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BINDING OF α-GALACTOSIDASE I FROM *VICIA FABA* TO POTATO STARCH GRANULES AND SHEEP ERYTHROCYTES

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Key Word Index—*Vicia faba*, Leguminosae, broad bean, α-galactosidase, lectin, seed

Abstract—α-Galactosidase I from *Vicia faba* seeds binds to potato starch and sheep erythrocytes With the aid of fluorescence microscopy and using 4-methylumbelliferyl α-D-galactoside as the substrate it has been demonstrated that the binding is via the lectin sites of the enzyme leaving catalytic sites free and detectable The lectin site is specific for D-glucose/D-mannose residues

INTRODUCTION

Three forms of α-galactosidases, I, II[1] and II[2] have been isolated from *Vicia faba* seeds and purified [1] All forms hydrolyse α-D-galactosides but they also behave as lectins with D-glucose/D-mannose specificities [2] There is evidence in the case of α-galactosidase I that these two activities reside in the same protein molecule but on separate loci [2] The 'classical' lectin, favin [3] which, again, is D-glucose/D-mannose specific, also occurs in *V faba* seeds, however, there are no indications that favin is a contaminant of purified α-galactosidase I The latter enzyme appears to be a unique example of a protein possessing separate catalytic and lectin sites In the case of other, so-called, enzyme-lectins, for example, α-galactosidase from *Vigna radiata* [4], both activities appear to reside on the same or same type of site [5]

In this communication we report the binding, via lectin sites, of α-galactosidase I to starch granules and sheep erythrocytes The mode of binding of this enzyme and perhaps others, to cell constituents is of considerable importance as it introduces a new concept of *in vivo* localisation of enzymes It has been postulated, for example, that α-galactosidases are compartmentalised in maturing seeds in order to separate enzyme from substrate (galactosyl-sucrose derivatives) [2, 6, 7] and also in the case of mature stachyose-exporting leaves of *Cucurbita* sp [8] In both cases it is conceivable that lectin binding of the enzyme to cellular constituents is involved [9]

RESULTS AND DISCUSSION

The haemagglutinin (lectin) activity of α-galactosidase I is clearly illustrated in Fig 1 which shows the agglutination of sheep erythrocytes by the enzyme The bound enzyme with free and functional catalytic sites can be observed by fluorescent light microscopy after the addition of 4-methylumbelliferyl α-D-galactoside to the agglutinated cells (Fig 2) This substrate is not itself fluorescent, but after enzymic hydrolysis the 4-methylumbelliferone released is strongly fluorescent in blue light

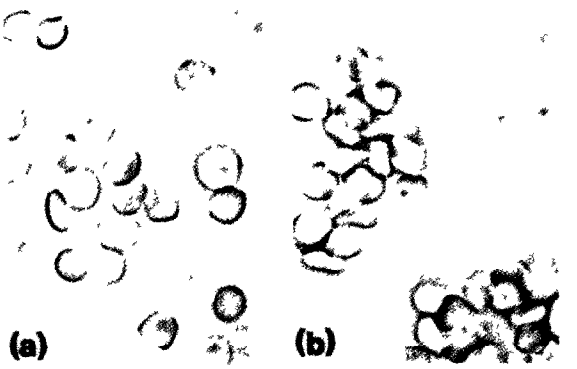

Fig 1 Agglutination of sheep erythrocytes, (a) sheep erythrocytes in PBS (× 500), (b) sheep erythrocytes agglutinated by incubating with α-galactosidase I from *V faba* (× 500)

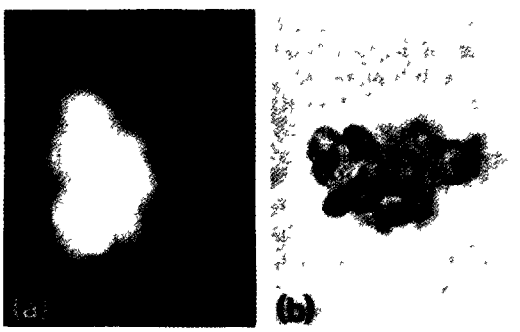

Fig 2 Display of fluorescence by α-galactosidase I agglutinated erythrocytes after 2 min (a) and 15 min (b) after addition of 4-methylumbelliferyl-α-D-galactoside solution

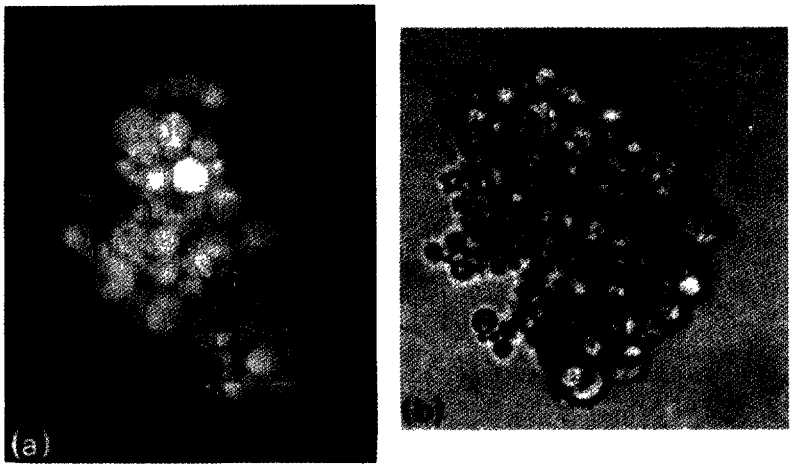

Fig 3 Display of fluorescence by potato starch granules after incubating (2 hr) with α-galactosidase I followed by the addition of 4-methylumbelliferyl-α-D-galactoside solution and leaving for (a) 2 min and (b) 15 min

