is estimated to afflict at least 10^6 people in South and Central America [1]. The causative agent of Chagas' disease is *Trypanosoma cruzi*, a parasitic protozoan transmitted by blood sucking reduviid bugs. *T. cruzi* has a complex life cycle and exists in three morphologically distinct forms: the epimastigote form which the organism takes when multiplying within the insect gut, the amastigote form taken during multiplication within mammalian cells and the trypomastigote, a non-dividing form released from the infected cells which circulates in the blood-stream [2].

Protective immunity against *T. cruzi* infection can be stimulated by immunising with either homogenates of epimastigotes [3] or subcellular fractions of epimastigotes enriched for flagellar and surface membrane components [4]. The immunity stimulated by these fractions can be interpreted to suggest that epimastigotes express antigens, probably cell surface in nature, which are also found on the other stages of the parasite found within the mammalian host. This study describes the identification of cell surface antigens by lactoperoxidase-catalysed ^{125}I iodination of different morphological forms of *T. cruzi*. Lectin affinity chromatography identified one major glycoprotein and this glycoprotein was present on the parasite cell surface throughout the life cycle.

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2. Materials and methods

2.1. *Parasites*

Epimastigotes used in this study were a clone (We Tryp Y_2C_1) derived from the Y_2 strain of epimastigotes by cloning using limiting dilutions on Terasaki plates (L. H., submitted). A number of other clones and strains of epimastigotes examined gave similar results to those reported for the clone used. Epimastigotes were cultured at 25°C in medium [5] containing 5% rabbit serum, penicillin (200 units/ml) and streptomycin (100 units/ml). Amastigotes were grown in vitro at 37°C in the presence of the mouse muscle sarcoma cell line S2 in Dulbecco's modified Eagles medium containing 10% foetal calf serum, penicillin and streptomycin. These cultures were initiated with Y strain trypomastigotes harvested aseptically from mouse blood (L. H., D. S., submitted). Y strain trypomastigotes were obtained from CBA/T6 mice infected by serial transfer of infected blood.

2.2. *Lactoperoxidase-catalysed iodination*

Cells were washed twice with PBS by centrifuging at 400 × *g* for 10 min before labelling. ^{125}I labelling was performed by incubating 5×10^7 cells at room temperature for 10 min in 100 μl PBS containing lactoperoxidase (20 μg/ml), glucose oxidase (0.2 units/ml), 5 mM glucose and 200 μCi carrier-free $Na^{125}I$ (Radiochemical Centre, Amersham). Labelled cells were subsequently washed twice with PBS before solubilisation. Radioactivity incorporated into the cells under these conditions has been shown by

electron microscope autoradiographs to be associated with the cell surface membrane (unpublished data).

2.3. *SDS–polyacrylamide gel electrophoresis*

Polyacrylamide gel electrophoresis was performed in 0.1% SDS on 10% (w/v) acrylamide slab gels using a 5% (w/v) acrylamide stacking gel and the Tris–glycine Laemmli buffer system [6]. Samples were dissolved in 2% (w/v) SDS, 10% (v/v) glycerol, 80 mM Tris–HCl (pH 6.8), 0.1 M dithiothreitol, 0.02% (w/v) bromophenol blue, 2 mM phenylmethylsulphonyl fluoride by heating at 100°C for 2 min. The following proteins were used as molecular weight markers: β galactosidase 130 000; phosphorylase 90 000 bovine serum albumin 67 000; ovalbumin 45 000; chymotrypsinogen 25 000. Proteins were visualised by staining with 0.02% (w/v) Coomassie blue in methanol–acetic acid–water (41:7:52, by vol.). Radioactively labelled components were detected by autoradiography using Kodirex X-ray film (Kodak Ltd) after the polyacrylamide gel had been immersed in a 1% (v/v) glycerol, 10% (v/v) acetic acid solution for 1 h and dried onto chromatography paper.

2.4. *Affinity chromatography*

Affinity chromatography for isolation of glycoproteins was as in [7]. The lectin used was isolated from *Lens culinaris* seeds and linked to cyanogen bromide-activated Sepharose 4B at 1 mg protein/ml gel. Samples for chromatography were dissolved in 2% (v/v) Nonidet P40, 0.15 M NaCl, 0.01 M Tris–HCl (pH 7.4) and centrifuged at 15 000 × *g* for 30 min. Lectin columns (5 × 1 cm) were eluted with the above buffer and glycoproteins bound to the lectin were subsequently eluted with 2% (w/v) methyl-α-D-mannopyranoside, 1% (v/v) Nonidet P40, 0.15 M NaCl, 0.01 M Tris–HCl (pH 7.4). Samples recovered from columns to be analysed by SDS–polyacrylamide gel electrophoresis were precipitated by the addition of 3 vol. ethanol and incubation at –20°C for 48 h.

