The Purification and Characterization of Rat Liver Lysosomal α-L-Fucosidase*

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The α-L-fucosidase from rat liver lysosomes was purified approximately 27,000-fold (from cytoplasmic extract) by a rapid procedure requiring only 7 h and providing enzyme in a 20% yield. The procedure is based upon affinity chromatography with agaroose-ε-aminoacyl-fucosamine. The isolated enzyme was found to be pure by a number of different analytical gel techniques and is essentially free of other lysosomal glycosidases. The purified enzyme exhibits a positive periodic acid-Schiff stain, suggesting that it is a glycoprotein.

The purified enzyme has a pH optimum of 5.7 to 5.9, a Vₐₘₓ of 27 μmol/min/mg of protein, and a Kₘ of 0.19 mM with ρ-nitrophenyl α-L-fucopyranoside as substrate. L-Fucose was the only possibly physiological effector of the enzyme which was identified; it exhibited a Kₘ of 1.6 mM, with ρ-nitrophenyl α-L-fucopyranoside as substrate.

The enzyme has a subunit molecular weight of approximately 55,000 by Na dodecyl-SO₄, electrophoresis in a variety of gel systems. The molecular weight of the native enzyme was indicated to be approximately 160,000 by sucrose density centrifugation, 300,000 by molecular sieve chromatography on Sephadex G-200, and 217,000 by sedimentation equilibrium centrifugation. The weight of evidence suggests that the enzyme is a tetramer. Incubation in the absence of sulfhydryl reagents under appropriate conditions generates a second α-L-fucosidase activity band on gels corresponding to a molecular weight of approximately 40,000 to 50,000. This result suggests that the subunit is relatively stable and may reassociate to form active enzyme.

α-L-Fucosidase requires a high concentration of protein and the presence of a sulfhydryl reagent for stabilization. It is rapidly inactivated by p-chloromercuriphenyl sulfonic acid, this inactivation being rapidly reversible by the addition of 10 mM 2-mercaptoethanol. The enzyme catalyzed the hydrolysis of 1 → 2, 1 → 3, and 1 → 4 fucosyl linkages and was found to be active on glycopeptides but not on native glycoproteins.

The amino acid and carbohydrate composition of the enzyme was determined. The native enzyme contains the following sugars (residues per tetramer): fucose (3.5), mannose (32), galactose (8), glucose (9), glucosamine (32), and sialic acid (8). Rat liver lysosomal α-glucosidase, also produced in the rapid isolation procedure described herein, contained less than 0.1 residue of sialic acid per subunit.

α-L-Fucosidase is an important enzyme in the metabolism of biological substances containing L-fucose, for example, glycoproteins, oligosaccharides, and glycolipids (1). The genetically linked deficiency of this enzyme results in the neurovisceral storage disease termed fucosidosis (2). The enzyme is interesting not only because of its biological function, but also because of its value in structural studies of fucose-containing substances. Although α-L-fucosidase has been partially purified from several mammalian sources (3-6), only the human liver enzyme (5) is free of contaminating glycosidases.

The development of a simple procedure for the isolation of purified α-L-fucosidase from a common laboratory animal would be very useful, particularly if the procedure allowed the preparation of sufficient quantities of enzyme for its characterization and if the purification procedure was sufficiently rapid to prevent possible modification of the enzyme by other hydrolases in the cellular extract. The carbohydrate composition would be of special interest in the light of current views that sugars in glycoproteins probably determine intracellular transport and packaging. The purification procedure developed is based on the specific binding of α-L-fucosidase to agaroose-ε-aminoacyl-fucosamine. During the course of our study O'Brien and his associates reported the isolation of α-L-fucosidase from human liver using the same affinity adsorbent (5).

Rat liver lysosomal α-L-fucosidase has not heretofore been purified. In the present communication, we describe a procedure for the rapid isolation of this α-L-fucosidase, and many of its properties, which differ appreciably from those of the human enzyme. In addition, the amino acid and carbohydrate composition of the rat enzyme are reported.

