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I-CELL DISEASE: ISOELECTRIC FOCUSING, CONCANAVALIN A-SEPHAROSE 4B BINDING AND KINETIC PROPERTIES OF HUMAN LIVER ACID β-D-GALACTOSIDASES

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Summary

Isoelectric focusing of the acid β-D-galactosidases (β-D-galactoside galactohydrolase, EC 3.2.1.23) in normal crude liver supernatant fluids demonstrated multiple isoelectric forms in the pH range 4.58—5.15, while corresponding I-cell disease samples showed an absence of isoelectric forms in the pH range 4.99—5.15. Concanavalin A-Sepharose 4B chromatography of the I-cell disease mutant C.A. demonstrated a 31% and 37% decrease in the binding of 4-methylumbelliferyl-β-D-galactosidase and G_{M1} β-D-galactosidase activities, respectively, when compared to normal samples. Isoelectric focusing profiles of the concanavalin A-Sepharose 4B α-methyl-D-mannoside effluents containing normal and I-cell disease acid β-D-galactosidase were generally similar, but the unadsorbed I-cell disease enzyme from concanavalin A-Sepharose 4B demonstrated more activity in the pH range 4.21—4.49 than normals. Normal and I-cell disease acid β-D-galactosidase "A" and "B", separated by gel column chromatography, were found to have similar properties with respect to apparent molecular weights, pH vs. activity profiles and apparent K_m values for the 4-methylumbelliferyl-β-D-galactopyranoside, G_{M1}-ganglioside and asialofetuin (ASF) substrates. However, the apparent V values for the ICD samples were consistently reduced when compared to the results obtained with the corresponding normal fractions. The greatest decreases in apparent V were obtained for acid β-D-galactosidase activities in I-cell disease crude supernatant fluids, and for the separated I-cell disease "B" enzyme. The differences in the isoelectric focusing profiles, the altered binding to concanavalin A-Sepharose 4B, and the reduced V values with natural and synthetic substrates may be related to changes in carbohydrate composition of I-cell disease acid β-D-galactosidase.

Abbreviations: G_{M1}-ganglioside, galactosyl-*N*-acetylgalactosaminyl-(*N*-acetylneuraminyl)-galactosylglucosyl-ceramide; Con A-Sepharose 4B, concanavalin A bound covalently to Sepharose 4B.

Introduction

I-cell disease or mucolipidosis II is an inherited childhood neurometabolic disorder characterized by severe psychomotor retardation, early cessation of growth in stature, joint contractures, absence of excessive excretion of mucopolysaccharides in urine, and the presence of numerous cytoplasmic granular inclusion bodies in I-cell disease cultured skin fibroblasts and autopsied tissues [1—9]. Studies have shown that these inclusions stain positive with periodic acid-Schiff reagent and Sudan Black, suggesting that this material may be composed of glycolipid and glycoprotein [3—5,7,10].

Biochemical studies of I-cell disease cultured fibroblasts have demonstrated a severe reduction or absence of acid β-D-galactosidase (β-D-galactoside galactohydrolase, EC. 3.2.1.23), α-L-fucosidase and neuraminidase activities, while the enzyme activities of *N*-acetyl β-D-hexosaminidase, β-D-glucuronidase, α-L-iduronidase, arylsulfatase A, α-D-galactosidase and α-D-mannosidase were partially reduced [8,11—20]. Examination of autopsied I-cell disease tissues have revealed only a marked decrease in the activity of β-D-galactosidase [2,4,13]. Studies of the supernatant medium of cultured fibroblasts and extracellular fluids (plasma, cerebrospinal fluid) obtained from I-cell disease patients have revealed elevated activity levels of a number of lysosomal hydrolases including β-D-glucuronidase, α-L-iduronidase, *N*-acetyl-β-D-glucosaminidase and arylsulfatase A [21—25]. Electrophoretic studies on cellulose acetate of *N*-acetyl-β-D-hexosaminidase, arylsulfatase A, β-D-glucuronidase, and α-L-fucosidase excreted by I-cell disease cultured fibroblasts have shown an increase in electronegative charge for these hydrolases which was attributed to the presence of increased sialic acid residues [26]. It has also been reported that starch gel electrophoresis has demonstrated a slower anodally migrating 4-methylumbelliferyl acid β-D-galactosidase "A" isolated from I-cell disease liver and brain [27,28].

