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PLASMA α-GALACTOSIDASE A: PROPERTIES AND COMPARISONS WITH TISSUE α-GALACTOSIDASES

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Summary

The human plasma form of α-galactosidase A (α-D-galactoside galactohydrolase, EC 3.2.1.22) was highly purified and exhibited apparent K_m values of 1.9 mM with 4-methylumbelliferyl-α-D-galactopyranoside and 0.23 mM with globotriglycosylceramide. Its inhibition with *myo*-inositol (K_i = 0.29 M) was similar to that observed with α-galactosidase A from various tissues. The plasma form of this lysosomal enzyme has a lower molecular weight of 96 600, a lower pI of 3.7 and faster electrophoretic mobility in polyacrylamide gels than the enzyme obtained from human liver. These data and the increased pI obtained after neuraminidase treatment suggest that the plasma form is an isoenzyme with a more highly sialylated carbohydrate moiety than the tissue isoenzymes.

Introduction

Metabolism of neutral glycosphingolipids of the "globo"-family is disturbed in patients with Fabry's disease, an X-linked disorder characterized by deficiency of the lysosomal glycosidase, α-galactosidase A (α-D-galactoside galactohydrolase, EC 3.2.1.22) [1]. Accumulation of globotriglycosylceramide (ceramide trihexoside) is observed in vascular endothelial cells in these patients and increased amounts of galabiosylceramide (galactosyl-(α1 → 4)-galactosylceramide) occurs in kidney, pancreas and urinary sediment cells [1]. Blood group B-specific glycosphingolipid accumulation has been reported in a patient with B specificity [2].

The purification and properties of α-galactosidase A have been reported with preparations from human placenta [3—5] and liver [6,7]. Our preceding paper reported a method for purifying an α-galactosidase from frozen Cohn fraction

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Abbreviations: 4-MU-Gal, 4-methylumbelliferyl-α-D-galactoside; $GbOse_3Cer$, globotriglycosylceramide.

IV-1 to a specific activity about 7000 times greater than that of fresh human plasma [8]. Further purification of the plasma form of α-galactosidase A has been achieved by hydroxyapatite and Octoyl-Sepharose chromatography; these data and the kinetic and molecular weight properties of the product are described in this report.

Materials and Methods

Chemicals. Hydroxyapatite was obtained from Clarkson Chemical Company, Inc., Williamsport, Pa., U.S.A.; *myo*-inositol from Sigma Chemical Company, St. Louis, Mo., U.S.A.; and Octoyl Sepharose from Pharmacia Fine Chemicals, Piscataway, N.J., U.S.A.

The source of plasma α-galactosidase and the sources of ingredients for enzymatic assays were listed in a previous paper [8]. 4-Methylumbelliferone (practical grade) was purchased from Sigma Chemical Co. All other chemicals were of analytical reagent grade. Human liver and splenic preparations of α-galactosidase A were obtained from K.J. Dean and R.J. Desnick, respectively.

α-Galactosidase assays. The assays using globotriglycosylceramide ($GbOse_3$-Cer) and 4-methylumbelliferyl-α-D-galactopyranoside (4-MU-Gal) were described previously [8]. The artificial substrate activities were measured at 4.3 mM 4-MU-Gal and the natural substrate activities were measured at 0.023 mM $GbOse_3Cer$ (corrected to 0.5 mM using the Michaelis-Menten equation, assuming a K_m of 0.45 mM).

Calculated V values were obtained with the K_m values determined herein and the integrated Michaelis-Menten equation, $V = (K_m \ln (a_o/a) + a_o - a)/t$, where a_o is concentration at zero time and a is concentration at time t (20 min). All ceramide trihexosidase values are reported in this way, whereas 4-MU-Gal substrate values were obtained as in the previous paper [8], unless indicated as integrated values.

Sucrose density centrifugation. The molecular weight of plasma α-galactosidase A was estimated by the sucrose density sedimentation method of Martin and Ames [9]. Gradients were prepared 1—2 h before use from 5 and 20% sucrose solutions containing 25 mM sodium phosphate (pH 6.0). The samples were centrifuged at 45 000 rev./min in a Beckman centrifuge (50.1 Ti rotor) for 16.5 h at 4°C. A Pharmacia P-3 peristaltic pump (Pharmacia Fine Chemicals, Piscataway, N.J., U.S.A.) was used to unload the gradients by pumping 50% w/v sucrose plus Blue Dextran 2000 (Pharmacia) into the bottom of the tube. 172-μl fractions were removed from the top; the blue color aided in the identification of the last fraction.

