BBA 63303

Separation of an α-galactosidase from rat uterus

Although extensive studies have been made of β-galactosidase from various origins, α-galactosidases are relatively unexplored α-Galactosidases have been reported in yeasts[1], molds[1], plant seeds[2], bacteria[3], earthworms[4], seminal plasma[5], epididymis[6], pancreas[6], and kidney[6] The presence of this enzyme in uterine tissue has not been previously reported

The enzyme assay mixture of 1 0 ml total volume contained 1 0 μmole of *o*-nitrophenyl-α-D-galactopyranoside, enzyme, and citrate buffer (pH 5 0), with a final ionic strength of 0.05 This buffer is hereafter referred to as citrate buffer After 30 min incubation at 37°, the reaction was terminated by the addition of 1 0 ml of 0.01 M Na_2CO_3 and the liberated nitrophenolate ion was determined at 420 mμ Protein was determined by the method of LOWRY *et al* [7] The unit of activity is defined as μmoles of nitrophenol liberated per min and specific activity is defined as units/mg protein.

A preliminary investigation demonstrated that diestrus uteri contained approximately twice the specific activity of full vaginal estrus uteri, thus only diestrus uteri from adult Holtzmann albino rats were used The uteri were stripped of excess fatty and vascular tissues, slit, rinsed with deionized water and a 10% (w/v) homogenate was made in 0 9% NaCl by blending in an all glass homogenizer The homogenate was centrifugated at 12 000 × *g* for 20 min at 5° This temperature was maintained throughout the fractionation procedure The clear supernant was slowly brought to 35% saturation with respect to solid $(NH_4)_2SO_4$ and centrifuged as above. The precipitate was dissolved in citrate buffer (0 1 the volume of homogenate) and dialyzed against the same buffer. This preparation was reprecipitated by $(NH_4)_2SO_4$ at 42% saturation, dissolved and dialyzed as before. This solution was concentrated by ultrafiltration in a collodion bag to give a concentration of 30–40 mg/ml

A sample of the concentrated protein solution was placed on a Bio-Gel P-200 column (4.5 cm × 48 cm packed volume) which had been equilibrated with the citrate buffer Fractions of 1 5 ml were collected by slow elution with the citrate buffer The α-galactosidase activity emerged in the first protein peak The active fractions were pooled, concentrated to 1 0 ml, and further fractionated by using CM-cellulose column chromatography The CM-cellulose column (1 cm × 25 cm packed volume) was equilibrated with citrate buffer The active protein was eluted with 0 1 M NaCl in citrate buffer After passage of a protein fraction devoid of α-galactosidase activity, the active protein was eluted in 6–8 fractions of 1.5 ml each

Results of a typical purification are shown in Table I These procedures resulted in 174-fold purification with a 12% recovery of the total activity. The preparation lost activity very rapidly at room temperature and upon freeze-thawing Partial protection was observed in the presence of 1 0 mM cysteine. Imidazole–HCl and Tris–HCl buffers with ionic strengths of 0.01 appeared to be inhibitory. The pH optima were found to be 5.2 in citrate buffer, having an ionic strength of 0.05 LINEWEAVER–BURK[8] plots of melibiose and D-galactose illustrate competitive inhibition with respect to *o*-nitrophenyl-α-D-galactopyranoside concentration A K_m of 4 mM for the uninhibited reaction was determined and the inhibited reactions yielded K_i values of 1.64 mM and 0 75 mM for melibose and D-galactose, respectively.

TABLE I

PURIFICATION OF RAT UTERINE α-GALACTOSIDASE

The units of enzyme activity refer to μmoles of nitrophenol liberated per min from *o*-nitrophenyl-α-D-galactopyranoside, specific activity expressed as units/mg protein.

Fraction	*Total protein (mg)*	*Total enzyme units*	*Specific activity*	*Purification*	*Recovery of enzyme (%)*
10% homogenate extract	3132	23 49	0 0075	(1)	100
First $(NH_4)_2SO_4$ ppt	80	10 81	0 135	18	46
Second $(NH_4)_2SO_4$ ppt	74	10 57	0 141	19	45
Bio-Gel P-200	14	6 34	0 451	60	27
CM-cellulose	2	2 82	1 307	174	12

By use of paper chromatographic methods of LI, LI AND SHETLAR[3] and LI AND SHETLAR[4], the enzyme preparation demonstrated the liberation of galactose from *o*-nitrophenyl-α-D-galactopyranoside, raffinose, melibiose, and stachyose The relative rates of hydrolysis were found to be· *o*-nitrophenyl-α-D-galactopyranoside, 100, raffinose, 14; melibiose, 8; and stachyose, 5. The preparation appeared to be capable of transgalactosylation since incubation of 0 25 M melibiose with 100 units of uterine α-galactosidase in the citrate buffer for 3 h produced three new spots on the chromatogram All three of these new spots had less mobility than melibiose, galactose and glucose

The *o*-nitrophenyl-α-D-galactopyranoside substrate of the enzyme assay mixture was replaced by 1.0 μmole of *o*-nitrophenyl-β-D-galactopyranoside, *p*-nitrophenyl-α-D-glucopyranoside or *p*-nitrophenyl-β-D-glucopyranoside. The uterine enzyme preparation was found to be free of β-galactosidase and β-glucosidase activities and contain a relatively small amount of α-glucosidase activity

This work was supported in part by a grant (HD-00136) from the U S. Public Health Service

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Received December 5th, 1967