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ACID GLYCOHYDROLASE IN CHINESE HAMSTER WITH SPONTANEOUS DIABETES

I. DEPRESSED LEVELS OF RENAL α-GALACTOSIDASE AND β-GALACTOSIDASE

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Summary

The activities of α- and β-galactosidases (α-D-galactoside galactohydrolase, EC 3.2.1.22; β-D-galactoside galactohydrolase, EC 3.2.1.23) were significantly lower in the kidneys of diabetic XA line than those in the nondiabetic M line Chinese hamsters. The depression of these enzymes was found only in the kidney but not in liver, spleen, hind leg muscle, cheek pouch or spinal cord. In young XA animals before onset of glycosuria, renal α-galactosidase level was similar to that in age-matched M animals; whereas, their renal β-galactosidase activity was about 90% of those in the M animals. Partial purification and separation of these enzymes were achieved by chromatography on DEAE-Sepharose CL-6B columns. β-galactosidase was separated into two isozymes and depression of activity in the XA kidneys was evident in both. α-galactosidase was recovered in a single peak. The pH optima of these enzymes from XA and M animals were identical. With *p*-nitrophenyl glycosides as substrates, the Michaelis constants of these enzymes were also the same for XA and M animals. Molecular weight estimation by gel filtration on Sepharose 6B yielded similar results between M and XA samples: $2.4 \cdot 10^5$ for α-galactosidase and $1.6 \cdot 10^5$ and $1.9 \cdot 10^5$ for β-galactosidase isozymes. The data suggest that the diabetic animals had lower concentrations of α- and β-galactosidase in their kidneys, probably as a consequence of hyperglycemia.

Introduction

It was reported that a hydroxylysine-linked α-glucosyl-β-galactose disaccharide unit was significantly increased in renal glomerular basement membrane isolated from diabetic human patients [1]. Such an increase could arise from

enhanced synthesis and/or decreased degradation of the disaccharide units. Decrease in kidney β-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) has been reported in diabetic rats chemically induced by streptozotocin injection [2]. The fact that streptozotocin also showed cytotoxicity to the kidney [3,4] raised the question whether the depression of renal β-galactosidase in these animals was directly related to diabetes. A study with hereditary diabetic animals will provide answer to this question.

This paper presents study on the levels of β-galactosidase as well as α-galactosidase (α-D-galactoside galactohydrolase, EC 3.2.1.22), α-glucosidase (α-D-glucoside glucohydrolase, EC 3.2.1.20) and β-glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21) in the kidney of Chinese hamster (*Cricetulus griseus)* with spontaneous diabetes. The significantly lower activities of α- and β-galactosidase observed in the kidneys of the diabetic Chinese hamsters as compared to the nondiabetics led to further investigations to determine if the decreased activities were due to depressed enzyme levels or variant forms of enzymes and if the depressions were related to hyperglycemia and limited only to the kidney.

Materials and Methods

Animals

The Chinese hamster colony was established to produce animals which would breed true for the diabetic or nondiabetic trait using glycosuria as the phenotypic endpoint for selection [5]. The animals in the XA line belonged to the 14th—17th generations of selective inbreeding between diabetic siblings and 95% of the animals in these generations had shown glycosuria at the age of 4—8 weeks, whereas the M line had produced only aglycosuric offspring in the past 14—16 generations of continuous inbreedings. The animals were all males and age-matched. The age of animals in the study on the levels of four glycosidases (Table I) were between 9—57 weeks, the pre-diabetic groups (Table II) were 20—23 days old and those in the last group (Tables III and IV and all Figures) were 11—23 months old. The levels of renal α- and β-galactosidase did not appear to vary much with age.

