Carbohydrate Research, 178 (1988) 23–28
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

BIOSYNTHESIS OF CANCER-ASSOCIATED SIALYL-X ANTIGEN BY A (1→3)-α-L-FUCOSYLTRANSFERASE OF HUMAN AMNIOTIC FLUID*

FRANZ-GEORG HANISCH, ARISTOTELIS MITSAKOS, HORST SCHROTEN, AND GERHARD UHLENBRUCK
Abteilung für Immunbiologie, 1. Medizinische Universitätsklinik (Direktor: Prof. Dr. V. Diehl), Kerpener Straße 15 D-5000 Köln 41 (Federal Republik of Germany)

(Received July 30th, 1986; accepted for publication, September 29th, 1986)

ABSTRACT

Activity of a hitherto unknown (1→3)-α-L-fucosyltransferase that acts on IV^3-α-NeuAc-nLcOseCer as acceptor substrate was demonstrated in human amniotic fluid. The ^{14}C-labelled product IV^3-α-NeuAc-III^3-α-Fuc-nLcOseCer was detected autoradiographically after t.l.c. and identified after desialylation by immunostaining with monoclonal antibody Leu M1. The enzyme is assumed also to catalyze the last step in the biosynthesis of sialyl-X antigen carried by mucins in human amniotic fluid.

INTRODUCTION

Since introduction of the hybridoma technique, evidence is accumulating for sialylated derivatives of blood group determinants (sialyl-Le^a, sialyl-X) being expressed in association with various human tumors[1,2]. Biosynthesis of these carbohydrate antigens requires the existence of variant glycosyltransferases having broader substrate specificities than those of the known enzymes from nonmalignant tissue. The (1→4)- and (1→3)-α-L-fucosyltransferases isolated from human milk[3] or saliva[4], for instance, are assumed not to use carbohydrates having a terminal (2→3)-linked sialyl group as acceptor substrates. Correspondingly, the known sialyltransferases from various sources do not act on β-D-Gal*p*-(1→3 or 4)-[α-L-Fuc-(1→4 or 3)]-D-GlcNAc termini[5,6]. The biosynthesis of the sialyl-Le^a antigen has been established to proceed *via* sequential sialylation and fucosylation, the latter step being catalyzed by a variant Le-gene-coded (1→4)-α-L-fucosyltransferase in colon carcinoma cells SW 1116 (ref. 7). Similarly, experimental evidence has been obtained for the biosynthesis of the isomeric sialyl-X antigen in human lung carcinoma cells, which has been demonstrated also to proceed *via* sialylation of Type 2 chain, followed by (1→3)-α-L-fucosylation[8,9].

The respective fucosyltransferase would be expressed not only in tumor cells, but also in normal cells like human granulocytes[10] or epithelial cells of the prostatic

*Dedicated to Professor Walter T. J. Morgan.

0008-6215/88/$ 03.50

gland[11] and amnion[12], as judged from chemical carbohydrate analyses of membrane-bound *N*-glycans[10] or secreted mucins[11,12]. We now report on the biosynthesis of sialyl-X antigen by an (1→3)-α-L-fucosyltransferase in human amniotic fluid.

EXPERIMENTAL

Materials. — Monoclonal antibody Leu M1 was purchased from Becton Dickinson, (Mtn. View, U.S.A.). IV^3-α-NeuAc-nLcOseCer was isolated from membranes of human erythrocytes[13] and purified by t.l.c.[14] using the solvent system cited below. GDP-L-[^{14}C]fucose (3.7–7.4 GBq/mmol) was from Amersham Buchler (Braunschweig, FRG), anti mouse-IgG-enzyme conjugates, *p*-nitrophenyl phosphate, and 3,3′,4,4′-diaminobenzidine from Sigma (München, FRG). IV^3-α-NeuAc-III^3-α-Fuc-nLcOseCer was prepared from human kidneys according to Mansson *et al.*[15]. Glycosylalditol FW3 was isolated from human amniotic mucin as described by Hanisch *et al.*[12] and its structure was established as α-NeuAc*p*-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→6)-[α-NeuAc*p*-(2→3)-β-D-Gal*p*-(1→3)]-D-GalNAcol[16].