2.5. *Peptide mapping*

Fractions for proteolysis were precipitated by 3 vol. ethanol at –20°C for 48 h. BSA (100 μg) was added to samples before precipitation to act as a carrier and attempt to maintain a constant protein : enzyme ratio during proteolysis. Limited proteolytic cleavage was carried out as in [8]. Samples were incubated for 30 min at 37°C in 0.125 M Tris–HCl (pH 6.8), 0.5% (w/v) SDS, 10% (v/v) glycerol containing 80 μg/ml *Staphylococcus aureus* protease V8 (Miles Labs Ltd). Digestion was stopped by increasing SDS to 2% and heating at 100°C for 2 min. Bromophenol blue and dithiothreitol were added to samples which were analysed by SDS–polyacrylamide gel electrophoresis.

3. Results

A comparison by SDS–polyacrylamide gel electrophoresis of the cell surface proteins of epimastigotes, amastigotes and trypomastigotes labelled by lactoperoxidase-catalysed ^{125}I iodination is shown in fig.1. Many of the proteins labelled appear to be common to more than one morphological stage of the parasite.

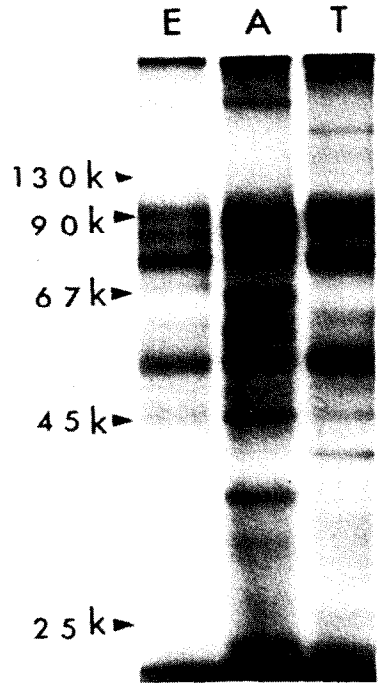

Fig.1. Autoradiographs of SDS–polyacrylamide gel electrophoresis patterns for ^{125}I lactoperoxidase labelled cell surface proteins of *T. cruzi*: E, epimastigote forms; A, amastigote forms; T, trypomastigote forms. Migration positions of standard proteins are indicated by their molecular weights.

The pattern of bands obtained however is characteristic for each morphological stage and although no protein specific to epimastigotes was identified, possible amastigote specific and trypomastigote specific proteins with app. mol. wt 30 000 and 180 000, respectively, were found.

Cell surface glycoproteins were separated from non-glycosylated proteins by affinity chromatography using the lectin from *Lens culinaris* which has specificity for glucose and mannose. The lectin retarded (glycoprotein) fraction represented 25% of the total trichloroacetic acid-precipitable radioactivity incorporated by lactoperoxidase into epimastigotes, 21% of that into amastigotes and 19% of that into trypomastigotes. The Nonidet P40 extract of the labelled cells used for affinity chromatography was shown by SDS–polyacrylamide gel electrophoresis to solubilise all of the labelled cell surface proteins. After lectin affinity chromatography only one of these cell surface components was eluted from the column with methyl α-D-mannopyranoside in the glycoprotein fraction. A comparison of the glycoprotein fraction recovered from each morphological stage is shown in fig.2. In each instance one major glycoprotein with an app. mol. wt 90 000 was found.

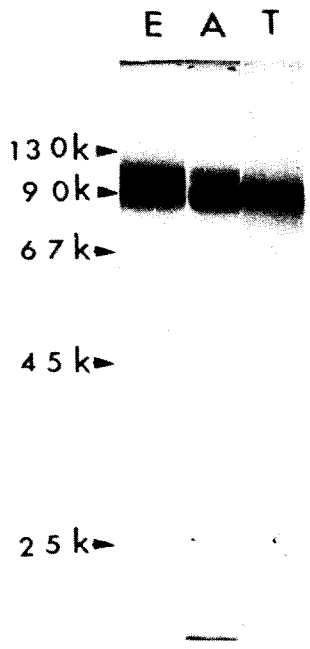

Fig.2. Autoradiographs of SDS–polyacrylamide gel electrophoresis patterns of the glycoprotein from *T. cruzi* obtained by *Lens culinaris* lectin affinity chromatography from the different morphological forms of *T. cruzi*: E, epimastigote; A, amastigote; T, trypomastigote.