Preparation of kidney 12 000 × g supernatant and precipitates

The animals were exsanguinated through the orbital sinus and a 20-μl aliquot of blood was diluted with 1 ml 0.6 M NaF for sugar determination. The kidneys were taken out immediately, rinsed in cold saline, blotted on filter paper, halved, decapsulated, weighed and homogenized in 9 vols. (tissue weight) of chilled buffer containing 0.45 M sucrose, 0.68 mM Na_2EDTA, pH 7.0 in a Potter-Elvejhem homogenizer with a Teflon pestle. The homogenates were centrifuged at 12 000 × *g* for 20 min. The supernatants were siphoned off and the pellets washed once with 5 ml buffer and resuspended in 9 vols. (tissue weight) of buffer. The suspensions were frozen in solid CO_2/acetone and thawed. The process was repeated twice and the suspensions were homogenized in a Polytron™ homogenizer/disintegrator at a setting of 7 to 15 s. The resultant homogenates were frozen and assayed for activity of α- and β-galactosidase and α- and β-glucosidase within a week.

Preparation of Triton-X100 extracts of various tissues

After exsanguinating the animals, the tissues were excised, rinsed in cold saline, blotted, weighed and homogenized in a solution containing 0.1% Triton X-100, 0.45 M sucrose, 0.68 mM Na_2EDTA, pH 7.0. The volumes of the solutions varied with tissues: liver, kidney, muscle and cheek pouch (9 vols. of fresh tissue weight), spleen (24 vols.) and spinal cord (19 vols.). The homogenates obtained with Polytron™ homogenizer/disintegrator (30 s at setting 7) were centrifuged at 12 000 × *g* for 20 min and the supernatants were used to assay α- and β-galactosidase.

DEAE-Sepharose CL-6B column chromatography

8 or 9 ml of pooled kidney 12 000 × *g* supernatant was chromatogrammed on a DEAE-Sepharose CL-6B column (0.9 × 30 cm), equilibrated in 0.01 M NaH_2PO_4/Na_2HPO_4, 0.1% NaN_3, pH 6.0, and eluted with a linear gradient made by mixing 100 ml each of 0 and 0.25 M NaCl in the same buffer. Fractions of 2 ml were collected and 0.1-ml aliquots of each even-numbered tube were assayed for α- and β-galactosidase for 1 h at 37°C and absorbance due to proteins was measured at 280 nm. The fractions containing α- and β-galactosidase were pooled and concentrated in Minicon™ macrosolute concentrators.

Determination of molecular weights on Sepharose 6B column

0.3 ml of partially purified α- or β-galactosidase was filtered through a Sepharose 6B column (0.9 × 60 cm) equilibrated in 0.01 M NaH_2PO_4/Na_2HPO_4, 0.1% of NaN_3 and 5 mM NaCl, pH 6.0. 1-ml fractions were collected and 0.1-ml aliquots of each fraction were assayed for enzyme activities. The column was calibrated with Blue Dextran 2000, glutamate dehydrogenase (EC 1.4.1.3), pyruvate kinase (EC 2.7.1.40) and aldolase (EC 4.1.2.7).

Enzyme assay

Enzyme activities were measured with *p*-nitrophenyl derivatives as substrates [6]. *p*-Nitrophenyl glycosides were dissolved in buffer (0.1 M Na_2HPO_4 adjusted to desired pH with 0.05 M citric acid) at a concentration of 5.26 mM. α- and β-galactosidase were assayed at pH 4.5, α-glucosidase at 6.5 and β-glucosidase at 5.5, which were selected as the optimal condition from preliminary pH dependence curves. The activities were determined from a linear time-course in 1 ml mixture at 37°C. Reaction was terminated with 0.2 ml cold 30% trichloroacetic acid and, after centrifugation, 0.2 ml 5 M 2-amino-2-methyl-propanediol was added to the supernatant. The products were measured at 415 nm with *p*-nitrophenol as standards and activity was calculated from a standard curve carried out simultaneously in each assay.

Miscellaneous

Blood sugar was determined by the ferricyanide method on an Autoanalyzer [7] and proteins according to the method of Lowry et al. [8]. Substrates and enzymes used in Sepharose 6B column calibration were purchased from Sigma, Blue Dextran 2000, Sepharose 6B and DEAE-Sepharose CL-6B from Pharmacia and Minicon™ concentrators from Amicon.