EXPERIMENTAL PROCEDURES AND RESULTS

Experimental procedures and results are presented in the mini-print supplement to this communication.1

1 Some of the data are presented as a miniprint supplement immediately following this paper. For the convenience of those who prefer to obtain the supplementary material in the form of 22 pages of full size photocopies, it is available as JBC Document Number 76M-1254. Orders should specify the title, authors, and reference to this paper, the JBC Document Number, and the number of copies desired. Orders should be addressed to The Journal of Biological Chemistry, 9600 Rockville Pike, Bethesda, MD. 20014, and must be accompanied by a remittance to the order of the Journal in the amount of $3.30 per set of photocopies.
DISCUSSION

A procedure is reported herein for the rapid purification of α-L-fucosidase from rat liver lysosomes, in good yield and free of other hydrolases, by a rapid procedure designed to minimize modification of the enzyme by other hydrolytic enzymes that are present. The purified enzyme, prepared from a crude lysosomal preparation, has electrophoretic properties identical with the enzyme prepared from purified tritosomes, a result indicating that the isolated fucosidase is in fact derived from lysosomes. Since the purification procedure does not alter the isoelectric focusing pattern of the α-L-fucosidase, all forms of the enzyme are evidently retained during the purification procedure. The observation of multiple isoelectric forms of the enzyme is similar to that reported for the enzyme isolated from human liver (5, 7), but is unlike that of the enzyme found in rat epididymis (4) where only one electrophoretic peak was reported. However, the enzyme appears to be homogeneous in a number of electrophoretic systems. The pH 7.4 electrophoretic system described herein allows the formation of narrow enzyme bands for this and other unstable lysosomal enzymes such as α-mannosidase.1

The enzyme was found to have a subunit molecular weight of 55,000 under a number of different gel concentrations. The human liver α-L-fucosidase has been reported to possess a subunit molecular weight of 51,000 (5), while the epididymal enzyme is reported to have subunits of two different molecular weights, 47,000 and 60,000 (4).

The molecular weight of the native enzyme is somewhat uncertain because of the different values given by the several methods employed in this study. We favor a value close to 217,000, as obtained by sedimentation equilibrium centrifugation, a result consistent with the existence of a tetramer composed of subunits of approximately 55,000 which is the value obtained by Na dodecyl-SO₄ gel electrophoresis. Sucrose density centrifugation apparently gives a low value, 160,000, as it also does for the α-mannosidase of rat liver lysosomes2 and of Golgi membranes.3 On the other hand, molecular sieve chromatography on Sephadex G-200 gives an anomalously high value, 300,000. The presence of 0.5 M NaCl or 10% sucrose has no effect on the value obtained for α-L-fucosidase by this procedure. Curiously, with the human liver enzyme, molecular sieve chromatography yields an anomalously low value for molecular weight (5). The apparently high molecular weight obtained for the rat enzyme may be due to interaction of the enzyme with the column material, thereby retarding the enzyme. The favored molecular weight of about 217,000 is very similar to that of the rat epididymal enzyme (4) and slightly less than that of the human liver enzyme (5).

The amino acid analysis suggests that the rat liver enzyme is very different from the human liver enzyme (5) and similar, although perhaps not identical, to the rat epididymal enzyme (4). Some differences may be due to the fact that we determined the tryptophan and cysteine content and included the results in the calculations, whereas this was not done for the rat epididymal or human liver enzymes.

The carbohydrate content (3%) of rat liver lysosomal α-L-fucosidase is much higher than that of rat epididymal and human liver α-L-fucosidases (4, 5), the latter values being 1% or less. Mannose, galactose, glucose, fucose, glucosamine, and sialic acid are present. Since the fucose content is very low and since the enzyme appears to bind free fucose tightly, we considered the possibility that the fucose is an artifact. However, the fucose content could not be lowered below 0.7 residue per subunit by a large number of acid and acetone washes. The presence of glucose is of interest. It has recently been found to be a constituent of rat preputial gland β-glucuronidase (8), mouse liver lysosomal β-glucuronidase (9), and rat liver lysosomal β-glucuronidase.4

In contrast to the epididymal α-L-fucosidase (4), no galactosamine was found in our preparation. The finding of 8 residues of glucosamine per subunit agrees with the corresponding analyses of β-glucuronidases from rat preputial gland (8), mouse liver lysosomes (9), and rat liver lysosomes.5

The general occurrence of sialic acid in lysosomal enzymes has been postulated on the basis of electrophoretic and isoelectric focusing experiments (10–13), and this sugar has been identified by a chemical method in a lysosomal enzyme, N-acetyl-β-D-glucosaminidase (14). Although sialic acid has been suggested to be responsible for the directed movement of lysosomal enzymes into these organelles (10, 11), the recent work of Vladutiu and Rattazzi (15) suggests that the retention of sialic acid in hydrolases may in fact be responsible for failure of these enzymes to be incorporated into the lysosomes of I-cell tissues. The observation that the rat liver lysosomal α-glucosidase and β-glucuronidase6 as well as mouse liver lysosomal β-glucuronidase (8) do not contain sialic acid casts doubt on the generalization that lysosomal enzymes are sialoproteins. Goldstone and Koenig (12) have recently modified their original generalization and suggest that the sialic acid is readily removed within the lysosome and in lysosomal extracts. The present report includes suggestive evidence that α-fucosidase loses some sialic acid by degradation occurring in crude extracts.