Enzyme assay. — The reaction mixture of the biosynthetic assay contained ten-fold concentrated human amniotic fluid (200 μL), pure glycolipid (100 μg), and GDP-L-[^{14}C]fucose (18.5 kBq) in Tris·HCl buffer (25mM), pH 7.5, with 0.1% Triton X-100, 10mM NaN_3, 7.5mM $MgCl_2$, and 10mM ATP. Aliquots (40 μL) were withdrawn at various time intervals, dried in a desiccator, and extracted with 1:1 (v/v) chloroform–methanol (200 μL). The extracts were chromatographed on Silica Gel 60-coated t.l.c. plates (Merck, Darmstadt, FRG) in 60:40:9 (v/v) chloroform–methanol–0.02% $CaCl_2$ as solvent system. After development, the plates were dried and analyzed chemically with the orcinol reagent, or autoradiographically on X-Omat X-ray film (Eastman Kodak, Heidelberg, FRG) with exposure times of several days at −20°. Enzyme immunostaining of the reaction product on Bakerflex IB 2-plates (Baker Chemicals, Gross Gerau, FRG) was performed according to Magnani *et al.*[17], as modified by Higashi *et al.*[18]. Prior to enzyme immunostaining, the plates were sprayed and soaked with 50mM acetate buffer and 9mM $CaCl_2$, pH 5.5, and incubated for 1 h at 37° with *Vibrio cholerae* sialidase (Behringwerke AG, Marburg, FRG) in the same buffer (10 mL). Assays with the glycosylalditol FW3 as substrate were performed accordingly, except that the reaction mixture was spotted directly onto t.l.c. plates after the protein had been precipitated in 50% methanol. The plate was developed with 100:30:10:10:3 (v/v) ethanol–water–butanol–pyridine–acetic acid, and analyzed by autoradiography.

Quantitative analysis of sialyl-X synthesis was performed similarly by enzyme immunoassay on Immulon plates (Dynatech, Plochingen, FRG) according to Voller *et al.*[19] or, alternatively, by radio-immunoassay.

The time course of enzyme activity was measured for 100 μg of IV^3-α-NeuAc-nLcOseCer and 9 kBq of GDP-L-[^{14}C]fucose in a total volume of 100 μL. Aliquots (10 μL) were withdrawn at various time intervals from 0 to 24 h, mixed with an

equal volume of methanol and dried. After extraction with 1:1 (v/v) chloroform–methanol, residual GDP-L-[^{14}C]fucose was separated by paper chromatography with water as solvent. The start zones were cut and the radioactivity was measured in Bray solution (10 mL) with a β-scintillation counter. Activity is expressed in units/mL (one unit corresponding to one pmol/h).

RESULTS AND DISCUSSION

Analysis of (1→3)-α-L-fucosyltransferase activity on sialylparagloboside (IV^3-α-NeuAc-nLcOseCer) in human amniotic fluid revealed formation of one major reaction product (Fig. 1). As demonstrated with a substrate-free control assay (Fig. 1, lane b) the ^{14}C-labelled product detected by autoradiography on t.l.c. plates was a derivative of the exogenous substrate with $R_{\text{sialylparagloboside}}$ 0.83. The compound comigrated with authentic IV^3-α-NeuAc-III^3-α-Fuc-nLcOseCer isolated from human kidneys (Fig. 1, lane f), and was identified by immunostaining with X-hapten-specific Leu M1 antibody after sialidase treatment (Fig. 1, lanes d, e).

Within the variability of the assay, the enzyme source and the specific radioactivity of the cosubstrate activity of (1→3)-α-L-fucosyltransferase was measured for sialylparagloboside as substrate to be 2–4 units/mL. The time course of the reaction process is shown in Fig. 2.

Results obtained with the amniotic mucin-derived glycosylalditol FW3 as substrate (Fig. 1, lane g) suggested that the same enzyme responsible for formation of