Results

Activities of kidney glycosidases in hyperglycemic XA and age-matched M animals

The XA animals were hyperglycemic (383 ± 30 mg sugar/100 ml blood) as compared to the M animals (117 ± 5 mg/100 ml) (Table I). The protein concentrations in the 12 000 × *g* supernatant and precipitate fractions of kidney extracts were not significantly different between the two lines of animals. The activities of kidney α- and β-glucosidase of M and XA animals were also similar in both supernatant and precipitate fractions and these enzymes were found mainly in the 12 000 × *g* supernatant. α- and β-galactosidase, on the other hand, were present in both soluble and particulate fractions and their activities were significantly lower in the kidneys of hyperglycemic XA animals than in those of the matched M animals (Table I). The decrease was about 30% in β-galactosidase of both fractions and in α-galactosidase of the particulate fractions and 44% in α-galactosidase in the soluble fraction.

Activities of α- and β-galactosidase in prediabetic XA and age-matched M animals

Table II shows the results of similar studies on young animals, i.e. 20—23 days, before the XA animals showed any signs of glycosuria by TesTape™. Although these XA animals were normoglycemic (111 ± 8 mg/100 ml) and aglycosuric, they showed some abnormal characteristics commonly found in hyperglycemic XA animals such as splenomegaly (26.7 ± 0.9 vs. 20.0 ± 1.2 mg,

TABLE I

KIDNEY α- AND β-GLUCOSIDASE AND α- AND β-GALACTOSIDASE ACTIVITY IN AGE-MATCHED MALE M- AND XA-LINE CHINESE HAMSTERS AFTER DEVELOPMENT OF GLYCOSURIA IN XA ANIMALS

One unit is defined as the amount of enzyme which liberated 1 nmol of *p*-nitrophenol per min. All values are mean ± S.E.M. (No. of animals). "N.S." denotes not significant, i.e. $P > 0.05$ according to Student's *t*-test.

Measurements	Unit	M	XA	*P*
Blood sugar	mg/100 ml	117 ± 5 (11)	383 ± 30 (11)	<0.001
Kidney protein concentration	mg/g kidney			
12 000 × *g* supernatant		92.1 ± 2.0 (11)	87.7 ± 1.6 (11)	N.S.
12 000 × *g* precipitate		83.5 ± 3.6 (11)	89.3 ± 3.1 (11)	N.S.
Kidney α-glucosidase	unit/g kidney			
12 000 × *g* supernatant		97.4 ± 8.6 (11)	105.0 ± 9.5 (11)	N.S.
12 000 × *g* precipitate		34.0 ± 4.6 (11)	35.3 ± 4.4 (11)	N.S.
Kidney β-glucosidase	unit/g kidney			
12 000 × *g* supernatant		947 ± 41 (11)	896 ± 34 (11)	N.S.
12 000 × *g* precipitate		50.2 ± 4.3 (5)	42.2 ± 2.3 (5)	N.S.
Kidney α-galactosidase	unit/g kidney			
12 000 × *g* supernatant		665 ± 41 (11)	376 ± 26 (11)	<0.001
12 000 × *g* precipitate		480 ± 25 (11)	348 ± 20 (11)	<0.005
Kidney β-galactosidase	unit/g kidney			
12 000 × *g* supernatant		1181 ± 58 (11)	851 ± 40 (11)	<0.001
12 000 × *g* precipitate		866 ± 56 (11)	622 ± 31 (11)	<0.005

TABLE II

KIDNEY α- AND β-GALACTOSIDASE ACTIVITY IN AGE-MATCHED MALE M- AND XA-LINE CHINESE HAMSTERS BEFORE ONSET OF GLYCOSURIA IN XA ANIMALS

For details see Table I.

