Biochimica et Biophysica Acta, 524 (1978) 373—384

BBA 68444

THE PURIFICATION, PROPERTIES AND CHARACTERIZATION OF THREE FORMS OF α-L-FUCOSIDASE FROM MONKEY BRAIN

TAUSIF ALAM and A.S. BALASUBRAMANIAN

Neurochemistry Laboratory, Department of Neurological Sciences, Christian Medical College and Hospital, Vellore - 632004 (India)

(Received October 31st, 1977)

Summary

α-L-Fucosidase (α-L-fucoside fucohydrolase, EC 3.2.1.51) has been purified to apparent homogeneity (about 22000-fold over the crude homogenate) from monkey brain. Values of kinetic constants for the purified enzyme were as follows: pH optimum, 5.0; K_m, 0.22 mM; V, 913 μmol/mg per h. α-L-Fucose was a competitive inhibitor (K_i, 0.275 mM) of the enzyme. Evidence for the involvement of sulphydryl group(s) and carboxyl group containing amino acid(s) in the catalytic process is presented. The purified enzyme was a tetramer of molecular weight of 285000 of identical subunits of 73500 held together by non-covalent forces. Gel filtration studies revealed the presence of three molecular forms of the activity in the purified preparation which appeared to be the tetramer, dimer and monomer. The existence of three types of activities was also supported by a triphasic heat inactivation profile of the enzyme at 50 or 55°C and the distinctly different pH activity profiles of the differentially heat-inactivated enzymes.

Immunodiffusion studies using antibody developed against purified monkey brain α-L-fucosidase showed that the monkey brain enzyme had only partial immunological identity with the enzymes from the non-neural tissues of monkey as well as the human and rat liver and the rat brain. However, the monkey brain and liver enzymes appeared to be similar to the human brain and liver enzymes, respectively.

Introduction

The enzyme α-L-fucosidase (α-L-fucoside fucohydrolase, EC 3.2.1.51) is one of the major enzymes initiating the hydrolysis of oligosaccharide chains of complex glycoconjugates. The importance of this enzyme in the normal functioning of the cell became more apparent after its reported absence in fucosidosis [1], an inheritable neurovisceral storage disease. In recent years this

enzyme has been purified to varying degrees from various mammalian sources [2—10], some of them to apparent homogeneity [2—5]. The first report on multiplicity of this enzyme came from Wiederschain and Rosenfeld [6] demonstrating the existence of two forms of the enzyme from pig kidney separable by gel filtration and thereafter a growing number of reports have indicated the multiplicity of this enzyme from mammalian sources with respect to isoelectric point [3—5,11—13]. The presence of two forms of α-L-fucosidase differing in molecular size, surface charges, thermal stability or pH activity profiles has also been noticed from various human tissues [7—9]. Knowledge regarding the relationship between the various naturally occurring molecular forms of the enzyme is, however, limited. This communication presents besides the purification, subunit structure and kinetic parameters of the enzyme α-L-fucosidase isolated from monkey brain, evidence for three forms of the enzyme on the basis of gel filtration, pH activity profile and thermal stability and the possible inter-relationship between the multiple forms. The immunological characteristics of the enzyme are also presented.

Materials and Methods

α-L-Fucose; *p*-nitrophenyl derivatives of α-L-fucoside, α-D-glucoside and α-D-galactoside; α-methylglucoside; *p*-chloromercuribenzoate; mol. wt. markers (crystals): cytochrome *c*, trypsin inhibitor, bovine serum albumin, γ-globulin and urease were obtained from Sigma Chemical Co. (U.S.A.). The *p*-nitrophenyl derivatives of α-D-mannoside, β-D-glucoside, β-D-galactoside and 2-deoxy-2-acetamidoglucosaminide were products of Koch Light Laboratories (U.K.). *N*-Ethylmaleimide, iodoacetamide, dithiothreitol and ethyldimethylaminopropyl carbodiimide were purchased from Schwarz Bioresearch (U.S.A.), Nutritional Biochemical Corp. (U.S.A.), Seikagaku (Japan) and Eastman Kodak (U.S.A.), respectively. The protease-free *Vibrio cholerae* neuraminidase and crystalline ovalbumin were obtained from Behringwerke (Germany) and Centron Research Laboratory (India), respectively. CM-Sephadex C-50, DEAE-Sephadex A-50, Sephadex G-200 and Sepharose 4B were obtained from Pharmacia Fine Chemicals, Uppsala (Sweden). Aquacide II was purchased from Calbiochem (U.S.A.). Complete Freunds adjuvent and agar were products of Difco Laboratories (U.S.A.). All the other chemicals used were of the highest grade of purity available. Adult monkey (*Macaca radiata*) brains were removed under nembutal anaesthesia and stored frozen at −20°C after the removal of meningis.

