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## Purification and properties of β-*N*-acetylglucosaminidase from bovine uterus

The occurrence of β-*N*-acetylglucosaminidase (β-2-acetamido-2-deoxy-D-glucoside acetamidodeoxyglucohydrolase, EC 3.2.1.30) in mammalian tissues was first described by WATANABE[1], and has since been studied in relation to other glycosidases by LINKER, MEYER AND WEISSMANN[2] and BUDDECKE AND WERRIES[3,4]. CONCHIE AND FINDLAY[5] have reported the presence of this enzyme in the rat uterus. Although the β-*N*-acetylglucosaminidase in male reproductive tissues has been the subject of several investigations[6–8], the preparation and properties of the enzyme from the female reproductive tissues have not been investigated.

Bovine uterine tissues from a local slaughter house were stripped of excess fatty and vascular tissues, slit, and rinsed with deionized water. The tissues were minced in a food grinder, pooled, shell frozen, and lyophilized. The dried material was put through a Wiley Mill, using a 40-mesh screen. The enzyme assay mixture of 1.0 ml total volume contained 0.1 ml of 0.01 M p-nitrophenyl-*N*-acetyl-β-D-glucoaminide, 0.005–0.1 ml enzyme preparation and citrate buffer. The buffer used throughout the isolation and assay procedures, unless otherwise stated, was 0.05 M sodium citrate–citric acid, pH 4.5. After 5 min incubation at 37°, the reaction was terminated by addition of 2.0 ml of 0.2 M $Na_2CO_3$. Protein was estimated either colorimetrically[9] or spectrophotometrically[10] using bovine serum albumin as a standard. The unit of activity is defined as μmoles of *p*-nitrophenol liberated per min at 37° at 420 mμ; specific activity is defined as units per mg protein.

An extract (5%, w/v) of the 40-mesh pass powdered uteri was made in cold citrate buffer. After blending for 2–4 minutes in a Servall Omni-Mixer, the mixture was centrifuged at 16 000 *g* for 20 min. The supernatant was designated as S-I. The clear S-I was divided into small volumes which were heated rapidly to 55°, then cooled immediately to 10–15°. After centrifugation as above, the supernatant was designated S-II. S-II was slowly brought to 45% saturation with respect to solid $(NH_4)_2SO_4$. The precipitate was collected by centrifugation and dissolved in cold citrate buffer (0.1 the volume of S-I). This dissolved precipitate was dialyzed overnight against the citrate buffer at 4° and after centrifugation the supernatant was labeled S-IV. S-IV was concentrated by ultrafiltration in a collodion bag to give a concentration of 150–200 mg protein/5 ml. This concentrated protein solution was termed E-1.

A sample of E-1 (not exceeding 225 mg protein) was placed on a Bio-Gel P-200 column (4.5 cm × 48 cm packed volume) equilibrated with the citrate buffer at 25°. Fractions of 1.5 ml were collected. Enzyme activity was eluted as a single broad peak after passage of 260 ml of citrate buffer. Those fractions which had high activity and low protein content were pooled, concentrated to 1 ml by ultrafiltration, and labeled E-2. A sample of E-2 (15 mg protein) was fractionated on a carboxymethyl-cellulose column (1 cm × 25 cm packed volume) equilibrated with citrate buffer. The active protein was eluted with 0.1 M NaCl in citrate buffer. A sharp peak was contained in 3 or 4 fractions of 1.2 ml each. The pooled fractions were termed E-3.

Results of a typical purification are shown in Table I. These fractionation

TABLE I

PURIFICATION OF BOVINE UTERUS β-*N*-ACETYLGLUCOSAMINIDASE

The units of enzyme activity refer to μmoles of *p*-nitrophenol liberated per min from *p*-nitrophenyl-β-D-glucosaminide.

