EVIDENCE FOR THE PRESENCE OF D-GALACTOFURANOSE IN THE LIPOPEPTIDOPHOSPHOGLYCAN FROM *TRYPANOSOMA CRUZI*

Modification and tritium labeling

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1. Introduction

The structure of a lipopeptidophosphoglycan (LPPG) from *Trypanosoma cruzi* is under investigation [1–3]. A glycophosphoceramide structure was demonstrated for the compound. Mannose, galactose, glucose and glucosamine are the sugar components of an oligosaccharide chain linked to the ceramide through inositol phosphate.

The plasma membrane localization of this glycoconjugate was shown [4,5]. However, it could not be labelled by sequential treatment of the cellls with galactose oxidase–KB^3H_4.

This communication presents evidence that the galactose units of LPPG have a furanoic ring. Considering that the exocyclic glycol would selectively be cleaved by periodate a method was developed to modify and label the LPPG.

2. Experimental

Epimastigote forms of *T. cruzi* (Y strain) were cultivated in LIT medium [6]. LPPG was isolated from the glycoconjugate complex and purified as in [2].

Polyacrylamide gel electrophoresis, analysis of neutral sugar, total phosphorus and P_i were as in [1]. Monoesterified phosphate was determined with *Escherichia coli* alkaline phosphatase (Sigma) at pH 8 in 0.2 M Tris–HCl buffer. At the end of the reaction P_i was determined. Descending paper chromatography was performed on Whatman no 1 paper with ethyl acetate/acetic acid/formic acid/water (18:3:1:4) as solvent. Gas–liquid chromatography (GLC) was carried out with a Hewlett-Packard 5830 A gas chromatograph equipped with glass columns (183 × 0.3 cm) packed with 3% ECNSSM on Gas-Chrom Q, with nitrogen at a flow rate of 20 ml/min; *ti* 220°C; *td* 220°C; *tc* 180°C.

Partial acid hydrolysis was performed a follows: a sample of LPPG containing 818 μg total sugar was hydrolysed with 0.02 N trifluoroacetic acid (1.6 ml) at 100°C. Aliquots were taken at different times and the neutral sugars quantitatively analysed by GLC of the alditol acetates. Another sample was hydrolysed with 0.05 N trifluoroacetic acid for 3 h at 100°C, evaporated in vacuo and chromatographed on a Bio-Gel P-2 column (1.5 × 90 cm). Elution was made with water. Fractions of 2 ml were collected and anlysed for sugar content.

Periodate oxidation was performed by treating the LPPG (7.3 mg) with 3 ml 0.05 N $NaIO_4$ in water for 30 min at room temperature in the dark. The excess oxidant was decomposed after acidification with 1 N sulfuric acid, by addition of 0.5 M sodium arsenite. The mixture was dialysed and the formaldehyde produced was determined in the dialysate by the chromotropic acid method [7].

Another sample (7.5 mg) was dissolved in 0.5 ml 0.1 M sodium phosphate buffer (pH 7.2) and oxidized with 0.34 ml 0.05 M sodium metaperiodate for 20 min. The oxidation was stopped by addition of ethylene glycol and the solution was then treated with an excess sodium borohydride at room temperature. After 2 h the solution was acidified to pH 4 with 0.02 M acetic acid and chromatographed on a Bio-Gel

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P-6 column with water. The excluded peak was lyophylized and a sample was hydrolysed with 1 N HCl for 2 h at 100°C. The solution was evaporated in vacuo and the sugars reduced and analysed by GLC.

Labeling of isolated LPPG was performed by oxidation with sodium metaperiodate as above and subsequent reduction was achieved with 1 mg KB^3H_4 (final spec. act. 0.2 mCi/mg) in two steps with 5 min interval between them. After 10 min, cold sodium borohydride (5 mg) was added and the reduction was allowed to continue for another 30 min. Excess reagent was decomposed with acetic acid and the mixture was loaded on a column (1 X 50 cm) of Bio-Gel P-10 and eluted with water. A sample of the excluded peak was hydrolysed as above and analysed by paper chromatography. The paper was cut into 1 cm horizontal strips and counted in toluene with PPO (5 g/l) and POPOP (0.1 g/l).

