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A COMPARISON OF THE α-L-FUCOSIDASE ACTIVITIES OF HUMAN LIVER AND SERUM

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Summary

Human liver α-L-fucosidase (α-L-fucoside fucohydrolase, EC 3.2.1.51) has been separated into four components by chromatography on Sephadex G-150 or DEAE-cellulose. These components differ in their relative stability to heat and acid treatment, and their response to neuraminidase.

The serum enzyme was devoid of high molecular weight activity and probably contained more sialic acid residues than the corresponding enzyme from liver.

All the liver components tested were able to liberate fucose from 2'-fucosyllactose but not fucose from other oligosaccharides.

Introduction

The presence of α-L-fucosidase (α-L-fucoside fucohydrolase, EC 3.2.1.51) has been demonstrated in several human tissues [1—8]. In the liver, two forms of the enzyme (α-L-fucosidase I and II) were separated by gel chromatography [8,9] and two forms by ion-exchange chromatography [9]. However, six to nine α-L-fucosidase components were observed using starch gel electrophoresis or isoelectric focusing [10,11]. In serum, separation into two forms was achieved by gel chromatography [12] and up to seven forms were observed on isoelectric focusing [13] although only a diffuse pattern of enzymic activity was obtained using starch gel electrophoresis [14]. Since little information is available regarding the individual components of the enzyme, both in tissues and body fluids, a comparative study of some of these forms has been carried out.

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Experimental Procedure

Serum and liver tissue. Sera from ten normal individuals, and five post-mortem liver samples from patients without known liver disease were stored at −20° C until required. Unless otherwise stated, all experiments were carried out at 4° C.

A 20% homogenate of liver tissue in 10 mM sodium phosphate buffer, pH 6.0, was prepared using a Potter-Elvehjem glass homogeniser. The homogenate was centrifuged at 35 000 × *g* for 15 min and the resulting supernatant was used.

Column chromatography. 2 ml of serum or liver supernatant were dialysed overnight against 2 l of 10 mM sodium phosphate buffer, pH 6.0, before application to a column (15 × 0.9 cm) of Whatman DEAE-cellulose (type DE-52, from Balston, Maidstone, U.K.) pre-equilibrated with 10 mM sodium phosphate buffer, pH 6.0. The same buffer was used to elute 4 ml fractions at a flow rate of 55 ml/h for 40 min, after which a linear NaCl gradient in the eluting buffer was applied.

Gel chromatography was performed in a column (2.6 × 95 cm) of Sephadex G-150 (superfine) with narrow particle size [15] in 10 mM sodium phosphate buffer, pH 5.5, containing 1 mM EDTA. 1 ml of serum or liver supernatant was applied, and 1.5-ml fractions were collected at a flow rate of 5 ml/h.

Treatment with neuraminidase. 2 ml of liver supernatant, or 2 ml of serum mixed with 2 ml of 10 mM sodium phosphate buffer, pH 6.0, were incubated at 37° C for 3 h with 1 mg of neuraminidase from *Clostridium perfringens* type VI (Sigma, St. Louis, Mo.). This enzyme was free of α-L-fucosidase and proteinase activity under the conditions used. No merthiolate was present [16]. After incubation the mixtures were dialysed overnight against 2 l of 10 mM sodium phosphate buffer, pH 6.0, and chromatographed as described. Control experiments without addition of neuraminidase were also performed.

Effect of heat. 200 µl aliquots of ion-exchange column fractions were heated at 55° C for different lengths of time, cooled, and the enzyme activity measured.

pH inactivation. Prior to enzyme assay, 25 µl aliquots of the most active peak fractions obtained after DEAE-chromatography were mixed with 25 µl of the citrate-phosphate buffer and incubated at various pH values and 37° C for 3 h. The pH was then brought to 5.5 using 25 µl of 1 M citrate buffer and assayed with the substrate in water for 3 h as described below.

Enzyme assay. α-L-Fucosidase activity was routinely assayed at pH 4.0 and 5.5. The sample to be assayed was mixed with 100 µl of a 1 mM solution of 4-methylumbelliferyl-α-L-fucopyranoside (Koch-Light, Colnbrook, U.K.) in water or citrate/phosphate buffer (200 mM Na_2HPO_4 added to 100 mM citric acid). This mixture was incubated at 37° C for either 90 min or 3 h. Specific conditions are given in the individual experiments. The reaction was stopped by adding 1 ml of 250 mM glycine/NaOH buffer, pH 10.4, and the fluorescence measured using an Aminco-Bowman spectrofluorimeter (excitation wavelength 348 nm, emission wavelength 450 nm).

