Phytochemistry, Vol 23, No 1, pp 55–58, 1984
Printed in Great Britain

0031–9422/84 $3 00 + 0 00

TOMATO α-GALACTOSIDASES: CONVERSION OF HUMAN TYPE B ERYTHROCYTES TO TYPE O

RUSSELL PRESSEY

Richard B Russell Agricultural Research Center, USDA, ARS, P O Box 5677, Athens, GA 30613, U S A

(*Revised received* 6 *April* 1983)

Key Word Index—*Lycopersicon esculentum*, Solanaceae, tomato, α-galactosidase

Abstract—Tomato fruit contained a high level of α-galactosidase activity, and the two components found responsible for this activity, α-galactosidases I and II, were separated by chromatography on DEAE-Sephadex A-50 α-Galactosidase II, in particular, was highly purified by subsequent chromatography on a column of melibiose–agarose The two enzymes differed markedly in MW and in inhibition by mercuric compounds but less so in other properties α-Galactosidase II converted type B erythrocytes to type O

INTRODUCTION

α-Galactosidases are widely distributed in higher plants, but most earlier studies on these enzymes were made with seeds [1–6] Their physiological functions may be to hydrolyse α-linked galactose residues in some storage polysaccharides, such as the galactomannan in certain seeds [7], and in oligosaccharides of the raffinose series, which are major components of soybeans and other seeds [8] There is additional interest in plant α-galactosidases because some of them can modify the B specificity of intact human erythrocytes by removing α-1,3-linked galactose residues from cell surfaces [9] This reaction was first demonstrated with the α-galactosidase from green coffee beans [9, 10], but has since been shown with the enzymes from figs [11] and soybeans [5] Preliminary tests indicated that ripe tomatoes are a rich source of α-galactosidase that can modify B-type blood cells, therefore the study reported here was undertaken to isolate the enzyme and characterize its activity

RESULTS AND DISCUSSION

Separation and purification of tomato α-galactosidases

The amount of α-galactosidase activity present in a crude protein extract of ripe tomatoes indicated that the fruit contained *ca* 0 15 unit of the enzyme/g fresh wt This extract was chromatographed on DEAE-Sephadex A-50 (Fig 1), and a plot of the collected fractions vs their α-galactosidase activity showed the presence of two enzymes The earlier eluted enzyme was designated α-galactosidase I (fractions 22 through 28) and the later eluted enzyme, α-galactosidase II (fractions 59 through 68) The fractions corresponding to each enzyme were pooled and concentrated to 10 ml by ultrafiltration Each enzyme was then chromatographed on a column of Sephadex G-100 The fractions containing α-galactosidase were pooled and concentrated to 10 ml Each enzyme solution was adjusted to pH 4 and chromatographed on a column of melibiose–agarose (Fig 2) Although this step did not greatly increase the specific activity of α-galactosidase I, it did serve to remove the contaminant invertase Chromatography on melibiose–agarose was very effective in purifying α-galactosidase II (Fig 2) It should be noted that this enzyme was simply retarded by the melibiose–agarose and that it was eluted by the equilibrium buffer without added melibiose The specific activity of α-galactosidase II was increased 8-fold, and the solution was devoid of invertase, β-galactosidase, α-mannosidase and α-lucosidase Summaries of the overall purification procedures are shown in Table 1

Many seeds contain multiple forms of α-galactosidase [2, 4, 6], and often the forms differ markedly in molecular size such that they can easily be separated by gel filtration [4] The mung bean [12] and soybean [6] α-galacto-

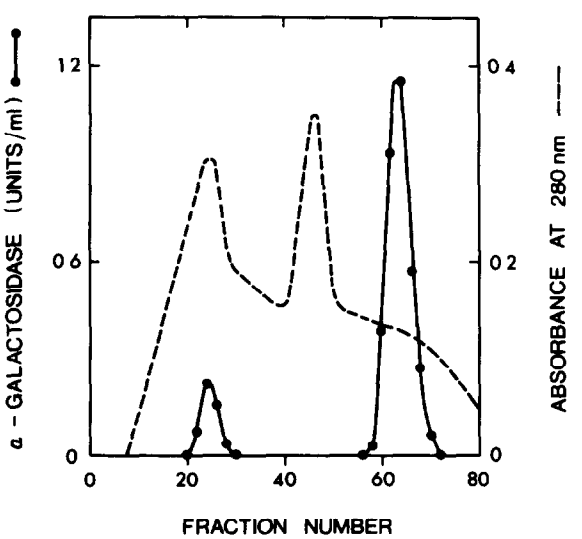

Fig 1 Separation of tomato α-galactosidases by chromatography on DEAE-Sephadex A-50 equilibrated with 0 05 M sodium acetate (pH 5 5) containing 0 1 M sodium chloride Protein was eluted with the equilibration solution at a flow rate of 30 ml/hr

