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DIFFERENTIAL CONCANAVALIN A BINDING OF CYSTIC FIBROSIS AND NORMAL LIVER α-L-FUCOSIDASE

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SUMMARY: A novel technique has been employed to demonstrate that α-L-fucosidase purified from cystic fibrosis and control livers exhibits differential binding to the lectin Concanavalin A. The concentration of α-CH_3-mannoside necessary to prevent 50% binding of α-L-fucosidase to Concanavalin A is considerably lower for the cystic fibrosis enzyme (13.5 vs. 33.3 mM). Comparable results were found when binding studies were done on crude supernatant α-L-fucosidase from 8 cystic fibrosis and 8 control livers (5.6 ± 0.4 mM and 13.2 ± 3.4 mM, respectively), without any overlap of values between the cystic fibrosis and control livers. These results suggest that comparative lectin binding studies on cystic fibrosis and normal glycoproteins from readily available tissues might result in an assay for detecting the cystic fibrosis genotype.

Cystic fibrosis (C.F.), one of the most common genetic diseases among Caucasian children, manifests itself as a generalized disorder primarily affecting exocrine glands and the pulmonary and gastrointestinal systems (1,2). Death usually occurs before or in early adulthood, most often as a result of chronic pulmonary disease (1,2). Since C.F. appears to be transmitted as an autosomal recessive trait (2,3), it is reasonable to postulate that the disease probably results from a single gene defect. Despite considerable biochemical research, the basic biochemical defect of C.F. is not known and no adequate biochemical marker for detecting C.F. patients and heterozygotes is yet available.

Many previous studies have suggested that glycoprotein metabolism is abnormal in C.F. tissues (1,2,4). Recently, the glycoprotein α-L-fucosidase (fucoside-fucohydrolase EC 3.2.1.51.) has been purified by affinity chromatography to apparent homogeneity from two C.F. livers (5). Characterization of the two C.F. α-L-fucosidases indicated that they were very similar to normal liver α-L-fucosidase (6) with regard to pH optima profiles, Michaelis constants (Km's), subunit structure and antigenicity. However, gas liquid chromatographic analysis re-

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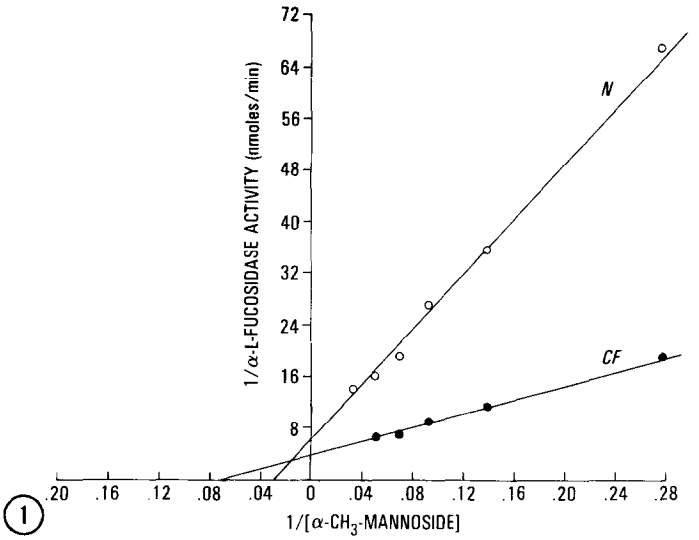
vealed altered carbohydrate compositions for both C.F. α-L-fucosidases. The three major sugars found in normal purified liver α-L-fucosidase (mannose, N-acetylglucosamine, sialic acid) (7) were reduced in both C.F. α-L-fucosidases, on the average, to 51%, 44% and 32%, respectively, of their normal amounts. This altered carbohydrate composition of C.F. α-L-fucosidase probably accounts for its previously reported altered isoenzyme patterns (8) and may be useful as a biochemical marker in detecting C.F. patients and heterozygotes.

In the present investigation we have employed a novel technique to study the inhibition of binding of purified and crude liver α-L-fucosidase to the lectin Concanavalin A in the presence of varying concentrations of α-CH_3-mannoside. This study has found decreased binding of C.F. α-L-fucosidase when compared to the normal enzyme.