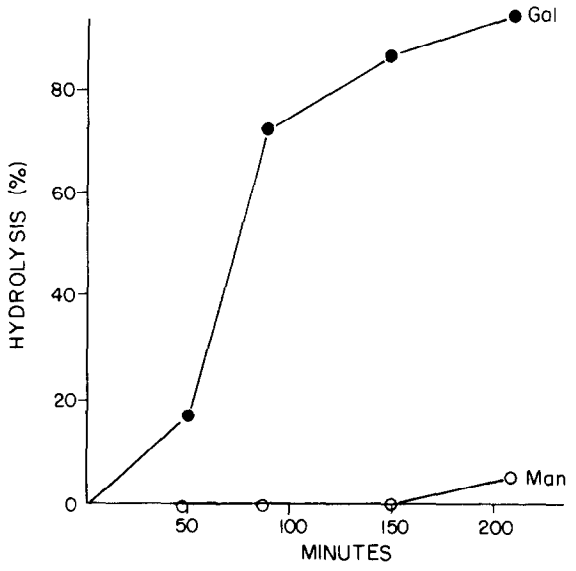

Fig.1. Differential release of galactose and mannose during hydrolysis of LPPG with 0.02 N trifluoroacetic acid at 100°C. The values are expressed as the % of the total individual sugar present.

3. Results

After 210 min of mild hydrolysis with 0.02 N trifluoracetic acid, LPPG selectively released 95.4% of the total amount of galactose present in the sample. Comparatively, only 5% of mannose was released (fig.1). This greater lability to acid of the galactoside linkages could be explained by a furanoic structure. Terminal galactoses linked to a main chain through 1-phosphate would also be preferentially cleaved by acid. However, the latter hypothesis is insufficient to explain the acid lability of all galactose residues. In fact, fractionation of the products of partial acid hydrolysis of LPPG on a Bio-Gel P-2 column gave two main carbohydrate peaks (fig.2). Peak I contained no galactose and 85% of the total phosphorus from the sample. Only 25% of this phosphorus could be released with alkaline phosphatase. This would amount to 1 μmol phosphorus for 11 μmol hexose found in peak II. As a control it must be stressed that the enzyme hydrolysed only 2% of the total phosphorus from the original LPPG. These results suggest that one, or more, furanoic galactose chains might be linked to the main carbohydrate structure by phosphodiester bonds.

Short term periodate treatment cleaves the primary hydroxyl group of furanosyl hexose, with free groups at C-5 and C-6. The amount of formaldehyde determined corresponded to 90% of the galactose present. Sequential mild oxidation and borohydride reduction of the LPPG gave a modified product which showed in 15% SDS–polyacrylamide gel electrophoresis one Schiff positive band with a slightly higher mobility (R_M 0.70) than the LPPG (R_M 0.66). Acid hydrolysis and analysis of the neutral sugar components by GLC showed arabinose, mannose, glucose, a trace of galactose and inositol (fig.3). Labeling of the LPPG was achieved when the oxidized product was reduced with tritiated borohydride.

Radioactive modified LPPG was excluded from

Fig.2. Bio-Gel P-2 chromatography in water, of a partial acid hydrolysate (0.05 N trifluoroacetic acid, 3 h, at 100°C) of the LPPG.

Fig.3. Gas-liquid chromatographic analysis of sugars as alditol acetates, on 3% ECNSSM on Gas-Chrom Q at 180°C. (A) After selective periodate oxidation of the LPPG (7.5 mg), KBH_4 reduction and hydrolysis with 1 N HCl for 2 h at 100°C: 5.44 arabinose; 14.34, mannose; 16.74, galactose; 18.93, glucose; 24.25, inositol. (B) Acid hydrolysate of LPPG (1.7 mg) with 2 N trifluoroacetic acid for 3 h at 100°C: 14.48, mannose; 16.95, galactose.