Enzyme assay. The final assay volume of 0.2 ml contained 0.2 μmol of *p*-nitrophenyl-α-L-fucoside, 20 μmol of acetate buffer, pH 5.0, 100 μg of bovine serum albumin and the enzyme. The incubation was carried out at 37°C for 30 min and terminated by the addition of 0.8 ml of 0.4 M glycine/NaOH buffer, pH 10.5. The liberated *p*-nitrophenol was spectrophotometrically estimated at 400 nm after centrifuging, if necessary. The other glycosidases, namely α-D-mannosidase, α-D-glucosidase, β-D-glucosidase, α-D-galactosidase, β-D-galactosidase and *N*-acetyl-β-D-hexosaminidase were assayed as reported earlier [14]. One unit of the enzyme activity is defined as 1 μmol of the *p*-nitrophenol liberated per h under the standard assay conditions. The reaction rate was

linear with respect to time and protein concentration in all the assays.

Protein estimation was done by the method of Lowry et al. [15] using crystalline bovine serum albumin as standard. Concanavalin A-Sepharose was prepared as described earlier [14] except that a higher amount of cyanogen bromide (50 mg/ml packed Sepharose 4B) was used for activation. The final preparation contained 14.5 mg concanavalin A per ml of the packed gel.

Purification of the enzyme

All the operations were performed at 0—4°C unless otherwise stated.

The frozen monkey brain (600 g) was homogenized in 0.02 M potassium phosphate buffer, pH 7.0 (5.4 l) in a Waring blendor in batches of 50 g tissue, for 5 min at the maximum speed. The homogenate (5720 ml) was stirred for 30 min and centrifuged at $67000 \times g$ for 30 min. The supernatant was collected. The homogenate had a total of 59050 mg protein and 1990 enzyme units and the supernatant had a total of 14501 mg protein and 1556 enzyme units.

Ammonium sulphate fractionation. To the supernatant (4988 ml) solid $(NH_4)_2SO_4$ (1868 g) was added with constant stirring to bring the solution to 60% saturation level. After an additional stirring of 30 min the precipitated protein was collected by centrifuging the suspension at $12000 \times g$ for 30 min. The precipitated protein was suspended in a small volume (100 ml) of 0.02 M phosphate buffer, pH 7.0, containing 0.15 M NaCl and dialyzed against the same buffer (9 l) for 12 h with two changes. After removing any sedimentable material at $12000 \times g$ for 30 min the dialyzed enzyme (205 ml) had a total of 8050 mg protein and 1292 enzyme units. It was adjusted to pH 6.0 by the addition of a suitable amount of 1 M KH_2PO_4 and the concentration of NaCl was raised to 0.5 M by addition of solid NaCl.

Concanavalin A-Sepharose affinity chromatography. The enzyme obtained from the previous step was loaded on a concanavalin A-Sepharose column (1.7 × 14.3 cm) equilibrated with 0.02 M phosphate buffer, pH 6.0, containing 0.5 M NaCl at a flow rate of about 25 ml/h. The column was washed by the equilibrating buffer (600 ml) followed by 0.02 M phosphate buffer, pH 7.5, containing 0.5 M NaCl (300 ml) when the effluent was almost free of the protein. Elution of the enzyme was carried out by 0.5 M α-methylglucoside in 0.02 M phosphate buffer, pH 7.5, containing 0.5 M NaCl at 25 ± 1°C at a flow rate of 12 ml/h. Fractions of 5 ml were collected. Active fractions (4—13) were pooled and dialyzed against 0.02 M ammonium acetate buffer, pH 5.0 (4 l) for 4 h with three changes. The precipitated protein was discarded by centrifugation and the supernatant was collected. (total protein 32.8 mg; enzyme units 883.)

CM-Sephadex C-50 ion-exchange chromatography. The dialyzed supernatant of the concanavalin A-Sepharose eluate (78 ml) was loaded on a CM-Sephadex C-50 column (1.1 × 20 cm) equilibrated with 0.02 M ammonium acetate buffer, pH 5.0, at a flow rate of 20 ml/h. The column was washed successively with 100 ml each of 0.02 M ammonium acetate buffers of pH 5.0 and 6.0. The enzyme was eluted by passing 0.02 M phosphate buffer, pH 6.5, at a flow rate of 20 ml/h. Fractions of 5 ml were collected in tubes containing 0.2 ml of 4 M NaCl solution. The active fractions (7—13) were pooled (total protein 7.1 mg;

enzyme units 461) and concentrated by aquacide II to a volume of 2.5 ml.