The following enzymes were used as molecular weight standards: mitochondrial pig heart malate dehydrogenase (Boehringer Mannheim Biochemicals, Indianapolis, IN., U.S.A.) M_r = 33 500 (subunit) [10,11] *; pig heart glutamate-oxaloacetate transaminase (Boehringer) M_r = 94 000 [12,13]; pig heart diaphorase (Sigma type III, Sigma Chemical Co., St. Louis, MO., U.S.A.) M_r =

* The first reference is for the molecular weight value and the second one is for the assay method.

100 000 [14,15]; rabbit muscle lactate dehydrogenase (Sigma type II) M_r = 139 000 [16,17]. The molecular weight was determined from a plot of migration distance vs. $M_r^{2/3}$ [9].

Neuraminidase treatment of spleen and plasma α-galactosidases. Neuraminidase (from *Clostridium perfringens*) bound to agarose beads was obtained from Sigma Chemical Co. It had an activity of 0.6 μmol/min per ml by the 2-(3′-methoxyphenyl)-*N*-acetyl-α-neuraminic acid assay of Palese et al. [18]. It had no detectable protease activity in either sodium acetate (pH 5) or sodium phosphate (pH 7) buffers when assayed by a modification of the method of Schwabe [19]. In this assay, methylated gelatin was used as substrate instead of succinylated hemoglobin. The minimum detectable protease activity was equivalent to 0.5 μg/ml trypsin.

Agarose-bound neuraminidase (1 ml packed volume) was washed three times with 4-ml aliquots of 0.1 M sodium acetate (pH 5.0). To the washed, packed beads, 1 ml spleen or plasma α-galactosidase A in 150 mM NaCl/25 mM sodium phosphate (pH 6.0) was added and the suspension mixed gently for 3 h at 25°C. The suspension was poured into a small column and washed with 0.2 M NaCl/50 mM sodium phosphate buffer (pH 6.0). The 1-ml flow-through and following 1-ml wash were combined to give the neuraminidase-treated enzyme. The recovery of activity after this treatment was 60 and 94% for the spleen and plasma enzymes, respectively.

Isoelectric focusing. The procedure for small tube isoelectric focusing was described by Behnke et al. [20]. Ampholytes from LKB, Rockville, Md., U.S.A. were used in the following final concentrations: pH range 2.5—4.0, 1.4%; pH 3—10, 0.86%. Approx. 1000 units (4-MU-Gal substrate) of either spleen or plasma α-galactosidase A activity were added. Focusing was carried out at 4°C for 6 h, initially at 100 V to limit the maximum current to 5 mA/tube and later at 250 V. The tube size was 0.8 × 15 cm and the fraction size during column elution was 0.2 ml.

Purification of plasma α-galactosidase A. The purification procedure outlined in the preceding paper [8] consistently gave a product with a specific activity of 1000—1500 4-MU-Gal units/mg protein. Higher purity could be obtained by pooling narrower segments of the elution volume, at a sacrifice of yield. Prior to further characterization of the enzyme, additional purification procedures were investigated.

Hydroxyapatite quantitatively adsorbed plasma α-galactosidase A, provided that the ionic strength of the column buffer and enzyme solution was not greater than 1 mM sodium phosphate (pH 6.0). This was accomplished by dialysis of the enzyme solution in a hollow-fiber beaker unit (b/HFU-1, Dow Chemical Co., Midland, Mich., U.S.A.) against 5 l buffer. The bound enzyme was eluted from the hydroxyapatite column by a step gradient over the range of 5—20 mM sodium phosphate (pH 6.0). The elution molarity was dependent on the units of plasma α-galactosidase A applied and on the purity of the enzyme, so that a reduced-scale column (0.75 × 10 cm) was used in pilot studies to determine the optimum elution conditions.

Substantial additional purification was obtained by hydrophobic chromatography. At moderate ionic strength, plasma α-galactosidase A bound to Octoyl-Sepharose while most of the other proteins did not. Elution of the enzyme

activity was accomplished by reducing the molarity of sodium phosphate and increasing the concentration of ethylene glycol, in opposing gradients.

Results

Validity of enzyme assays. The enzymatic hydrolysis of 4-MU-Gal was linear up to 5 nmol 4-methylumbelliferone generated per assay. A standard curve of 4-methylumbelliferone vs. relative fluorescence also showed non-linearity above 5 nmol per assay, apparently due to fluorescence quenching. The assay was linear over the range of protein concentration used in the experiments and was linear with time for at least 30 min with an enzyme purity of 1000 U/mg protein.