Measurements	Unit	M	XA	P
Blood sugar	mg/100 ml	105 ± 4 (6)	111 ± 8 (6)	N.S.
Kidney protein concentration	mg/g kidney			
12 000 × *g* supernatant		81.3 ± 5.7 (6)	80.4 ± 4.3 (6)	N.S.
12 000 × *g* precipitate		109 ± 6.5 (6)	101 ± 9.1 (6)	N.S.
Kidney α-galactosidase	unit/g kidney			
12 000 × *g* supernatant		564 ± 36 (6)	623 ± 25 (6)	N.S.
12 000 × *g* precipitate		589 ± 22 (6)	567 ± 15 (6)	N.S.
Kidney β-galactosidase	unit/g kidney			
12 000 × *g* supernatant		880 ± 60 (6)	798 ± 41 (6)	N.S.
12 000 × *g* precipitate		881 ± 42 (6)	774 ± 23 (6)	<0.05

$P < 0.005$); the degree of these abnormalities was much less severe in these young 'prediabetic' than in the older diabetic animals, however. The 'prediabetic' XA animals showed similar protein contents in both supernatant and particulate fractions of the kidney extracts as the age-matched M animals. The activity of kidney α-galactosidase in the young XA animals was also not different from that in the age-matched M animals. These normoglycemic XA animals, however, showed slightly lower β-galactosidase activity in their kidneys, i.e. about 90% of that in the M animals; the difference, however, was statistically significant only in the particulate, but not in the supernatant fraction (Table II).

Activities of α- and β-galactosidase in 0.1% Triton X-100 extracts of various tissues of hyperglycemic XA and age-matched M animals

The extracting buffer containing 0.1% Triton X-100 was able to solubilize 90% of total tissue α-galactosidase and 85% β-galactosidase in the kidney. Table III shows the activities of α- and β-galactosidase in 0.1% Triton X-100 extracts of kidney, liver, spleen, hind leg muscle, cheek pouch and spinal cord of the glycosuric and hyperglycemic (237 ± 29 mg sugar/100 ml blood) XA animals and their age-matched M animals (70 ± 5 mg/100 ml). Significantly lower activities of both enzymes were again observed in the kidney extracts of the XA animals than in those of the M animals and the decrease was about 25% in α-galactosidase and 30% in β-galactosidase. The activities of these enzymes in other tissue extracts, however, were not significantly different between XA and M animals. The tissue contents (unit/g fresh tissue weight) of these two enzymes were parallel and followed this order of decreasing activity: kidney > spleen > liver > spinal cord > cheek pouch > hind leg muscle. Hind-leg muscle showed higher α- than β-galactosidase activity, whereas kidney, spleen, and cheek pouch showed the reverse. Liver and spinal cord had similar activities in α- and β-galactosidase. XA and M animals showed similar patterns of these enzymes in their tissues.

TABLE III

ACTIVITIES OF α- AND β-GALACTOSIDASE IN 0.1% TRITON X-100 EXTRACTS FROM VARIOUS TISSUES OF AGE-MATCHED MALE M- AND XA-LINE CHINESE HAMSTERS AFTER DEVELOPMENT OF GLYCOSURIA IN THE XA ANIMALS

For details see Table I.

Tissue	α-galactosidase			β-galactosidase		
	Unit/g tissue			Unit/g tissue		
	M	XA	*P*	M	XA	*P*
Kidney	1068 ± 79 (7)	796 ± 73 (7)	<0.05	1860 ± 57 (7)	1285 ± 131 (7)	<0.01
Liver	123 ± 10 (7)	141 ± 11 (7)	N.S.	112 ± 8 (7)	122 ± 14 (7)	N.S.
Spleen	450 ± 29 (7)	553 ± 43 (7)	N.S.	756 ± 34 (7)	848 ± 53 (7)	N.S.
Hind-leg muscle	15.9 ± 1.5 (7)	16.7 ± 3.5 (7)	N.S.	7.3 ± 0.8 (7)	7.6 ± 1.4 (7)	N.S.
Cheek pouch	35.4 ± 3.9 (7)	38.7 ± 3.0 (7)	N.S.	46.3 ± 3.5 (7)	67.2 ± 11.3 (7)	N.S.
Spinal cord	111 ± 19 (7)	84 ± 6 (7)	N.S.	73.0 ± 10.7 (7)	93.0 ± 13.7 (7)	N.S.