Sephadex G-200 gel filtration. The concentrated CM-Sephadex eluate was chromatographed on a Sephadex G-200 column (1.65 × 94.4 cm) equilibrated with 0.02 M phosphate buffer containing 0.15 M NaCl. Fractions of 5 ml were collected at a constant flow rate of 18 ml/h and monitored for the enzyme activity and protein. The two fractions of highest activity (18 and 19, Fig. 1) were pooled for further purification (total protein 0.58 mg; enzyme units 170).

DEAE-Sephadex A-50 ion-exchange chromatography. Pooled enzyme from the previous step (10 ml) was dialyzed against 0.02 M phosphate buffer, pH 7.5 (1.5 l) with two changes over a period of 3 h. The dialyzed enzyme was loaded on a column of DEAE-Sephadex A-50 (1.1 × 4.2 cm) equilibrated with 0.02 M phosphate buffer, pH 7.5, at a flow rate of 8 ml/h. The column was washed with the equilibrating buffer (20 ml) at a flow rate of 15 ml/h and subsequently eluted with the same buffer containing 0.07 M NaCl at a flow rate of 6 ml/h. Fractions of 2 ml were collected. The active fractions (2—20) were pooled and dialyzed against 0.02 M phosphate buffer, pH 7.0, containing 0.15 M NaCl (total protein 0.11 mg; enzyme units 82). It was stored at 0—4°C after concentrating to about 1 ml with aquacide II.

Neuraminidase treatment of the enzyme. An aliquot of 0.1 ml containing 25 μg of the purified enzyme in 0.05 M acetate buffer, pH 5.0, and 0.01 M $CaCl_2$ was incubated with 10 enzyme units (20 μl) of neuraminidase for 2 h at 37°C.

Polyacrylamide gel electrophoresis of the purified enzyme. Polyacrylamide gel electrophoresis of the purified native enzyme before and after neuraminidase treatment was performed on gels of 7% acrylamide according to the method of Davis [16] with modifications in the pH of buffers used. Since the enzyme was found to be highly unstable above pH 8.5, the running gel was polymerized at pH 7.5 and electrophoresis was done in 0.05 M Tris/glycine buffer, pH 7.4, at 2 mA/tube for 5 h when the tracking dye, bromophenol blue, reached the end

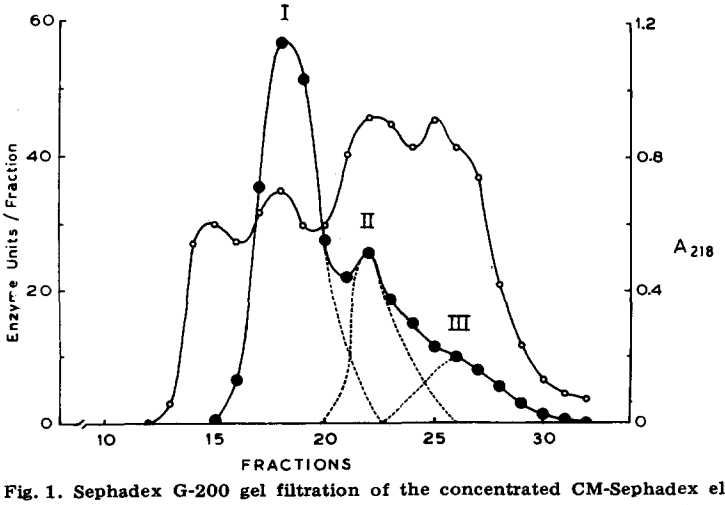


Fig. 1. Sephadex G-200 gel filtration of the concentrated CM-Sephadex eluate. Details are mentioned under Materials and Methods. ●, α-L-fucosidase activity; ○, absorption at 218 nm; - - - - -, graphical resolution of individual peaks.

of the gel. After electrophoresis the gel was cut into two longitudinal halves, one half was sliced for assay of the enzyme activity and the other stained for protein by Coomassie Brilliant Blue R.

Molecular weight determination. Molecular weight was determined by gel filtration chromatography on Sephadex G-200 as described by Andrews [17].

Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis. SDS-polyacrylamide gel electrophoresis of the purified enzyme with and without β-mercaptoethanol treatment was performed according to the method of Weber and Osborn [18] and the molecular weight of the SDS-treated enzyme was determined using marker proteins.

pH stability. pH stability of the enzyme was studied by assaying the activity under standard conditions after preincubating the enzyme for 30 min at 37°C at various pH ranging from 3.5 to 8.0 using 0.04 M acetate or phosphate buffers in the presence of 0.15 M NaCl.

