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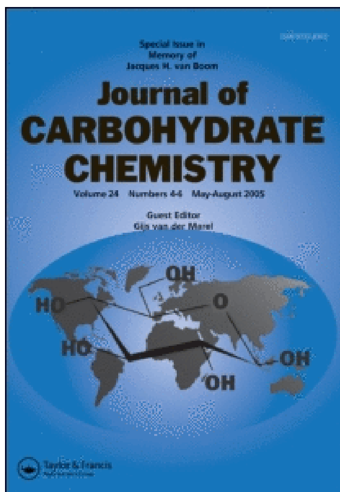

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CHARACTERIZATION OF A GLYCOSYLPHOSPHATIDYLINOSITOL-ANCHOR IN A CELL ADHESION MOLECULE, CSA, FROM *DICTYOSTELIUM DISCOIDEUM* [1]

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ABSTRACT

The cell adhesion molecule, csA, treated with lysyl endopeptidase led to a peptide fragment with a molecular weight of 31 kDa (31-kDa csA). It was shown after the treatment of the 31-kDa csA with phosphatidylinositol-specific phospholipase C and following extraction with chloroform using TLC that the 31-kDa csA contained a component of glycosylphosphatidylinositol (GPI), and it was also confirmed that the cell adhesion molecule, csA, consisted of ceramide instead of diacylglycerol. The results from GC-MS suggested that hydrocarbons of C_{16}- C_{33} interacted with the GPI-anchor region.

INTRODUCTION

The cell adhesion molecule, csA, from *Dictyostelium discoideum* is a glycoprotein with an apparent molecular weight of 80 kDa, including two kinds of carbohydrates: one is carbohydrate I, modified with sulfate,[2] and the other is carbohydrate II, identified using wheat germ agglutinin.[3] The csA glycoprotein is involved in cell adhesion during the aggregation stage,[4] and is anchored to the

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plasma membrane by means of a glycosylphosphatidylinositol (GPI)-linkage, with ceramide instead of diacylglycerol.[5] Stadler *et al.* have reported that the GPI-anchor region of the csA glycoprotein was not cleaved with phosphatidylinositol-specific phospholipase C (PI-PLC). This suggested that the GPI-anchor region of the csA glycoprotein might consist of a unique component or structure.

Since a common mode of membrane attachment via the GPI-anchor was clarified,[6] over one hundred membrane proteins have been found to be anchored to the membrane by a GPI-linkage. The breakdown products of phosphoinositides are produced with the treatment of PI-PLC. This has led to the idea that the cleavage of the GPI-anchor might be a part of receptor-mediated triggering reactions.[7] However, the role of the GPI- anchor in signal transduction remains unclear.

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The properties of the GPI-anchor in the csA glycoprotein were initially investigated, in order to clarify the relationship between the cell adhesion activity and the signaling mechanism by phosphatidylinositol produced with PI-PLC. In this paper, we report the properties of the GPI-anchor in the csA glycoprotein.

RESULTS AND DISCUSSION

After treating the 31-kDa csA with PI-PLC, a sample extracted with chloroform was resolved using thin-layer chromatography (TLC) to identify the lipid components in the GPI-anchor of the 31-kDa csA. Three spots appeared on the TLC plate (Figure 2). Of them, the middle spot was identified as ceramide. This reconfirmed the fact that the GPI-anchor consisted of ceramide instead of diacylglycerol.[5] Ceramide-based anchors have been found in *Saccharomyces cerevisiae*, *Tetrahymena pyriformis* and *Trypanosoma cruzi*.[8-10] In *S. cerevisiae*, the Golgi lipid-exchange enzyme converts diacylglycerol-based anchors to ceramide-based anchors during transportation from the endoplasmic reticulum (ER) to the Golgi apparatus.[11] The significance of the lipid-exchange is unknown. Recently, it has been reported that ceramides might function as a second messenger in various biological

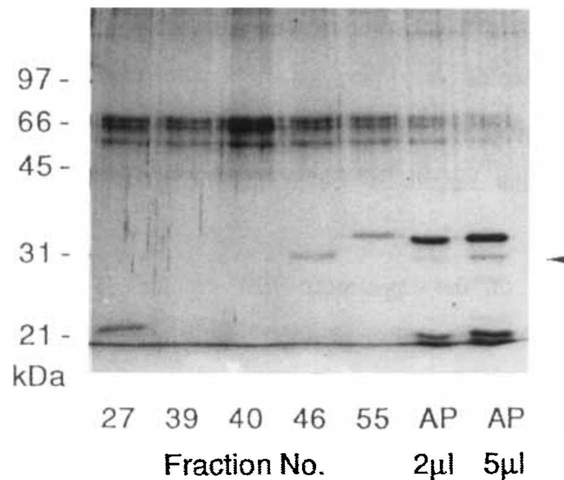

Figure 1 . Isolation of the 31-kDa csA by preparative electrophoresis system. Arrow head indicates the position of the 31-kDa csA. AP is the sample before application to preparative electrophoresis system. AP 2 μL corresponds to 0.2 μg and AP 5 μL to 0.5 μg in the amount of protein. Fraction No. shows the number of fractions eluted from the preparative electrophoresis system. A few observed bands, at molecular weights of 44-70 kDa, were evaluated as artificial; this is because they were observed even though no sample was applied.

