# SELECTIVE INHIBITION OF α-GALACTOSIDASES BY MYOINOSITOL

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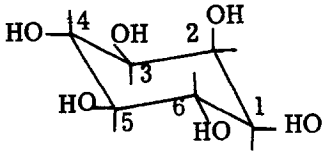
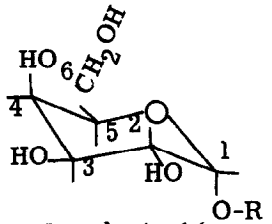
Summary: Evidence is presented that myoinositol is a potential, highly stereospecific and competitive inhibitor of α-galactosidases.

## INTRODUCTION

Glycosidases are inhibited by a variety of substances. Aldonolactones, for example, inhibit their corresponding α-, and β-glycosidases (1, 2, 3, 4). Polyols inhibit glucosidases and galactosidases from the germinating barleys and almond emulsion (5). Recently, Lee (6) has reported that D-galactal is a highly stereospecific inhibitor of β-galactosidases. However, the effect of D-galactal on α-galactosidase was not determined by him. Presumably, no inhibitor is known that would inhibit selectively α-galactosidase without affecting the activity of β-galactosidase. Even the aldonolactones which are considered stereospecific inhibitors of glycosidases inhibit both α-, and β-glycosidases. This communication presents the evidence indicating that myoinositol (I) is a potent inhibitor of α-D-galactosidases (α-D-galactoside galactohydrolase, E.C. 3.2.1.22) with no apparent effect on the activity of β-D-galactosidases (E.C. 3.2.1.23) or on other glycosidases studied.




Myoinositol (I)




α-D-galactoside (II)
(R = p-Nitrophenyl-)

## MATERIALS AND METHODS

Chicken peas, peanuts, cottonseeds, and pinto beans were obtained locally. p-Nitrophenyl-glycosides were obtained from Pierce Company (Rockford, Ill.). p-Nitrophenyl-N-Acetyl-β-D-glucosaminide and myoinositol were obtained from Sigma Chemical Company (St. Louis, Mo.).

Preparation of Glycosidases: All operations during extraction and purification were carried out at 4°C unless otherwise specified. Glycosidases used in this study were obtained from the germinating chicken peas, pinto beans, peanuts, and glandless cottonseeds by a procedure as described by Bahl and Agrawal (7). The partially purified enzyme preparation obtained above contained the following enzymes:

α-galactosidase (α-D-galactoside galactohydrolase, E.C. 3.2.1.22),
β-galactosidase (β-D-galactoside galactohydrolase, E.C. 3.2.1.23),
α-mannosidase (α-D-mannoside mannohydrolase, E.C. 3.2.1.24),
α-glucosidase (α-D-glucoside glucohydrolase, E.C. 3.2.1.20),
β-glucosidase (β-D-glucoside glucohydrolase, E.C. 3.2.1.21), and
β-N-acetyl-glucosaminidase (β-2-acetamido-2-deoxy-D-glucoside acetamidoglucohydrolase, E.C. 3.2.1.30).

This enzyme preparation was used throughout the study for enzyme assay with or without the presence of inhibitor.

Enzyme Assay: The method of enzyme assay with and without myoinositol was the same as described by Bahl and Agrawal (7). Each enzyme was assayed at optimum pH in 0.05M sodium citrate buffer containing 250 μmoles of appropriate p-nitrophenyl-D-glycopyranoside as substrate, 0.2 ml of enzyme solution (0.5-1 mg of protein). The reaction mixture was incubated for 20 min. at 30°C. The enzyme action was stopped by the addition of 1.5 ml of 2% $Na_2CO_3$ solution to a total volume of 2.5 ml of the enzyme reaction mixture and the resulting yellow color was measured at 420 mμ by a Beckman Model DU spectrophotometer.

The unit of enzyme was defined as the amount which would liberate 1 μ mole of p-nitrophenol per min. at 30°C. The specific activity

of the enzyme was expressed in units per mg of protein. Protein was estimated by the method of Lowry et al. (8) with bovine serum albumin as standard.