| *Fraction* | *Total protein (mg)* | *Total enzyme units* | *Specific activity (μmoles/mg protein per min)* | *Purification* | *Recovery of enzyme (%)* |
|---|---|---|---|---|---|
| S-I: PCU extract | 5142 | 277 | 0.054 | (1.0) | 100 |
| S-II: Heat treatment | 3867 | 243 | 0.063 | 1.2 | 88 |
| S-IV: Dialyzed supernatant after 0–40% $(NH_4)_2SO_4$ precipitation | 1138 | 228 | 0.200 | 3.7 | 82 |
| E-1: After concentration | 869 | 196 | 0.226 | 4.2 | 71 |
| E-2: Gel filtration | 54 | 97 | 1.796 | 33.4 | 35 |
| E-3: CM-cellulose | 4 | 40 | 10.003 | 185.9 | 15 |

procedures resulted in almost a 200-fold purification with 15% recovery of the total activity. Fig. 1 shows the effect of pH on the hydrolysis of substrate using 0.05 M citrate buffers, pH 3 to 6, with two optima observed at pH 4.5 and 4.8. The experimental points shown by WALKER, WOOLLEN AND HEYWORTH[11] are in agreement with these observations. No inflection of the pH activity curve was found in 0.05 M acetate buffers, pH 4 to 6. The purified preparation had no β-glucuronidase activity nor did the addition of fraction S-IV (which had β-glucuronidase activity) stimulate the β-*N*-acetylglucosaminidase activity of E-2. BUDDECKE AND HOEFELE[12] showed that albumin or β-*N*-acetylglucosaminidase stimulated β-glucuronidase activity and altered the double pH optima of β-glucuronidase.

The two products of the β-*N*-acetyl-D-glucosaminidase reaction (*p*-nitrophenol and *N*-acetylglucosamine) were determined[13] at various time intervals and found to be formed in equimolecular quantity. A $K_m$ of 1.25 mM and a $v_{max}$ of 7.7 μmoles/min per mg protein were determined. At inhibitor concentrations from 10 to 50 mM, the following $K_i$ values were determined: acetamide, 4.2 mM; acetate, 7.2 mM; and *N*-acetylglucosamine, 41.6 mM. The data for acetate and acetamide are in agreement

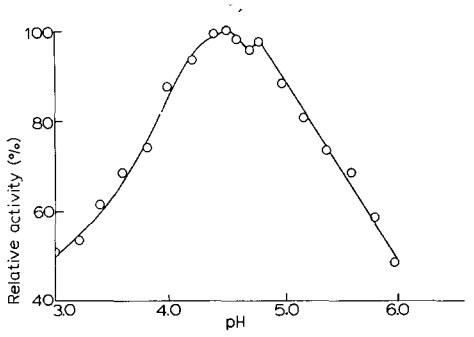


Fig. 1. Effect of pH on the hydrolysis of 1 mM substrate in 0.05 M citrate buffers by β-*N*-acetylglucosaminidase. Assay conditions are described in the text.

with values determined from other enzyme sources[4,8,12] while the value for *N*-acetylglucosamine differs by an order of magnitude[8,11].

At a final concentration of 4 mM, $Mn^{2+}$, $Ca^{2+}$, and EDTA had no effect on enzyme activity, while the same concentration of $Mg^{2+}$, $Cu^{2+}$, $Pb^{2+}$, and $Co^{2+}$ reduced activity by approximately 14%. No enzyme activity could be detected in the presence of 1 mM of $Ag^{+}$ or $Hg^{2+}$. At 10 mM, $CN^{-}$ reduced activity 14%, and NaCl enhanced activity by 10%. Bovine serum albumin (0.01%) stimulated activity by 25%. In general, there was little to no effect on β-*N*-acetylglucosaminidase activity by most of the metals tested. An exception appeared among the heavy metals, where the enzyme was relatively insensitive to $Pb^{2+}$ as compared to $Ag^{+}$ and $Hg^{2+}$. The sensitivity of the uterine enzyme to various metals was found to be similar to that of spleen and arterial tissues (*cf.* BUDDECKE AND WERRIE[3,4]).

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## Some aspects of the apparent glucose-6-phosphatase activity in the pancreatic islets of mammals

On the basis of staining histochemistry it has been claimed that glucose-6-phosphatase (D-glucose-6-phosphate phosphohydrolase, EC 3.1.3.9) occurs in the pancreatic islets of several species[1,2]. There are, however, no biochemical data available to corroborate this view. On the contrary, it has been shown in toadfish[3] that glucose 6-phosphate is hydrolyzed at a lower rate than glycerophosphate in the