Vol. 114, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 18, 1983 Pages 119-125

PRESENCE OF GLYCEROL AND FATTY ACIDS IN THE C-TERMINAL END OF A VARIANT SURFACE GLYCOPROTEIN FROM TRYPANOSOMA EQUIPERDUM

G. Duvillier*, A. Nouvelot**, C. Richet*, T. Baltz*** and P. Degand*

*Unité INSERM N°16, Place de Verdun, 59045 LILLE Cédex (France)

**Laboratoire de Physiopathologie des Lipides,
Faculté de Pharmacie, 3 rue du Professeur Laguesse,
59045 LILLE Cédex (France)

***Laboratoire d'Immunologie et Biologie Parasitaire,
Université Bordeaux II, 146 rue Léo Saignat,
33076 BORDEAUX Cédex (France)

Received May 27, 1983

SUMMARY - The composition of the C-terminal end of a variant surface glycoprotein from Trypanosoma equiperdum (BoTat-1 VSG) has been examined. It has been reported for two Trypanosoma brucei VSGs (Holder, A.A., Biochem. J. (1983), 209, 261-262) that ethanolamine was involved in binding the C-terminal amino acid to an oligosaccharide side chain. Tryptic glycopeptides were prepared from BoTat-1 VSG and analyzed. One of them was found to contain ethanolamine and consequently was assumed to be C-terminal. It was shown that the glycopeptide also included phosphate, glycerol and fatty acids. The fatty acid composition was representative of that of glycerolipids. All the results suggest that the end of the molecule is a core of phosphatidylethanolamine.

African trypanosomes can evade the immunological defense of their mammalian host by the sequential expression of antigenically distinct surface glycoproteins (for review, 1-2).

In Trypanosoma brucei, isolated specific antigens have been characterized (3-6) and analyzed for N-terminal amino acid sequence (7). The complete polypeptidic structure of one glycoprotein (VSG 117) has been reported (8) and also the complete nucleotide sequence of its corresponding cDNA (9). Partial cDNA sequences are also available for other variants (10-14). The comparison between the two types of structural data (8-11) has shown that the protein is initially synthesised with N- and C-terminal hydrophobic extensions which are not found on the purified

Abbreviations :
VSG : Variant Surface Glycoprotein. BoTat : Bordeaux Trypanozoon-antigenic type.

0006-291X/83 $1.50

glycoprotein. There are two types of oligosaccharide side chains on VSGs : asparagine-linked or attached to the C-terminal residue (15) through ethanolamine which is linked to the carboxyl group of the amino acid by an amide bond (16). The C-terminal oligosaccharide is involved in the immunological cross reacting determinant found in different VSGs (15).

In a clone of T. equiperdum, several VSGs have been isolated (17), characterized (18-19), and shown to be phosphorylated (20). Partial amino acid sequence has been reported for one glycoprotein (BoTat-1 VSG) (21).

MATERIALS AND METHODS

Preparation of purified glycoproteins from cloned variants. The clone specific glycoprotein was purified by affinity chromatography on Concanavalin-A Sepharose as described previously (17).

Isolation of tryptic glycopeptides. Native BoTat-1 VSG was digested with trypsin in 0.1 M ammonium bicarbonate pH 8.5, for 24 h at 37°C, in an enzyme/substrate molar ratio of 1 : 100. The cleavage products were applied to a Bio-Gel P60 column (150 x 2 cm, 100-200 mesh) equilibrated with 0.1 M acetic acid. The collected fractions were analyzed by absorption at 230 nm and by ninhydrin and orcinol reactions (22). A second purification was performed on a Bio-Gel P10 column (150 x 2 cm, 100-200 mesh) in the same conditions.

Amino acid composition. Samples were hydrolysed in sealed tubes for 24 h in 5.6 M HCl at 105°C under vacuum. Amino acids, hexosamines, and ethanolamine were separated on a single column of DC-6A resin (Dionex Corporation Sunnyvale Calif 94086) on a Beckman 119 CL amino acid analyzer according to the buffer system described by Fauconnet and Rochemont (23).

Carbohydrate composition. Gas-liquid-chromatographic analyses of neutral and 2-amino-2-deoxyhexose were performed according to Reinhold (24) using a 5840A Hewlett Packard gas-chromatograph equipped with a dual flame ionization detector.