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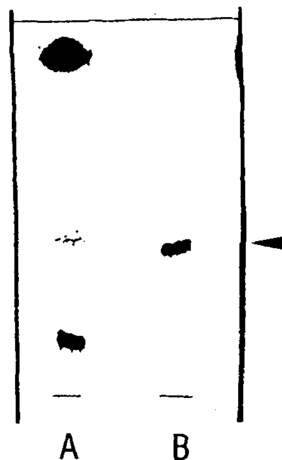

Figure 2 . Identification of lipid components in the 31-kDa csA by TLC.
A: chloroform extracts of PI-PLC treated 31-kDa csA. B: a standard of ceramide. Development and staining were carried out as described in the Experimental.

phenomena including programed cell death.[12] In ceramide-based anchors cleaved with PI-PLC, the free ceramide may play an important role in the signal-transduction system. Despite a number of reports demonstrating biological activity for ceramide *in vitro*, the direct cellular targets which mediate ceramide activity have yet to be elucidated.[12,13]

The contents of the uppermost spot on the TLC plate (Figure 2) were extracted with chloroform and subsequently subjected to gas chromatography-mass spectroscopy (GC-MS). The uppermost spot was detected irrespective of PI-PLC treatment (data not shown). The results from the GC-MS indicated the existence of hydrocarbons of C_{16}-C_{33} (Figure 3A). The highest peak in the GC-MS spectrum was identified as an unsaturated hydrocarbon of $C_{28}H_{56}$ (Figure 3B). These results suggest that hydrocarbons interact with the GPI-anchor region of the csA glycoprotein through hydrophobic bonds. This is the first known paper to report the interaction between a hydrocarbon and a GPI-anchor region. It is unlikely that these hydrocarbons are derived from fatty acids. Treatment to release hydrocarbons from fatty acids was not applied in the preparation stage before the sample was subjected to TLC. It is also unlikely that the csA glycoprotein was contaminated with hydrocarbons, because purified csA glycoprotein was used as the starting material. In addition, an isolation step of the 31-kDa csA was carried out. There is evidence to suggest that GPI-anchor proteins interact with cholesterol in the caveola which is involved in endocytosis.[14] It is necessary to investigate the role of hydrocarbons in the GPI-anchor region.

EXPERIMENTAL

Purification of the csA glycoprotein and the 31-kDa csA.

Purified csA glycoprotein was prepared as described in a previous paper.[15] Purified csA glycoprotein shows up as a single band when stained using SDS-polyacrylamide gels with silver according to Oakley *et al.*[16] Purified csA glycoprotein was suspended in 0.1% SDS, after which lysyl endopeptidase (Wako

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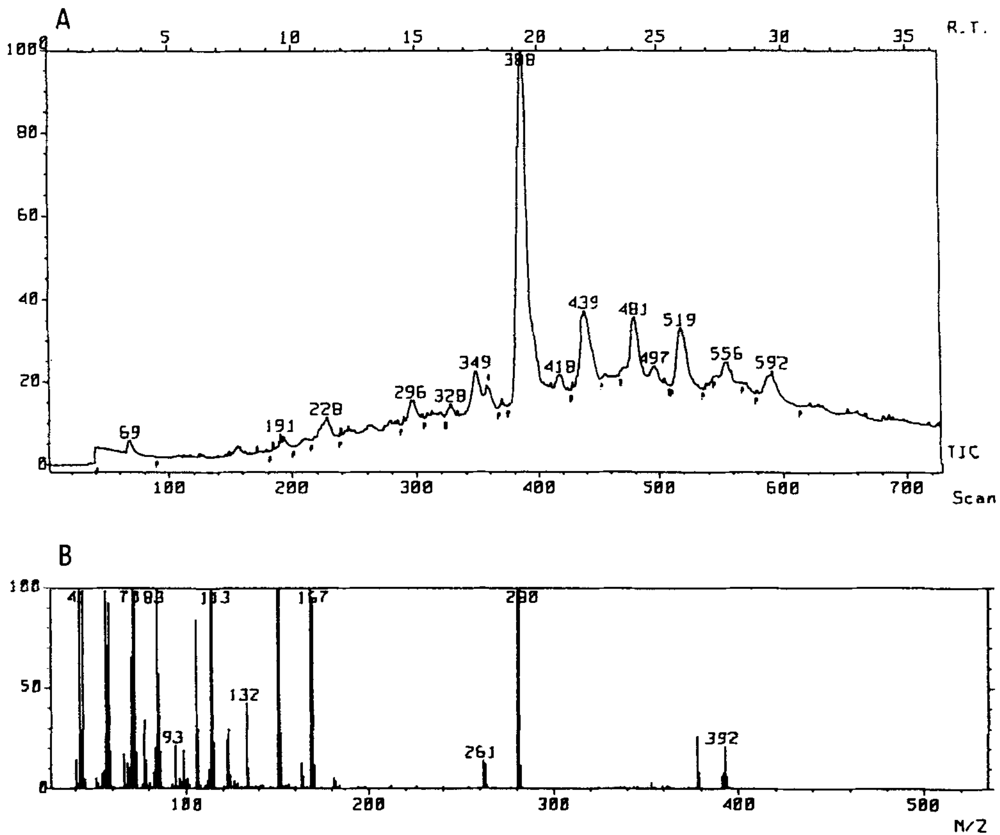