In the studies using oligosaccharides, 100 µl of the above buffer at pH 4.0 or 5.5 were incubated at 37° C for 18 h with 100 µl of column fraction and 50 µl

of a 50 mM aqueous solution of the following sugars: 2′-fucosyllactose, lacto-difucotetraose, lacta-*N*-fucopentaose II, lacto-*N*-difucohexaose I, A-pentasaccharide, A-trisaccharide *, and α-L-fucopyranosyl-(1—6)-β-3-acetamino-2-deoxy-D-glucopyranosyl-Asn [17—19], all isolated from human urine. Control experiments using 100 μl of water instead of enzyme were also performed. After incubation, the mixtures were applied to Whatman No. 1 papers and developed in ethyl acetate/pyridine/water (10 : 4 : 3, v/v/v) for 18 h. Sugars were visualized using alkaline silver nitrate [20].

Results

Ion-exchange chromatography of serum

The fractionation of serum α-L-fucosidase on DEAE-cellulose, both before and after neuraminidase treatment, is shown in Fig. 1. Untreated serum (Fig. 1A) gave one major peak of α-L-fucosidase activity which was equally active at pH 4.0 and 5.5. A shoulder of exclusively neutral (pH 5.5) activity preceded the mean peak in all the serum samples examined. The main peak completely disappeared after neuraminidase treatment (Fig. 1B), but two other major peaks of activity appeared, one unadsorbed to the column (DS_1) and one which was less tightly bound to the column than the original main peak (DS_3) and was not affected further even by incubation with 2 mg neuraminidase for 18 h. DS_1 had relatively more neutral and DS_3 more acidic (pH 4.0) α-L-fucosidase activity than the untreated enzyme. By using half the amount of neuraminidase it was also possible to detect a third component (DS_2) which had acid and neutral α-L-fucosidase activity in the same proportion as DS_1. Control experiments demonstrated that these conversions were due only to the action of neuraminidase.

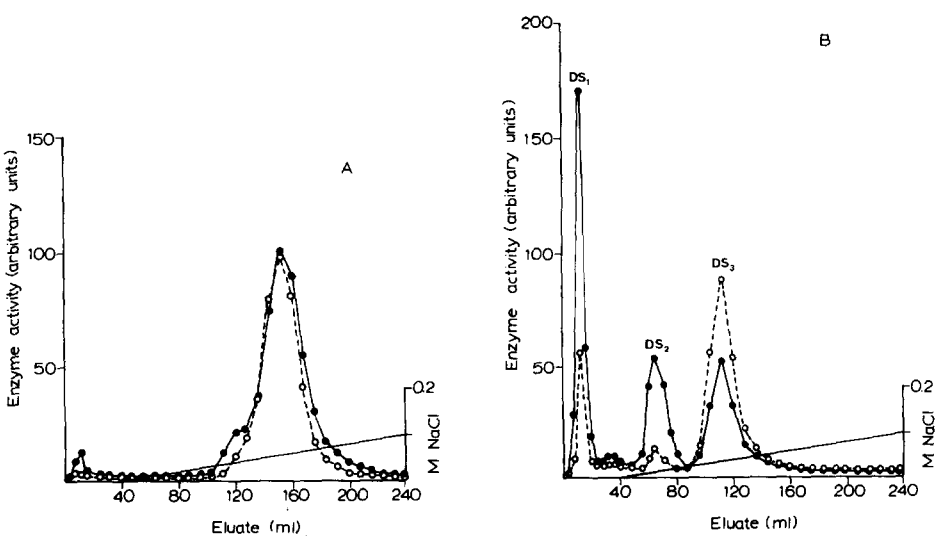


Fig. 1. DEAE-cellulose chromatography of serum before (A) and after (B) neuraminidase treatment. α-L-Fucosidase activity was measured by incubating 200 μl of column fractions with substrate in buffer for 90 min at pH 4.0 (○- - - - -○) and pH 5.5 (●———●).

