Delineation of Fucosyltransferase Activities with Thiol Reagents

By David KESSEL and Ta-Hsu CHOU

Department of Oncology, Wayne State University School of Medicine,
Harper-Grace Hospitals, 3990 John R. Street, Detroit, MI 48201, U.S.A.

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The thiol reagent dithiothreitol inhibits the activity of a core GDP-fucose-*N*-acetylglucosaminide α -6-fucosyltransferase in plasma and blood-cell homogenates, while promoting the activity of α -2- and α -3-fucosyltransferases. The latter enzymes catalyse transfer of fucose on to terminal galactose and subterminal *N*-acetylglucosamine residues respectively. A thiol-blocking reagent *N*-ethylmaleimide does not affect the activity of the α -6-fucosyltransferase, but inhibits the other two enzymes. These results indicate the presence of a critical disulphide linkage in the α -6-fucosyltransferase, and provide a means of delineation of different fucosyltransferases.

At least three fucosyltransferases have been detected in human plasma or serum and these are also found in marrow and mature blood cells. An α -2-fucosyltransferase, specified by the H gene, catalyses transfer of fucose on to galactose residues of an appropriate acceptor (Bosmann *et al.*, 1968; Schenkel-Brunner *et al.*, 1972; Munro & Schachter, 1973; Pacuszka & Kosielak, 1974; Chester *et al.*, 1976; Mulet *et al.*, 1977; Schachter & Tilley, 1978). An α -3-fucosyltransferase mediates formation of a Fuc α 1 $\rightarrow$ 3GlcNac linkage involving an *N*-acetylglucosamine residue subterminal to galactose (Schenkel-Brunner *et al.*, 1972; Watkins, 1978). Most investigators have measured α -2- and α -3-fucosyltransferase simultaneously by using an acceptor with Gal α 1 $\rightarrow$ 4GlcNac configuration, e.g. *N*-acetyl-lactosamine (Schenkel-Brunner *et al.*, 1972; Munro & Schachter, 1973) or a desialylated high-molecular-weight glycoprotein, e.g. fetuin or α ₁-acid glycoprotein (Bosmann *et al.*, 1968; Bella & Kim, 1971; Munro & Schachter, 1973; Munro *et al.*, 1975; Kessel & Chou, 1976; Jabbal & Schachter, 1977; Chatterjee & Kim, 1978). Phenyl β -galactoside was identified as a specific acceptor for α -2-fucosyltransferase activity (Chester *et al.*, 1976). A third plasma enzyme requires an acceptor with a terminal *N*-acetylglucosamine residue (Munro & Schachter, 1973). This enzyme has also been found in tissues and blood cells (Bosmann *et al.*, 1968; Bella & Kim, 1971; Kessel & Chou, 1976; Jabbal & Schachter, 1977; Chou *et al.*, 1977; Chatterjee & Kim, 1978) and was found to catalyse transfer of fucose on to the 6'-position of an asparagine-linked *N*-acetylglucosamine residue in the glycoprotein core (Wilson *et al.*, 1976).

We reported preferential inhibition of α -2- and α -3-fucosyltransferases of plasma by the thiol-

blocking reagent *N*-ethylmaleimide; activity of the α -6-fucosyltransferase was unaffected (Chou *et al.*, 1977). This result indicated a method for delineation of *N*-ethylmaleimide-sensitive and -resistant fucosyltransferases in plasma, even in the presence of endogenous acceptors (Kessel *et al.*, 1978; Khilanani *et al.*, 1977, 1978). However, the α -2- and α -3-fucosyltransferases in blood-cell and marrow homogenates were found to be relatively resistant to *N*-ethylmaleimide. This study was initiated to determine whether other thiol reagents might affect the fucosyltransferase activities in such homogenates. The results also provide information on the nature of disulphide and thiol groups in the different enzymes.

Materials and Methods

GDP-L-[¹⁴C]fucose (175 Ci/mol) was purchased from Amersham/Searle, Arlington Heights, IL, U.S.A. and New England Nuclear Corp., Boston, MA, U.S.A. Acceptors were obtained from Calbiochem Corp., Los Angeles, CA, U.S.A. Terminal sialic acid and subterminal galactose were removed from the glycoproteins as described by Spiro (1964). Blood was anti-coagulated with EDTA, erythrocytes and platelets were removed by centrifugation, and the plasmas were stored at -70°C . Platelets from normal donors, lymphocytes from patients with chronic lymphocytic leukaemia, and peripheral and marrow myeloblasts from patients with acute myelogenous leukaemias were homogenized in 4 vol. of 0.05 M-Hepes buffer, pH 7.0, containing 0.1% Triton X-100.

