# SELECTIVE INHIBITION OF A PLASMA FUCOSYLTRANSFERASE BY N-ETHYLMALEIMIDE

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SUMMARY Two fucosyltransferases have been found in plasma: the blood group *H*-dependent GDP-L-fucose:galactoside 2'fucosyltransferase and a GDP-L-fucose:N-acetylglucosaminide fucosyltransferase. The presence of endogenous acceptors for both enzymes in plasma from normal donors and leukemia patients has complicated measurement of levels of the individual enzymes. We have found that the sulfhydryl reagent N-ethylmaleimide, at 3 mM, inhibits the *H*-gene specified enzyme without affecting the other. Both enzymes have been partly characterized here with regard to $K_m$, Mg requirement and sensitivity to inhibitors.

INTRODUCTION The term fucosyltransferase describes the activity of a group of enzymes which catalyze transfer of the sugar fucose from GDP-fucose onto appropriate glycoprotein, glycolipid or low molecular-weight acceptors. At least two fucosyltransferases in plasma have been identified. Fucosyltransferase I ($F_1$) catalyzes transfer of fucose onto N-acetylglucosamine residues; fucosyltransferase II ($F_2$), specified by the *H*-gene, transfers fucose onto terminal galactose (1,2). Presence in plasma of a 3'fucosyltransferase, which also utilizes N-acetylglucosamine residues as an acceptor, has also been reported (2), but at relatively low levels (1). We had previously (3) described elevation in total fucosyltransferase activity in plasma of cancer patients. But presence of endogenous acceptors of both $F_1$ and $F_2$ activity made determination of levels of the individual enzymes difficult. Our efforts to remove this endogenous acceptor activity led to substantial loss of plasma fucosyltransferases. We therefore sought a method for preferentially inhibiting activity of one of the two major

plasma fucosyltransferases without altering activity of the other.

MATERIALS AND METHODS GDP-L-[$^{14}$C]fucose (174 Ci/mole) was purchased from the New England Nuclear Corp., fetuin from Calbiochem Corp., other chemicals(of reagent grade or better) from Sigma Chemical Co. Asialo-fetuin and asialo-agalacto-fetuin were prepared as described by Spiro (4). Plasmas were obtained from normal donors and leukemia patients using EDTA as the anticoagulant. Red cells were removed by low-speed centrifugation; platelets by centrifugation at 10,000 x g for 10 min. A plasma sample from a 'Bombay' donor was provided by Dr. William Kuhns, New York University Medical Center. All plasmas were stored at -70°.

Enzyme assays were carried out in a 200 µl volume containing 50 µl of plasma (4 mg of protein), 0.5 mg of fetuin derivative, 50 mM cacodylate buffer pH 7.0, 10 mM EGTA, 10 mM $MgCl_2$ and 1 µM of labeled GDP-fucose,N-ethylmaleimide was added, as specified.

Fucosyltransferase I activity was measured in the presence of 3.3 mM N-ethylmaleimide, with asialo-agalactofetuin as the fucose acceptor. Fucosyltransferase II was determined using asialo-fetuin as acceptor, using duplicate incubation mixtures, one containing 3.3 mM N-ethylmaleimeide. Activity was calculated by difference, compensating for any endogenous $F_1$ acceptor activity.

After incubation at 37° for 60 min, the reaction mixtures were washed through 0.5 x 1 cm columns of Dowex 1 (hydroxide) with two column-volumes of water. This procedure resulted in selective elution of glycoprotein, but not of free fucose or of GDP-fucose. The radioactive product was measured by liquid scintillation counting. In initial experiments, we verified the presence of radioactive fucose following acid hydrolysis of product as described in Ref. 1. Similarly, we used the alkaline hydrolysis procedure to identify products of $F_1$ and $F_2$ activity (1). Results are reported in terms of counts/minute of product formed per 50 µl of plasma during 60 min incubations at 37°.

RESULTS AND DISCUSSION We found that addition of graded amounts of N-ethylmaleimide to an incubation mixture containing normal plasma and $F_1$ enzyme acceptor asialo-agalactofetuin led to an inhibition of of only 40% of the incorporation of fucose from GDP-[$^{14}$C]fucose into glycoprotein. When the inhibitor level was increased to almost 14 mM, no further inhibition of incorporation occurred. In contrast, >90% fucose incorporation was inhibited by 1 mM N-ethylmaleimide using the $F_2$ enzyme acceptor asialo-fetuin (Fig. 1).

Interpretation of these data was aided by a study of the effect of N-ethylmaleimide on fucosyltransferase activity of a 'Bombay' plasma containing no $F_2$ enzyme activity (1,2). Using the $F_1$ enzyme acceptor asialo-agalactofetuin, we found no inhibition of fucosyltrans-

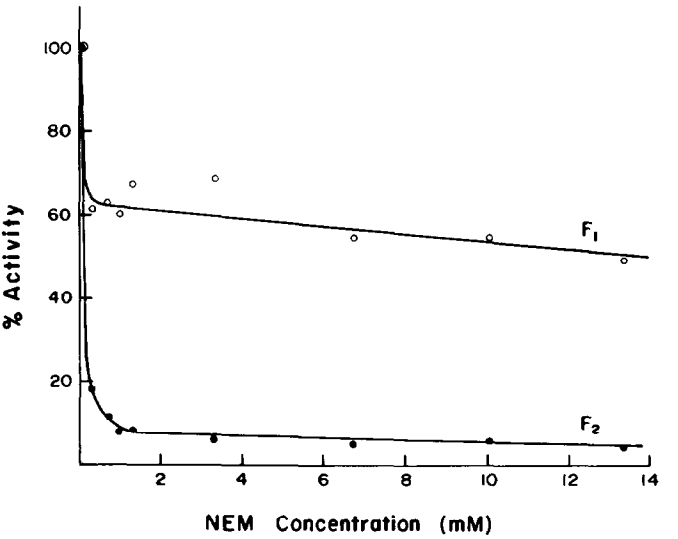


Fig. 1: Effects of graded levels of N-ethylmaleimide on fucosyltransferase activity in normal plasma: (o) asialo-agalactofetuin as acceptor ($F_1$) and (●) asialofetuin acceptor ($F_2$). Data are shown as % control value measured in the absence of inhibitor.