The present report describes studies of the residual acid β-D-galactosidase activity from I-cell disease liver which include altered isoelectric focusing patterns, alterations in binding profiles of acid β-D-galactosidases to Con A-Sepharose 4B, decreased *V* values with respect to glycoprotein, glycolipid and synthetic substrates, and an evaluation of these findings as they relate to the molecular basis for I-cell disease. A preliminary report of these results has been previously presented [29].

Materials and Methods

General

All procedures were carried out at 0—4°C unless otherwise stated. Buffer I contained 5 mM sodium phosphate, 10 mM sodium chloride and 0.02% (w/v) sodium azide at pH 7.0. Buffer II at pH 7.0 contained 10 mM sodium phosphate, 100 mM sodium chloride, 1 mM EDTA and 0.02% (w/v) sodium azide. 4-methylumbelliferyl-β-D-galactopyranoside was from Koch-Light. Concanavalin A-Sepharose 4B (Con A-Sepharose 4B), Sepharose 6B and Sephadex G-150 were from Pharmacia, and DEAE-cellulose (DE-52) was from Whatman. Sodium boro[^{3}H]hydride (870 mCi/mmol) was obtained from Amersham-

Searle, and Riafluor scintillant was from New England Nuclear. Galactose oxidase (EC 1.1.3.9) was obtained from A.B. Kabi, Stockholm, Sweden. Protein determinations were carried out according to the method of Lowry et al. [30] using bovine serum albumin as the standard.

Preparation of crude liver supernatant fluids

Normal and I-cell disease livers (C.A. and S.V.) were obtained at autopsy and stored frozen(−20° C). Autopsied livers from controls appeared normal upon gross pathological inspection. Normal and I-cell disease livers were homogenized in a 20% (w/v) ratio of Buffer I using a Duall glass homogenizer. The homogenates were centrifuged at 40 000 × *g* for 30 min, and in the gel filtration experiments sodium chloride was added to the resulting crude supernatant fluids to final concentration of 100 mM.

Enzyme assays

Acid β-D-galactosidase, neutral β-D-galactosidase and β-D-glucosidase were assayed as previously described [31], using 4-methylumbelliferyl-β-D-galactopyranoside and 4-methylumbelliferyl-β-D-glucopyranoside, respectively. G_{M1}-ganglioside β-D-galactosidase was assayed using G_{M1}-ganglioside, purified and specifically tritiated in the terminal galactose moiety, as described by Norden and O'Brien [32]. ASF was prepared by mild acid hydrolysis of fetuin (Sigma) according to Spiro [33]. Specific tritiation of the terminal galactosyl residues was carried out according to Morrell et al. [34], with the following modifications. Asialofetuin (1% w/v) was incubated in the presence of galactose oxidase (22 units/mg asialofetuin) for 25 h at 37° C. A unit of enzyme activity will produce a change in A_{125} of 1.0 per min at pH 6.0 and 25° C in a peroxidase and *o*-tolidine system. After an overnight incubation with sodium boro[^{3}H]hydride, unlabelled sodium borohydride was added (5 mg per 1 mg tritiated sodium borohydride) and left at 25° C for 1 h. The reaction mixture was placed in an 80° C water bath for 1 h in order to inactivate the galactose oxidase. The mixture was exhaustively dialyzed at 0° C against glass distilled water (30 l) to remove the unreacted boro[^{3}H]hydride. The labelled asialofetuin was subjected to DEAE-cellulose column chromatography (0.2 ml bed volume DEAE per mg asialofetuin). The column was equilibrated with 10 mM sodium phosphate, pH 7.0, and asialofetuin was eluted with the same buffer containing a gradient of 0—400 mM sodium chloride. The fractions containing labelled asialofetuin were combined, concentrated and dialyzed against glass distilled water (20 l for 72 h) at 0° C. The asialofetuin was lyophilized and stored at 0° C. ASF β-D-galactosidase activity was assayed according to the method of Norden et al. [35].

A unit of enzyme activity is defined as the hydrolysis of 1.0 nmol of substrate per min at 37° C.

Starch gel electrophoresis

Vertical starch gel electrophoresis and slicing zymograms were carried out as previously described by Holmes et al. [27]. Electrophoresis was routinely performed for 14—16 h at 10 mA and 160—220 V. Samples subjected to starch gel electrophoresis included: (1) normal and I-cell disease isoelectric focusing peak tubes, and (2) normal and I-cell disease concentrated α-methyl-D-mannoside effluents from Con A-Sepharose 4B chromatography.