Plasma fucosyltransferases were measured as described by Chou *et al.* (1977). In some experiments, 20 mM-dithiothreitol or 5 mM-*N*-ethylmaleimide was added to incubation mixtures. Enzyme activities in homogenates were measured in a 200 μ l volume

Abbreviation used: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

containing 25 μ l of homogenate, 0.1% Triton X-100, 20 mM-Hepes, pH 7.0, 10 mM-MgCl₂, 1 μ M-labelled GDP-fucose (50000 d.p.m.), 0.5 mg of fetuin-derived acceptor (omitted where indicated) and (as indicated) 5 mM-thiol-blocking reagent. After 60 min at 37°C, incubations were terminated by addition of 0.5%

phosphotungstic acid in 0.5 M-HCl; the precipitates were washed with 10% (w/v) trichloroacetic acid, containing 2 M-NaCl (Hoflack *et al.*, 1978) and ethanol/diethyl ether (1:1, v/v); radioactivity was then measured by liquid-scintillation counting, with Aquasol (New England Nuclear Corp.) as scintillant.

Table 1. *Effects of thiol reagents on blood-cell fucosyltransferase activity*

Homogenates of lymphocytes (from a patient with chronic lymphocytic leukaemia), myeloblasts (from a patient with acute myelogenous leukaemia) or platelets (from a normal donor) were incubated as described in the text with the indicated acceptor and, as specified, thiol reagent (5 mM). The data describe incorporation of [¹⁴C]fucose from a GDP-fucose donor into acceptor in terms of d.p.m./h per mg of cell protein. Enzyme activity was calculated as described in the text. The data represent the average of five determinations, and were reproducible to $\pm 5\%$.

Cell type	Thiol reagent	Fucosyltransferase activity				
		Acceptor added			Net activity	
		None	Asialo-fetuin	Asialo-agalactofetuin	α -2/3	α -6
Lymphocyte	None	505	655	2610	245	2505
	N-Ethylmaleimide	495	510	2590		
	Dithiothreitol	95	245	105		
Myeloblast	None	495	1245	2055	1180	1615
	N-Ethylmaleimide	500	1260	2040		
	Dithiothreitol	455	1180	440		
Platelet	None	345	410	18300	340	18050
	N-Ethylmaleimide	355	400	12805		
	Dithiothreitol	305	340	250		

Table 2. *Effects of thiol reagents on plasma fucosyltransferase activities*

Plasmas were incubated and enzyme activities calculated as described in the text. Values represent incorporation of radioactive fucose into the specified acceptor from a GDP-[¹⁴C]fucose donor in the presence of 5 mM-N-ethylmaleimide or 20 mM-dithiothreitol. Units are d.p.m./h per 50 μ l of plasma. The data represent averages of three determinations, and were reproducible to $\pm 5\%$.

Donor status	Thiol reagent	Fucosyltransferase activity of plasma				
		Acceptor added			Net activity	
		None	Asialo-fetuin	Asialo-agalactofetuin	α -2/3	α -6
Normal	None	145	310	340	265	250
	N-Ethylmaleimide	50	45	255		
	Dithiothreitol	90	265	90		
Normal ('Bombay' blood group)	None	20	30	700	15	685
	N-Ethylmaleimide	13	25	685		
	Dithiothreitol	10	15	15		
Acute myelogenous leukaemia (90% marrow blasts)	None	1495	2095	1490	2010	50
	Dithiothreitol	1405	2010	1440		
Acute myelogenous leukaemia (stored 18 months)	None		920			
	N-Ethylmaleimide		85			
	Dithiothreitol		1680			
Acute myelogenous leukaemia (remission)	None	195	295	1800	250	1650
	N-Ethylmaleimide	45	45	1650		
	Dithiothreitol	150	250	150		

Proteins were measured as described by Lowry *et al.* (1951), with bovine serum albumin as standard.

Results and Discussion

The data shown in Table 1 provide an indication of the types and activities of fucosyltransferase in blood-cell homogenates. In all cases, endogenous acceptor activity was found to be present. Addition of the asialo-fetuin acceptor (terminal *N*-acetylglucosamine) strongly promoted transfer of fucose in the lymphocyte and platelet homogenates, indicating substantial α -6-fucosyltransferase activity in these preparations. In the myeloblast homogenate, fucose incorporation was stimulated by both asialoagalactofetuin and asialo-fetuin acceptors, indicating activity of both α -6- and α -2/3-fucosyltransferases. In contrast with results obtained with plasma, the activity detected with the asialo-fetuin acceptor was not inhibited by *N*-ethylmaleimide, but addition of dithiothreitol abolished α -6-fucosyltransferase in both plasma and cell homogenates.