* A-, blood group A-active- [17—19].

The effect of a greater variation in pH on the α-L-fucosidase activity of these different components is shown in Fig. 2A. Above pH 5.0 the activities of all three components were similar, but a sharp decrease in activity was observed at acidic pH and was due to enzyme inactivation as shown using the method described under Experimental Procedure. Form DS_3 was relatively more acid stable, and as shown in Fig. 3A this form was also the most heat stable. Analysis of serum stored at −20° C for up to 1 year did not give significantly different results.

Ion-exchange chromatography of liver supernatant

The fractionation of liver α-L-fucosidase on DEAE-cellulose, both before and after neuraminidase treatment, is shown in Fig. 4. Untreated liver supernatant (Fig. 4A) was fractionated into four enzyme forms, three being predominantly neutral and the fourth (DL_4) having slightly more acidic than neutral activity and closely resembling the serum component (DS_3) obtained after neuraminidase treatment. When the liver samples were treated with neuraminidase (Fig. 4B) the distribution of α-L-fucosidase activity after DEAE-chromatography was similar to the pattern obtained with serum in which only two major components occurred, one unadsorbed to the column and the other eluted at a

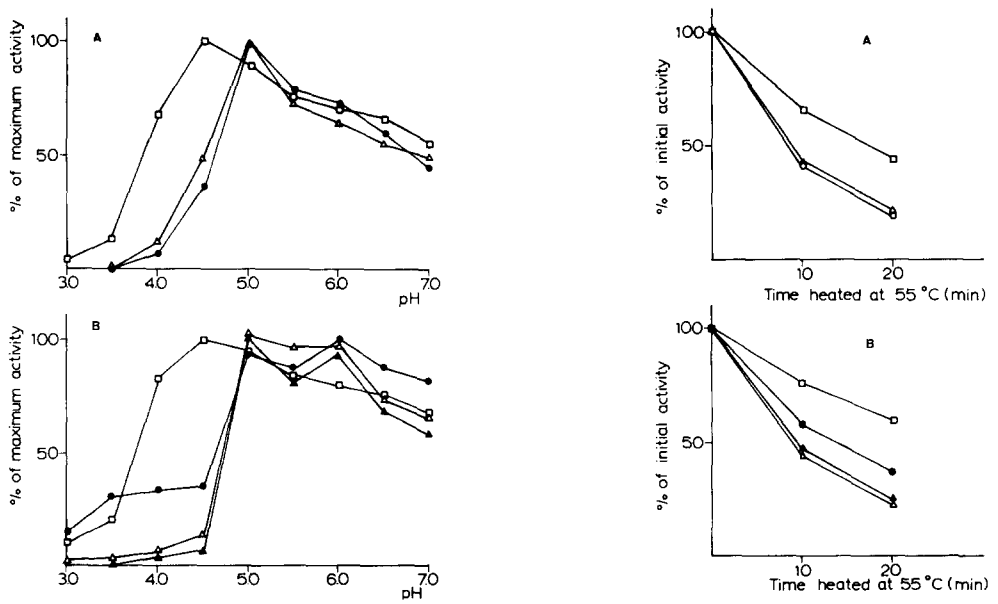


Fig. 2. Dependence of α-L-fucosidase activity on pH in components separated by DEAE-cellulose chromatography. α-L-Fucosidase activity was measured using 25 μl of the most active fraction from each peak, 25 μl of the citrate-phosphate buffer, and substrate in water, incubated for 3 h. (A) Serum; peak DS_1 (△——△), peak DS_2 (○——○), peak DS_3 (□——□). (B) Liver; peak DL_1 (●——●), peak DL_2 (△——△), peak DL_3 (▲——▲) and peak DL_4 (□——□).

Fig. 3. Heat inactivation of α-L-fucosidase activity in components separated by DEAE-cellulose chromatography. α-L-Fucosidase activity was measured using 100 μl of the most active fraction from each peak and 100 μl of substrate in buffer, pH 4.0 and 5.5, incubated for 90 min at 37° C. Identical results were obtained at both pH values. (A) Serum; peaks DS_1 (△——△), peak DS_2 (○——○), peak DS_3 (□——□). (B) Liver; peak DL_1 (●——●), peak DL_2 (△——△), peak DL_3 (▲——▲) and peak DL_4 (□——□).