## RESULTS

The effect of myoinositol on the kinetics of the chicken peas α-galactosidase activity was investigated in presence of varying concentrations of p-nitrophenyl- α-D-galactoside (0.1 mM to 0.60 mM) at optimum pH. Lineweaver-Burk plots show straight line relationships and indicate that myoinositol acts as a competitive inhibitor (Fig. 1). The $K_i$ and $K_m$ values as determined by Dixon plots (9) and Lineweaver-Burk plots respectively were found to be $0.24 \times 10^{-2}$ M and $0.33 \times 10^{-3}$ M

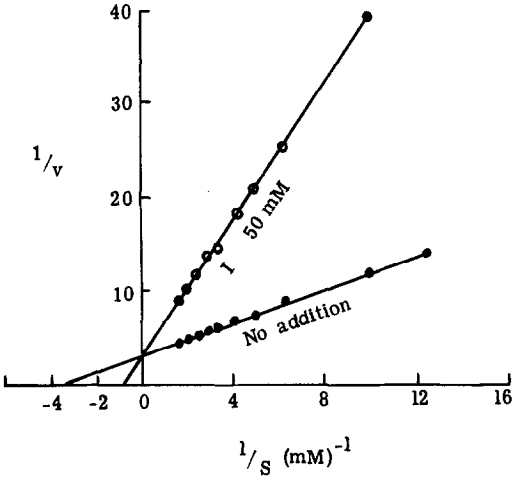



Fig. 1. Effect of myoinositol on the hydrolysis of p-nitrophenyl-α-D-galactoside catalyzed by α-galactosidase in the presence of varying concentrations of substrate. The reaction mixture contained the following in a volume of 2.5 ml: 125 μ moles of sodium citrate buffer (pH 5.5), 0.2–1.5 μmole of p-nitrophenyl-α-D-galactoside, 125 μmoles of myoinositol, and 0.56 mg of the enzyme preparation from chicken peas. The incubation was carried out at 30°C for 20 min. p-Nitrophenol liberated was measured according to standard assay procedure described in "Methods and Materials".

respectively. The results of inhibition of α-galactosidase activity by varying concentrations of myoinositol (0.0-250 mM) at 0.1 mM and 0.2 mM substrate concentrations are shown in Fig. 2-a. The curves are obtained

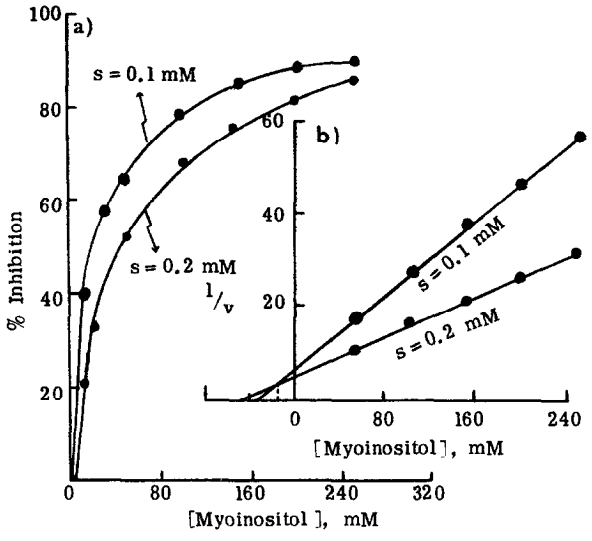



Fig. 2a). The percentage inhibition of chicken peas α-galactosidase by varying concentrations of myoinositol (0 - 250 mM) at 0.1 mM and 0.2 mM substrate concentrations. 2b). Dixon Plots of 1/v versus myoinositol at 0.1 mM and 0.2 mM substrate concentrations. Conditions of incubation and assay were similar to those in Fig. 1.

by plotting the percent inhibition against the inhibitor concentration. The plots are typical hyperbolic curves and not sigmoid-shaped ones observed in case of allosteric inhibition (10). Dixon plots of $\frac{1}{V}$ versus [myoinositol] are shown in Fig. 2-b. The curves show a linear relationship and provide a further confirmation for the competitive type of inhibition of chicken peas α-galactosidase by myoinositol.

The effect of myoinositol on other glycosidases of chicken peas was also studied. The experiments were performed on unpurified enzyme preparations to be certain that all the active glycosidases were included. The specific activities of glycosidases present in the enzyme preparation and the effect of myoinositol on their activities are given in Table 1. These results clearly show that myoinositol inhibits selectively and powerfully the α-galactosidase and under identical conditions exhibit almost no effect on the activity of other glycosidases tested including β-galactosidase. These results indicated that the chicken peas α--galactosidase is different from the rest of glycosidases reported here.

An examination of the effects of myoinositol on glycosidases

Table 1. The Effects of Myoinositol on the Hydrolysis of p-Nitrophenyl Glycosides by Enzyme Extract of Cicer Arietinum[1]

| Enzyme | Specific Activity (Units/mg) | | |
|---|---|---|---|
| | Without Myoinositol | With Myoinositol | Inhibition % |
| α-galactosidase (pH 5.5) | 8.33 | 1.57 | 81.2 |
| β-galactosidase (pH 4.6) | 2.76 | 2.76 | 0.0 |
| α-glucosidase (pH 3.2) | 0.11 | 0.11 | 0.0 |
| β-glucosidase (pH 5.5) | 1.00 | 1.00 | 0.0 |
| α-mannosidase (pH 5.1) | 0.675 | 0.675 | 0.0 |
| β-N-Acetyl-glucosaminidase (pH 5.2) | 5.26 | 5.26 | 0.0 |

[1]The pH values in the parentheses are the optinum pH levels at which the various activities were measured. One unit is defined as the amount of enzyme which releases 1 μmole of p-nitrophenol per min. The assay conditions and reaction mixture were those as described in the legend for Figure 1.