Phosphate analysis. The sample was ashed according to Ames (25). The inorganic phosphate was characterized by a micromethod using Malachite green (26).

Determination of glycerol. The sample was hydrolyzed in 2 M HCl at 125°C for 48 h according to the method described by Renkonen for the hydrolysis of glycerophospholipids (27). The glycerol content in the acid hydrolysate was obtained from the reaction of phosphorylation with glycerokinase and ATP, combined with the estimation of the amount of ADP obtained in the assay ; ADP was phosphorylated with phosphoenolpyruvate and pyruvate-kinase, and the pyruvate obtained was converted into lactate by means of $NADH_2$. The reaction was carried out on a centrifuge analyzer (Rotochem) (28).

Lipid analysis. The release of fatty acids was obtained by hydrolysis in sealed tubes for 24 h in 5.6 M HCl at 105°C under vacuum. The lipids were extracted by the procedure of Folch et al. (29) and converted into methyl esters (30). After extraction with heptane, the methyl esters of each extract were identified by gas-liquid chromatography on a carbowax 20 M (2 %) column using the equivalent chain length method.

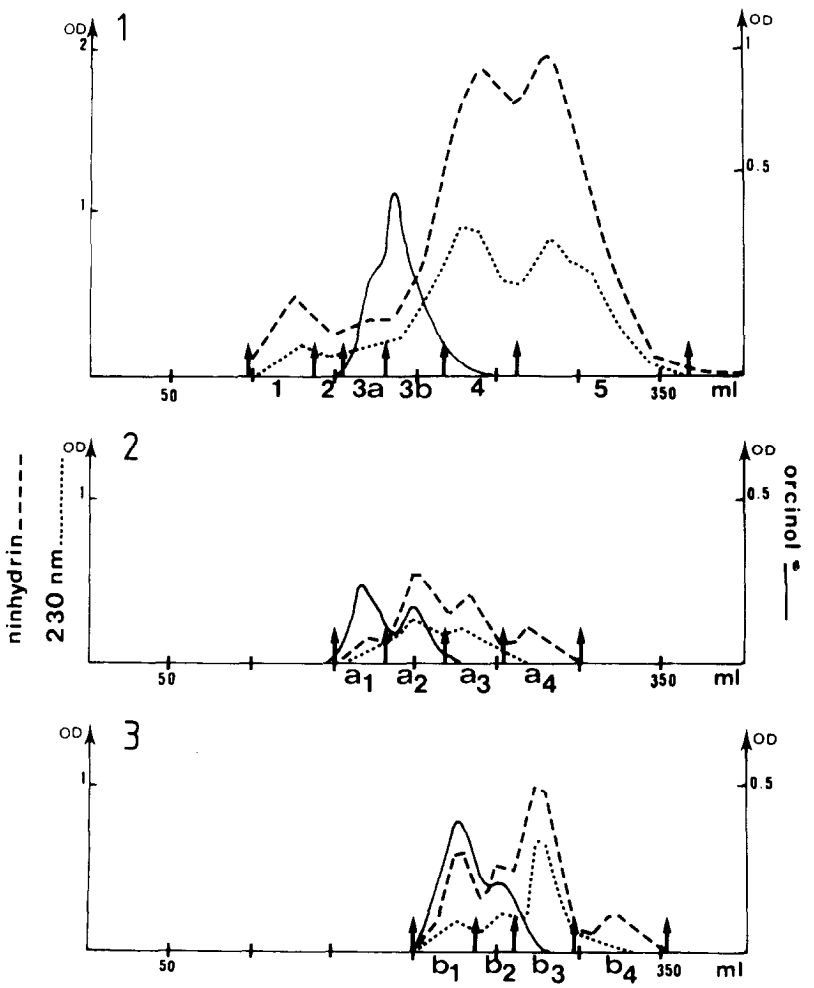

Figure 1 : Purification of the tryptic glycosylated fractions. The digest was applied to a Bio-Gel P60 column (1). The collected fractions 3a and 3b were further purified on Bio-Gel P10 (respectively 2 and 3).

RESULTS

Preparation and amino acid composition of the tryptic glycosylated fractions

Native BoTat-1 VSG was extensively digested with trypsin and the enzymatic hydrolysate was purified by two steps of gel filtration chromatography, as shown in Figure 1. The glycosylated fractions were analyzed in amino acid composition (Table I). Ethanolamine was found in the fraction b1 in an approximate molar ratio of 1 : 1 relative to glycine. According to the results reported by Holder (16), that fraction appeared to be only constituted with the tryptic C-terminal glycopeptide.