$GbOse_3Cer$ hydrolysis was linear up to 50 μg protein per assay with a sharp reduction of hydrolytic rates at higher protein concentration. The net cpm liberated in galactose were not corrected for utilization of substrate during the assay, resulting in a slight departure from linearity below 50 μg. The rapid decrease in activity above this point may have been due to the interaction of protein with substrate micelles, as suggested by Gatt et al. [21]. The amount of $[^3H]GbOse_3Cer$ hydrolyzed was linear with time for 20 min with 19 μg ceramide trihexosidase (61 U/mg protein).

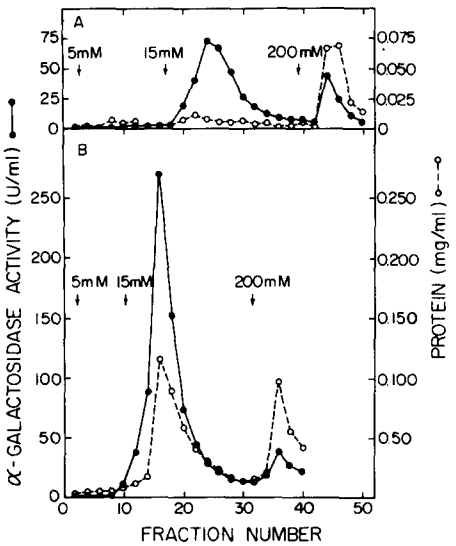
Purification of plasma α-galactosidase. Further purification of plasma α-galactosidase A from material purified through the Sephadex G-200 step is outlined in Table I. Two Sephadex G-200 products were each purified by hydroxyapatite chromatography with an average purification of 3.1-fold and 58% yield. The elution patterns for this step are presented in Fig. 1. At this stage in the purification, concentration of the two hydroxyapatite products using hollow-fiber units gave complete recovery of activity (Table I).

The purification obtained by Octoyl-Sepharose chromatography with opposing gradients of sodium phosphate and ethylene glycol is illustrated in Fig. 2. This step gave a 29-fold purification with a 60% yield (Table I). The pooled fractions were diluted 1 : 1 with 10 mM sodium phosphate (pH 6.0) and concentrated with a 43% loss in activity and a 40% reduction in purity (Table I). The sample was diluted to minimize deterioration of the hollow-fiber units by the

TABLE I

ADDITIONAL PURIFICATION OF PLASMA α-GALACTOSIDASE A FROM PARTIALLY PURIFIED ENZYME

Step		Volume (ml)	Protein (mg)	4-MU-Gal activity (KU)	Specific activity of II a + b (U/mg)	Purification (-fold)	Yield (%)
I	a. Sephadex G-200	50	11.2	20.5	1 830	—	100
	b. Sephadex G-200	24	63.4	43.4	686	—	100
II	a. Hydroxyapatite	220	1.65	10.5	6 360	3.5	51
	b. Hydroxyapatite	320	14.8	28.1	1 890	2.7	65
III	Hollow fiber concentration	26.5	15.2	38.3	2 530	3.0	60
IV	Octoyl sepharose	397	0.313	23.1	73 800	86	36
V	Hollow fiber concentration	23.4	0.296	13.2	44 600	52	21



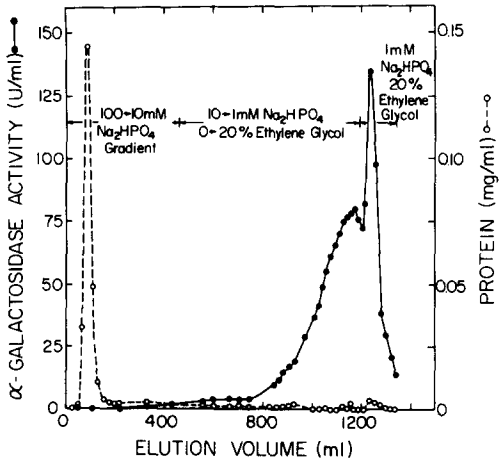


Fig. 1. Hydroxyapatite chromatography of plasma α-galactosidase A. Enzyme in 1 mM sodium phosphate, pH 7.0, was applied to a 3.1 × 10 cm column of hydroxyapatite (equilibrated against the same buffer) at a flow-rate of 0.4 ml/min at 4°C. The column was eluted with pH 7.0 phosphate buffer at the ionic strengths and times indicated at a flow rate of 4.0 ml per min. The fraction size was 20 ml. (A) The 50-ml sample (Table I, Ia) was applied over a period of 1 h. Fractions 22—23 were combined. (B) The 25 ml sample (Table I, Ib) was applied over a period of 1 h and Fractions 10—25 were pooled. For the 4-MU-gal assay, 39 mM Na_2HPO_4, 23 mM citric acid (final pH 4.6), and 4.3 mM 4-MU-gal was used. The enzyme solution was 25 μl and the final volume was 175 μl. The reaction was stopped with 2.33 ml of 0.1 M ethylene diamine. The protein assays were carried out as described in the previous paper [8].