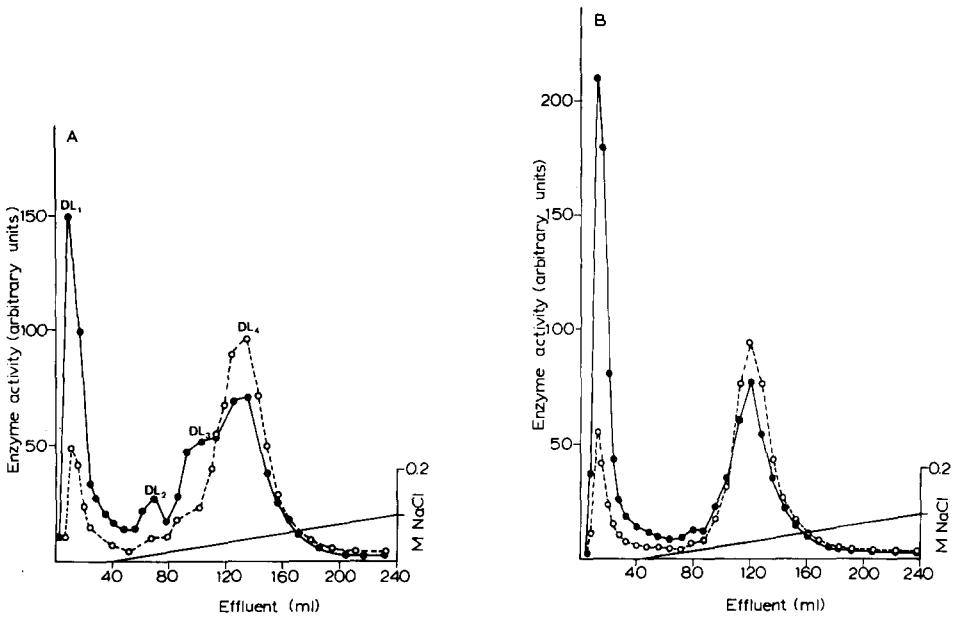


Fig. 4. DEAE-cellulose chromatography of liver before (A) and after (B) neuraminidase treatment. Activity measured at pH 4.0 (o- - - - -o) and pH 5.5 (●———●). Other conditions as given in Fig. 1.

slightly lower salt concentration than DL_4. The peaks DL_2 and DL_3 (Fig. 4A) disappeared and were probably converted to forms no longer adsorbed to the column, since the proportion of neutral activity in DL_1 increased.

The pH profiles of the four liver components which separated without neuraminidase treatment are shown in Fig. 2B. As with serum the rapid decrease in activity at acidic pH was due to enzyme inactivation. The heat sensitivity of these four forms is demonstrated in Fig. 3B. The predominantly neutral α-L-fucosidase activities of liver and serum behaved similarly, being more sensitive to acidic pH and heat than the acidic α-L-fucosidase activities from both sources. Furthermore, liver component DL_1, whilst having mainly neutral activity, contained some acidic characteristics as shown by its intermediate α-L-fucosidase activity when subjected to acidic pH (Fig. 2B) and heat (Fig. 3B).

No significant differences in these α-L-fucosidase activities were detected after storage of the tissue at −20° C for up to 1 year.

Gel chromatography of liver extract and serum

Chromatography of liver supernatant on Sephadex G-150 (Fig. 5A) suggested the presence of four peaks of α-L-fucosidase activity. The two components of highest molecular weight (GL_1 and GL_2) exhibited both acidic and neutral activity but the two lower molecular weight components were essentially neutral. Gel chromatography of serum (Fig. 5B) gave a simpler pattern with a major component corresponding to GL_2 plus a partially separated lower molecular weight form corresponding to GL_3. Component GL_1 of liver, which was completely absent from serum had a pH profile (stability) and heat stability similar to those observed for peak DL_1 (Fig. 2B and 3B). Material from