TABLE I
Amino acid composition of the tryptic glycosylated fractions

	a_1	a_2	b_1	b_2
Asx	300	769	399	424
Thr	447	722	741	441
Ser	200	563	203	414
Glu	27	161	42	226
Pro	-	308	36	100
Gly	214	400	247	172
Ala	47	1544	99	730
Cys	-	-	-	-
Val	18	95	19	130
Met	-	30	-	-
Ile	8	48	13	32
Leu	17	467	37	615
Tyr	6	12	10	-
Phe	10	202	24	176
His	9	82	9	11
Lys	21	174	20	53
Arg	-	101	17	208
GlcN	392	247	473	430
ethanolamine	140	-	203	22

results are expressed in µmol/g

Chemical composition of the C-terminal glycopeptide

The chemical composition of the tryptic C-terminal glycopeptide is given in Table II in molar ratio relative to glycine. The carbohydrate moiety is composed of galactose, mannose and glucosamine.

It was also shown that the glycopeptide contained a phosphate group (Table II).

The glycopeptide was hydrolyzed under suitable conditions for characterization of glycerol from glycerophospholipids. The hydrolysate gave a positive reaction with glycerokinase and ATP. The glycerol content, under these conditions, was estimated to be one mol per mol of the glycopeptide.

Presence of fatty acids bound to the C-terminal glycopeptide

Gas-liquid chromatographic analyses gave a set of peaks that could be identified through their retention time in reference to the elution of standard

TABLE II
Chemical composition of the tryptic C-terminal glycopeptide

Asx	1.6
Thr	3.0
Ser	0.8
Gly	*1*
GlcN	1.9
ethanolamine	0.8
Galactose	6.0
Mannose	6.1
Phosphate	0.9

results are expressed in molar ratio relative to glycine

fatty acids and to the method of equivalent length chain. The relative percentage of each fatty acid is given in Table III, and the results have been compared to that obtained with the native variable antigen under the same conditions. Fatty acids compositions, here reported, result from investigations on stored lyophilized material.

DISCUSSION

The present analysis has shown that the structure of the tryptic C-terminal glycopeptide from BoTat-1 VSG of T. equiperdum included the following elements : (1) ethanolamine, (2) phosphate group, (3) glycerol and (4) fatty acids.

Bound fatty acids have been found in transmembranal virus glycoproteins, and a linkage to a serine residue has been described (reviewed in 31).

In the murein lipoprotein of the E. coli outer membrane, glycerol has been characterized as a binding element between some lipids and the protein. The

TABLE III
Relative repartition of total fatty acids (moles %) in the native BoTat-1 VSG and in its tryptic C-terminal glycopeptide.

Fatty acid	BoTat-1 VSG	C-terminal glycopeptide
C 16 : 0	38.4	42.4
C 18 : 0	37.5	34.2
C 18 : 1	24.1	23.4

N-terminus consists of S-glycerylcysteine thioether which is an attachment site for one amide-linked fatty acid and for two ester-linked fatty acids (32). Further studies have brought evidence that the glycerol derived from the nonacylated glycerol moiety of phosphatidylglycerol (33, 34) and the fatty acids from the acyl moieties of phospholipids (35).

The fatty acid composition of the C-terminal part of BoTat-1 VSG resembles that of glycerolipids. Furthermore, the presence of phosphate and ethanolamine strongly suggests that the linkages between ethanolamine, phosphate glycerol and fatty acids constitute the core of a phosphatidyl ethanolamine. This hypothesis would suppose a rather particular attachment of the C-terminal oligosaccharide.

ACKNOWLEDGMENTS

This work was supported by grants from the Institut National de la Santé et de la Recherche Médicale C.R.L. N°813022, the U.E.R. de Médecine Université de Lille II, and the Fondation pour la Recherche Médicale Française. We thank for technical assistance : Marie-Chantal MULLET and Claude VANDEPERRE. We also thank Drs G. SEZILLE and S. MOULIN for helpful discussions. The authors are greatly indebted to Pr Raymond PAUTRIZEL, Christiane GIROUD and Dominique BALTZ for the supply of the purified VSG.