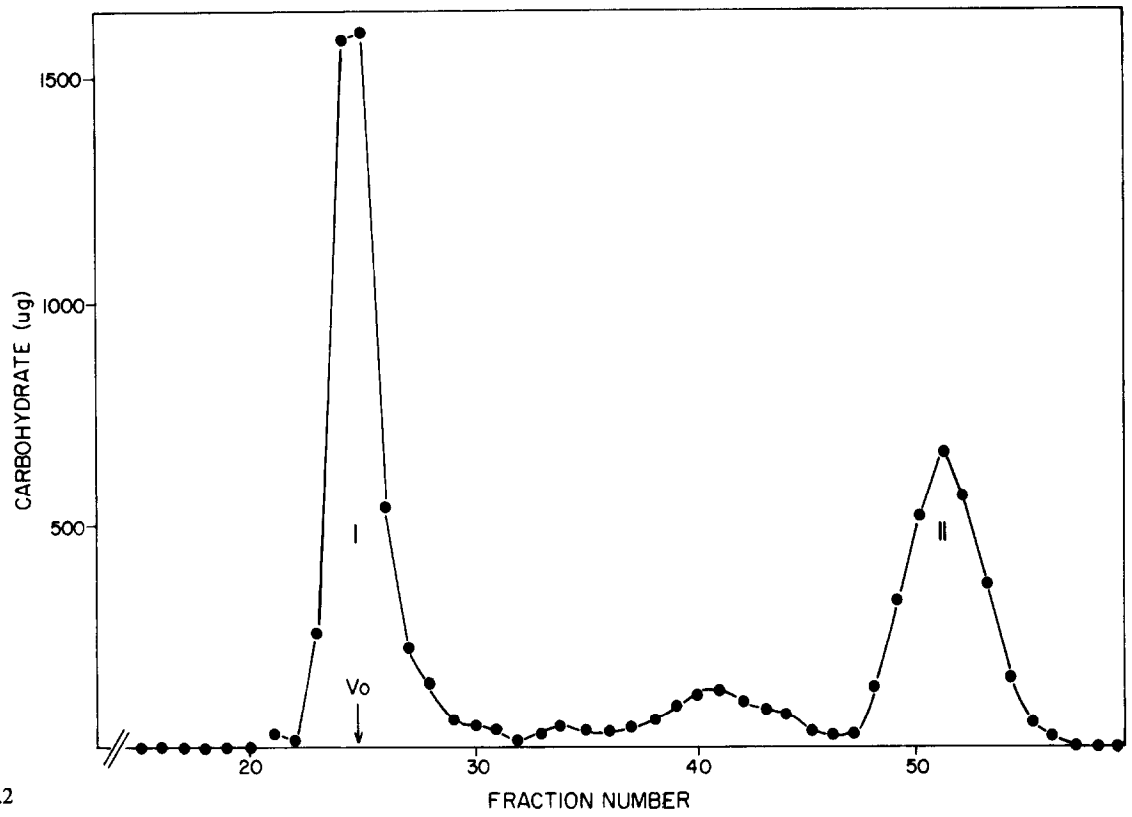
1500
1000
500
CARBOHYDRATE (ug)
I
II
Vo
20
30
40
50
FRACTION NUMBER

Fig.2

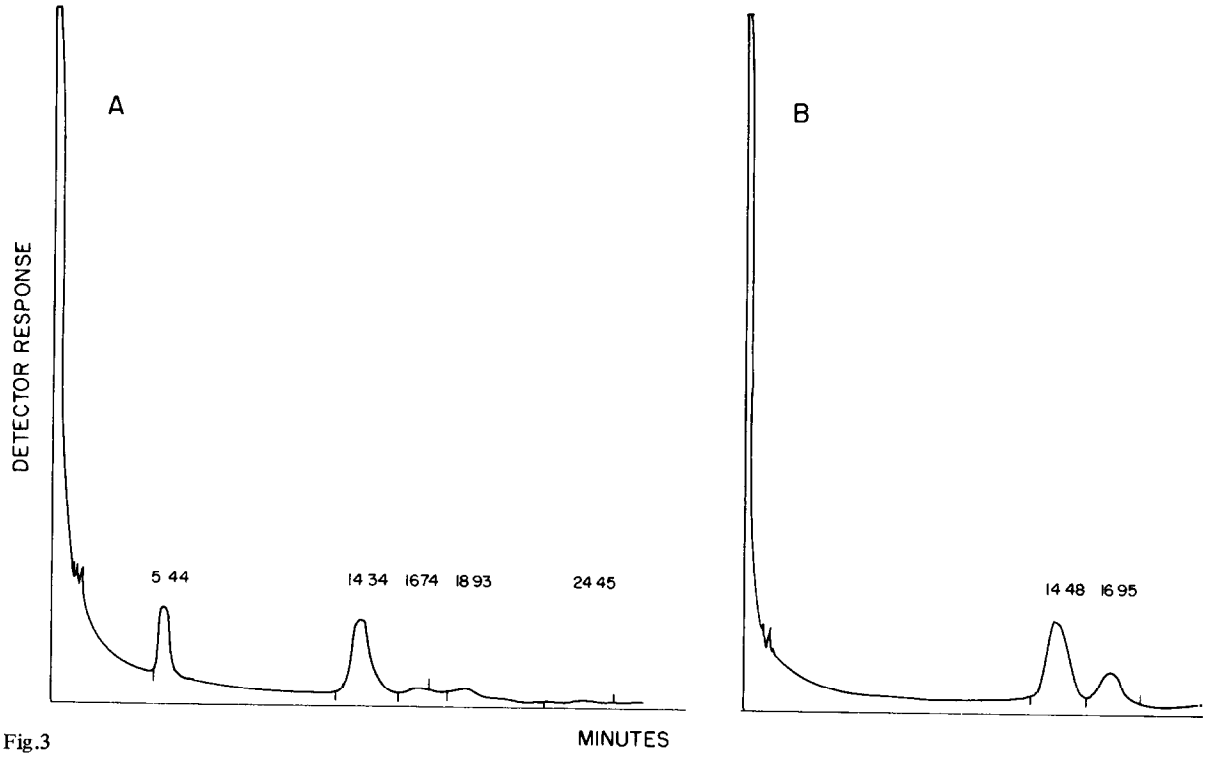
A
B
DETECTOR RESPONSE
5 44
14 34
16 74
18 93
24 45
14 48
16 95
MINUTES