DEAE-Sepharose CL-6B column chromatography of XA and M α- and β-galactosidase

Fig. 1 shows the elution profiles of α- and β-galactosidase and total proteins in the pooled 12 000 × *g* supernatant fraction of M or XA kidney extracts. Although the profiles were qualitatively similar, i.e. identical retention times, the amount of activities present in each peak was much lower in the XA sample. The tubes containing enzyme activities were pooled and concentrated; the collected samples were designated as β-galactosidase A (fraction I), and B (fraction II) and α-galactosidase (fraction III) (Fig. 1). Table IV summarizes the yields and purification factors of these enzymes after the one-step operation. About 20-fold purification in α-galactosidase was obtained for both lines, and the yields were 21.2% for M and 45.5% for XA. The purification factors for β-galactosidase were much lower, 1.33—1.52 for A and 2.57—3.12 for B and the yields of combined A and B activities were 25.3% for M and 39.4% for XA.

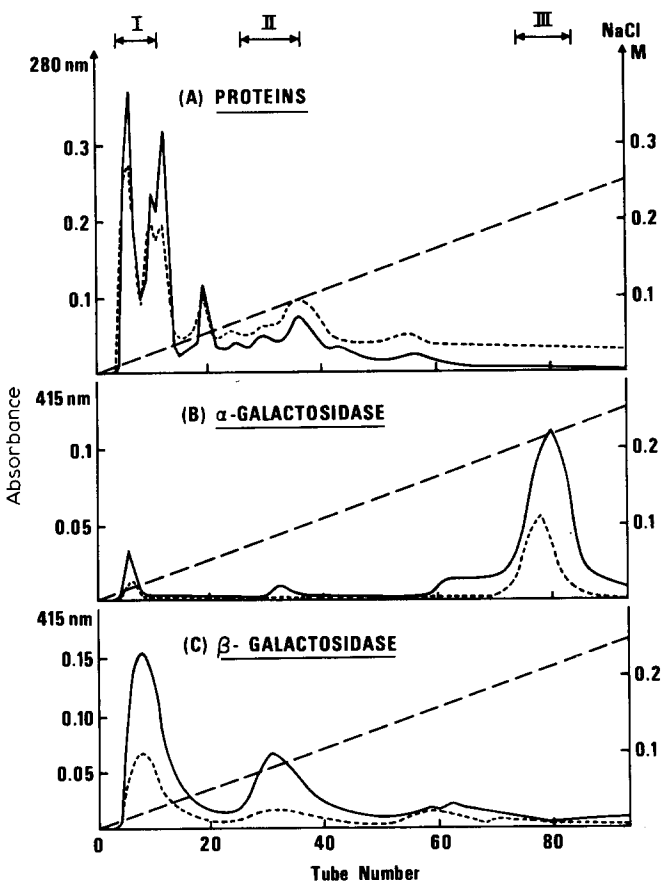

Fig. 1. Elution profiles of proteins, α-galactosidase and β-galactosidase in renal 12 000 × *g* supernatant of M and XA animals on DEAE-Sepharose CL-6B column. Column size was 0.9 × 30 cm. Each tube contained 2.0 ml fractions. Solid lines, M; dotted lines, XA animal sample; dashed lines, NaCl concentrations. Fractions I and II contained mainly β-galactosidase activity; Fraction III, α-galactosidase.

TABLE IV

RECOVERY AND PURIFICATION OF KIDNEY α- AND β-GALACTOSIDASE OF AGE-MATCHED MALE M- AND XA-LINE CHINESE HAMSTERS AFTER DEVELOPMENT OF GLYCOSURIA IN XA ANIMALS ON DEAE-SEPHAROSE CL-6B COLUMN CHROMATOGRAPHY

See Fig. 1 for fraction designation and Table I for unit definition.