Con A-Sepharose 4B chromatography

Normal and I-cell disease crude supernatant fluids were subjected to column chromatography on Con A-Sepharose 4B according to a modification [36] of the method of Norden and O'Brien [37]. Crude liver supernatant fluids from normal (12.3 ml, 193 mg protein) and I-cell disease (C.A. 12.3 ml, 118 mg protein; and S.V. 4.7 ml, 47 mg protein) were applied to different 2-ml bed volume columns of Con A-Sepharose 4B. The unadsorbed Con A-Sepharose 4B effluents and the α-methyl-D-mannoside eluted samples were concentrated to 6 mg/ml protein and 2 mg/ml protein, respectively, by ultrafiltration at 40—50 lb/inch^2 using an Amicon concentrator fitted with UM-10 Diaflo membrane.

Isoelectric focusing

General. Isoelectric focusing was performed according to the method of Haglund [38], using an LKB 8101 electrofocusing column (110 ml) and 1% (v/v) ampholytes (pH 4.0—6.0) in a gradient of 0—67% (w/v) sucrose. Initially 165 V was applied to establish a current of 1 mA. After 20 h the voltage was increased to 320 V in order to restore the current to 1 mA. After a total of 62 h focusing time had elapsed, approximately 210 column fractions were collected in the following manner: 15-drop fractions were collected in the area where acid β-D-galactosidase activity was present (fractions Nos. 40—120), while 20 drops were collected for all other fractions. The pH of each fraction was measured on a Beckman digital pH meter at 0—2° C.

Crude supernatant fluids. 2-ml aliquots of normal and I-cell disease crude supernatant fluids were applied separately to isoelectric focusing columns. These normal samples contained 350—400 units of acid 4-methylumbelliferyl-β-D-galactosidase and 28 mg of protein, while 35—40 units of acid 4-methylumbelliferyl-β-D-galactosidase and 23—32 mg of protein were used for the I-cell disease samples.

Post-Con A-Sepharose 4B α-methyl-D-mannoside effluents. Prior to isoelectric focusing of the concentrated α-methyl-D-mannoside effluents from Con A-Sepharose 4B chromatography, the normal and I-cell disease samples were dialyzed for 6 h against 3 changes (3.3 l per change) of a buffer containing 10 mM sodium phosphate, 100 mM NaCl, and 0.02% (w/v) sodium azide at pH 7.0. Aliquots of 0.1 ml of normal α-methyl-D-mannoside effluents (400 units acid β-D-galactosidase activity, 2.5 mg protein) or 0.1 ml of I-cell disease α-methyl-D-mannoside effluents (19—35 units acid β-D-galactosidase activity, 2.5—2.7 mg protein) were applied to the isoelectric focusing column. Bovine serum albumin (6 mg) was added to each of two tubes containing the aforementioned normal and I-cell disease samples in order to stabilize the acid β-D-galactosidase activity during isoelectric focusing.

Post-Con A-Sepharose 4B unadsorbed effluents. The post Con A-Sepharose 4B unadsorbed samples were dialyzed for 6 h against 3 changes (3.3 l per change) of Buffer I. Aliquots of 4 ml of normal Con A-Sepharose 4B unadsorbed effluent (40 units acid β-D-galactosidase activity, 140 mg protein) or 2.5 ml of I-cell disease (C.A.) Con A-Sepharose 4B unadsorbed effluent (37 units acid β-D-galactosidase activity, 110 mg protein) were applied to the isoelectric focusing columns.

Isoelectric focusing enzyme assays. Acid 4-methylumbelliferyl-β-D-galactosid-

ase, neutral 4-methylumbelliferyl-β-D-galactosidase and 4-methylumbelliferyl-β-D-glucosidase activities were measured in the isoelectric focusing column fractions by incubating 20-μl aliquots from each fraction with 100 μl of the appropriate 4-methylumbelliferyl-substrate solution. The incubation time for each set of assays depended on the amount of acid β-D-galactosidase originally placed on the column (i.e. 1 h for 350—400 units and 2 h for less than 100 units of enzyme activity). G_{M1}-ganglioside β-D-galactosidase was measured according to the method of Norden and O'Brien [32] after incubating 25 μl of isoelectric focusing column fractions with 25 μl of the buffered substrate solution at 37° C for 2 h.

Sepharose 6B and Sephadex G-150 column chromatography

Sepharose 6B column chromatography (55 cm bed volume, flow rate 5.5 ml per h) or normal and I-cell disease crude supernatant fluids was carried out according to the procedure of Norden and O'Brien [32] using Buffer II. The column was calibrated using the following protein standards of known molecular weight: rabbit muscle aldolase (Pharmacia), α-chymotrysinogen-A, bovine serum albumin, and thyroglobulin (all from Sigma). Apparent molecular weights of the acid β-D-galactosidases were determined within experimental error [39] on Sepharose 6B.