Fig. 2. Octoyl-Sepharose chromatography of plasma α-galactosidase A. See Table I for the sample (III) and product (IV) for this column. The column was 4.1 × 10 cm and the beads were equilibrated with 100 mM Na_2HPO_4, pH 7.0 before sample application. The flow rate for all stages was 10 ml per min and the fraction size was approximately 20 ml. Fractions 42—63 were pooled.

ethylene glycol. However, since a later transfer of the product to small test tubes resulted in a further loss of activity, it is suspected that the highly diluted enzyme adsorbed to glass and possibly to plastic surfaces. This would explain a previously unaccounted for loss of activity in the dilute CM-cellulose product (see ref. 8 for procedure) upon transfer to a 2-l Nalgene-graduated cylinder. Aside from the activity loss experienced in transfers, the more highly purified enzyme was quite stable for periods of at least one month at 4°C or −20°C.

pH optimum with the $GbOse_3Cer$ Substrate. Enzyme with specific activities of 221 $GbOse_3Cer$ units per mg protein and 1180 4-MU-Gal units (integrated) per mg protein [8] gave a pH optimum of about 3.8 (Fig. 3). The observed activity was decidedly lower with citrate as buffer, as was pointed out in the preceding paper [8]. Since citric acid caused the enzyme to precipitate at pH 4.3 [8], the reduction in activity might have been due to denaturation of the enzyme by this ion. A more extended pH profile showed only one pH optimum. In acetate buffer (pH 3.5—5.5) the peak of activity for the more highly purified sample of α-galactosidase was at pH 4.05. The pH values at half-height for the peak were 3.75 and 4.35. With phosphate buffer (pH 5.5—7.0) and Tris buffer (pH 7.0—8.0) the activity was never greater than 5% of the peak at pH 4.05. The specific activity of this enzyme sample (Step V, Table I) was 8800 $GbOse_3Cer$

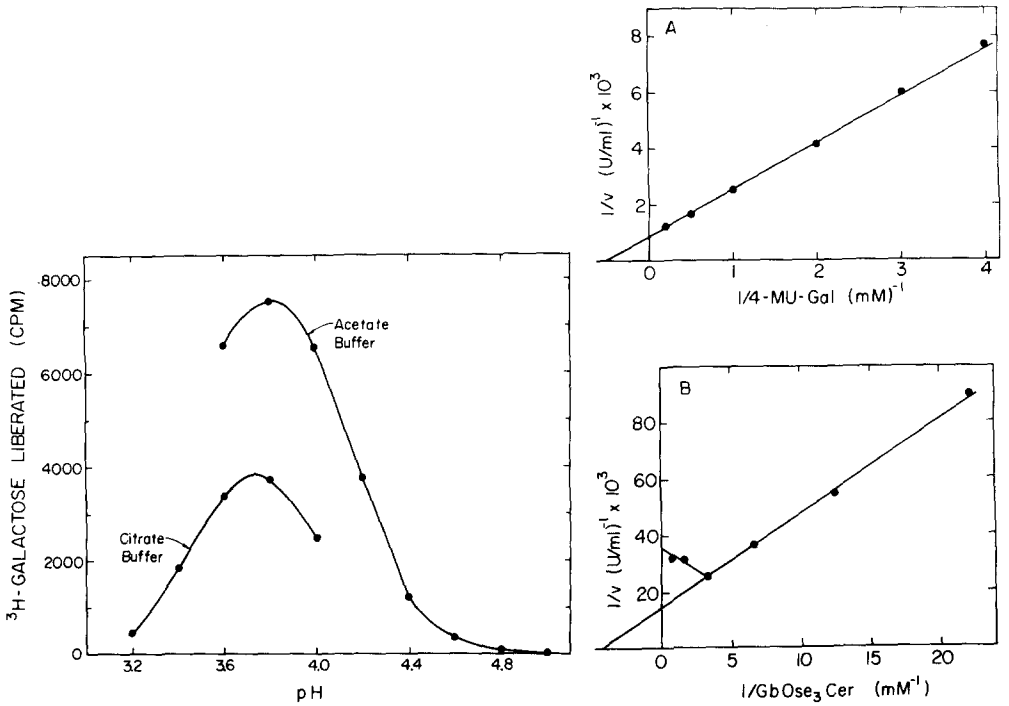


Fig. 3. Ceramide trihexosidase pH optimum. The purity of the enzyme used was 221 U/mg protein ($GbOse_3Cer$ substrate) and the incubation time was 30 min at 37°C. In 100 μl total volume were 50 mM sodium citrate or sodium acetate, 9.3 mM sodium taurocholate, 22 μM $GbOse_3Cer$ (50 000 cpm, 22 800 cpm/nmol) and 41 μg protein per assay.

