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MECHANISM OF ACTION OF α-GALACTOSIDASE

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Key Word Index—*Cocos nucifera*; Palmae; coconut; α-galactosidase; mechanism.

Abstract—The effect of pH on K_m and V_{max} values of coconut α-galactosidase indicates the involvement of two ionizing groups with pK_a values of 3.5 and 6.5 in catalysis. Chemical modification has indicated the presence of two carboxyl groups, a tryptophan and a tyrosine, at or near the active site of α-galactosidase. Based on these facts a new mechanism of action for α-galactosidase is proposed in which the ionizing group with a pK_a of 3.5 is a carboxyl group involved in stabilizing a carbonium ion intermediate and the ionizing group with a pK_a of 6.5 is a carboxyl group perturbed due to the presence of a hydrophobic residues in its vicinity which donates a H^+ ion in catalysis.

INTRODUCTION

Carbohydrates containing α-D-galactosyl linkages are widely distributed in nature [1]. Deficiency of α-galactosidase (EC 3.2.1.22) lead to Fabry's disease in humans [2] and may prevent germination in coconut [3]. α-Galactosidase is used in industry in the production of beet sugar [4] and soybean milk [5].

Kinetic studies on the mechanism of action of α-galactosidase from sweet almond [6] and *Vicia faba* [7] indicate that a carboxyl group and an imidazole group are involved in its catalysis and a mechanism of action has been proposed based on these results [6]. Our studies on the chemical modification of α-galactosidase indicate the presence of two carboxyl groups, tyrosine and a tryptophan and the absence of an imidazole group, at or near the active site of α-galactosidase [8]. A new mechanism of action is proposed based on the chemical modification experiments and kinetic studies.

RESULTS AND DISCUSSION

The effects of pH on K_m and V_{max} were determined using Lineweaver–Burk plots. The treatment of results according to Dixon [9] indicates that the dissociable groups in the active site have pK_a values of 3.8 and 6.5 (Fig. 1). The protonated form of the group with a pK_a of 6.5 is required for catalysis as the affinity decreases with increase in pH. On the same basis the ionized form of the group with a pK_a of 3.8 is required as the affinity increases with increase in pH (Fig. 1). The same ionizing groups (pK_as of 3.8 and 6.3) are observed in a log (V_{max}/K_m) graph indicating their presence in the free enzyme. The effect of pH on V_{max} is constant over the pH range 2.5–6.5 (Fig. 1). This graph indicates only one ionizing group with a pK_a of 6.9. From these results we can conclude there are two ionizable groups with pK_as of 3.8 and 6.5 involved in binding and one group in the catalysis. Similar results have been obtained with α-galactosidase from sweet almond [6] and *Vicia faba* [7]. The group with a pK_a of 3.8 has to be a carboxyl group (pK_a 3.0–4.7) [10] required as a $-COO^-$ group for catalysis. The group with a pK_a of 6.5 can be an imidazolium group of histidine (pK_a 5.6–7.0) [10] or a perturbed carboxyl group as in lysozyme [11]. But it cannot be an imidazole group, as chemical modification shows that an imidazole group is not present at or near the active site [8].

No studies have been done on the bond fission of α-galactosidases. But considering other glycosidases [12–14] it is likely that galactose oxygen bonds are cleaved. NMR and polarimetry studies show that the liberated galactosyl residues possess the same anomeric configuration as the substrate [6]. Retention of configuration will occur if there are two successive inversions at the C-1 carbon or if there is a carbonium ion intermediate that can be attacked by a nucleophile on one side only. Retention of configuration is therefore consistant with a multistep reaction involving an intermediate, as in lysozyme [15].

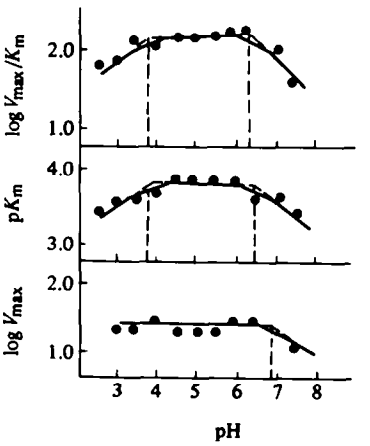

Fig. 1. K_m and V_{max} as a function of pH for the hydrolysis of *p*-nitrophenyl-α-D-galactopyranoside by α-galactosidase.

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In the proposed mechanism of action of α-galactosidase (Fig. 2) the group with a pK_a of 3.8 is a carboxyl group present in the ionized form to stabilize a carbonium ion intermediate and the presence of this ionized group protects the carbonium ion from attack by a nucleophile from one direction so that the product retains the same configuration as the substrate. The galactose molecule forms the half chair conformation when the carbonium ion is formed. The group with a pK_a of 6.5 is a perturbed carboxylic group that is present in the protonated form and is involved in donation of a proton. This carboxyl group is perturbed due to the hydrophobic environment produced by the presence of tryptophan and tyrosine residues in its vicinity [8].

EXPERIMENTAL

Analytical grade Sigma chemicals were used. Sepharose 4B was purchased from Pharmacia.

Purification of α-galactosidase. α-Galactosidase from coconut kernel was purified by hydrophobic chromatography using Sepharose 4B-capranilide [16].

Enzyme assay. α-Galactosidase was assayed using *p*-nitrophenyl-α-D-galactopyranoside [17].

Effect of pH on K_m and V_{max}. McIlvaine buffers [18] of the pH range 2.5–7.5 were used to determine the K_m and V_{max} values for the hydrolysis of *p*-nitrophenyl-α-D-galactopyranoside. The substrate concn was varied from 1×10^{-4} to 5×10^{-4} M and the enzyme activity determined. Lineweaver–Burk plots were constructed for each pH value and K_m and V_{max} values were calculated.

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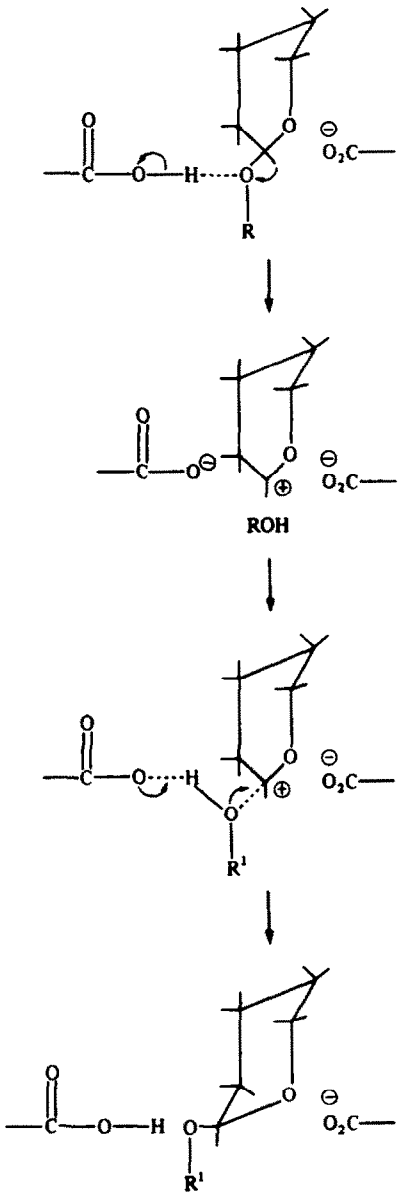

Fig. 2. Mechanism of action of α-galactosidase.

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