Acid β-D-galactosidase "A" and "B" used for kinetic studies were obtained by Sephadex G-150 gel chromatography (490 ml bed volume) in Buffer II, according to the method of Ho et al. [40]. The separated acid β-D-galactosidases "A" and "B" were concentrated by ultrafiltration at 50 lb/inch^2 using an Amicon concentrator fitted with a UM-10 (Diaflo) membrane. Protein concentrations for I-cell disease and normal samples were similar for all kinetic experiments. Kinetic studies employing the glycolipid, glycoprotein and synthetic substrates were carried out using normal and I-cell disease crude liver supernatant fluids, and the gel chromatography separated, combined, and concentrated acid β-D-galactosidase "A" and "B" enzymes. These samples were dialyzed against 1 l of 10 mM sodium phosphate containing 100 mM sodium chloride and 0.02% (w/v) sodium azide at pH 7.0 for 4 h.

Results

Table I reveals that the normal crude liver supernatant fluids and one I-cell disease case (S.V.) demonstrated similar adsorption and elution characteristics from Con A-Sepharose 4B, with respect to the G_{M1}-ganglioside and acid 4-methylumbelliferyl-β-D-galactosidase activities. The second I-cell disease (C.A.) crude supernatant fluid showed a decreased binding to the Con A-Sepharose 4B column for the G_{M1}-ganglioside, β-D-galactosidase (37.2% unadsorbed) and acid 4-methylumbelliferyl-β-D-galactosidase (30.8% unadsorbed) activities when compared to the corresponding normal samples.

Fig. 1 contrasts the isoelectric focusing profiles of acid 4-methylumbelliferyl-β-D-galactosidase present in the normal and I-cell disease crude liver supernatant fluids with the α-methyl-D-mannoside effluents from Con A-Sepharose 4B chromatography. Although the normal and I-cell disease 4-methylumbelliferyl acid β-D-galactosidase from crude supernatant fluids exhibit multiple

TABLE I

CONCANAVALIN A-SEPHAROSE 4B COLUMN CHROMATOGRAPHY OF NORMAL AND I-CELL DISEASE β-D-GALACTOSIDASES

	Percent of total units applied	
	4-MU * acid β-D-galactosidase	G_{M1}-ganglioside β-D-galactosidase
Unadsorbed		
Normal	3.3	0.6
ICD ** (C.A.)	30.8	37.2
ICD (S.V.)	4.3	7.0
α-Methyl-D-mannoside effluent		
Normal	73.1	73.4
ICD (C.A.)	33.0	41.1
ICD (S.V.)	84.9	95.0

* 4-methylumbelliferyl.

** I-cell disease.

isoelectric forms, the I-cell disease samples demonstrate an absence of isoelectric forms in the pH range 4.99—5.15 (Fig. 1, Panel A). Isoelectric focusing profiles of the acid β-D-galactosidase activity present in the normal and I-cell disease α-methyl-D-mannoside effluents from Con A-Sepharose 4B chromatography (Fig. 1, Panel B) were different when compared to their respective crude