All procedures were carried out at 0-4°C unless otherwise stated. α-L-Fucosidase activity was assayed using 4-methylumbelliferyl-α-L-fucopyranoside (Koch-Light, Ltd.) as previously described (9). Concanavalin A was purchased from Miles Biochemical and α-CH_3-mannoside (Grade II) from Sigma Chemical Co. Human livers were obtained at autopsy from persons who had cystic fibrosis or from controls whose livers appeared normal on gross pathological inspection. All livers were frozen (-20°C) immediately after autopsy and stored frozen until used. The procedures which involved human tissues were approved by the Committee on Investigations/Activities Involving Human Subjects of the School of Medicine, University of California, San Diego. Liver supernatant fluids were prepared as follows: 0.2 grams of liver were homogenized in 1.0 ml 0.1 M pH 4.0 citric acid-sodium citrate buffer using ground glass homogenizers. The homogenates were centrifuged for 30 min at 23,500 x g and the resultant supernatant fluids contained approximately 94% of the fucosidase activity present in the homogenate. This supernatant, as well as purified normal and C.F. α-L-fucosidase from previous studies (5,7), was used for the Concanavalin A-α-L-fucosidase binding studies.

The Concanavalin A binding studies were run modified after the method of Bishayee and Bachhawat (10). The exact conditions of the assay were arrived at empirically after independently varying the pH and concentrations of NaCl, α-CH_3-mannoside and Concanavalin A. The final assay was run in 0.4 ml microfuge tubes (Cole Scientific) containing the following components brought to a total volume of 150 µl with 0.1 M, pH 4.0 citric acid-sodium citrate buffer: 0.6 mg Concanavalin A, 0.16 M NaCl, 2.0-4.0 units (nmoles/min at 37°C) of purified or crude liver supernatant α-L-fucosidase and varying amounts of α-CH_3-mannoside to give final concentrations of 3.8-40.2 mM. All additions were made in the order listed above, in 0.1 M, pH 4.0 citric acid-sodium citrate buffer. The contents of each tube were vortexed for 15 sec, preincubated at 37°C for 1 hr and centrifuged for 1 hr at 39,000 x g to precipitate the Concanavalin A-α-L-fucosidase complex. The supernatant fluids were immediately transferred to different test tubes being careful not to disturb the precipitated complex. Aliquots of the supernatant were assayed in duplicate for α-L-fucosidase activity using conditions which were linear with respect to time (5-20 min) and enzyme amount (5-10 µl of supernatant). The concentra-



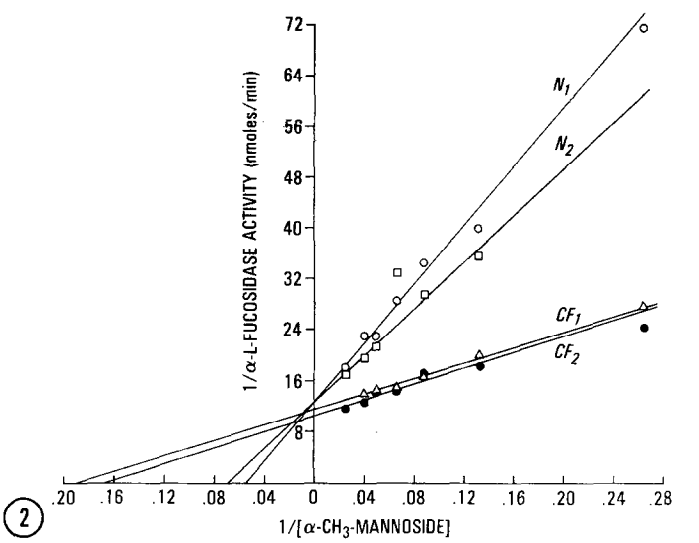


Figure 1. Double reciprocal plot of α-CH_3-mannoside concentration vs. unbound α-L-fucosidase activity from purified normal and cystic fibrosis liver α-L-fucosidases. See Methods section for details.