Preparation of rabbit antifucosidase immunoglobulin fraction. A normal healthy rabbit was injected subcutaneously with 40 μg (0.6 ml) of the purified fucosidase mixed with an equal volume of complete Freunds adjuvent. A booster dose of 20 μg of the enzyme was given after 1 week without the adjuvent. The rabbit was bled after the third week of the first injection and the serum obtained (16 ml) was raised to 33% ammonium sulphate saturation at pH 6.5. The precipitated protein was washed twice by 40% saturated ammonium sulphate solution, pH 6.5, as described [19]. The final preparation was dialyzed against 0.02 M potassium phosphte buffer, pH 7.5, containing 0.15 M NaCl and stored over melting ice in the presence of 0.02% sodium azide.

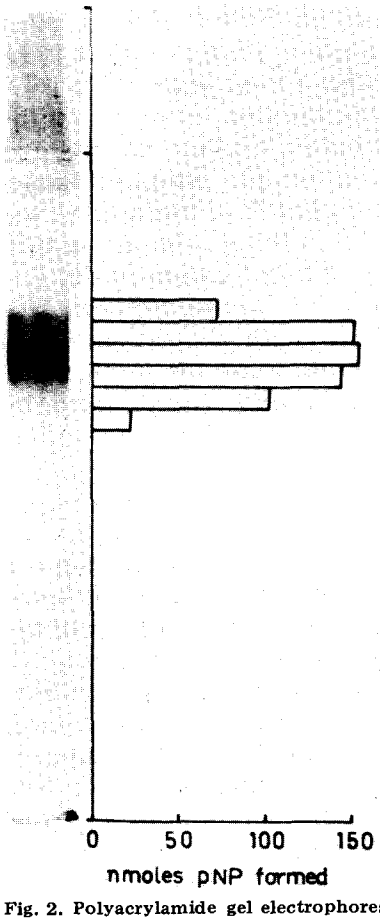
Preparation of the enzyme from various tissues for immunodiffusion. For immunological studies, the enzyme from various sources was extracted and subjected to ammonium sulphate fractionation as described for the monkey brain. The dialyzed ammonium sulphate fractions were concentrated by aquacide II, if necessary.

Results

The purification procedure of the monkey brain α-L-fucosidase resulted in a 22000-fold increase in specific activity over the homogenate with an overall recovery of 4.1%. The purified enzyme did not show the presence of any of the glycosidases tested (mentioned in Materials and Methods) except *N*-acetyl-β-hexosaminidase which was less than 1% of the fucosidase activity. The purified enzyme when stored under conditions mentioned earlier did not show any loss of activity for a period of at least 4 months. The presence of NaCl in the purified enzyme was found to significantly enhance its stability on storage. The purified enzyme had an optimum pH of 5.0, K_m of 0.22 mM and V of 913 μmol/mg per h. Addition of albumin increased the activity of the purified enzyme and a maximum of 100% increase in activity was observed with the addition of 100 μg albumin. It is presumable that albumin has a protective effect against inactivation of the enzyme. The enzyme was competitively inhibited by α-L-fucose with an inhibition constant (K_i) of 0.275 mM as determined by the Dixon plot [20]. Cu^{2+}, Ag^{2+} and Hg^{2+} were highly inhibitory causing almost a total inhibition at 1 mM.

Gel electrophoresis of enzyme

The purified native enzyme when subjected to polyacrylamide gel electrophoresis at pH 7.4 gave six very closely moving distinct bands of protein. Enzyme activity appeared to be associated with all the bands (Fig. 2). This is similar to observations made by others [4] on the occurrence of seven forms of the purified enzyme on isoelectric focusing. The mobility of these bands did not undergo any detectable change by neuraminidase treatment. SDS-polyacrylamide gel electrophoresis, however, showed the presence of a single band of a molecular weight of 73500 as obtained from the calibration curve using various marker proteins.



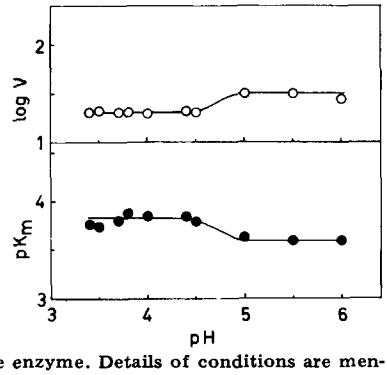


Fig. 2. Polyacrylamide gel electrophoresis of the purified native enzyme. Details of conditions are mentioned under Materials and Methods. Left: The gel stained for protein. Right: the activity profile. 1.5-mm thick slices of the gel were incubated overnight at 30°C with the substrate under conditions of standard assay system of the enzyme.