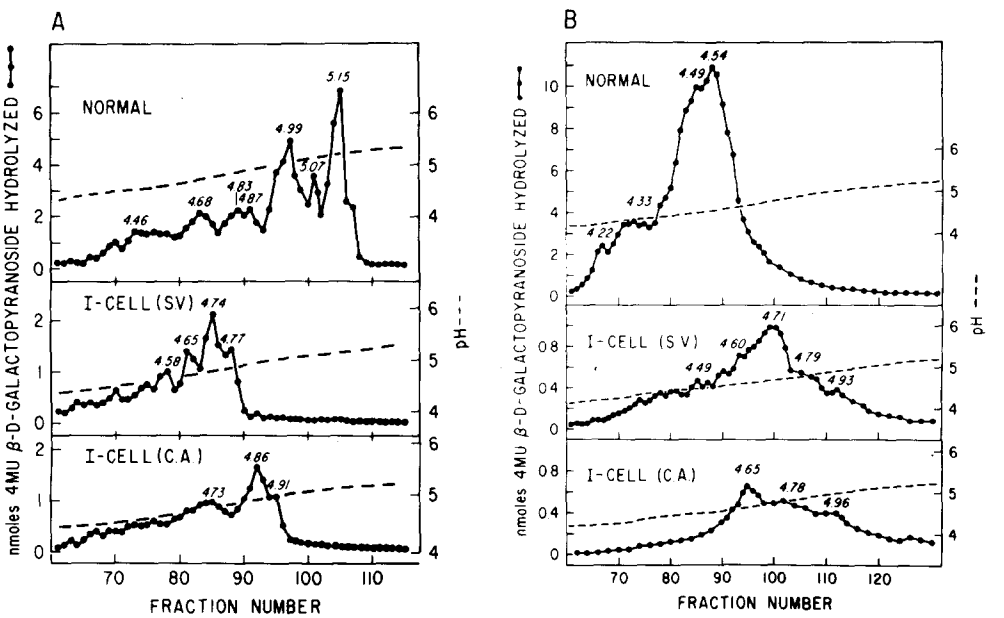

Fig. 1. Panel A: Isoelectric focusing patterns of acid β-D-galactosidase from normal and I-cell disease crude liver supernatant fluids. Panel B: Isoelectric focusing patterns of normal and I-cell disease Con A-Sepharose 4B concentrated and dialyzed α-methyl-D-mannoside eluted acid β-D-galactosidase. All samples were prepared and focused as described under Materials and Methods. Aliquots of 20 μl fom each column fraction were assayed at 37°C with 100 μl of 0.5 mM 4-methylumbelliferyl-β-D-galactopyranoside at pH 4.35. Normal fractions were incubated for 1 h while I-cell disease fractions were incubated for 2 h. ●———●, acid 4-methylumbelliferyl-β-D-galactosidase; - - - - - -, pH gradient in column fractions.

supernatant samples. Acid β-D-galactosidase activity present in the normal α-methyl-D-mannoside effluents exhibited a predominant form at pH 4.54 with shoulders at pH 4.33 and pH 4.22. In contrast, the I-cell disease isoelectric focusing profiles exhibited: a shift of the predominant isoelectric forms to more neutral pH values of 4.65 and 4.71, enzyme activity in the pH range 4.78—4.93, and an absence of significant enzyme activity in the pH range 4.22—4.49 for the mutant C.A. The presence of bovine serum albumin in the focused fractions did not alter the isoelectric focusing profiles obtained for the normal and I-cell disease acid β-D-galactosidases.

Starch gel slicing zymograms of the coincident isoelectric forms of acid β-D-galactosidase from normal and I-cell disease crude supernatant fluids revealed the distribution of acid β-D-galactosidases "A" and "B" enzyme activities. The normal and I-cell disease acid β-D-galactosidases activities in the pH range 4.58—4.63 migrated as equivalent mixtures of "A" and "B", while "B" was the predominant enzyme activity present in the isoelectric forms from pH 4.65—4.79. Starch gel electrophoresis of the normal isoelectric forms in the pH range 4.99—5.15 revealed the presence of only the "B" enzyme.

Starch gel electrophoresis and a slicing zymogram of the normal and I-cell disease α-methyl-D-mannoside effluents from Con A-Sepharose 4B chromatography, and the respective peak activity tubes from isoelectric focusing, revealed that prior to focusing, the acid β-D-galactosidase activity migrated as an equivalent mixture of the "A" and "B" enzyme activities. After isoelectric focusing, the starch gel slicing zymogram demonstrated the presence of only the "B" enzyme activity in both the normal and I-cell disease samples.

The I-cell disease (C.A.) acid β-D-galactosidase activity which did not adsorb to Con A-Sepharose 4B was also focused, and the resulting isoelectric focusing profile was compared to the normal enzyme activity of 3.3% which did not adsorb to Con A-Sepharose 4B (Fig. 2). The I-cell disease mutant C.A. lacked significant acid β-D-galactosidase activity within the pH range 5.10—5.46, but demonstrated more enzyme activity than the normal in the acidic pH range 4.21—4.49. The β-D-galactosidase activity shown at pH 4.67 may be attributed to β-D-glucosidase which focuses as a single peak at this pH. Ho and O'Brien [41] have previously shown that β-D-glucosidase exhibited a "nonspecific" β-D-galactosidase activity. This enzyme activity was enriched 6-fold in the concentrated normal and post-Con A-Sepharose 4B unadsorbed sample when compared to the normal crude supernatant fluid.

The apparent molecular weights of normal human acid β-D-galactosidase were determined to be 85 000 ± 8500 for the "A" enzyme and 500 000 ± 50 000 for the "B" enzyme after Sepharose 6B column chromatography. The apparent molecular weight of the I-cell disease acid β-D-galactosidase "A" ranged from 82 000 to 85 000, while the apparent molecular weight of I-cell disease acid β-D-galactosidase "B" ranged from 530 000 to 550 000.