Fig. 4. K_m determinations with artificial and natural substrates. (a) Varying amounts of 4-Mu-gal were used with the assay conditions described in the legend for Fig. 1. The purity of the enzyme used was 1040 U/mg (4-MU-gal) and 5.2 units were used per assay. (b) Varying levels of unlabeled $GbOse_3Cer$ were used with 102 000 cpm [^{3}H]$GbOse_3Cer$ (22 800 cpm/nmol) under the assay conditions described for Fig. 3 except that the incubation time was 10 min. The purity of the enzyme was 8800 U/mg ($GbOse_3Cer$) and 5.6 units were used per assay.

U/mg and 64 000 4-MU-Gal U/mg protein (integrated).

K_m determinations. The K_m for plasma α-galactosidase A with 4-MU-Gal was 1.9 mM (Fig. 4a) while with $HbOse_3Cer$ it was 0.23 mM (Fig. 4b). Using the K_m values determined for both substrates, the ratio of $V_{4\text{-MU-Gal}}/V_{GbOse_3Cer}$ for 15 different enzyme samples ranging in purity from 660—44 600 U/mg protein was 7.0 ± 1.3 S.D.).

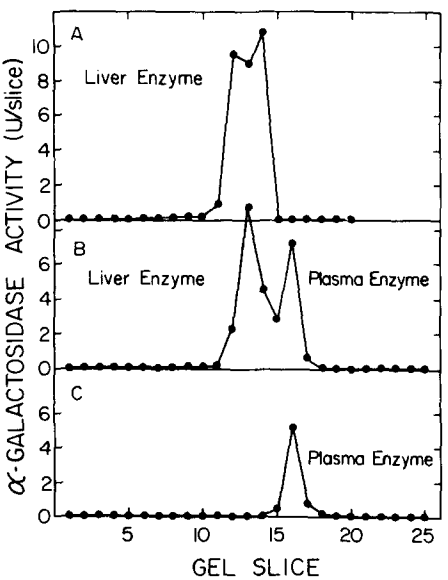
Inhibition by myo-inositol. The inhibition of plasma α-galactosidase by graded levels of *myo*-inositol was studied at two substrate concentrations. In the absence of *myo*-inositol the average maximal velocity (V) was 1250 U/ml (integrated) with 4-MU-Gal. Dixon plots for the two substrate concentrations, which co-intersect with the $1/V$ line above the i axis, establish that the inhibition is neither non-competitive nor uncompetitive [22]. The K_i for *myo*-inositol with plasma α-galactosidase A was 0.29 M.

Molecular weight. The molecular weight of the enzyme was 96 600, as determined by the sucrose density sedimentation method of Martin and Ames [9]. In a less accurate determination on Sephadex G-200, the molecular weight was

estimated at 112 000 relative to human serum albumin monomer and dimer peaks.

Mobility of plasma and liver α-galactosidase in polyacrylamide gel electrophoresis. A pH 6.5 gel system was used to minimize denaturation of the enzyme [8]. The plasma form of α-galactosidase A was more anodic than that of human liver (Fig. 5b). Simultaneous electrophoresis of the separate enzymes in parallel gels confirmed this difference (Figs. 5a and c). While the recovery of activity in gel B was 94%, the recovery in gel C was lower (59%) and in gel A was higher (175%). This last result suggests that the doublet in Fig. 5a may be artifactual, as indicated by its absence in the same region in gel B. The fact remains that whether by virtue of size or charge, the liver enzyme is electrophoretically different from the plasma enzyme.