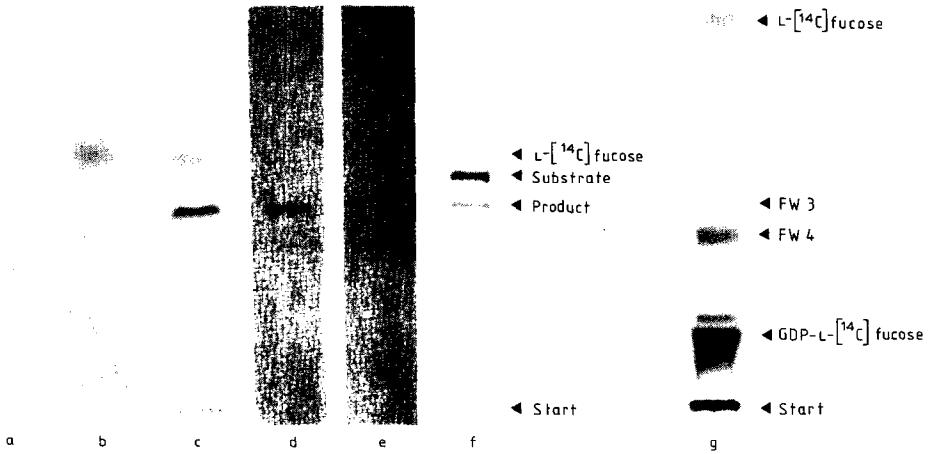

Fig. 1. T.l.c. analysis of products formed by (1→3)-α-L-fucosyltransferase from human amniotic fluid. Lanes a–c: Autoradiograms of ^{14}C-labelled reaction products; aliquots of enzymic assay with VI^3-α-NeuAc-nLcOseCer as substrate taken at time 0 (a) or 24 h (b, c); reaction products formed in the absence (b) or presence (c) of exogenous substrate. Lanes d, e: Enzyme-immunostain with Leu M1 antibody of sialidase-treated reaction products formed during 24 h (d) or at time 0 (e) in the presence of IV^3-α-NeuAc-nLcOseCer. Lane f: IV^3-NeuAc-III^3-α-FucnLcOseCer and IV^3-α-NeuAc-nLcOseCer visualized chemically with the orcinol reagent. Lane g: Autoradiogram of (1→3)-α-L-fucosyltransferase assay using oligosaccharide alditol FW3 as substrate.

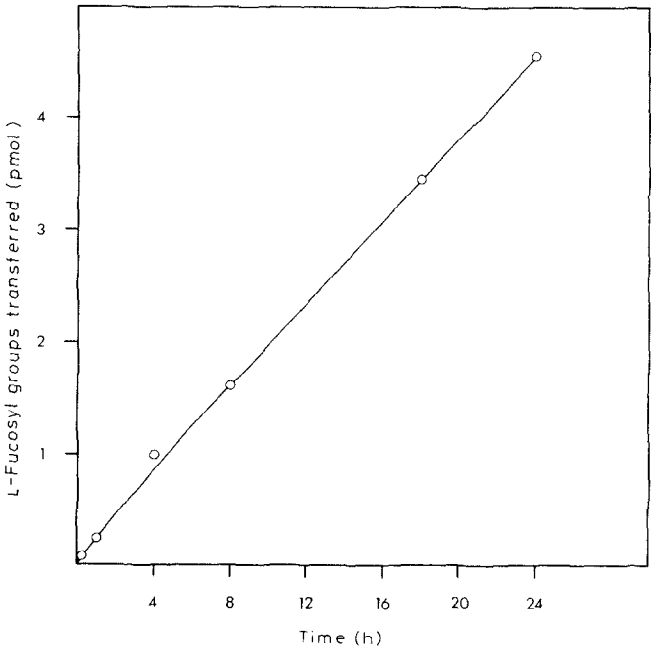

Fig. 2. Time course of the reaction process at 37°. Conditions were the same as described in the Experimental section.

β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→R or R′)

↓ CMP-NeuAc

α-D-NeuAc*p*-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→R or R′)

↓ GDP-Fuc

α-D-NeuAc*p*-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→R or R′)

3

↑

1

α-L-Fuc*p*

R = →3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-Cer

R′ = →6)-α-D-Gal*p*NAc*p*-Ser (Thr)

3

↑

1

α-NeuAc*p*-(2→3)-β-D-Gal*p*

Scheme 1. Postulated pathway for the biosynthesis of sialyl-X determinant carried by a ganglioside (R) from human kidney[20] or mucins (R′) from amniotic fluid[12].

the sialyl-X antigen carried by glycosphingolipids is also involved in the biosynthesis of mucin-carried oligosaccharide FW4, characterized previously to express this antigenic determinant[12]. These results indicated the existence in human amniotic fluid of an (1→3)-α-L-fucosyltransferase that is able to transfer an α-L-fucosyl group to O-3 of a subterminal 2-acetamido-2-deoxy-D-glucose unit in the acceptor sequence α-NeuAc*p*-(2→3)-β-D-Gal*p*-(1→4)-D-GlcNAc. An enzyme having a similar or identical substrate and reaction specificity has recently been described to occur in human lung carcinoma PC 9 cells[9]. Although the alternative reaction sequence cannot be excluded for the amniotic antigen, the available results imply that biosynthesis of sialyl-X determinants carried by Type 2-chain glycosphingolipids in lung cancer cells or mucins of amniotic fluid presumably follows the same sequence of glycosylation. Thus, sialylation of the respective precursor sugar chain precedes fucosylation by a cancer-associated or organ characteristic (1→3)-α-L-fucosyltransferase (Scheme 1).