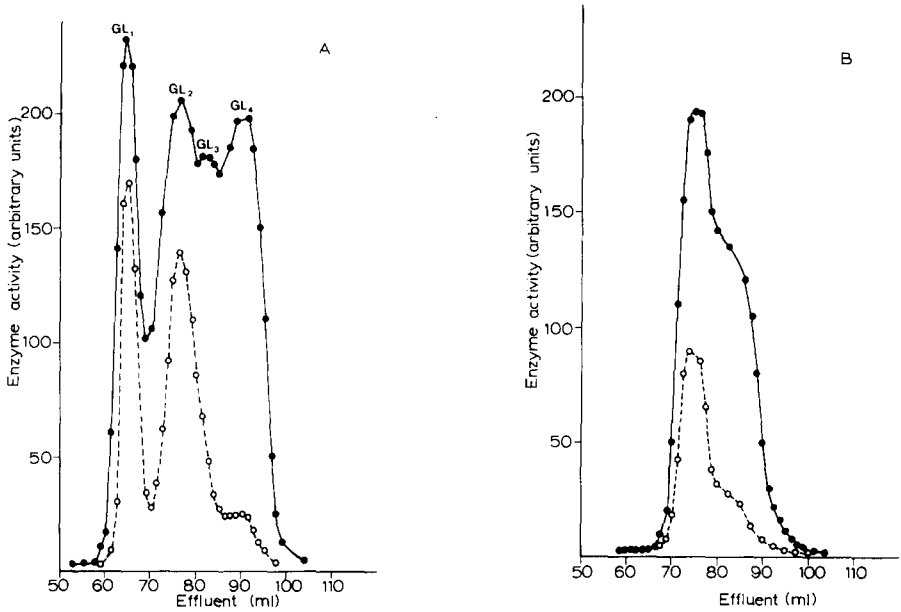


Fig. 5. Sephadex G-150 chromatography of (A) liver supernatant, (B) serum. Void volume 63 ml. Human serum albumin eluted after 92 ml. α-L-Fucosidase activity was determined as described in Fig. 1, at pH 4.0 (○- - - - -○) and 5.5 (●——●).

peaks GL_1, GL_2, and GL_4 from liver was subsequently chromatographed on DEAE-cellulose as described in Experimental Procedure. Peak GL_2 and the major part of GL_1 were eluted from the column in the same position as DL_4 (Fig. 4A). GL_4 and a small part of GL_1 were not adsorbed to the column (c.f. DL_1, Fig. 4A).

Whilst it was not possible to obtain α-L-fucosidase activity from peak GL_3 free from contamination by GL_2 and GL_4, DEAE-cellulose chromatography of material relatively rich in GL_3 indicated that peaks DL_2 and DL_3 (Fig. 4A) were probably derived from GL_3.

Action of α-L-fucosidase components on oligosaccharides

Aliquots from the most active fractions from peaks DL_1 and DL_4 (Fig. 4A) were incubated at pH 4 and 5.5 with the compounds listed in Experimental Procedure. No degradation of any of these compounds was observed except 2'-fucosyllactose, which showed 30—50% breakdown to fucose and lactose with both enzyme components at both pH values tested. No fucose or lactose was observed in the control sample.

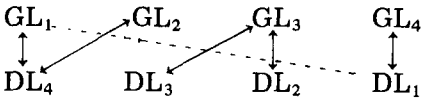
Discussion

Up to nine α-L-fucosidase components have been separated in both tissues and body fluids using analytical starch gel electrophoresis and isoelectric focusing [10,11,13,21]. However, using preparative techniques such as gel and ion-exchange chromatography [8,9] only two forms of the enzyme were isolated for further study. Affinity chromatography of liver [22] and placenta [23]

extracts resulted in a high degree of purification of α-L-fucosidase but subsequent polyacrylamide gel electrophoresis showed the presence of only 1 and 2 isoenzymes, respectively. A more detailed study of α-L-fucosidase in liver tissue was performed by Alhadeff et al. [24] who purified the enzyme 6300-fold by affinity chromatography, to apparent homogeneity. Polyacrylamide gel electrophoresis and isoelectric focusing demonstrated the presence of six forms of the enzyme, presumably differing only in charge, since a single species ($M_r \approx$ 230 000) was indicated after high speed sedimentation equilibrium analysis. These authors concluded that a single subunit ($M_r \approx$ 50 000) existed and that the active enzyme consisted of four identical subunits. Robinson and Thorpe [9] reported an equilibrium between α-L-fucosidase I, which was not retarded by a Sephadex G-200 column, and α-L-fucosidase II ($M_r \approx$ 50 000), but no such equilibrium was described by Alhadeff et al. [24] after Sepharose 4 B or high speed sedimentation equilibrium studies.