REFERENCES

1. Borst, P. and Cross, G.A.M. (1982) Cell 29, 291-303.

2. Englund, P.T., Hajduk, S.L., and Marini, J.C. (1982), Ann. Rev. Biochem. 51, 709-726.

3. Cross, G.A.M. (1975) Parasitology 71, 393-417.

4. Cross, G.A.M. (1977) Ann. Soc. Belge Med. Trop. 57, 389-399.

5. Johnson, J.G. and Cross, G.A.M. (1977) J. Protozool. 24, 587-591.

6. Johnson, J.G. and Cross, G.A.M. (1979) Biochem. J. 178, 689-697.

7. Bridgen, P.J., Cross, G.A.M and Bridgen, J. (1976) Nature 263, 613-614.

8. Allen, G., Gurnett, L.P. and Cross, G.A.M. (1982) J. Mol. Biol. 157, 527-546.

9. Boothroyd, J.C., Paynter, C.A., Coleman, S.L. and Cross, G.A.M. (1982) J. Mol. Biol. 157, 547-556.

10. Boothroyd, J.C., Cross, G.A.M., Hoeijmakers, J.H.J. and Borst P. (1980) Nature 288, 624-626.

11. Boothroyd, J.C., Paynter, C.A., Cross, G.A.M., Bernards, A. and Borst, P. (1981) Nucl. Acids Res. 9, 4735-4743.

12. Majumder, H.K., Boothroyd, J.C. and Weber, H. (1981) Nucl. Acids Res. 9, 4745-4753.

13. Matthyssens, G., Michiels, F., Hamers, R., Pays, E. and Steinert, M. (1981) Nature 293, 230-233.

14. Rice Ficht, A.C., Chen, K.K. and Donelson, J.E. (1981) Nature 294, 53-57.

15. Holder, A.A. and Cross, G.A.M. (1981) Mol. Biochem. Parasitol. 2, 135-150.

16. Holder, A.A. (1983) Biochem. J. 209, 261-262.

17. Baltz, T., Baltz, D. and Pautrizel, R. (1976) Ann. Immunol. (Institut Pasteur) 127C, 761-774.

18. Baltz, T., Baltz, D., Pautrizel, R., Richet, C., Lamblin, G. and Degand, P. (1977) Febs letters 82, 93-96.

19. Duvillier, G., Aubert, J.P., Baltz, T., Richet, C. and Degand, P. (1983) Biochem. Biophys. Res. Comm. 110, 491-498.

20. Baltz, T., Giroud, Ch., Baltz, D., Duvillier, G., Degand, P., Demaille, J. and Pautrizel, R. (1982) EMBO J. 1, 1393-1398.

21. Duvillier, G., Richet, C., Briand, G., Baltz, T. and Degand, P. (1983) Mol. Biochem. Parasitol. in press.

22. Demaille, J., Dautrevaux, M., Havez, R. and Biserte, G. (1965) Bull. Soc. Chim. Fr. 12, 3506-3511.

23. Fauconnet, M. and Rochemont, J. (1978) Anal. Biochem. 91, 403-409.

24. Reinhold, V.N. (1972) Methods Enzymol. 25, 244-249.

25. Ames, B.N. (1966) Methods Enzymol. 8, 115-118.

26. Itaya, K. and Michio, Ui. (1966) Clin. Chim. Acta, 14, 361-366.

27. Renkonen, O. (1962) Biochim. Biophys. Acta, 56, 367-369.

28. Ziegenhorn J. (1975) Clin. Chem. 21, 1627-1629.

29. Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497-509.

30. Hagenfeldt, L. (1966) Clin. Chim. Acta 13, 266-268.

31. Schlesinger, M.J. (1981) Ann. Rev. Biochem. 50, 201-203.

32. Hantke, K. and Braun, V. (1973) Eur. J. Biochem. 34, 284-296.

33. Chattopadhyay, P.K. and Wu, H.C. (1977) Proc. Natl. Acad. Sci. USA 74, 5318-5322.

34. Chattopadhyay, P.K., Lai, J.S., Wu, H.C. (1979) J. Bacteriol 137, 309-312.

35. Lai, J.S., Philbrick, W.M., Wu, H.C. (1980) J. Biol. Chem. 255, 5384-5387.