Fig. 3. Plots of pK_m and log V against pH. Details are described in the text. Every point represents an average of two values.

Studies on the involvement of carboxyl and sulphydryl group containing amino acids in the ctalytic activity of the enzyme

K_m and V values were determined at various pH values ranging from 3.5 to 7.0 for the purified native enzyme. Plots of pK_m against pH and log V against pH suggested two bends at pH 4.5 and 5.0 (Fig. 3) which correspond to the ionization of carboxyl groups [21]. An attempt to explore the involvement of carboxyl group(s) in the catalytic process was made by blocking them by the ethyldimethylaminopropyl carbodiimide-mediated reaction with ethanolamine at pH 4.75 as described [22]. Due to the instability of the enzyme below pH 5.5 the control itself lost 70% of the total activity but the blocking of carboxyl groups resulted in a further loss of 78% of the control activity indicating the involvement of carboxyl group(s) in the catalytic activity of the enzyme.

p-Chloromercuribenzoate inhibited the enzyme at very low concentrations (50% inhibition at 0.15 μM). This inhibition could be totally reversed by the preincubation of *p*-chloromercuribenzoate-treated enzyme with 4 mM dithiothreitol for 20 min at 37°C. Iodoacetamide and *N*-ethylmaleimide were, however, not so effective and caused only about 30% inhibition at 1 mM.

Gel filtration profile

It is noteworthy that the enzyme upon gel filtration on Sephadex G-200 gave a major peak at fractions 18—19, a small peak at fraction number 22 and a distinct shoulder around fraction number 26 (Fig. 1). Considering the entire region of activity as a composite picture of three peaks, an approximate hypothetical resolution of the major and two minor peaks could be worked out, as shown by dotted lines (Fig. 1). Of the total activity recovered in the gel filtration step 64% was peak I, 25% peak II and 11% peak III. When the peak I was rechromatographed under identical conditions, once again the same pattern was observed with a slight difference in the distribution of the activity (59% peak I, 17% peak II and 24% peak III). When peak II (fraction 22, Fig. 1) was subjected to gel filtration under identical conditions, as much as 91% of the activity appeared at the position corresponding to peak III. These results indicated that the peak I enzyme tends to get converted to peak III through the intermediate formation of peak II. Molecular weights of these three peaks as determined by gel filtration on Sephadex G-200 were 285000, 145000 and 74000 for peaks I, II and III, respectively.

Heat inactivation studies

A triphasic heat inactivation profile was obtained by heating the purified native enzyme at 50 or 55°C for varying periods of time (Fig. 4A) suggesting the presence of three forms of the enzyme differing in the susceptibility to the heat inactivation. When the peak III enzyme, isolated by gel filtration, was subjected to a similar kind of heat treatment at 55°C, a continuous rapid loss of activity upto 90% of the total activity was observed (Fig. 4B) corresponding to the most heat sensitive form of the native enzyme shown in Fig. 4A.

pH activity profile

The pH activity profile of the purified native enzyme showed a pH optimum of 5.0 and a shoulder around pH 6.0 (Fig. 5A). In the highly acidic region of

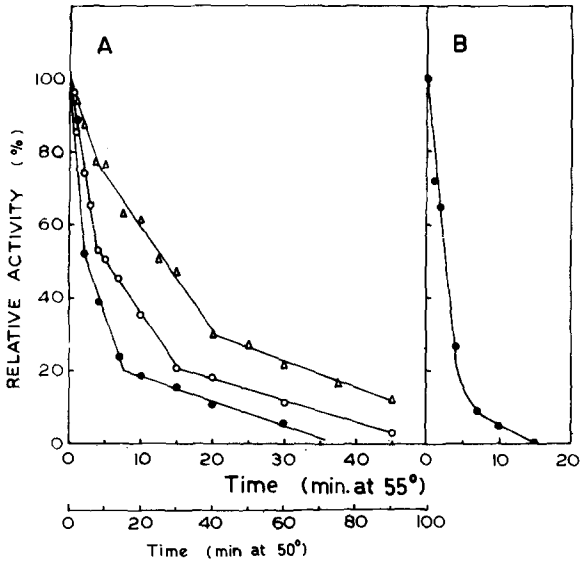


Fig. 4. Heat inactivation profiles of the purified native enzyme and the peak III enzyme. The enzyme preparations were heated at 50 or 55°C for the indicated period of time in the presence of 0.02 M phosphate buffer, pH 7.0, containing 0.15 M NaCl. (A) Purified enzyme: △, protein concentration 50 μg/ml, temperature 50°C; ○, protein concentration 50 μg/ml, temperature 55°C; ●, protein concentration 8 μg/ml, temperature 55°C. (B) Peak III enzyme: ●, protein concentration 10 μg/ml, temperature 55°C.