Figure 2. Double reciprocal plot of α-CH_3-mannoside concentration vs. unbound α-L-fucosidase activity from crude normal and cystic fibrosis liver α-L-fucosidases. See Methods section for details.

tion of α-CH_3-mannoside necessary to prevent 50% binding of α-L-fucosidase activity ($Ki_{50\%}$) was graphically determined by double reciprocal plots of α-CH_3-mannoside concentration versus unbound α-L-fucosidase activity after preincubation with Concanavalin A and centrifugation (see Figs. 1 and 2).

Table 1

Concentration of α-CH_3-Mannoside Necessary to Prevent 50% Binding ($Ki_{50\%}$'s) of α-L-Fucosidase to Concanavalin A.

LIVER (Number Studied)	Average $Ki_{50\%}$'s (mM α-CH_3-mannoside) ± Mean Deviation	
Normals (8)	13.2 ± 3.4	Range 8.4-20.1
Cystic Fibrosis (8)	5.6 ± 0.4	Range 4.8-6.1

Figure 1 depicts double reciprocal plots of α-CH_3-mannoside concentration vs. unbound α-L-fucosidase activity (after preincubation with Concanavalin A and centrifugation) from C.F. and control livers (5). From this figure it can be seen that the concentration of α-CH_3-mannoside necessary to prevent 50% binding of α-L-fucosidase to Concanavalin A ($Ki_{50\%}$; inhibition of binding constant) is considerably lower for the purified C.F. liver α-L-fucosidase (13.5 mM) than for the control liver enzyme (33.3 mM). Comparable results were found when binding studies were done on crude supernatant α-L-fucosidase from C.F. and control livers (Fig. 2). Table 1 summarizes the $Ki_{50\%}$'s for the 8 normal and 8 C.F. livers investigated in the present study. The individual values are the average of at least two determinations and are expressed as mM concentration of α-CH_3-mannoside necessary to prevent 50% binding of α-L-fucosidase to Concanavalin A. Although only a limited number of livers (and no obligate heterozygotes) were available for study, the average ± mean deviation appears to be significantly lower for the C.F. when compared to the normal liver α-L-fucosidase (5.6 ± 0.4 mM and 13.2 ± 3.4 mM, respectively). Furthermore, there is no overlap between the range of normal and C.F. values. The lower $Ki_{50\%}$'s for the crude when compared to the purified α-L-fucosidases may be due to the presence of other glycoproteins in the crude preparations competing with α-L-fucosidase for Concanavalin A. The presence of α-CH_3-mannoside alone (i.e., with out Concanavalin A) did not have any effect on either C.F. or normal liver α-L-fucosidase activity.

Several studies have suggested that α-L-fucosidase may be altered in C.F. tissues. Hosli and Vogt (11) have demonstrated a decreased intracellular concentration of α-L-fucosidase activity in C.F. fibroblasts and speculated that the basic defect in C.F. may lead to abnormal recognition sites on enzymes and in consequence to the leakage of these enzymes into the extracellular space. On the other hand, Scanlin et al. (12) have reported that α-L-fucosidase is elevated in C.F. skin fibroblasts when compared to controls. However, they also have proposed that an abnormal distribution of α-L-fucosidase, possibly due to altered carbohydrate content, is involved in the pathogenesis of C.F. This is consistent with our previous finding of an altered carbohydrate composition for C.F. liver α-L-fucosidase in which decreased amounts of mannose, N-acetylglucosamine and sialic acid were found when compared to the normal liver enzyme (5). Scanlin and Glick (13) have also found decreased amounts of mannose, N-acetylglucosamine and sialic acid in membrane glycopeptides from C.F. skin fibroblasts when compared to controls.

All of the above findings suggested to us that lectins might be useful probes in searching for altered C.F. glycoproteins, one of which might be useful as a biochemical marker for the C.F. genotype. Our present findings of decreased binding of purified and crude liver C.F. α-L-fucosidase to Concanavalin A, a lectin with specificity for mannose and glucose containing glycoconjugates (14), are consistent with our previous findings of decreased mannose content in C.F. α-L-fucosidase and further suggest that defective glycosylation may be involved in the pathophysiology of C.F. Although our results are not directly applicable for diagnostic purposes, they suggest that further comparative lectin binding studies of various C.F. and normal glycoproteins from readily available tissues (e.g., serum, urine, cultured skin fibroblasts) might reveal a biochemical marker for detecting C.F. patients and possibly even heterozygous carriers.

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