Sepharose 6B column chromatography revealed that smaller quantities of the I-cell disease "B" enzyme activity were eluted when compared with the normal "B" activity. Integration of the areas under each enzyme peak from the Sepharose 6B elution profile demonstrated that the ratio of "A" to "B" enzyme activity was 7.0 for the normal tissue and 9.0—15.0 for the I-cell disease crude liver supernatant fluids.

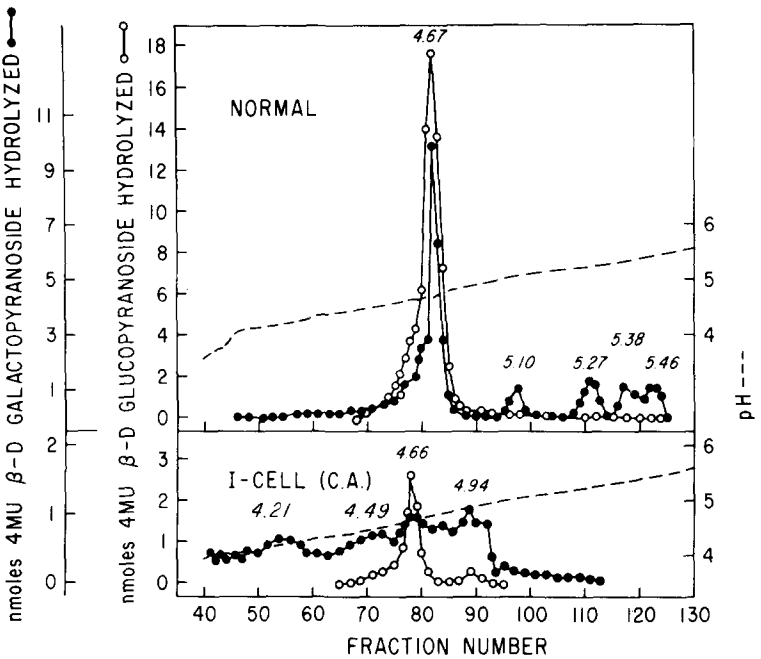

Fig. 2. Isoelectric focusing patterns of normal and I-cell disease concentrated post-Con A-Sepharose 4B unadsorbed acid β-D-galactosidase activity. All samples were prepared and focused as described under Materials and Methods. Aliquots of 20 μl from each column fraction were assayed with 100 μl of 0.5 mM 4-methylumbelliferyl-β-D-galactopyranoside at pH 4.35, and 100 μl of 1.0 mM 4-methylumbelliferyl-β-D-glucopyranoside at pH 5.8. Normal and I-cell disease acid β-D-galactosidases and I-cell disease β-D-glucosidase were incubated for 2 h at 37°C, while normal β-D-glucosidase was incubated for 1 h at 37°C. ●———●, acid 4-methylumbelliferyl-β-D-galactopyranosidase; ○———○, 4-methylumbelliferyl-β-D-glucopyranosidase; - - - - - -, pH gradient in eluted fractions. MU = methylumbelliferyl.

The pH activity profiles of the separated acid β-D-galactosidases were similar for normal and I-cell disease liver tissues, with respect to the 4-methylumbelliferyl-β-D-galactopyranoside, G_{M1}-ganglioside and asialofetuin substrates. A small but consistent difference was observed for the pH optima of the "A" (pH 4.2) and "B" (pH 4.0) enzymes with respect to the 4-methylumbelliferyl-β-D-galactopyranoside, and G_{M1}-ganglioside substrates (Table II). The pH activity profile of the separated "A" and "B" enzymes from normal livers displayed a major activity peak at pH 4.2, and a minor peak of activity at pH 3.7 with respect to asialofetuin. The absence of this minor pH optimum for acid β-D-galactosidase in the I-cell disease samples may be due to the low total asialofetuin activity.