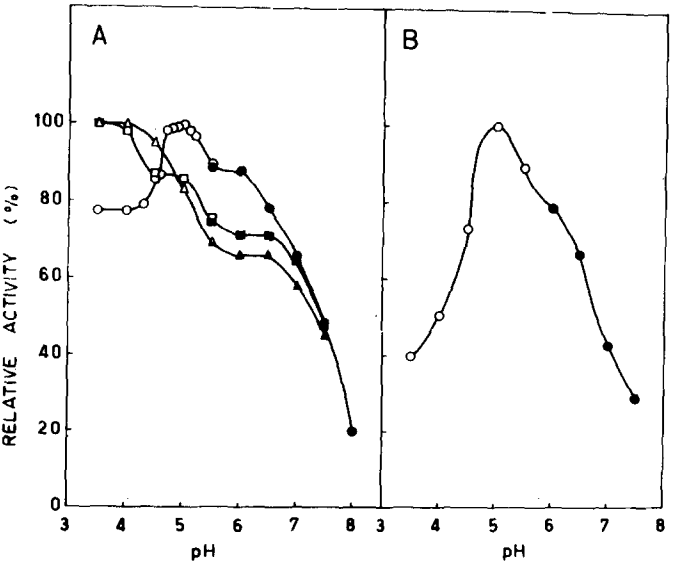


Fig. 5. pH activity profiles. The enzyme preparations containing about 0.03—0.05 enzyme units were incubated under identical standard assay conditions except that buffers of different pH, as mentioned, were used. (A) Purified native enzyme: ○, acetate buffer; ●, phosphate buffer; native enzyme free of the most heat sensitive form (heated for 9 min at 55°C, protein concentration 50 μg/ml); □, acetate buffer; ■, phosphate buffer; the least heat sensitive form of the enzyme (heated for 24 min at 55°C, protein concentration 50 μg/ml); △, acetate buffer; ▲, phosphate buffer. (B) Peak III enzyme isolated by gel filtration: ○, acetate buffer; ●, phosphate buffer.

pH 3.5—4.0 the enzyme showed a relatively low but constant activity. The pH profiles of the differentially heat-inactivated enzymes were also studied. When the native enzyme was removed of its most heat sensitive form by heating for 9 min at 55°C it yielded a pH profile in which the optimum activity was in the region of pH 3.5—4.0, a shoulder at pH 5.0 and a second shoulder around pH 6.0—6.5 (Fig. 5A). With the least heat-sensitive form of the enzyme (obtained by heating the native enzyme at 55°C for 24 min), the pH activity profile showed optimum activity at pH 3.5—4.0 and a disappearance of the shoulder at pH 5.0 (Fig. 5A). The results suggested that the most heat-sensitive form of the enzyme (which in turn appears to be the same as peak III enzyme mentioned earlier) has a pH optimum around 5.0 and the other two forms have a pH optimum around 3.5—4.0, one of them (the enzyme with intermediate heat sensitivity) exhibiting a shoulder at pH 5.0. The peak III enzyme from gel filtration gave a pH profile with a sharp optimum at pH 5.0 (Fig. 5B) with much less activity in the pH 3.5—4.0 region, once again indicating the possibility that the peak III enzyme and the most heat-sensitive form of the enzyme are the same. The possibility of these differences in pH activity profiles due to differences in pH stability was ruled out by studying the pH stability profiles of the native and differentially heat-inactivated enzymes. The same pattern of pH stability was observed for the native and partially heat-inactivated forms of the enzyme in the range of pH 3.5—8.0, the pattern showing lesser stability below pH 5.5 and a uniform stability between pH 5.5—8 for all the enzymes.

