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THE PROPERTIES OF SYNOVIAL FLUID β-MANNOSIDASE ACTIVITY

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SUMMARY

The aim of the present study was to evaluate the β-mannosidase activity (β-D-mannoside mannohydrolase, EC 3.2.1.25) of human synovial fluid. It was shown to have a pH requirement of 3.5–4.0 and a K_m value of $3.4 \cdot 10^{-3}$ M using a *p*-nitrophenyl-β-mannopyranoside as the substrate. β-Mannosidase is significantly more heat-labile than mammalian α-mannosidase; substrate competition studies suggest it is an independent glycosidase; and *p*-nitrophenyl-β-galactoside is observed to inhibit this enzyme. A wide distribution of β-mannosidase activity in mammalian tissue was demonstrated.

INTRODUCTION

Several of the lysosomal acid hydrolases have been studied in synovial fluid and tissue. Of these the glycosidases evaluated in synovial fluid include β-glucuronidase[1,2], β-*N*-acetylglucosaminidase[2–5], β-galactosidase[2], and α-mannosidase[6]. The purpose of the present study is to evaluate β-mannosidase activity in synovial fluid and to (1) develop a microassay system for this enzyme, (2) delineate its kinetic properties in human synovial fluid, and (3) show that β-mannosidase activity is independent of the activity of other lysosomal hydrolytic glycosidases.

MATERIAL AND METHODS

Enzyme sources

The source of enzyme activity consisted of freshly aspirated synovial fluid from patients with osteoarthritis or rheumatoid arthritis. The samples were frozen at −18 °C and used for study as desired. Synovial fluid proved to be a stable source of β-mannosidase activity with eight repeated freezing–thawing procedures over a period of two weeks showing a variation of enzyme activity of less than ± 10%. These results were not influenced by detergents, *e.g.* Triton X-100. Samples from mouse organs and human synovial tissue or cell preparations were surveyed for β-mannosi-

dase activity. These samples were homogenized in a Ten-Brock glass homogenizer, frozen and thawed, re-homogenized and centrifuged at 35 000 × *g* to produce a cell-free crude enzyme preparation.

Material used

The compounds used in this investigation, *p*-nitrophenyl-β-mannopyranoside, mannono-(1→4)-lactone, *p*-nitrophenyl-β-*N*-acetylglucosaminide, *p*-nitrophenyl-β-galactopyranoside, *p*-nitrophenyl-β-glucuronide and *p*-nitrophenyl-α-mannopyranoside were obtained commercially.

Assay conditions

The optimal microassay system had a volume of 125 µl containing: 25 µmoles of sodium acetate buffer, pH 3.5; 1.5 µmoles, *p*-nitrophenyl-β-mannoside; and 10–40 µl, synovial fluid. The incubation time was 30–120 min at 37 °C. The reaction was terminated with 2% Na_2CO_3 in 0.1 M NaOH; and the chromophore of free *p*-nitrophenol was measured at 400 nm in a Beckman DU-2 spectrophotometer. Enzyme activity was expressed as µmoles of *p*-nitrophenol released per ml of synovial fluid, or 10 mg of protein, per h of incubation at 37 °C. Protein concentrations were determined by the method of Lowry *et al.*[7].

RESULTS

In developing the optimal microassay system, the kinetic properties of synovial fluid β-mannosidase were evaluated. Fig. 1 illustrates the pH and buffer system requirements of this enzyme. No difference was noted between sodium and potassium salts or their concentration, and no metal ion requirement was observed (Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+}, or Co^{2+}). The enzyme activity was unaffected by the addition of EDTA. Under these optimal assay conditions, a linear response was noted for