Figure 3. GC-MS of the uppermost spot in TLC.
Abscissa, scan number in A; mass number in B. *Ordinate*, peak intensity (relative abundance). A: total ion chromatogram of the uppermost spot in TLC (Figure 2). B: mass spectrum of scan number 388 (highest peak) in A.

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pure chemical) was added until the enzyme to substrate ratio (w/w) was 1/100 to 1/500. The reaction was carried out at 37 °C for 16 h in 0.01 M phosphate buffer, pH 7.8. The samples treated with lysyl endopeptidase were applied to a preparative electrophoresis system (biophoresis III, Atto). The samples were eluted with 0.37 M Tris-HCl buffer, pH 8.8 containing 5% glycerol, and collected at 0.5 mL per fraction. The 31-kDa csA showed a single band of staining in the SDS-polyacrylamide gels with silver (Figure 1). Fractions containing the 31-kDa csA were pooled, precipitated using acetone and dried under reduced pressure.

Preparation of lipid components from the GPI-anchor. The acetone-dried sample of the 31-kDa csA was suspended in 800 μL of 0.1% deoxycholate and a reaction with PI-PLC from *Bacillius cereus* (a final conc. of 10 unit/mL) was carried out at 37 ℃ for 20 h in 0.05 M Tris-HCl buffer, pH 7.5. The sample treated with PI-PLC was extracted three times with chloroform. The chloroform phase was removed by the processes of centrifugation, pooling, and evaporation under reduced pressure. The sample was kept overnight in a desiccator and resolved using TLC. TLC was developed in chloroform/methanol (9:1, v/v). TLC plates were sprayed with orcinol/H_2SO_4, and heated at 110 ℃. The sample was dissolved in 0.5 mL of pyridine using vortexing. For trimethysilylation, 0.1 mL of hexamethyldisilazane and 0.05 mL of trimethylchlorosilane were added to the sample with vortexing and the sample was kept at 65 ℃ for 5 min. After cooling, 3.0 mL of chloroform was added and washed four times with 2.0 mL of deionized water. The lower phase was removed after centrifugation and evaporation under reduced pressure. The dried sample was dissolved in chloroform and subjected to GC-MS.

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REFERENCES

1. Presented at the *XVIII th International Carbohydrate Symposium*, Milan, Italy, July 21-26, 1996.
2. J. Stadler, G. Gerisch, G. Bauer, C. Suchanek and W. B. Huttner, *EMBO J.*, **2**, 1137 (1983).
3. M. Yoshida, J. Stadler, G. Berthold and G. Gerisch, *EMBO J.*, **3**, 2663 (1984).

4. H. Beug, F. E. Katz and G. Gerisch, *J. Cell Biol.*, **56**, 647 (1973).
5. J. Stadler, T. W. Keenan, G. Bauer and G. Gerisch, *EMBO J.*, **8**, 371 (1989).
6. M. A. Ferguson and A. F. Williams, *Annu. Rev. Biochem.*, **57**, 285 (1988).
7. M. G. Low, *Biochim. Biophys. Acta*, **988**, 427 (1989).
8. A. Conzelmann, H. Riezman, C. Desponds and C. Bron, *EMBO J.*, **7**, 2333 (1988).
9. K. Kaya, C. S. Ramesha and G. A. Thompson, Jr., *J. Lipid Res.*, **25**, 68 (1984).
10. R. M. de Lederkremer, C. Lima, M. I. Ramirez and O. L. Casal, *Eur. J. Biochem.*, **192**, 337 (1990).
11. A. Conzelmann, A. Puoti, R. L. Lester and C. Desponds, *EMBO J.*, **11**, 457 (1992).
12. Y. A. Hannun, *J. Biol. Chem.*, **269**, 3125 (1994).
13. International Symposium on Sphingolipid Function, Seattle, USA, August 20-26, 1995.
14. K. G. Rothberg, J. E. Heuser, W. C. Donzell, Y. Ying, J. R. Glenney and R. G. W. Anderson, *Cell*, **68**, 673 (1992).
15. M. Yoshida, *J. Biochem.*, **101**, 1233 (1987).
16. B. R. Oakley, D. R. Kirsch and N. R. Morris., *Anal. Biochem.*, **105**, 361 (1980).

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