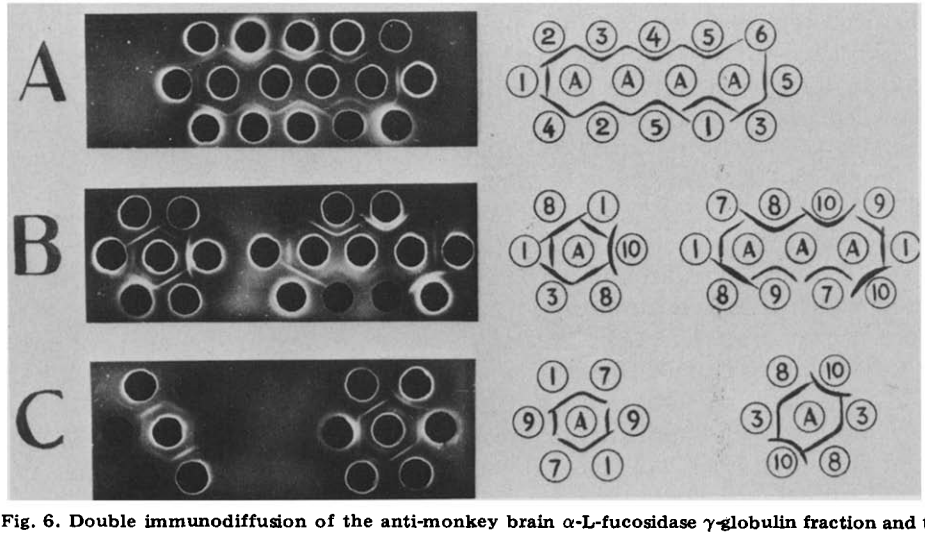


Fig. 6. Double immunodiffusion of the anti-monkey brain α-L-fucosidase γ-globulin fraction and the ammonium sulphate fraction of the enzyme from various sources. Immunodiffusion was performed in 1% agar gel in 0.02 M barbitone buffer, pH 8.6. The mg protein followed by the enzyme units used in each well is given in parentheses. 1—6, various tissues of monkey, 1, brain (0.9, 0.15); 2, intestine (0.9, 1.9); 3, liver (3.5, 1.8); 4, kidney (6.0, 3.7); 5, spleen (2.6, 2.9); 6, heart (3.7, 0.8); 7, human brain (2.2, 0.4); 8, human liver (1.3, 0.7); 9, rat brain (4.2, 0.8); 10, rat liver (6.0, 0.5). Antibody wells (A) were filled with 1.0 mg protein.

Immunodiffusion studies

The anti-monkey brain fucosidase immunoglobulin fraction appeared to be monospecific giving a single precipitin line with the monkey brain enzyme. The precipitin lines for monkey intestine, liver, kidney and spleen enzymes merged with each other but the brain enzyme showed the presence of spur with the rest of the enzymes. Heart enzyme under our experimental conditions did not cross-react with the brain enzyme (Fig. 6A). The antibody was also tested against the enzymes isolated from the brain and liver of human and rat. There was spur formation between enzymes from brain and liver tissues from both the species. A total merging of the precipitin lines between the monkey and human brain enzymes and monkey and human liver enzymes (Figs. 6B and 6C) was observed. However, the rat brain and liver enzymes showed spur formation with the enzymes from the other species (Figs. 6B and 6C).

Discussion

The purified α-L-fucosidase from monkey brain gave a single protein band on SDS gel electrophoresis with a molecular weight corresponding to 73500. Gel filtration of the native enzyme on Sephadex G-200 gave evidence for three peaks of enzyme activity with molecular weights corresponding to 285000 (peak I), 145000 (peak II) and 74000 (peak III). These results indicate that the peak I enzyme is a tetramer of identical subunits of molecular weight 73500 associated with non-covalent forces and the SDS treatment results in the complete dissociation of the enzyme to the monomer level. The rechromatography of peaks I and II on Sephadex G-200 indicates a tendency for the conversion of the tetramer to the monomer via the dimer. The tetrameric nature of α-L-fucosidases consisting of identical subunits from human liver [3] and rat liver [5] has been reported, whereas rat epididymis enzyme has been found to consist of two types of subunits [2]. The human brain enzyme has recently been reported to have a single type of subunit [4] while the number of subunits in the native enzyme is not known.

Although the earlier reports [7,9] a bound and unbound form of α-L-fucosidase based on DEAE-cellulose chromatography at pH 6.5 was described, we found that at pH 7.5 the monkey brain enzyme was completely retained by DEAE-Sephadex.

From Fig. 3 the involvement of carboxyl groups in the catalytic process is indicated which is further supported by a marked loss of activity upon masking of free carboxyl groups. It is noteworthy that amino acid analysis data of purified α-L-fucosidases from different mammalian sources reported so far [2,3,5] show an unusually large abundance of glutamic and aspartic acid residues. The monkey brain enzyme appeared to be similar to the rat liver enzyme [5] but different from rat brain enzyme [10] in having sulphydryl group(s) that are important for catalytic activity.