TABLE 1

EFFECT OF N-ETHYLMALEIMIDE AND DIFFERENT ACCEPTORS

ON APPARENT FUCOSYLTRANSERASE ACTIVITY

| | Fetuin SG Acceptor | | Fetuin S Acceptor | | Enzyme Activity | |
|---|---|---|---|---|---|---|
| Plasma Source | - NEM | + NEM | - NEM | + NEM | $F_1$ | $F_2$ |
| Normal donor | 340 | 250 | 250 | 50 | 250 | 200 |
| 'Bombay' donor | 700 | 700 | 25 | 20 | 700 | 5 |
| Leukemia #1 | 2500 | 2100 | 1200 | 120 | 2100 | 1080 |
| Leukemia #2 | 1650 | 750 | 1350 | 150 | 750 | 1200 |

Fetuin - SG = asialo-agalactofetuin; Fetuin - S = asialofetuin acceptor. Data are in terms of counts/min of radioactive product formed per 50 µl of plasma during a 60 min incubation. NEM; N-ethylmaleimide, present at a 3.3 mM concentration where specified. $F_1$ and $F_2$ enzyme activities are calculated as described in the text.

TABLE 2

EFFECT OF INHIBITIORS ON FUCOSYLTRANSFERASE ACTIVITY

| Assay Conditions | $F_1$ | $F_2$ |
|---|---|---|
| Control | 100 | 100 |
| - $Mg^{++}$ | 94 | 47 |
| - $Mg^{++}$ + EDTA | 54 | 27 |
| + EDTA | 90 | 38 |
| - EGTA | 88 | 44 |
| + Fucose | 87 | 87 |
| + GDP | 57 | 14 |

Data are shown as % control values. Where specified, concentrations of additions were 10 mM EDTA, 13 mM L-fucose and 0.03 mM GDP. Other components: 50 µl plasma and 1 mM radioactive GDP fucose in a 200 µl volume containing 0.5 mg asialo-agalactofetuin ($F_1$) or asialo-fetuin ($F_2$) 50 mM cacodylate pH 7.0, 10 mM EGTA, and mM $MgCl_2$.

ferase activity by N-ethylmaleimide (Table 1). This finding suggests that N-ethylmaleimide preferentially inhibits $F_2$ enzyme activity. Although plasma may contain both endogenous $F_1$ and $F_2$ enzyme acceptors, we can determine levels of each enzyme activity using this inhibitor. Total (endogenous + exogenous) $F_1$ enzyme is measured in the presence of asialo-agalactofetuin + N-ethylmaleimide; $F_2$ enzyme is determined by measuring apparent fucosyltransferase in the presence *vs.* absence of inhibitor, with asialo-fetuin as acceptor. The data of Table 1 show comparisons of results using plasma samples from several patients with acute leukemia demonstrating varying levels of both enzymes.

Both enzymes showed a broad pH optimum, extending from 6.0-8.0. We found (Table 2) $F_2$ enzyme activity to be more $Mg^{++}$-dependent and

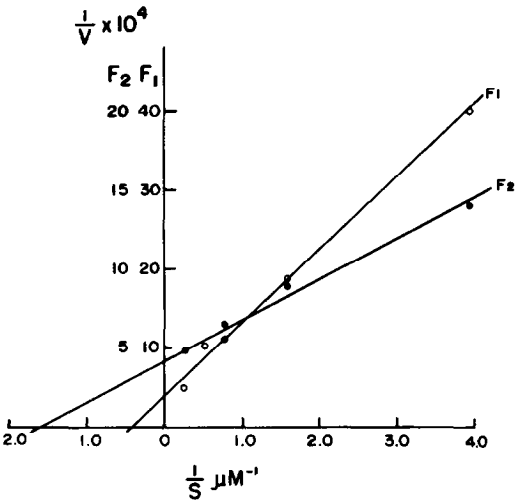


Fig. 2: Lineweaver-Burk plot of enzyme activity as a function of GDP-fucose concentration: (o) asialo-agalactofetuin acceptor ($F_1$) and (●) asialofetuin acceptor ($F_2$). N-ethylmaleimide was present at a level of 3.3 mM for the assay of $F_1$ enzyme activity. The plasma sample was provided by a normal donor.

more sensitive to presence of serum calcium that the $F_2$ enzyme. The product, GDP, was a more potent inhibitors of $F_2$ activity than of $F_1$. We also measured apparent $K_m$ values of the GDP-fucose substrate (Fig. 2), finding 2.5 μM for $F_1$ and 0.6 μM for $F_2$. The former value agrees closely with data reported by Bella and Kim (5) but both values are higher than those reported by Bosmann (6).

These findings described in this paper should enable us to measure relative levels of the two major fucosyltransferase activities present in plasma, or in other tissues. One also can estimate levels of endogenous enzyme acceptors in the samples. We are presently characterizing these two major enzymes further in both normal and cancer patients.

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