Measurement	Unit	α-Galactosidase		β-Galactosidase		
		12 000 × *g* Supernatant	Fraction III	12 000 × *g* Supernatant	Fraction I	Fraction II
M-line sample						
Volume	ml	8.0	6.0	8.0	4.5	5.0
Protein conc.	mg/ml	9.59	0.23	9.59	2.13	0.52
Specific activity	unit/mg	7.02	154.7	11.8	15.7	30.4
Yield	%	100	21.2	100	16.6	8.7
Purification	-fold	1	22.1	1	1.33	2.57
XA-line sample						
Volume	ml	9.0	4.5	9.0	5.5	5.2
Protein conc.	mg/ml	9.18	0.42	9.18	2.23	0.86
Specific activity	unit/mg	5.71	113.6	7.03	10.7	21.9
Yield	%	100	45.5	100	22.6	16.8
Purification	-fold	1	19.9	1	1.52	3.12

The effect of pH on XA and M α- and β-galactosidase activity

Fig. 2 shows pH-dependence of α-galactosidase from both M and XA kidneys. The optimal pH values for α-galactosidase from these lines were identical and fell around 4.5. The pH-dependence curves of β-galactosidase isozymes were also similar between A and B and between samples of M and XA lines; all four curves peaked at pH 4.0 (Fig. 3). Unlike α-galactosidase which showed a distinct peak at pH 4.5 and drastically reduced activities at either end of pH extremes, β-galactosidase still retained considerable activities at pH values as low as 2.5.

The effect of substrate concentration on XA and M α- and β-galactosidase activity

The substrate-dependent initial rates of α-galactosidase were run from 0.526 to 5.26 mM *p*-nitrophenyl-α-D-galactoside at pH 4.5 and the results were plotted according to the system of Lineweaver and Burk [9] as shown in Fig. 4. Both M and XA kidney α-galactosidases yielded the same Michaelis constant, 2.78 mM for *p*-nitrophenyl-α-D-galactoside. Similar studies with β-galactosidase isozymes A and B from M and XA kidneys also yielded identical intercepts at the abscissa of the Lineweaver-Burk plot, i.e. 4.75 mM^{-1} (Fig. 5). The K_m for these β-galactosidases with *p*-nitrophenyl-β-D-galactoside was estimated to be 0.21 mM.

Molecular weight determination of XA and M α- and β-galactosidase

To determine the size of α- and β-galactosidase, the partially purified fractions were chromatogrammed on Sepharose 6B column and the filtration profiles showed coincidental peaks of these enzymes between M and XA kidney