Acid β-D-galactosidase from normal and I-cell disease crude supernatant fluids and separated "A" and "B" enzymes yielded linear Lineweaver-Burk plots. Similar apparent K_m values were found for the normal and I-cell disease enzymes with respect to the individual substrate tested, i.e., 4-methylumbelliferyl-β-D-galactopyranoside (0.22—0.30 mM), G_{M1}-ganglioside (0.10—0.15 mM), and asiafetuin (0.71—0.90 mM). However, the I-cell disease acid β-D-galactosidases in crude supernatant fluids and the separated "A" and "B" enzymes consistently showed decreased apparent V values, when compared to the corresponding normal liver fractions. The decreased apparent V values for

TABLE II

pH OPTIMUM OF SEPHADEX G-150 SEPARATED LIVER ACID β-D-GALACTOSIDASES "A" AND "B"

	4-MU **-β-D-Galactopyranoside	G_{MI}-ganglioside	Asialofetinin
β-D-Galactosidase "A"			
Normal	4.2	4.2	4.2, 3.7
ICD *	4.2	4.2	4.2
β-D-Galactosidase "B"			
Normal	4.0	4.0	4.2, 3.7
ICD *	4.0	3.8	4.1

* Average of the two I-cell disease liver enzyme values.
** 4-methylumbelliferyl.

TABLE III

RATIO OF *V* NORMAL TO *V* I-CELL DISEASE

	4-MU ***-β-D-galactopyranoside	G_{M1}-ganglioside	Asialofetinin
Crude supernatant			
ICD † (C.A.)	7.0	6.3	11.8
ICD (S.V.)	7.2	8.6	N.D. *
Separated ** acid β-D-galactosidases			
ICD "A" (C.A.)	2.5	2.7	5.6
ICD "A" (S.V.)	2.3	7.2	N.D. *
ICD "B" (C.A.)	7.0	7.4	20.0
ICD "B" (S.V.)	11.2	11.4	N.D. *

* Not determined.
** Obtained by Sephadex G-150 column chromatography.
*** 4-methylumbelliferyl.
† I-cell disease.

the I-cell disease acid β-D-galactosidases are depicted as an elevated ratio of normal to I-cell disease *V* in Table III. Generally, the largest increase in the ratio of normal to I-cell disease *V* occurred with the "B" enzyme. An exception to this observation was the I-cell disease "A" enzyme (S.V.) with respect to the G_{M1}-ganglioside.

Discussion

The most consistent finding in samples of I-cell disease autopsied tissues with respect to lysosomal enzyme activity levels is a decrease in acid β-D-galactosidase activity [2,4,13]. The relationship of this reduced activity to the molecular defect in I-cell disease is not well understood. The present report examines specific properties of this residual acid β-D-galactosidase activity from I-cell disease liver including isoelectric focusing, Sepharose 6B column chromatography, binding characteristics to Con A-Sepharose 4B, and kinetic studies using natural and synthetic substrates.

Human liver acid β-D-galactosidase occurs as the "A" protein (mol. wt.

60—70 · 10^3) and the "B" protein (mol. wt. 600—800 · 10^3) which share common antigenic determinants [35]. Isoelectric focusing profiles of the enzyme activity present in I-cell disease liver crude supernatant fluids demonstrated an absence of enzyme activity in the pH range 4.99—5.15 (Fig. 1, Panel A), which is thought to be due to a specific loss in the "B" enzyme. Evidence for this comes from starch gel electrophoresis of the normal enzyme where activity in this pH range is comprised of only the β-D-galactosidase "B" enzyme. However, it is difficult to evaluate the significance of the absent isoelectric forms in I-cell disease crude supernatant fluids, since different isoelectric focusing profiles were obtained with partially purified enzyme (Fig. 1, Panels A and B). This latter observation is consistent with the literature to date concerning isoelectric focusing of crude, partially purified and purified acid β-D-galactosidase from other sources [42,43].

Recent experiments in our laboratory suggest that in I-cell disease the absence of enzyme activity in the pH range 5.0—5.15 represents a decrease in the association of the "B" enzyme with constituents present in crude liver supernatant fluids. Aliquots of partially purified normal acid β-D-galactosidase and the unadsorbed post Con A-Sepharose 4B effluent were combined, dialyzed, and focused as outlined for crude liver supernatant fluids under Materials and Methods. The isoelectric focusing profile of the combined samples was comparable to that obtained for acid β-D-galactosidase present in normal crude supernatant fluids (Fig. 1, Panel A). However, if the focused fractions were subjected to centrifugation and reassayed, a severe reduction in the enzyme activity within the pH range above 5.00 ("B" enzyme) was seen, while no significant change was detected in the fractions below pH 5.00. Resuspension of the pellets in the focused fractions was accompanied by a return of enzyme activity to levels seen with the original focused fractions. Evidence for a specific decrease in the β-D-galactosidase "B" enzyme activity was also demonstrated in kinetic studies, where the apparent V for the I-cell disease "B" enzyme was consistently more reduced when compared to the I-cell disease "A" enzyme with respect to the 4-methylumbelliferyl-β-D-galactopyranoside, G_{M1}-ganglioside and asialofetuin substrates.