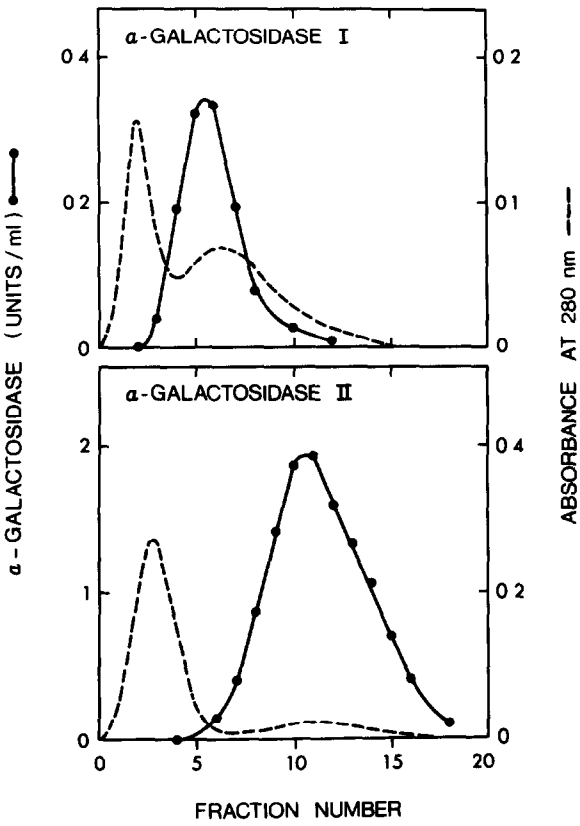

Fig 2 Purification of tomato α-galactosidases by chromatography on melibiose–agarose equilibrated with 0 05 M sodium acetate (pH 4) containing 0 1 M sodium chloride Protein was eluted with the equilibration solution at a flow rate of 20 ml/hr

sidases convert reversibly from a tetramer at pH 4 to a monomer at pH 7 Possibly α-galactosidases in other seeds exhibit similar reversible aggregation An important difference between the tomato enzymes and those in most of the other sources studied is that the tomato α-galactosidases had relatively low MWs (see below) Also, the smaller form (α-galactosidase II) predominated in tomato extracts, whereas the larger enzyme was the major form in most other sources [2, 4] The MWs of the tomato enzymes suggest a monomer–dimer relationship, but no interconversion between the enzyme forms could be brought about by changing the pH of the enzyme solutions

Properties of the α-galactosidases

α-Galactosidase I was most stable at pH 5 2, and heating solutions of the enzyme at this pH for 5 min at 70° resulted in 50% inactivation α-Galactosidase II was most stable at pH 4 5, and heating 5 min at only 55° resulted in 50% inactivation The MWs of the enzymes were determined by gel filtration on Sephadex G-100 The standard proteins and their elution volumes were as follows cytochrome *c*, 364 ml, ovalbumin, 261 ml, bovine serum albumin monomer, 231 ml, and bovine serum albumin dimer, 174 ml The elution volumes and calculated MWs for α-galactosidases I and II, respectively, were 266 ml and 44 000 and 335 ml and 19 000

The tomato α-galactosidases hydrolysed *p*-nitrophenyl-α-galactoside, raffinose, stachyose, methyl-α-galactoside and locust bean galactomamman, which contains α-linked galactose [7] The K_m values and relative maximum velocities are presented in Table 2 In contrast to other α-galactosidases [2, 3], the tomato enzymes were not affected by high substrate concentrations, not even 7 6 mM *p*-nitrophenyl-α-galactoside When this galactoside was substrate, α-galactosidases I and II were active well into the acid range with a pH optimum at 5 7 and 5 2, respectively, and a second, smaller, pH optimum near pH 3.5 (Fig 3) Similar double pH optima have been reported for *Vicia faba* [2], *Pisum sativum* [4] *and Glycine max* [5, 6], α-galactosidases acting on *p*-nitrophenyl-α-galactoside The tomato α-galactosidases further resembled the above enzymes in that they each had a single pH optimum with raffinose as the substrate (Fig 3)

The activities of the tomato α-galactosidases were not affected by the nature of the buffer Nor were they affected by 1 mM Mn^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+}, or Ag^{+} Mn^{2+} has been reported to activate weakly the α-galactosidase from *Vicia sativa* [3] Tomato α-galactosidase I was not inhibited by 1 mM Hg^{2+}, however, α-galactosidase II was extremely sensitive to this cation, with total inhibition by 1 4 μM $HgCl_2$ as well as by 0 3 μM *p*-hydroxymercuribenzoate α-Galactosidase II was not activated by either 2 mM EDTA or 2 mM cysteine Galactose was a relatively potent inhibitor of the tomato α-galactosidases The concentrations of this sugar required to inhibit α-galactosidases I and II by 50% were 2 0 and 1 2 mM, respectively Glucose, mannose and arabinose at 14 mM levels were not inhibitory