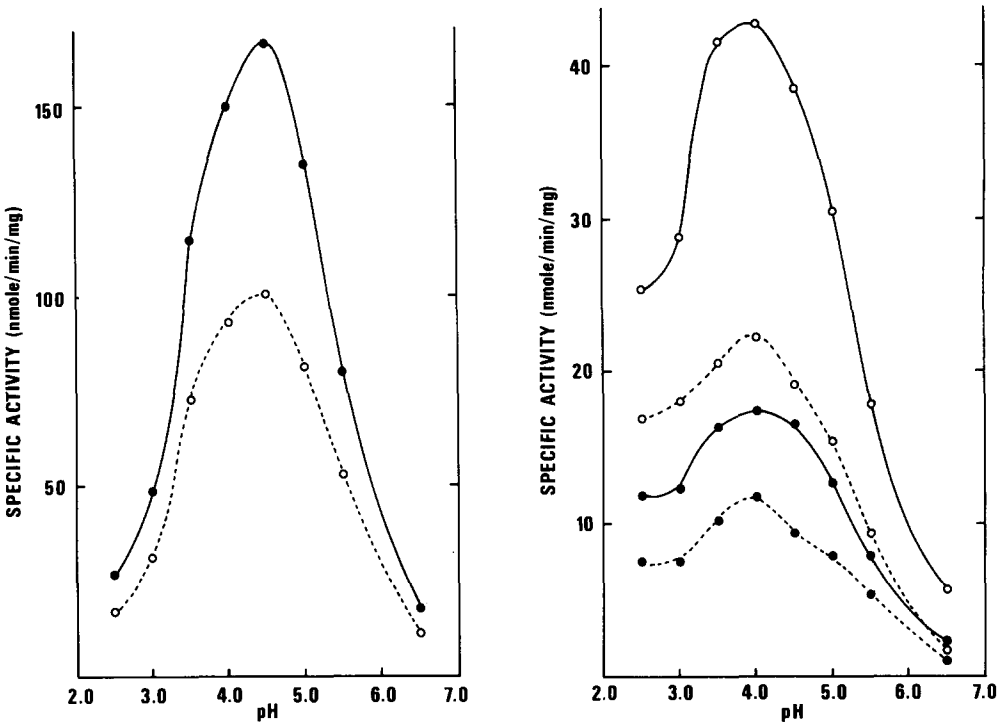

Fig. 2. Activity vs. pH of Chinese hamster renal α-galactosidase. Buffers were made by adjusting 0.1 M Na_2HPO_4 with 0.05 M citric acid to desired pH. ●———●, M line; ○- - - - -○, XA line.

Fig. 3. Activity vs. pH of Chinese hamster renal β-galactosidase. Buffers were made by adjusting 0.1 M Na_2HPO_4 with 0.05 M citric acid to desired pH. ●———●, M line isozyme A; ○———○, M line isozyme B; ●- - - - -●, XA line isozyme A; ○- - - - -○, XA line isozyme B.

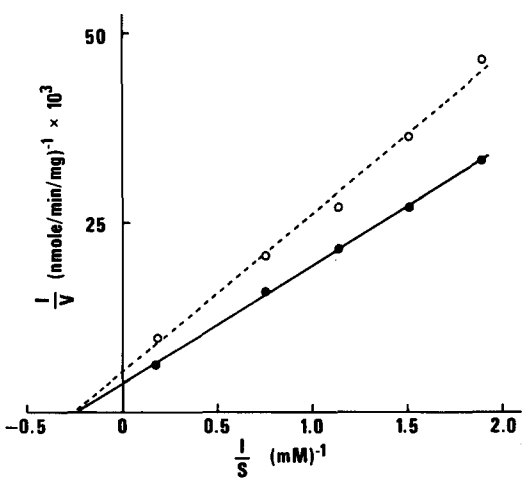

Fig. 4. Activity vs. substrate concentrations of Chinese hamster renal α-galactosidase. Activity was measured at pH 4.5. ●———●, M line; ○- - - - -○, XA line.

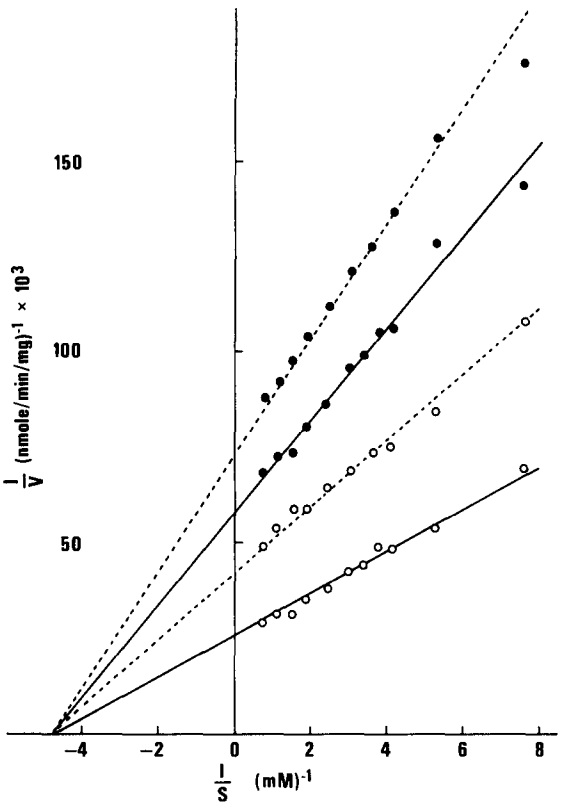

Fig. 5. Activity vs substrate concentrations of Chinese hamster β-galactosidase. Activity was measured at pH 4.5. Lines and symbols as in Fig. 3.

samples. The column was calibrated with Blue Dextran 2000 for void volume and 3 enzymes of known molecular weights [10]: glutamate dehydrogenase ($3.5 \cdot 10^5$), pyruvate kinase ($2.4 \cdot 10^5$) and aldolase ($1.6 \cdot 10^5$). The molecular weights of renal α-galactosidase and β-galactosidase isozymes A and B were estimated to be $2.4 \cdot 10^5$, $1.6 \cdot 10^5$ and $1.9 \cdot 10^5$ respectively and they were the same for enzymes from M and XA animals.