It is questionable, however, whether these enzymes represent isoenzymes or variants of the known (1→3)-α-L-fucosyltransferases with distinct structural and functional properties. Hence, it is noteworthy that the purified (1→3)-α- and (1→3/4)-α-L-fucosyltransferases from human plasma and milk have been demonstrated to utilize 3'-sialyllactosamine as acceptor[21]. This finding may be explained by a less restricted substrate specificity of the respective enzymes or, alternatively, by a possible molecular heterogeneity of the enzyme preparation, assuming copurification of iso-enzymes with different acceptor specificities. Preliminary results on the isolation and partial characterization of the respective (1→2)-α-L-fucosyltransferase in human amniotic fluid indicate that a similar enzyme may exist also in human granulocytes and human Hodgkin cell lines[22].

ACKNOWLEDGMENTS

This investigation was supported by the Deutsche Forschungsgemeinschaft grant Uh 8/14-2. The authors are obliged to Prof. Dr. A. Bolte from the University Clinic of Gynecology and Obstetrics, Cologne, for kindly providing the samples of human amniotic fluid. The presented work is part of the thesis performed by A. Mitsakos.

REFERENCES

1 J. L. MAGNANI, B. NILSSON, M. BROCKHAUS, D. ZOPF, Z. STEPLEWSKI, H. KOPROWSKI, AND V. GINSBURG, *J. Biol. Chem.*, 257 (1982) 14 365–14 369.
2 K. FUKUSHIMA, M. HIROTA, P. J. TERASAKI, A. WAKISAKA, H. TOGASHI, D. CHIA, N. SUYAMA, Y. FUKUSHI, E. NUDELMAN, AND S. HAKOMORI, *Cancer Res.*, 44 (1984) 5279–5285.
3 J. P. PRIEELS, D. MONNOM, M. DOLMANS, T. BEYER, AND R. L. HILL, *J. Biol. Chem.*, 256 (1981) 10 456–10 463.
4 P. M. JOHNSON, A. D. YATES, AND W. M. WATKINS, *Biochem. Biophys. Res. Commun.*, 100 (1981) 1611–1618.
5 J. WEINSTEIN, U. DE SOUZA-E-SILVA, AND J. C. PAULSON, *J. Biol. Chem.*, 257 (1982) 13 845–13 853.
6 R. KORNFELD AND S. KORNFELD, *Annu. Rev. Biochem.*, 54 (1985) 631–664.

7 G. C. HANSSON AND D. ZOPF, *J. Biol. Chem.*, 260 (1985) 9388–9392.
8 S. HAKOMORI, *Cancer Res.*, 45 (1985) 2405–2414.
9 E. H. HOLMES, G. K. OSTRANDER, AND S. I. HAKOMORI, *J. Biol. Chem.*, 261 (1986) 3737–3743.
10 M. FUKUDA, E. SPOONCER, J. E. OATES, A. DELL, AND J. C. KLOCK, *J. Biol. Chem.*, 259 (1984) 10 925–10 935.
11 F.-G. HANISCH, H. EGGE, J. PETER-KATALINIC, G. UHLENBRUCK, C. DIENST, AND R. FANGMANN, *Eur. J. Biochem.*, 152 (1985) 343–351.
12 F.-G. HANISCH, H. EGGE, J. PETER-KATALINIC, AND G. UHLENBRUCK, *FEBS Lett.*, 200 (1986) 42–46.
13 J. R. WHERRETT AND B. L. BROWN, *Neurology*, 19 (1969) 489–493.
14 H. KUBO AND M. HOSHI, *J. Lipid Res.*, 26 (1985) 638–641.
15 J. MANSSON, P. FREDMAN, O. NILSSON, L. LINDHOLM, J. HOLMGREN, AND L. SVENNERHOLM, *Biochim. Biophys. Acta*, 834 (1985) 110–117.
16 F. G. HANISCH, G. UHLENBRUCK, J. PETER-KATALINIC, AND H. EGGE, unpublished results.
17 J. L. MAGNANI, D. F. SMITH, AND V. GINSBURG, *Anal. Biochem.*, 109 (1980) 399–402.
18 H. HIGASHI, Y. FUKUI, S. UEDA, S. KATO, Y. HIRABAYASHI, M. MATSUMOTO, AND M. NAIKI, *J. Biochem. (Tokyo)*, 95 (1984) 1517–1520.
19 A. VOLLER, D. BIDWELL, AND A. BARTLETT, in M. P. ROSE AND H. FRIEDMANN (Eds.), *Manual of Clinical Immunology*, American Society of Microbiology, Washington DC, 1976, pp. 506–512.
20 H. RAUVALA, *J. Biol. Chem.*, 251 (1976) 7517–7520.
21 P. H. JOHNSON AND W. M. WATKINS, *Biochem. Soc. Trans.* 13 (1985) 1119–1120.