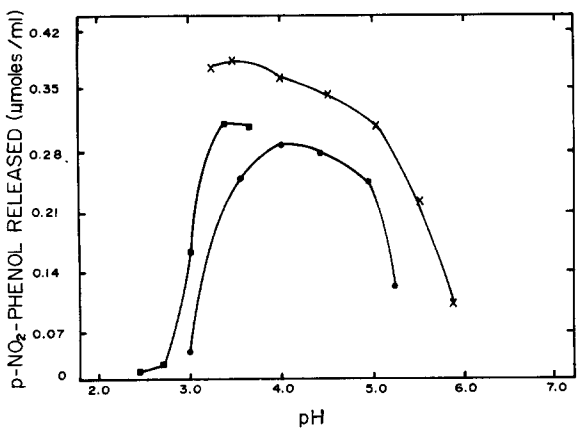

Fig. 1. Buffer and pH requirements of β-mannosidase activity. Three buffer systems were evaluated; sodium acetate–acetic acid (×); glycine–HCl (■); and sodium citrate–citric acid (●). The incubation mixtures were as indicated under methods except that 25 µmoles of the various buffers were used. Product formation is expressed as µmoles of *p*-nitrophenol released per ml of incubation mixture per h of incubation.

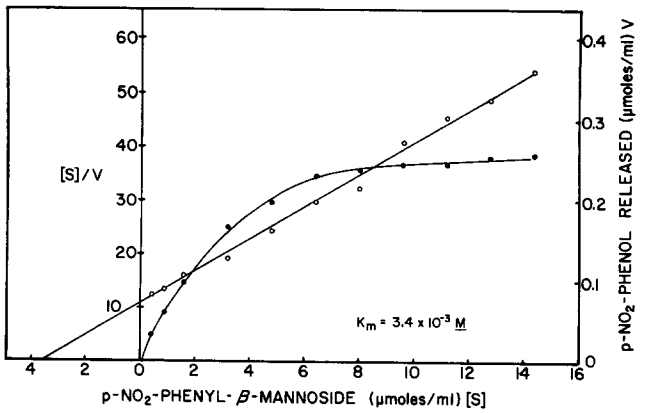

Fig. 2. The relationship of substrate concentration to product formation, (●), with a superimposed K_m plot of $[S]/V$ *versus* $[S]$, (○). Product formation V is expressed as μmoles of *p*-nitrophenol released per ml of incubation mixture per h of incubation. Incubation conditions are as under methods except for substrate concentration and individual control substrate incubations.

both time (at least 120 min) and enzyme concentration. The latter was unaffected by the modestly variable protein concentration of synovial fluid.

A substrate saturation curve is shown in Fig. 2 for β-mannosidase activity in synovial fluid. Superimposed is an $[S]/V$ *versus* $[S]$ plot for the determination of the K_m value by the method of Lineweaver and Burk[8].

The microassay system and kinetic properties make possible the characterization and quantitation of synovial fluid β-mannosidase but do not establish it as a distinct synovial fluid enzyme. To establish its individuality, indirect methods were employed including the heat stability properties of both the α- and β-mannosidases (Fig. 3). Substrate competition experiments are illustrated in Table I. The additive results suggest that synovial fluid β-mannosidase activity is independent of β-*N*-acetylglucosaminidase, β-glucuronidase and α-mannosidase activity, and that *p*-nitrophenyl-β-galactoside inhibits β-mannosidase activity. In a synovial fluid and a serum, both with negligible β-galactosidase activity, *p*-nitrophenyl-β-galactoside (8.0 mM) was found to inhibit β-mannosidase activity 82 and 76% respectively.

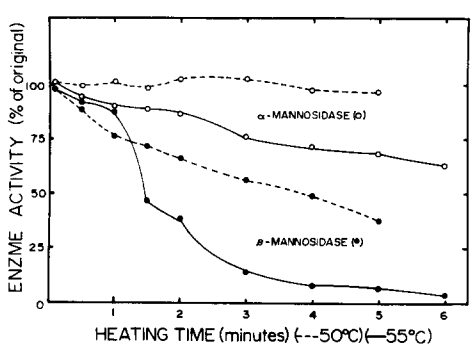

Fig. 3. Heat stability studies comparing α- and β-mannosidase activity of synovial fluid. Both studies were performed on the same enzyme preparation under optimal conditions.