Effect of neuraminidase on spleen and plasma α-galactosidase. The isoelectric points of untreated spleen and plasma α-galactosidase A fractions were 4.30 and 3.75, respectively (Fig. 6). Treatment of the plasma enzyme with neuraminidase bound to Agarose beads (Sigma Chemical Co.) raised the isoelectric point to pH 4.25 (Fig. 6b), approximately the same value as found for the untreated spleen enzyme. On the other hand, neuraminidase treatment of the



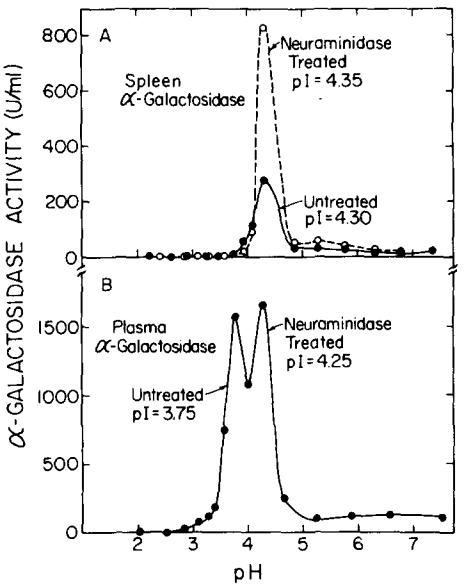


Fig. 5. Polyacrylamide gel electrophoresis of human liver and plasma α-galactosidase A. The preparation of the gels is outlined in the preceding paper [8]. After 90 min at 250 V each gel was frozen and cut into 47 26-μl slices. The slices were assayed whole according to the procedure found in the legend to Fig. 1. A fourth gel, identical to B, was stained with Coomassie Blue [34] and showed no bands beyond slice 18. Approximately 20 units human liver α-galactosidase A were applied to each of gels A and B. The plasma enzyme was 1660 U/mg protein (4-MU-Gal substrate) and about 12 units were applied to each of gels B and C.

Fig. 6. Effect of neuraminidase treatment on spleen and plasma α-galactosidase A isoelectric points. The procedures for neuraminidase treatment and isoelectric focusing are described in Materials and Methods. The assay is outlined in the legend to Fig. 1. (A) Treated and untreated spleen enzyme were focused simultaneously in two separate tubes. (B) A mixture of treated and untreated plasma enzyme was focused in the same tube. The purity of the plasma enzyme was 1040 U/mg protein (4-MU-Gal substrate).

TABLE II

THE EFFECT OF NEURAMINIDASE TREATMENT ON THE K_m VALUES OF PLASMA α-GALACTOSIDASE A

4-MU-Gal substrate. The statistic is a standard error based on the scatter in a single spot.

Enzyme	K_m (mM)
Plasma (untreated)	1.9 ± 0.1
Plasma (neuraminidase treated) *	2.6 ± 0.1
Spleen (untreated)	3.0 ± 0.2

* See Materials and Methods.

spleen enzyme changed its isoelectric point very little (Fig. 6a), suggesting relatively fewer or no hydrolyzable sialic acid groups in the splenic enzyme.

The neuraminidase treatment of plasma α-galactosidase A also increased the K_m of the enzyme slightly to a value closer to that of the untreated splenic enzyme (Table II).

Discussion

Ongoing studies with patients with Fabry's disease (Desnick, L.J., Dean, K.J., Grabowski, G.A. and Sweeley, C.C., unpublished) suggest that the in vitro fate of the plasma form of α-galactosidase A is somewhat different from that observed with enzyme from tissues [23]. It is important, therefore, to explore all purification possibilities and to maximize yield, in view of an expected demand of material for further clinical evaluation of enzyme replacement therapy with the plasma enzyme. In addition to the methods described in the previous paper [8], we report here a substantial further purification using hydroxyapatite and hydrophobic chromatography.

The overall procedure has not yet been optimized. For example, it may be better to perform hydroxyapatite chromatography before Sephadex G-200 gel filtration. This variation (tried once) gave an 11-fold purification and 80% yield in contrast to the 3-fold purification and 60% yield reported here when the hydroxyapatite step followed gel filtration. The additional purification was apparently due to the early elution (below 10 mM sodium phosphate) of protein which is also removed by gel filtration. Using hydroxyapatite first might therefore lead to better resolution in the gel filtration step due to a smaller protein load. The excellent results obtained by hydrophobic chromatography on Octoyl-Sepharose suggest the presence of a hydrophobic site on the enzyme. The yield could be improved by eliminating enzyme loss or inactivation experienced in this study during handling. Silicone coating or other treatment of containers as well as the maintenance of higher enzyme concentrations should be investigated.

Initial experiments showed quantitative adsorption of plasma α-galactosidase A onto concanavalin A-Sepharose beads (Pharmacia), suggesting that this might be a good purification step. This has recently been shown to be the case for the placental enzyme [5].

The current purification attained for plasma α-galactosidase A is approximately 700 000-fold over plasma levels [24] and at 74 000 U/mg (4-MU-Gal substrate) represents the highest purity achieved to date for the plasma enzyme. This is still impure if compared with placental [5] and liver [25] preparations, at 510 000 and 1030 000 U/mg, respectively. Even if the plasma enzyme has a lower turnover number than the tissue enzymes, it is clear that it is a very minor constituent of human plasma.

