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Review

α-L-Rhamnosidase: A review

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ABSTRACT

α-L-Rhamnosidase [E. C. 3.2.1.40] cleaves terminal α-L-rhamnose specifically from a large number of natural products. The enzyme has wide occurrence in nature and is reported from animal tissues, plants, yeasts, fungi and bacteria. It is a biotechnologically important enzyme due to its applications in debittering and clearance of citrus fruit juices, enhancement of wine aromas and derhamnosylation of many natural products containing terminal α-L-rhamnose to compounds of pharmaceutical interests. Though α-L-rhamnosidases have been investigated actively during recent years, there is no recent review on α-L-rhamnosidases. An attempt has been made to fill up this gap in this review. It consists of a brief introduction of α-L-rhamnosidase which is followed by a critical description of the methods used for assaying the enzyme activity. Purifications, characterizations and properties of α-L-rhamnosidases from different sources have been discussed and the available structural and molecular biological studies on the enzyme have been given. Biotechnological applications of this enzyme in different processes have been briefly described. The review concludes with the identification of areas which needs further extensive studies.

Contents

1. Introduction

α-L-Rhamnosidase [E. C. 3.2.1.40] cleaves terminal α-L-rhamnose specifically from a large number of natural products which include naringin, rutin, quercitrin, hesperidin, diosgene, terpenyl glycosides and many other natural glycosides containing terminal α-L-rhamnose [1–6] (Reaction Scheme 1). The enzyme has wide occurrence in nature and has been reported from animal tissues, plants, yeasts, fungi and bacteria.

This enzyme has turned out to be a biotechnologically important enzyme due to its applications in a variety of processes like debittering of citrus fruit juices [7–13], manufacture of prunin from naringin [14], manufacture of L-rhamnose by hydrolysis of natural glycosides containing terminal L-rhamnose [15], enhancement of wine aromas by enzymatic hydrolysis of terpenyl glycosides containing L-rhamnose [5,16], elimination of

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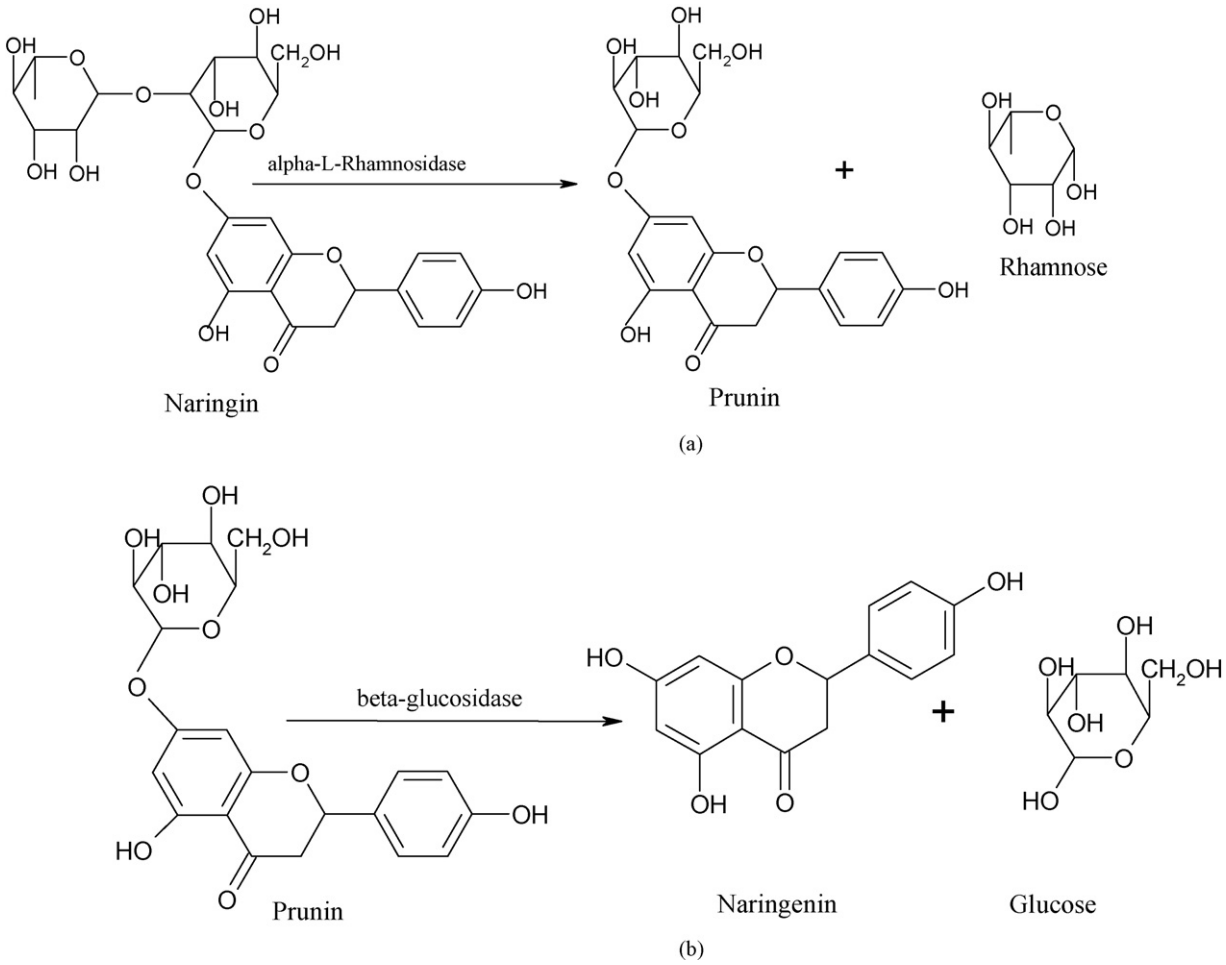