The heat inactivation and pH activity profile studies also suggest the presence of three forms of the enzyme and as described under Results a possible relationship between the three forms observed by gel filtration, heat inactivation and pH activity profile data emerges. Thus the most heat-sensitive form of the enzyme appears to be similar to the peak III (monomer form) of α-L-fuco-

sidase with a pH optimum of 5.0. The least heat-sensitive form of the enzyme has a pH optimum of 3.5—4.0 without a shoulder at pH 5.0 and it is presumable that the enzyme of intermediate heat stability also has a pH optimum around 3.5—4.0 with a shoulder at pH 5.0. A relationship between the peak I and peak II enzymes on the one hand and the enzymes with the least and intermediate heat sensitivity on the other could not be directly established because of the association of peak II and peak III enzymes with the peak I enzyme in varying proportions and a relatively rapid conversion of peak II to peak III (as evidenced by rechromatography of the peaks I and II on Sephadex G-200). It is however presumable that the peaks I and II enzymes correspond to enzymes of least and intermediate heat sensitivity. The following characteristics can thus be assisgned to the three forms of α-L-fucosidase of monkey brain.

Peak I enzyme tetramer:	Molecular weight 285 000; pH optimum 3.5—4.0	Less heat sensitive
Peak II enzyme dimer:	Molecular weight 145 000; pH optimum 3.5—4.0	Less heat sensitive
Peak III enzyme monomer:	Molecular weight 73 500; pH optimum 5.0	Most heat sensitive

Our results on immunological characterization of α-L-fucosidase from various tissues of monkey showed that the enzymes isolated from the non-neural tissues were similar with each other but possessed only a partial similarity with the brain enzyme. Studies on the enzymes isolated from the brain and liver of monkey, human and rat showed that only monkey and human brain enzymes were immunologically similar as were liver enzymes from these two species. There was only partial similarity between the brain and liver enzyme of all three species. In contrast to this is the finding of Alhadeff and Janowsky [4] who found the immunological similarity of human brain and liver fucosidases using antibody raised against the purified human liver α-L-fucosidase. Our results are suggestive of a strong tissue immunospecificity of the monkey brain enzyme as well as of a human primate relationship which may have evolutionary significance.

Acknowledgement

One of us (T.A.) is a Senior Research Fellow of the Council of Scientific and Industrial Research, India.

References

1 van Hoof, F. and Hers, H.G. (1968) Eur. J. Biochem. 7, 34—44
2 Carlsen, R.B. and Pierce, J.G. (1972) J. Biol. Chem. 247, 23—32
3 Alhadeff, J.A., Miller, A.L., Wenaas, H., Vedvick, T. and O'Brien, J.S. (1975) J. Biol. Chem. 250, 7106—7113
4 Alhadeff, J.A. and Janowsky, A.J. (1977) J. Neurochem. 28, 423—427
5 Opheim, D.J. and Touster, O. (1977) J. Biol. Chem. 252, 739—743
6 Wiederschain, G.Y. and Rosenfeld, E.L. (1971) Biochem. Biophys. Res. Commun. 44, 1008—1014
7 Di Matteo, G., Orfeo, M.A. and Romeo, G. (1976) Biochim. Biophys. Acta 429, 527—537
8 Wiederschain, G.Y., Kolibaba, L.G. and Rosenfeld, E.L. (1973) Clin. Chim. Acta 46, 305—310

9 Robinson, D. and Thorpe, R. (1973) Clin. Chim. Acta 47, 403—407
10 Bosmann, H.B. and Hemsworth, B.A. (1971) Biochim. Biophys. Acta 242, 152—171
11 Turner, B.M., Beratis, N.G., Turner, V.S. and Hirschhorn, K. (1974) Clin. Chim. Acta 57, 29—35
12 Alhadeff, J.A., Miller, A.L., Wenger, D.A. and O'Brien, J.S. (1974) Clin. Chim. Acta 57, 307—313
13 Thorpe, R. and Robinson, D. (1975) FEBS Lett. 54, 89—92
14 Alam, T. and Balasubramanian, A.S. (1978) J. Neurochem., in the press
15 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
16 Davis, B.J. (1964) Ann. N.Y. Acad. Sci. 121, 404—427
17 Andrews, P. (1965) Biochem. J. 96, 595—606
18 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406—4412
19 Stelos, P. (1967) in Handbook of Experimental Immunology (Wier, D.M., ed.), pp. 4—6, Blackwell Scientific Publications, Oxford
20 Dixon, M. and Webb, E.C. (1964) Enzymes, 2nd edn., pp. 315—331, Longmans Green, London
21 Dixon, M. and Webb, E.C. (1964) Enzymes, 2nd edn., pp. 116—145, Longmans Green, London
22 Carraway, K.L. and Koshland, Jr., D.E. (1972) in Methods in Enzymology (Hirs, C.H.W. and Timasheff, S.N., eds.), Vol. 25, pp. 616—623, Academic Press, New York