Explanations for the reduction in apparent V of both I-cell disease acid β-D-galactosidase "A" and "B" activities include: (a) a decreased rate of enzyme synthesis which could be accompanied by an increased rate of degradation, (b) presence of normal quantities of kinetically altered "A" and "B" enzymes, and (c) cell leakage of acid β-D-galactosidase coupled to a decreased rate of its synthesis. In addition, the more pronounced decrease of the apparent V of the I-cell disease "B" enzyme could result from (d) an increased level of degradation of the "B" enzyme, or (e) a post-translational alteration of the acid β-D-galactosidase "A" which would either decrease the ability of this monomer to associate to form the "B" enzyme (polymer), or cause formation of an unstable "B" enzyme which is subject to dissociation and subsequent proteolysis. Support for hypothesis (e) comes from studies using a mutant (Mod A) of the slime mold *Dictyostelium discoideum* [44]. This mutation involves a post-translational modification of several developmentally regulated lysosomal hydrolases. Results suggest that an alteration of a common post-translational modification system is responsible for the observed aberrant electrophoretic

properties for the hydrolases and differential effects on the intracellular levels of these multimeric enzymes.

In addition to altered isoelectric focusing profiles and *V* values, we have also obtained aberrant binding to Con A-Sepharose 4B. The specific binding of glycoproteins to Con A-Sepharose 4B has been shown to be influenced by the orientation of mannose residues within the oligosaccharide chain, the presence of additional mannose containing chains of oligosaccharide, the position of other sugars relative to mannose, and in certain cases by the presence of sialic acid residues [45—49]. The decreased binding to Con A-Sepharose 4B of the acid β-D-galactosidase from the I-cell disease mutant C.A. would be compatible with changes in either the accessibility, number, or orientation of the mannose residues. Altered Con A-Sepharose 4B binding has been observed for the 4-methylumbelliferyl-β-D-galactosidase isolated from another autopsied I-cell patient's liver (K.Z., 26% not adsorbed). In addition, we have demonstrated that 36% (S.V.) and 21% (J.G.) of the applied I-cell disease acid β-D-galactosidase from crude spleen supernatant fluids did not adsorb to Con A-Sepharose 4B as compared to less than 1% for the normal enzyme [50].

Isoelectric focusing profiles of the unadsorbed I-cell disease (C.A.) acid β-D-galactosidase from Con A-Sepharose 4B revealed more enzyme activity in the pH 4.21—4.49 when compared to corresponding normal profiles (Fig. 2). This could reflect an increased proportion of sialylated I-cell disease acid β-D-galactosidase and would be compatible with reports of an increased degree of sialylation of certain excreted lysosomal hydrolases from I-cell disease cultured fibroblasts [26], increased levels of sialyted oligosaccharides from I-cell disease urine [51], and increased levels of sialic acid containing molecules in I-cell disease cultured fibroblasts and autopsied liver [19,52]. All of the aforementioned results could be attributed to a deficiency of neuraminidase activity in I-cell cultured fibroblasts and leukocytes [19,51,53]. Molecules other than sailic acid, however, have not been ruled out as contributing to I-cell disease enzyme activity in the pH range 4.21—4.49.

In contrast, the reproducible shift of 0.12—0.17 pH units observed for the predominant isoelectric forms of the partially purified I-cell disease acid β-D-galactosidases (Fig. 1, Panel B), suggest that the subtle changes in charged group(s) responsible for the shift of isoelectric points were also responsible for the electrophoretic variation we observed on starch gel electrophoresis [27].

At the present time the molecular mechanism responsible for I-cell disease is not known. The evidence, however, suggests that an altered carbohydrate composition of lysosomal hydrolases may be involved in the disease process [23,54]. If a change in a common post-translational modification system (i.e. carbohydrate) had occurred in I-cell disease, it could explain many of the altered properties (electrophoretic mobility, sialylation, kinetic constants, "recognition" markers, and different intracellular levels of certain hydrolases) reported for the ICD hydrolases [19,23,26,27,51—54]. The expression of the defect may vary among the lysosomal hydrolases depending on their specific carbohydrate composition and the structural and biochemical function of the carbohydrate. Studies are now in progress to ascertain whether the altered properties observed for the I-cell disease β-D-galactosidases are the result of such a post-translational modification.

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