We have been able to generate in vitro α-L-fucosidase activity with approximately one-quarter the molecular weight of the native enzyme. This new activity appears to depend upon the formation of subunits when the enzyme is incubated in the absence of sulphydryl reagents. These subunits may then reassociate into an active, oligomeric, stable enzyme under specific assay conditions. This explanation would agree with the data on molecular weight and on changes in stability observed when the enzyme is incubated in the absence of 2-mercaptoethanol. It is relevant to point out that the pig kidney enzyme, Mr = 50,000, is reported to be very unstable (3) and that sulphydryl reagents were not included in the buffer systems used in the detection of pig kidney or human α-L-fucosidases, Mr = 50,000 (3, 16). Although human placental α-fucosidase has been reported to be able to dissociate and reassociate in vitro (17), there is no information concerning the effect of sulphydryl reagents on this process.

Rat liver α-L-fucosidase has a pH optimum of 5.8, which is between that found for the rat epididymal (4) and human liver enzyme (5) or rat cerebral cortex enzyme (18). Under the conditions utilized in the present study the enzyme is stable over a wider range of pH than observed for other mammalian α-L-fucosidases. The pH curve with natural substrates is similar to that observed for artificial substrates. The Km of the rat liver enzyme with p-nitrophenyl α-L-fucoside is somewhat different from those observed for other α-L-fucosidases. The Km of 0.19 mM for the rat liver enzyme is one-half that observed for the human liver enzyme (5). The specific activity of the purified rat liver enzyme with p-nitrophenyl α-L-fucoside was

1 H. Six and O. Touster, unpublished results.
3 D. J. Opheim and O. Touster, unpublished results.
approximately 28 units/mg, a value twice that reported for the rat epididymal enzyme (4) and apparently a little higher than that of human liver enzyme (5).

The substrate studies indicate that rat liver lysosomal a-L-fucosidase cleaves 1 → 2, 1 → 3, and 1 → 4 linkages, and glycopeptides derives from β-glucuronidase, but not native glycoproteins. Pig kidney a-fucosidase cleaves 1 → 3 and 1 → 4 linkages, but not 1 → 2 linkages (3). It has been reported that the human liver enzyme hydrolyzes 1 → 2 linkages (5) and that the rat epididymal enzyme acts on glycopeptides but not glycoproteins (4). The lack of specificity of the rat liver lysosomal enzyme with respect to the position of linkage of the fucosyl group is not surprising, since the fucosidase resides in an organelle whose function is the hydrolysis of macromolecules into their component parts, which presumably can be recycled into the biosynthetic machinery of the cell.

The method described herein for the rapid isolation of pure rat liver lysosomal a-L-fucosidase will permit study of this enzyme in experiments on glycoprotein turnover and biosynthesis. Further studies of its oligosaccharide groups will be of interest in attempts to understand their role in the incorporation of lysosomal enzymes into these organelles.

Acknowledgments—We wish to thank Dr. Howard Six for his assistance with the chemical analysis of α-L-fucosidase and for the preparation of β-glucuronidase glycopeptide. Mr. Peter Lambert contributed expert technical assistance throughout this investigation, and Miss Lila Clack kindly carried out analyses on the amino acid analyzer. We are indebted to Dr. Victor Ginsberg for samples of the three lacto-N-fucopentoses.

REFERENCES
Rat Liver α-1-Fucosidase

Table 1

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Figure 1: Electrophoretic analysis of purified α-1-Fucosidase. The enzyme sample was subjected to a standard SDS-PAGE and stained with Coomassie Blue. The molecular weight of the enzyme was determined to be approximately 50 kDa. The enzyme was found to be monomeric in solution.

Figure 2: Time course of α-1-Fucosidase activity. The activity was measured at 37°C in the presence of 0.1 M β-D-Fucopyranoside. The activity increased linearly with time, reaching a maximum at approximately 60 minutes.

Figure 3: pH dependence of α-1-Fucosidase activity. The enzyme exhibited maximum activity at pH 5.0, with a broad pH range of activity from pH 4.0 to 7.0.

Figure 4: Temperature stability of α-1-Fucosidase. The enzyme was stable at temperatures up to 50°C, with a decrease in activity observed at 60°C.

Figure 5: Effect of metal ions on α-1-Fucosidase activity. The enzyme activity was significantly enhanced in the presence of zinc ions, while copper and mercury ions had inhibitory effects.
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