The results presented in Fig. 5A show that human liver α-L-fucosidase activity exists in at least four components (GL_1—GL_4) of different molecular weight. DEAE-cellulose chromatography (Fig. 4A) also resulted in the separation of four components (DL_1—DL_4), in this case on the basis of their net charge, although after neuraminidase treatment components DL_2 and DL_3 were no longer adsorbed to the column (Fig. 4B).

DEAE-cellulose chromatography of the α-L-fucosidase activity from peaks GL_1—GL_4 (Fig. 5A) indicated the following interrelationship:



in which GL_1 is mainly an aggregate of the relatively acidic GL_2, but also contains a neutral component.

The gel chromatographic pattern obtained using serum was simpler than that obtained using liver, since only two components which correspond to liver components GL_2 and GL_3 were separated (Fig. 5B). Using the interrelationship postulated in the preceding paragraph, ion-exchange chromatography of serum would give peaks corresponding to DL_2, DL_3, and DL_4. This was observed except that the small neutral peaks DL_2 and DL_3 were missing. We suggest, however, that these components, through higher sialic acid content, coincide with the major peak of serum α-L-fucosidase activity. Evidence supporting this suggestion is: (a) the ratio of the acidic and neutral α-L-fucosidase activities of the combined peaks DL_2, DL_3, and DL_4 from liver is the same as that found in the serum peak in Fig. 1A; (b) neuraminidase treatment of serum resulted in the formation of an enzyme component which was not adsorbed to the DEAE-cellulose column (DS_1 in Fig. 1B); (c) milder treatment with neuraminidase formed a component DS_2 with similar characteristics to DL_2. These results demonstrate that in serum at least two α-L-fucosidase components exist, differing in molecular weight. The component of higher molecular weights has a $pI > 6.0$ after neuraminidase treatment and is relatively stable to heat and acidic pH, whereas the lower molecular weight component has a $pI < 6.0$ after neuraminidase treatment, is relatively unstable to heat, and is inactivated at acidic pH. Human liver α-L-fucosidase exhibits similar characteristics but has,

in addition, at least one extra low-molecular-weight neutral component and a high molecular weight form which is possibly an aggregate containing both acidic (GL_2) and neutral (GL_4) forms. These results suggest that at least two structurally different α-L-fucosidase units exist with additional diversity due to aggregation and sialic acid content. It is not possible, from the present results, to come to any firm conclusions on the interrelationship between the four liver components, however a situation more complex than a monomer-tetramer as suggested by Alhadeff et al. [24] is indicated. Of the four liver components only the two of intermediate molecular weight are present in serum and both of these are more sialylated than their liver analogues.

The metabolic function of α-L-fucosidase is to participate in the degradation of glycoproteins and glycolipids. In these compounds the fucose residue is linked either α-(1 → 2) to D-galactopyranosyl or α-(1 → 3), α-(1 → 4) or α-(1 → 6) to 2-acetamido-2-deoxy-D-glucopyranosyl residues. All the enzyme components tested were able to liberate fucose linked α-(1 → 2) to the terminal galactosyl residue in 2′-fucosyllactose but no fucosyl linkage in any other oligosaccharide. It is conceivable that the enzyme degrading other fucose linkages are extremely labile and hence may be denatured during the isolation procedure, in which case the α-L-fucosidase components studied here would all be isoenzymes of α-(1 → 2)-L-fucosidase. The inability of this α-L-fucosidase to liberate fucose from lactodifucotetraose or lacto-*N*-desfucohexaose I (which both contain fucose linked α-(1 → 2) to D-galactose) indicates that a specific, well defined degradative sequence exists in which the removal of adjacent α-(1 → 3) or α-(1 → 4) linked fucose residues is a prerequisite for α-(1 → 2) fucosidase action. Since glycoconjugate degradation is thought to take place in lysosomes it is also surprising that one of the α-L-fucosidase components is easily inactivated at a pH at which the majority of lysosomal enzymes function most effectively. Presumably the conditions prevailing in vivo, with the α-L-fucosidases structurally located in the lysosomes, are more conducive to efficient enzymic action.

Acknowledgements

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