Figure 2a shows that after a short incubation with substrate there is an intense fluorescence surrounding the agglutinated cells which is about 600-fold (estimated from the film exposure time) greater than the natural fluorescence displayed by untreated cells in a control experiment The dark background in Figure 2a indicates that no significant diffusion of newly liberated 4-methylumbelliferone from the surface of the agglutinated cells into the surrounding medium has taken place This should be contrasted with the light background, resulting after 15 min incubation, in Fig 2b in which 4-methylumbelliferone has diffused into the surroundings

When α-galactosidase I was incubated with potato starch granules the bound enzyme could again be demonstrated by fluorescence microscopy Unlike the erythrocytes, starch granules do not display a natural fluorescence Figure 3a represents an early stage during the incubation with 4-methylumbelliferyl α-D-galactoside and α-galactosidase I, with a dark background surrounding the fluorescing starch particles Furthermore, it was shown that the fluorescence of the granules was not produced by incubating them with 4-methylumbelliferone Again, at a later stage (Fig 3b) the fluorescence has spread into the background as a result of product diffusion It is assumed that the binding of α-galactosidase I to the granules occurred via the interaction between the lectin sites of the enzyme and the starch glucosyl residues while the substrate binding/catalytic sites remained free The production of granule fluorescence was inhibited by 0 1 M D-mannose in the incubation mixture In addition, studies with various hapten inhibitors on the distribution of α-galactosidase activity between starch granules and the suspension medium (Table 1) support this assumption D-Glucose and D-mannose and oligosaccharides with terminal non-reducing α-D-glucopyranosyl residues all inhibited the binding whereas melibiose and raffinose, with terminal α-D-galactopyranosyl residues, did not Furthermore, treatment of the starch granule–α-galactosidase complex with α-amylase released 80% of the bound enzyme into the supernatant Extensive lectin binding to glycan components of amyloplast membrane fragments on the granules was unlikely as washing the granules with acetone had no apparent effect on the degree of binding

Table 1 Effect of carbohydrates on the binding of α-galactosidase I to potato starch granules*

Carbohydrate (final conc 100 mM)	α-Galactosidase activity (%)	
	Starch granules	Supernatant
None	51 4	48 6
D-Glucose	19 7	80 3
D-Mannose	18 6	81 4
Maltose	35 4	64 6
Sucrose	37 7	62 3
Melibiose	54 5	45 6
Raffinose	56 6	43 6

*See Experimental section for details

These results indicate that α-galactosidase binds to the glycosyl residues of sheep erythrocytes/starch granules via its D-glucose/D-mannose specific lectin sites [2] leaving the catalytic site free and detectable This work also suggests the possibility of using enzyme-lectins as cytochemical reagents which can be very readily detected after binding to cell receptors

EXPERIMENTAL

Enzyme preparation The procedure used for the purification of α-galactosidase I from resting seeds of *Vicia faba* was the same as that described earlier [1] The enzyme activity was assayed using *p*-nitrophenyl-α-D-galactoside (PNPG) as the substrate [10, 11]

Qualitative observations using incident fluorescence light microscopy (a) Using potato starch (Me_2CO-washed)—four tubes were prepared containing 500 μl starch suspensions (10 mg/ml) in NaPi buffered saline, (PBS, 7 2 g NaCl, 1 42 g Na_2HPO_4, 0 43 g KH_2PO_4) pH 7 2, tube (iii) contained, in addition, 0 1 M mannose α-Galactosidase I (10 μl, 80 nkat/ml) was added to tubes (i), (ii) and (iii) and incubated for 2 hr No enzyme was added to tube

(iv) The suspensions were then mounted on microscope slides for observing fluorescence using a Leitz Epifluorescent system [Ploenopak incident fluorescent illumination with H_2 filter block, which provides violet and blue light (390–490 nm) for excitation] 4-Methylumbelliferyl α-D-galactoside soln (1 mg/ml, 10 μl) in PBS was added to the slides prepared from tubes (i), (iii) and (iv) and observed under the microscope immediately and at definite intervals Samples of tube (ii) and (iv) were taken as controls

(b) Using sheep erythrocytes—two tubes, (i) and (ii), were prepared with 500 μl of 2% (v/v) red blood cell suspension in PBS, pH 7 2 α-Galactosidase I (10 μl, 80 nkat/ml) was added to tube (i) and the sample was incubated at room temp for 2 hr Slides were prepared from both tubes for observation under the fluorescence microscope

Quantitative measurement of α-galactosidase I binding to starch Potato starch suspensions in PBS, pH 7 2 (500 μl, 1% w/v) were placed in microcentrifuge tubes containing 0 1 M D-mannose, D-glucose, melibiose, maltose, sucrose or raffinose one control reaction mixture contained only starch and PBS α-Galactosidase I (10 μl, 80 nkat/ml) was added to each tube and the suspensions incubated for 2 hr at 20° The tubes were shaken at intervals and finally centrifuged, each supernatant (50 μl) was assayed for α-galactosidase activity The concentration of the haptens in the assay was not inhibitory to the enzyme

In a separate experiment, α-galactosidase I–starch incubation mixture in PBS was treated with *Aspergillus niger* α-amylase (final concn, 25 units/ml) for 1 hr at 37° followed by centrifugation and assay for α-galactosidase activity in the supernatant

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