Plasma α-galactosidase isolated from Cohn Fraction IV-1 is shown here to be the A form, according to accepted criteria [25]. Consistent with this designation it is inhibited by *myo*-inositol (K_i = 0.29 M), has a K_m in the 10^{-3} range (1.9 mM) and is heat-labile [8]. These characteristics are similar to those of tissue α-galactosidases of the A form [3,6,26]. Other properties, however, are not shared with the tissue enzymes and establish the plasma α-galactosidase A as a structural isoenzyme of the tissue form(s). The molecular weight is 96 600—112 000, which is decidedly lower than the value of 150 000 found for placenta and liver α-galactosidase A [3,25]. Furthermore, the pI of the plasma enzyme is about 0.6 pH unit lower than that reported for the tissue enzymes [3,7], though Kano and Yamakawa [27] do report a minor kidney form with a pI of 3.7. These and perhaps other properties are reflected in the different electrophoretic mobilities of plasma and liver α-galactosidases on polyacrylamide gels. The plasma enzyme moves more rapidly to the anode which is consistent with either smaller size or higher net charge.

It is difficult to reconcile such divergent molecular weights between plasma and tissue enzymes, especially since they differ by a multiple of 3 and not 2. While proteins with trimeric subunit arrangements are not unheard of [28], they are rare enough to suggest that the solution lies elsewhere. The plasma and tissue enzymes may turn out to have similar weights if analyzed simultaneously. The 150 000 value for the placenta enzyme was determined by gel filtration only and may be different due to some anomaly in size or charge of the enzyme. Recently, hexosaminidase A was found to have a molecular weight of 100 000 by sedimentation equilibrium experiments while values of 130 000—140 000 were reported using gel filtration methods [29]. Also, Dean, K.J., (unpublished data) has found that human liver α-galactosidase A migrated slightly slower than hexosaminidase A on Sephadex G-200 even though according to the included protein standards the α-galactosidase had an apparent molecular weight of 150 000. Perhaps more attention should be paid to the diffuse SDS band at 47 000 daltons found by Mayes and Beutler for highly purified placental enzyme [5].

If the molecular weights of plasma and tissue isoenzymes prove to be similar, the different electrophoretic mobilities may be due to charge differences. This is supported here by the demonstration of neuraminidase-cleavable sialic acid groups on the plasma but not splenic α-galactosidase A. This finding is in agreement with the recent work of Willcox and Renwick [30] with crude extracts of plasma and liver α-galactosidase. Both studies support the concept of a general pattern of sialylated plasma hydrolases and asialylated tissue glycosyl hydrolases. Carbohydrate analysis of α-*N*-acetylgalactosaminidase (α-galactosidase B) from pig liver revealed the presence of mannose and glucosamine, but no galactose or sialic acid, in this tissue glycosidase [31].

The properties reported here for the plasma form of α-galactosidase A are not entirely in agreement with those reported by Mapes et al. [32]. The K_m values, molecular weights and isoelectric points were similar. The pH optimum was different from that of the acidic form reported previously [33], probably because different detergents were used. Furthermore, Mapes et al. [32] reported the presence of several forms of plasma ceramide trihexosidase, a finding which cannot be supported by our more recent studies [8]. There is, however, a major difference in the way the enzyme was isolated in the two studies. Butanol extraction and acetone precipitation, followed by affinity chromatography, were employed by Mapes et al. [32] while the present method uses aqueous extraction procedures, ion exchange chromatography and gel filtration.

Since this paper was submitted for publication, Jusiak et al. have reported on the purification and properties of α-galactosidase A from human placenta [35]. The molecular weight found for this tissue isoenzyme was 103 000 in contrast to an earlier reported value of 150 000 [3] and is in agreement with the value of 96 600 reported here.