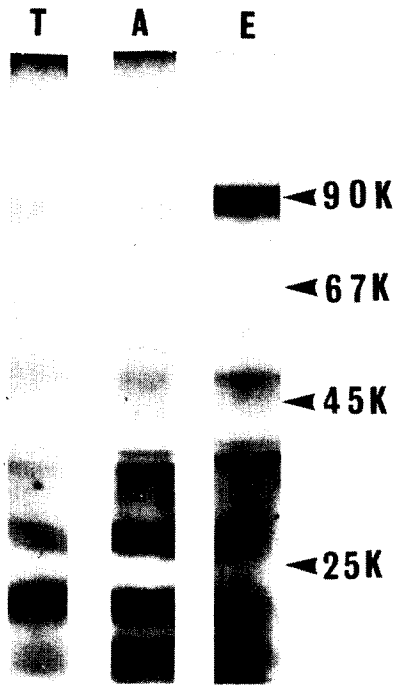

Fig.3. Limited proteolysis peptide maps of the glycoprotein from the different morphological forms of *T. cruzi*. Samples were digested by *S. aureus* protease V8: E, epimastigote; A, amastigote; T, trypomastigote.

The common identity of the glycoproteins recovered from each stage of the life cycle was demonstrated by limited proteolysis peptide maps (fig.3). ^{125}I lactoperoxidase-labelled glycoproteins were digested with *S. aureus* protease V8 and the intermediate breakdown products produced by limited proteolysis identified by SDS–polyacrylamide gel electrophoresis. The pattern of peptides produced is essentially the same for each glycoprotein although the persistance of more higher molecular weight material in the epimastigote glycoprotein fraction is probably evidence of variation in the enzyme to glycoprotein ratio. Variation of this type, which was found in each attempt at peptide maps of the glycoproteins, was interpreted to result from a variable efficiency in precipitation of the glycoprotein plus carrier BSA by ethanol.

4. Discussion

The cell surface labelling studies described suggest that many of the cell surface antigens found on *T. cruzi* are common throughout the life cycle. It is probable that the protection of the mammalian host by immunising with material of epimastigote origin is induced by these common antigens. The common expression of epimastigote antigens throughout the life cycle has subsequently been confirmed by absorption of antibody against epimastigotes by other stages of the organism (D. S., unpublished data). The identification of cell surface antigens which may be specific for amastigotes and trypomastigotes however adds further support to previous reports of the existence of stage specific antigens [9].

The presence of carbohydrate at the cell surface of *T. cruzi* has been shown for all stages of the organism by studies using Con A [10,11] (L. H., unpublished data). Although some disagreement on the presence of Con A binding sites on trypomastigotes has been reported [10,11], the present study provides further evidence for the presence of lectin binding sites at the trypomastigote cell surface. Phenol extraction of epimastigotes has resulted in the isolation of three glycoproteins as well as a complex molecule containing carbohydrate, phosphorus, amino acids and lipid [12,13]. These components have been shown to be located in the surface membranes of the epimastigote [14]. Amongst the three glycoproteins identified [12] may well have been the 90 000 mol. wt glycoprotein identified in this study, the other components having been formed by proteolysis of the major glycoprotein. Great care was needed during the handling of the epimastigotes or further glycoprotein components $< 90\,000$ mol. wt appeared. This proteolysis was induced by centrifuging the organisms at high speeds during harvesting and washing procedures, e.g., at $800 \times g$ or greater, or by not keeping detergent extracts on ice or frozen.

A number of pathogenic protozoa undergo antigenic variation, that is they have the ability to change the antigenic determinants expressed on their cell surfaces. Antigenic variation has been found with malaria [15], babesia [16] and has been demonstrated to occur in the major cell surface glycoprotein of the African trypanosome *T. brucei* [17,18]. It has been postulated that *T. cruzi* also undergoes antigenic variation [19] although no evidence for variation during infection [20] or within the major glycoprotein has been found (D. S., in preparation). However the presence of many different cell surface components on all stages of *T. cruzi* confirms that a glycoprotein coat of the type found on African trypanosomes [18,21] does not exist on *T. cruzi* [22].

Further studies are in progress to examine the structure and biological role of the major cell surface glycoprotein of *T. cruzi*.

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