Reaction Scheme 1. (a) Hydrolysis of naringin to prunin by α-L-rhamnosidase. (b) Hydrolysis of prunin to naringenin by β-D-glucosidase.

hesperidin crystals from orange juices [3,17], conversion of chloropolysporin B to chloropolysporin C [18], the derhamnosylation of many L-rhamnose containing steroids *for example*, diosgene, desglucoruscin, ginsenosides-Rg2, etc. whose derhamnosylated products have their clinical importance [4,19–21]. In spite of α-L-rhamnosidases being biotechnologically important enzymes, review article on α-L-rhamnosidases is not available in the literature. The α-L-rhamnosidase activities are associated with debittering enzymes which are commercially known as hesperidinases [20] and naringinases [22]. There are two reviews on naringinase: the one by Chandler and Nicol [23] which covers literature up to 1975 and the other by Puri and Banerjee [24] which covers literature up to 2000. In this article, an attempt has been made to review the recent literature on α-L-rhamnosidases.

2. Assay methods

A convenient method for assaying the activity of α-L-rhamnosidase has been a problem before the investigators working on this enzyme since the very beginning. This problem has been solved partly by Romero et al. [22] using synthetic substrate p-nitrophenyl-α-L-rhamnopyranoside and monitoring the liberation of p-nitrophenolate ion spectrophotometrically at 400 nm using molar extinction coefficient value of 21.44 mM^{-1} cm^{-1} (Reaction Scheme 2).

Though the synthetic substrate, p-nitrophenyl-α-L-rhamnopyranoside, is commercially available but it is expensive. The method for synthesizing p-nitrophenyl-α-L-rhamnopyranoside triacetate is available in the literature [25], but it requires expert synthetic organic chemists to control the experimental conditions for its preparation and it also is not convenient for biochemists, microbiologists and biotechnologists working on α-L-rhamnosidase.

Naringin is the most commonly used natural substrate for assaying the activity of α-L-rhamnosidase using the Davis method [26]. α-L-Rhamnosidase cleaves terminal α-L-rhamnose of naringin and converts it to prunin as shown in Reaction Scheme 1(a). If β-D-glucosidase activity is present along with α-L-rhamnosidase activity, prunin is converted into naringenin and glucose as shown in Reaction Scheme 1(b). Naringenin so produced reacts with the Davis reagent [26] to form much less color than naringin. Samples of the reaction mixture taken at intervals show a continuous decrease in color on reacting with the reagent. This makes it possible to follow the course of naringin hydrolysis. The concentration of naringin at different intervals of time could be determined by drawing calibration curve with the known concentrations of naringin. The difficulty with this method is that it measures the disappearance of either naringin or prunin or both and thus it is not specific for the assay of α-L-rhamnosidase activity. However, this is the only method which could be performed conveniently.

The α-L-rhamnosidase activity could also be determined by monitoring the formation of prunin from naringin as shown in Reaction Scheme 1(a) using HPLC as done by Romero et al. [22] but the method needs modification in the light of the method reported by Yusof et al. [27] in the context of determination of naringin contents in citrus fruits.