Acknowledgements

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References

1 Desnick, R.J., Klionsky, B. and Sweeley, C.C. (1978) in The Metabolic Basis of Inherited Disease (Stanbury, J.B., Fredrickson, D.S. and Wyngaarden, J.B., eds.), pp. 810—840, McGraw-Hill, New York
2 Wherrett, J.R. and Hakomori, S.-I. (1973) J. Biol. Chem. 248, 3046—3051
3 Beutler, E. and Kuhl, W. (1972) J. Biol. Chem. 247, 7195—7200
4 Johnson, W.G. and Brady, R.O. (1972) Methods Enzymol. 28, 849—856
5 Mayes, J.S. and Beutler, E. (1977) Biochim. Biophys. Acta 484, 408—416
6 Ho, M.W. (1973) Biochem. J. 133, 1—10
7 Romeo, G., DiMatteo, G., D'Urso, M., Li, S.-C. and Li, Y.-T. (1975) Biochim. Biophys. Acta 391, 349—360
8 Bishop, D.F., Wampler, D.E., Sgouris, J.T., Bonefeld, R.J., Anderson, D.K., Hawley, M.C. and Sweeley, C.C. (1978) Biochim. Biophys. Acta 524, 109—120
9 Martin, R.G. and Ames, B.N. (1961) J. Biol. Chem. 236, 1372—1379
10 Noyes, B., Glatthaar, B., Garavelli, J. and Bradshaw, R. (1974) Proc. Natl. Acad. Sci. 71, 1334—1338
11 Boehringer Mannheim GMBH (1973) Biochemica Information I, pp. 124—125, Boehringer Mannheim, GmbH, Mannheim, G.F.R.
12 Martinez-Carrion, M., Turano, C., Chiancone, E., Bossa, F., Giartoso, A., Riva, F. and Fasella, P. (1967) J. Biol. Chem. 242, 2397—2409
13 Boehringer Mannheim GMBH (1975) Biochemica Information II, pp. 84—85, Boehringer Mannheim GMBH, Mannheim, G.F.R.
14 Massey, V., Hofman, T. and Palmer, G. (1962) J. Biol. Chem. 237, 3820—3828
15 Massey, V. (1966) Methods Enzymol. 9, 272—278
16 Jaenicke, R. and Knof, S. (1968) Eur. J. Biochem. 4, 157—163
17 Stolzenbach, F. (1966) Methods Enzymol. 9, 278—288
18 Palese, P., Bucher, D. and Kilbourne, E.D. (1973) Appl. Microbiol. 25, 195—201
19 Schwabe, C. (1973) Anal. Biochem. 53, 484—490
20 Behnke, J.N., Dagher, S.M., Massey, T.H. and Deal, W.C., Jr. (1975) Anal. Biochem. 69, 1—9
21 Gatt, S., Barenholz, Y., Borkovski-Kubiler, I. and Leibovitz-Ben Gershon, Z. (1972) in Sphingolipids,

Sphingolipidoses and Allied Disorders (Volk, B.W. and Aronson, S.M., eds.), pp. 237—256, Plenum Press, New York
22 Segel, I.H. (1975) Enzyme Kinetics pp. 134—143, John W. Wiley and Sons, New York
23 Brady, R.O., Tallman, J.F., Johnson, W.G., Gal, A., Leahy, W.R., Quirk, J.M. and DeKaban, A.S. (1973) N. Eng. J. Med. 289, 9—123
24 Desnick, R.J., Allen, K.Y., Desnick, S.J., Mohanreddy, R.K., Bernlohr, R.W. and Krivit, W. (1973) J. Lab. Clin. Med. 81, 157—171
25 Dean, K.J. and Sweeley, C.C. (1977) in Practical Enzymology of the Sphingolipidoses (Glew, R.H. and Peters, S.P., eds.), pp. 173—216, Alan R. Liss, Inc., New York
26 Rietra, P.J.G.M., Van Den Bergh, F.A.J.T.M. and Tager, J.M. (1975) Clin. Chim. Acta 62, 401—413
27 Kano, I. and Yamakawa, T. (1974) J. Biochem. (Tokyo) 75, 347—354
28 Wood, W.A. (1977) Trends Biochem. Sci. 2, 223—226
29 Geiger, B. and Arnon, R. (1976) Biochemistry 15, 3484—3493
30 Willcox, P. and Renwick, A.G.C. (1977) Eur. J. Biochem. 73, 579—590
31 Sung, S.-J. (1976) Ph. D. Dissertation, Michigan State University, E. Lansing, Mich.
32 Mapes, C.A., Suelter, C.H. and Sweeley, C.C. (1973) J. Biol. Chem. 248, 2471—2479
33 Sweeley, C.C., Mapes, C.A., Krivit, W. and Desnick, R.J. (1972) in Sphingolipids, Sphingolipidoses and Related Disorders (Volk, B.W. and Aronson, S.M., eds.), pp. 287—304, Plenum Publishing Corp., New York
34 Blakesley, R.W. and Boezi, J.A. (1977) Anal. Biochem. 82, 580—582
25 Kusiak, J.W., Quirk, J.M., Brady, R.O. and Mook, G.E. (1978) J. Biol. Chem. 253, 184—190