Table 2. The Effect of Myoinositol of Glycosidases

| Enzyme | Enzyme Source and % Inhibition | | | |
|---|---|---|---|---|
| | C. Arietinum | P. Vulgaris | A. hypogaea | G. hirsutum |
| α-galactosidase | 74.3 | 69.0 | 60.0 | 75.6 |
| β-galactosidase | 0.0 | 1.0 | -1.0 | 0.5 |
| α-glucosidase | 0.0 | 1.1 | 0.0 | 0.0 |
| β-glucosidase | 0.6 | 0.0 | 2.0 | 2.0 |
| α-mannosidase | 0.0 | -2.0 | 0.0 | 1.5 |
| β-N-acetyl-glucosaminidase | 0.0 | 0.0 | 0.0 | 0.8 |

The assay conditions and the reaction mixture were those as described in the legend for Figure 1.

from other plant sources (peanuts, cottonseed, and pinto beans) revealed a similar type of effect as was observed in case of chicken peas glycosidases (Table 2). Thus the highly stereospecific inhibition of α-galactosidases by myoinositol seems to be common among plant α-galactosidases.

## DISCUSSION

It is characteristic of glycosidases thus far investigated that they are highly specific for the glycosyl residue and the anomeric configuration of glycosidic linkage regardless of the nature of aglycon (11, 12). According to Koshland (13) a glycosidase has two binding sites, a specificity site and a catalytic site. The glycosyl residue and the glycosidic linkage of the substrate bind at the specificity and catalytic sites of the enzyme respectively to make an active enzyme-substrate complex. For such a complex to be formed, specificity site and the catalytic site in glycosidase should be present in close proximity. Thus, as suggested by Kelemen and Whelan (5), selective inhibition by competitive mechanism requires that the inhibitor should be able to compete strongly with substrate both for specificity and catalytic sites in order to prevent the enzyme-substrate interaction. The inhibition of α-galactosidase by myoinositol seems to provide an excellent example for the above model because myoinositol is a powerful competitive inhibitor and has no effect on the β-galactosidase activity or any other glycosidases reported here. The very fact that β-galactosidase is not inhibited by myoinositol suggests that myoinositol does not interact at the catalytic site in β-galactosidase. In this respect, the pattern of inhibition of α-galactosidase by myoinositol is strikingly different from those reported for other potential inhibitors such as D-galactono-1,4-lactone (1), D-threitol, ribitol, xylitol, and D-arabitol (5). In all these cases both α- and β-galactosidases were inhibited. Furthermore, besides α-and β-galactosidases, the α- and β-glucosidases were also inhibited both by D-galactono-1,4-lactone (1) and polyols (5).

Complex effects of inhibitors have been reported (5, 14, 15) because of differences in species, differences in organs and multiforms of the same enzyme. Although, under identical conditions, the percent inhibition of α-galactosidases from various sources by myoinositol varies from 60 to 75%, (Table 2), it is important to note, however, that in each case α-galactosidase activity is powerfully inhibited with virtually no effect on β-galactosidase.

The selective inhibition of α-galactosidase by myoinositol (I) suggests that the orientation of OH-groups about carbon atoms C-1 and C-4 in D-galactopyranoside ring (II) and the conformation together with the orientation of OH-groups in myoinositol are critical. Orientation of OH-groups about carbon atoms C-2, C-3, and C-4 in myoinositol (I) is identical to that of carbon atoms C-4, C-3, and C-2, or C-1, C-2, and C-3 in α-D-galactopyranosyl residue (II) of the substrate respectively. If it is assumed that configuration about C-4 and C-1 in galactosyl residue is involved in binding of substrate at specificity and catalytic sites respectively, then the structural similarity between myoinositol and α-galactosyl residue supports, but by no means proves, that myoinositol prevents the enzyme-substrate interaction by competing with the substrate both for specific and catalytic sites. Thus, configurational similarity between the myoinositol and α-D-galactopyranoside seems to be responsible for the selective inhibition of α-galactosidase. This is in agreement with the findings of Kelemen and Whelan (5), and Heyworth and Walker (4).

Data presented in this report suggest a possible role of myoinositol in the regulation of galactose metabolism. In view of the present findings, it seems possible that other isomers of myoinositol may act as selective inhibitors of other glycosidases.

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