Table 1 Purification of tomato α-galactosidases

	α-Galactosidase I			α-Galactosidase II		
Step	Protein (mg)	Enzyme activity (units)	Specific activity (units/mg)	Protein (mg)	Enzyme activity (units)	Specific activity (units/mg)
Ammonium sulfate pellet	270	155*	—	270	155*	—
DEAE-Sephadex A-50	38	14	0 37	26	126	4 8
Sephadex G-100	13	12	0 92	12	118	9 8
Melibiose–agarose	4	9	2 3	1 3	106	82

*Total α-galactosidase activity in the ammonium sulfate pellet

Table 2 Michaelis constants and relative maximum velocities for several substrates

Substrate	α-Galactosidase I		α-Galactosidase II	
	K_m	V_{max}	K_m	V_{max}
p-Nitrophenyl-α-galactoside	0 54 mM	100	0 22 mM	100
Raffinose	1 8 mM	47	2 7 mM	19
Stachyose	2 4 mM	29	3 8 mM	5
Methyl-α-galactoside	5 3 mM	12	8 4 mM	2
Galactomannan	4 5 mg/ml	19	6 3 mg/ml	3

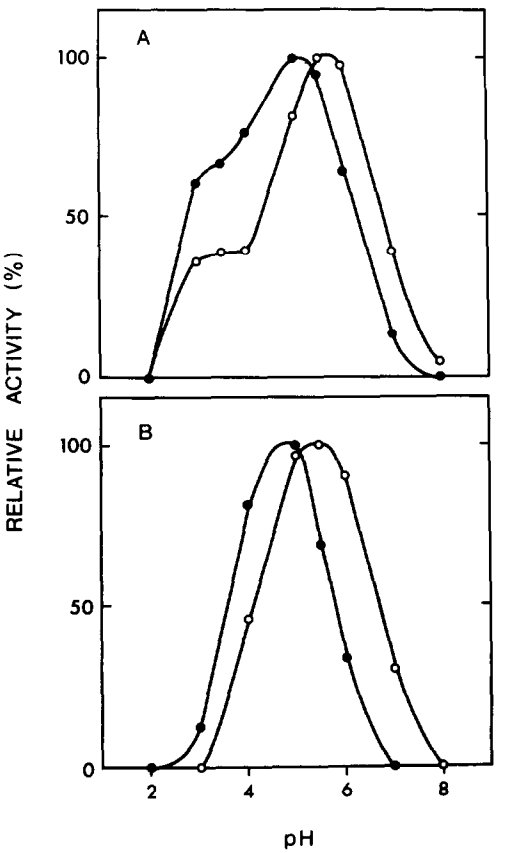

Fig 3 The pH profiles of α-galactosidases I and II with the substrates *p*-nitrophenyl-α-galactoside (A) and raffinose (B) ○—○, α-Galactosidase I, ●—●, α-galactosidase II

Effect on blood type B specificity

The studies with erythrocytes were conducted only with α-galactosidase II obtained by chromatography on the melibiose–agarose column because the yield and purity of α-galactosidase I were too low Specifically, the problem with α-galactosidase I was that concentrates of this enzyme added to blood cells caused them to agglutinate although this action may have been due to the high concentration of protein rather than a hemagglutinin [6] Sufficient α-galactosidase II for this study was accumulated by the purification procedure from three extracts of tomatoes Washed suspensions of human type B erythrocytes were incubated with α-galactosidase II at final enzyme concentrations of 50 and 100 units/ml of suspension A control sample was incubated without the enzyme There was a gradual loss of B activity in the samples containing the enzyme, the loss being complete after 6 0 and 3 5 hr for 50 and 100 units/ml of α-galactosidase II, respectively

Tomato α-galactosidase II can thus be added to the small number of α-galactosidases that hydrolyse the α-galactose moieties of blood group B substances The effectiveness of the tomato enzyme was similar to that of α-galactosidases from coffee beans [13] and soybeans [5] A possible advantage of tomatoes as a source of α-galactosidase is that they are readily available Furthermore, high yields of the enzyme can be obtained by a relatively simple procedure This enzyme, therefore, may be useful in converting group B erythrocytes to type O, a process that has drawn considerable interest recently [14] as a way of utilizing unused type B blood

EXPERIMENTAL

Enzyme extraction One kg fresh, ripe tomatoes (cv Better Boy) was homogenized with 1 l of cold H_2O Solid NaCl was added to the suspension to a final concn of 1 M, and the pH was adjusted to 5 5 with 1 M NaOH All subsequent steps were conducted at *ca* 3° The suspension was stirred for 2 hr and then centrifuged at 8000 *g* for 20 min The supernatant was made 80% saturated with $(NH_4)_2SO_4$ and centrifuged The pelleted proteins were collected, dissolved in 30 ml H_2O, and dialysed against 0 15 M NaCl for 16 hr This protein soln was clarified by centrifugation, and the supernatant represented the crude extract