We interpret the data shown in Table 1 to indicate the presence of endogenous acceptors of α -6-fucosyltransferase activity in the lymphocyte homogenate, but not in the other homogenates. With appropriate acceptors we can estimate total α -2/3-fucosyltransferase activity (asialo-fetuin acceptor, dithiothreitol present) and α -6-fucosyltransferase activity (asialoagalactofetuin acceptor, in the presence or absence of dithiothreitol).

Results obtained with different plasmas are shown in Table 2. Dithiothreitol (20mM) inhibited the α -6-fucosyltransferase activity in plasma from a 'Bombay'-blood-group donor and from an acute-leukaemia patient in drug-induced remission. Plasmas from such patients contain elevated α -6-fucosyltransferase activity (Khilanani *et al.*, 1978). The α -2/3-fucosyltransferase activity in plasma of an acute-myelogenous-leukaemia patient with more than 90% marrow myeloblasts was not affected by dithiothreitol. Table 2 also shows the striking inhibition of plasma α -2/3-fucosyltransferase activity by *N*-ethylmaleimide. This inhibitor does not affect the corresponding enzyme activity in blood-cell homogenates. *N*-Ethylmaleimide inhibits α -2-ectofucosyltransferase activity on the surface of the rat lymphocyte (Hoflack *et al.*, 1978). The ecto-enzyme therefore resembles the corresponding enzyme in plasma, but not in the lymphocyte homogenate, in *N*-ethylmaleimide sensitivity.

In another series of experiments, we found the rate of incorporation of fucose on to the different acceptors to be a linear function of incubation time, and of the amount of enzyme protein added. Liberation of fucose and fucose 1-phosphate, monitored by chromatography (Hoflack *et al.*, 1978), indicated less than 15% substrate degradation by plasma and homogenates during incubation.

The present study indicates the presence of an important disulphide linkage in the α -6-fucosyltransferase of cells and plasma that is sensitive to reduction (Cleland, 1964) by dithiothreitol, whereupon activity is lost. Moreover, the *N*-ethylmaleimide-sensitive α -2/3-fucosyltransferase activity of a plasma sample that had decreased during 18 months of storage at low temperature was restored by dithiothreitol (Table 2). This finding, together with the sensitivity of the α -2/3 enzymes to *N*-ethylmaleimide, indicates that free thiol groups are essential for their activity.

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References

- Bella, A., Jr. & Kim, Y. S. (1971) *Arch. Biochem. Biophys.* **147**, 753-761
- Bosmann, H. B., Hagopian, A. & Eylar, E. H. (1968) *Arch. Biochem. Biophys.* **128**, 470-481
- Chatterjee, S. K. & Kim, U. (1978) *J. Natl. Cancer Inst.* **61**, 151-162
- Chester, M. A., Yates, A. D. & Watkins, W. M. (1976) *Eur. J. Biochem.* **69**, 583-592
- Chou, T. H., Murphy, C. & Kessel, D. (1977) *Biochem. Biophys. Res. Commun.* **74**, 1001-1006
- Cleland, W. W. (1964) *Biochemistry* **3**, 480-482
- Hoflack, B., Cacan, R. & Verbert, A. (1978) *Eur. J. Biochem.* **88**, 1-6
- Jabbal, I. & Schachter, H. (1977) *J. Biol. Chem.* **247**, 5154-5161
- Kessel, D. & Chou, T. H. (1976) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **35**, 1442
- Kessel, D., Khilanani, P. & Chou, T. H. (1978) *Cancer Chemother. Rep. Part 1*, **62**, 147-149
- Khilanani, P., Chou, T. H., Lomen, P. L. & Kessel, D. (1977) *Cancer Res.* **37**, 2557-2559
- Khilanani, P., Chou, T. H. & Kessel, D. (1978) *Cancer Res.* **38**, 181-185
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Mulet, C., Cartron, J. P., Badet, J. & Salmon, C. (1977) *FEBS Lett.* **84**, 74-78
- Munro, J. R. & Schachter, H. (1973) *Arch. Biochem. Biophys.* **156**, 534-542
- Munro, J. R., Narasimhan, S., Wetmore, S., Riordan, J. R. & Schachter, H. (1975) *Arch. Biochem. Biophys.* **169**, 269-277
- Pacuszka, T. & Kosiellak, J. (1974) *FEBS Lett.* **41**, 348-351
- Schachter, H. & Tilley, C. A. (1978) *Int. Rev. Biochem.* **16**, 209-246
- Schenkel-Brunner, H., Chester, M. A. & Watkins, W. M. (1972) *Eur. J. Biochem.* **30**, 269-277
- Spiro, R. G. (1964) *J. Biol. Chem.* **239**, 567-573
- Watkins, W. M. (1978) *Rev. Fr. Transfus.* **21**, 201-228
- Wilson, J. R., Williams, D. & Schachter, H. (1976) *Biochem. Biophys. Res. Commun.* **72**, 909-916