TABLE I

SUBSTRATE COMPETITION EXPERIMENTS

The assay conditions used in this experiment are those optimal for synovial fluid β-mannosidase activity, and all the synthetic glycosides used were at substrate saturation concentrations for their individual enzyme activities. Control substrate incubations were performed in each case. Results are expressed as μmoles of *p*-nitrophenol released per ml of synovial fluid per h of incubation.

Competing substrate	*μmoles/ml substrate*	*p-Nitrophenyl-β-mannoside*	
		0 μmole/ml	*12 μmoles/ml*
—	—	0	1.60
p-Nitrophenyl-β-*N*-acetylglucosaminide	8	2.24	3.78
p-Nitrophenyl-β-glucuronide	6	2.26	3.78
p-Nitrophenyl-β-galactoside	4	0.72	1.10
p-Nitrophenyl-α-mannoside	8	1.27	2.79

TABLE II

β-MANNOSIDASE INHIBITION STUDIES

The synovial fluid enzyme preparation employed contained negligible β-galactosidase activity, and incubation conditions were as outlined under methods and in the text.

Inhibitor	*Concentration (μmoles/ml)*	*Enzyme activity (% of original)*
—	—	100
Mannono-(1→4)-lactone	67	4.5
Galactose	2.5	100
Mannose	2.5	98
Lactose	2.5	100
p-Nitrophenyl-β-galactoside	2	59
p-Nitrophenyl-β-galactoside	4	47
p-Nitrophenyl-β-galactoside	8	23

TABLE III

DISTRIBUTION OF β-MANNOSIDASE IN MAMMALIAN TISSUES

Enzyme activity is expressed as μmoles of *p*-nitrophenol released per 10 mg protein per h incubation. Assay conditions were as described in methods and were performed at two enzyme concentrations. (M) represents tissues obtained from mice while (H) denotes a human source. (RA) represents an average of four patients with rheumatoid arthritis and (PVNS) a patient with pigmented villous nodular synovitis.

Enzyme source	*Enzyme activity*	*Enzyme source*	*Enzyme activity*
Liver (M)	0.36	Lymphocytes (H)	0.71
Spleen (M)	0.92	Fibroblasts (H)	3.92
Kidney (M)	1.22	Polymorphonuclear cells (H)	0.43
Brain (M)	0.28	Synovium (RA) (H)	1.70
Lung (M)	0.62	Synovium (PVNS) (H)	3.65
Muscle (M)	0.05	Serum (H)	0.11

Levy and co-workers[9,10] demonstrated the glyconolactones [(1→4) and (1→5)] inhibit their corresponding glycosidases. Table II shows the results in experiments employing mannono-(1→4)-lactone and other potential inhibitors of the β-manno-

sidase activity. The inhibition with mannono-(1→4)-lactone was only apparent after pre-incubation with the enzyme (0.02–0.10 M for 10–20 min) prior to the addition of the substrate and buffer. The data demonstrate inhibition by mannono-(1→4)-lactone and *p*-nitrophenyl-β-galactopyranoside, but not by galactose, mannose or lactose.

β-Mannosidase activity is widely distributed in mammalian tissues and cell types, as shown in Table III, with its highest concentrations being present in synovial tissue, fibroblasts and kidney.

DISCUSSION

β-Mannosidase activity has been studied from various sources including mollusks[11–13], yeast[14], seminal fluid and sperm[15]; and recently hen oviduct[16]. Certain comparative observations are of interest. The K_m of synovial fluid β-mannosidase is similar to that from hen oviduct, but apparently different than was observed in mollusks. The present study suggests a somewhat lower pH requirement and a higher enzyme activity with the use of an acetate buffer. Heat stability studies were similar to those previously reported and non-identity with other synovial fluid glycosidases (substrate competition studies) was observed. Of particular interest was the concentration dependent inhibitory activity of *p*-nitrophenyl-β-galactoside not shared by galactose or lactose, and the wide distribution of β-mannosidase activity with the highest levels being found in synovium and fibroblasts. The possible role of β-mannoside in connective tissue metabolism or inflammatory joint disease deserves further study.

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