Enzyme assays α-Galactosidase was assayed by measuring the rate at which it hydrolysed *p*-nitrophenyl-α-galactoside The reaction mixture consisted of 0 5 ml McIlvaine buffer (pH 5 5), 0 4 ml 0 1% bovine serum albumin, 0 1 ml appropriately diluted enzyme soln (*ca* 0 01 unit) and 0 4 ml 13 mM substrate soln After 15 min at 37°, the reaction was terminated by the addition of 2 ml 0 2 M Na_2CO_3, and the liberated *p*-nitrophenol was measured at 415 nm Appropriate controls with boiled enzyme were included One unit of α-galactosidase was defined as that amount that hydrolysed 1 μmol of *p*-nitrophenyl-α-galactoside per min [5]

α-Galactosidase activity with the substrates raffinose, stachyose, methyl-α-D-galactoside and galactomannan was assayed by measuring the rate at which reducing groups were formed The reaction mixture consisted of 0 5 ml McIlvaine buffer (pH 5), 0 1 ml diluted enzyme soln and 1 ml 2% substrate soln After 30 min at 37°, the solns were analysed for reducing groups by the method of ref [15]

The protein content of chromatographic fractions was estimated by *A* at 280 nm, whereas those of concentrated crude and

purified solns were determined by the method of ref [16]

Column chromatography The following columns were used for separating, purifying and characterizing the α-galactosidases a 5 × 70 cm column of DEAE-Sephadex A-50 equilibrated with 0 05 M NaOAc (pH 5 5) containing 0 1 M NaCl, a 1 × 5 cm column of melibiose–agarose equilibrated with 0 05 M NaOAc (pH 4) containing 0 1 M NaCl, and a 2 5 × 90 cm column of Sephadex G-100 equilibrated with 0 15 M NaCl Each column was eluted with the equilibration soln The enzymes in the fractions were concd with Amicon ultrafiltration units by using PM-10 membranes

Human erythrocytes Human type B erythrocytes were prepared from freshly drawn blood from healthy donors according to ref [10] After the plasma and buffy coat were removed, the cells were washed 4 times with NaPi-buffered saline, pH 7 4 The packed cells were then washed 3 times with 4 vols of 0 1 M citric acid/0 2 M Na_2HPO_4 buffer, pH 5, containing 3% glycerol Aliquots (0 5 ml) of cell suspensions were added to 0 5 ml buffered glycerol soln containing α-galactosidase II and incubated at 37° Ten μl of reaction mixture was withdrawn at 30 min intervals and tested for B activity with anti-B blood grouping antiserum (American Dade)

Acknowledgements—I thank Jimmy K Avants and Patsy D Cole for excellent technical assistance, and Dr Huda S Lillard and Stephen E Craven for samples of type B blood

REFERENCES

1 Agrawal, K M L and Bahl, O P (1968) *J Biol Chem* **243**, 103
2 Dey, P M and Pridham, J B (1969) *Biochem J* **113**, 49
3 Petek, F, Villarroya, E and Courtors, J E (1969) *Eur J Biochem* **8**, 395
4 Barham, D, Dey, P M, Griffiths, D and Pridham, J B (1971) *Phytochemistry* **10**, 1759
5 Harpaz, N, Flowers, H M and Sharon, N (1977) *Eur J Biochem* **11**, 426
6 Del Campillo, E and Shannon, L M (1982) *Plant Physiol* **69**, 628
7 Meir, H and Reid, J S G (1977) *Planta* **133**, 243
8 Amuti, K S and Pollard, C J (1977) *Phytochemistry* **16**, 529
9 Zarnitz, M L and Kobat, E A (1960) *J Am Chem Soc* **82**, 3953
10 Harpaz, N, Flowers, H M and Sharon, N (1975) *Arch Biochem Biophys* **170**, 676
11 Hakomori, S I, Siddeqiu, B, Li, Y-T, Li, S-C and Hellergvist, C G (1971) *J Biol Chem* **246**, 2271
12 Del Campillo, E, Shannon, L M and Hankins, C N (1981) *J Biol Chem* **256**, 7177
13 Harpaz, N, Flowers, H M and Sharon, N (1974) *Biochim Biophys Acta* **341**, 213
14 Goldstein, J, Sivigilia, G, Hurst, R, Lenny, L and Reich, L (1982) *Science* **215**, 168
15 Nelson, N (1944) *J Biol Chem* **153**, 375
16 Bradford, M (1976) *Analyt Biochem* **72**, 248