Discussion

Decreased activity of β-galactosidase in the 10 000 × *g* supernatant fraction of kidney homogenates has been reported in the diabetic rats induced by streptozotocin injection [2]. The observations obtained in the present study that both the 12 000 × *g* supernatant and particulate fractions of the kidney extracts of Chinese hamsters with spontaneous diabetes had similar changes suggest that the lower activity of renal β-galactosidase was directly related to the development of diabetes. Furthermore, this study using partially purified enzyme preparations yielded data showing that renal β-galactosidase from the diabetic XA and the nondiabetic M line animals had identical properties with respect to molecular size, retention time on ion-exchange columns, effect of H^+ concentration on activity and affinity to *p*-nitrophenyl-β-D-galactoside and that the

decrease in renal β-galactosidase activity in the diabetic animal kidneys was found in both isozymes A and B. These lines of experimental evidence provided support that these animals had depressed levels of β-galactosidase in their kidneys.

The studies on the young XA animals before the onsets of hyperglycemia and glycosuria showed that these animals already had slightly lower level (90%) of renal β-galactosidase than those in the age-matched M animals, indicating that the depression of β-galactosidase was an early process and preceded the manifestation of hyperglycemia and glycosuria. On the other hand, one could argue that the level of tissue β-galactosidase was genetically controlled in a manner analogous to the *Bgs* locus in the mouse [6,11] and that the difference observed in the highly inbred M and XA line animals was a result of genetic variants of this specific locus. If it was the case, one would expect differences in β-galactosidase level between XA and M animals in other tissues besides the kidney as in mice carrying Bgs^h and Bgs^d alleles [6,11]. However, the observation that the XA animals did not show decreased level of β-galactosidase in liver, spleen, muscle, cheek pouch and spinal cord ruled out the possibility that allelism in *Bgs*-like locus was responsible for the depressed β-galactosidase in the kidney of the diabetic XA animals. Furthermore, the depression of renal β-galactosidase was exacerbated (70% of control) in the adult XA animals after the onset of hyperglycemia, again suggesting correlation between the level of renal β-galactosidase and hyperglycemia.

It is interesting that α-galactosidase level was also considerably depressed in the kidney, but not in other tissues, of the diabetic XA animals. There have been no reports on the level of this enzyme in the diabetic animals in the literature probably because most glycoproteins studied so far contained galactose mainly in the β-linkage [12]. Nevertheless, the depression of α-galactosidase in the kidneys of diabetic animals was more severe (44%) than that of β-galactosidase (30%) and its level was normal in the prediabetic XA kidneys, indicating that the onset of its depression probably followed the manifestation of hyperglycemia, unlike that of β-galactosidase.

The lack of difference in renal α- and β-glucosidase levels between XA and M animals was surprising but it further indicated specificity of diabetes-induced depression of renal α- and β-galactosidase. However, the physiological significance of this finding remained obscure and any discussion on this subject is speculative at best. In renal glomerular basement membrane isolated from diabetic humans with kidney complications, significant increase in an α-glucosyl-β-galactose disaccharide unit was reported [1]. If similar changes also occurred in the diabetic Chinese hamster kidneys, the depressed level of β-galactosidase may be one of the contributing factors leading to the accumulation of this disaccharide in the basement membrane. The removal of this unit from the basement membrane may proceed by sequential cleavage of the sugars or by the action of an endo-β-galactosidase. Therefore, whether the β-galactosidase studied here with artificial substrate also contained such endoglycosidase activity would be an interesting course to follow. Furthermore, the observed depression of renal α-galactosidase level signifies possible alterations in glycoproteins or glycolipids containing galactose in the α-linkage in the diabetic animals and calls for further studies on the compositional changes in renal glycoproteins and glycolipids of these animals.

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