Fig.3

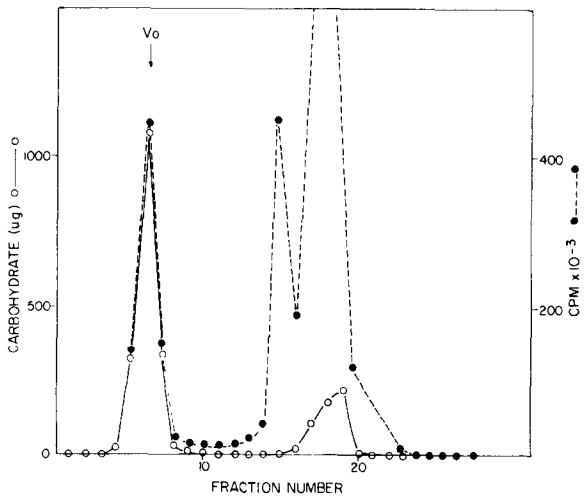

Fig.4. Bio-Gel P-10 chromatography in water of the modified LPPG, after sequential treatment with sodium periodate and KB^3H_4.

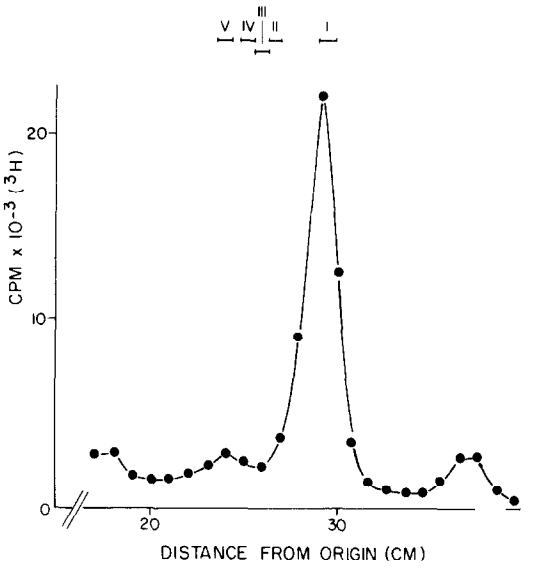

Fig.5. Paper chromatography of the sugars released after acid hydrolysis of the LPPG labeled with $NaIO_4$-KB^3H_4 as in section 2. Standard sugars: I, arabinose; II, mannose; III, glucose; IV, galactose; V, glucosamine.

Bio-Gel P-10 (fig.4). The incorporation determined was 410 cpm/μg sugar. Extraction with ether of an acid hydrolysate removed only 0.8% of the total radioactivity. This could be due to partial reduction of the ester bound fatty acids [2]. Paper chromatography of the aqueous phase showed that arabinose was the only radioactive manosaccharide (fig.5). The fact that galactose could be selectively degraded to arabinose provided a means to modify and label the LPPG.

4. Discussion

The plasma membrane localization of the LPPG was demonstrated [4,5]. However, this macromolecule containing ~20% of galactose could not be labeled with galactose oxidase–KB^3H_4 either in vivo or in vitro. Controversial reports on the oxidation of D-galactofuranose units by this enzyme were published. While galactofuranosyl glycerol and methyl α- or β-D-galactofuranoside could not be oxidised by the enzyme [8], the oxidation of galactofuranosyl residues of a galactan and a glycoprotein was reported [9]. The argument that the enzyme would need a substrate of large molecular weight does not agree with the high reactivity of methyl-β-D-galactopyranoside [10]. The importance of the size of the C-4 substituent on the enzyme action was reported [11]. In the furanoic configuration the hydroxyl at C-4 is involved in ring formation, and hindrance for the two carbon exocyclic groups to adopt an optimal conformation could explain the failure of some furanose structures to interact with the enzyme.

The furanoic configuration of the galactose in the LPPG is now demonstrated by the experiments on mild acid hydrolysis and periodate oxidation. The formation of formaldehyde is also evidence that OH-6 is not involved in linkages which would prevent the oxidation with galactose oxidase.

A modified tritiated LPPG could be obtained by treatment of the selectively oxidized product with KB^3H_4. The method has been used to label sialoglycoproteins [12,13] but apparently has not been used to label galactofuranose containing glycoconjugates.

Labeling of LPPG would facilitate the structural studies that are in progress which could eventually lead to the understanding of its biological function.

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