Chemical Modification of Saccharomycopsis fibuligera *R64 α-Amylase to Improve its Stability Against Thermal, Chelator, and Proteolytic Inactivation*

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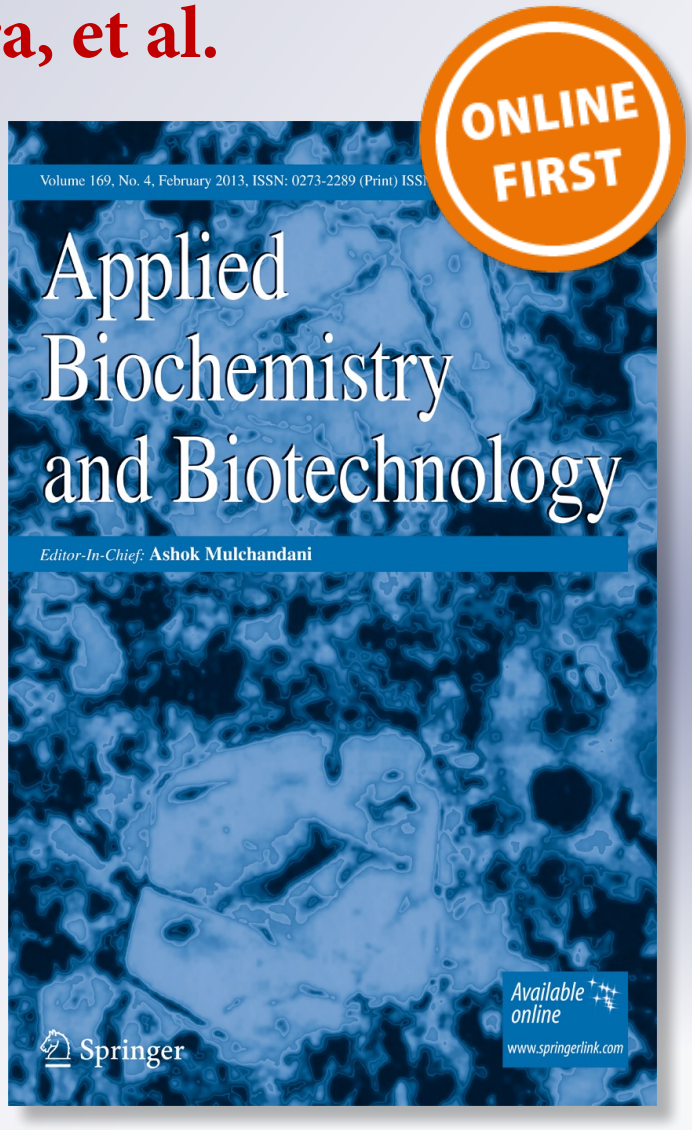

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Chemical Modification of *Saccharomycopsis fibuligera* R64 α-Amylase to Improve its Stability Against Thermal, Chelator, and Proteolytic Inactivation

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Abstract α-Amylase catalyzes hydrolysis of starch to oligosaccharides, which are further degraded to simple sugars. The enzyme has been widely used in food and textile industries and recently, in generation of renewable energy. An α-amylase from yeast *Saccharomycopsis fibuligera* R64 (Sfamy) is active at 50 °C and capable of degrading raw starch, making it attractive for the aforementioned applications. To improve its characteristics as well as to provide information for structural study ab initio, the enzyme was chemically modified by acid anhydrides (nonpolar groups), glyoxylic acid (GA) (polar group), dimethyl adipimidate (DMA) (cross-linking), and polyethylene glycol (PEG) (hydrophilization). Introduction of nonpolar groups increased enzyme stability up to 18 times, while modification by a cross-linking agent resulted in protection of the calcium ion, which is essential for enzyme activity and

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integrity. The hydrophilization with PEG resulted in protection against tryptic digestion. The chemical modification of Sfamy by various modifiers has thereby resulted in improvement of its characteristics and provided systematic information beneficial for structural study of the enzyme. An in silico structural study of the enzyme improved the interpretation of the results.

Keywords α-Amylase · *Saccharomycopsis fibuligera* · Tryptic digestion · Chemical modification · Enzyme engineering · Structure–function relationship

Abbreviations

Aotamy	*Aspergillus oryzae* taka-amylase
CC	Cyanuric chloride
DEAE	Diethylaminoethyl
DMA	Dimethyl adipimidate
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetate
HIC	Hydrophobic interaction chromatography
PAGE	Polyacrylamide gel electrophoresis
PEG	Polyethylene glycol
PEGylation	Modification with polyethylene glycol
SDS	Sodium dodecyl sulfate
Sfamy	*Saccharomycopsis fibuligera* α-amylase
TIM	Triosephosphate isomerase
TNBS	Trinitrobenzene sulfonate
TPCK	L-1-Tosylamido-2-phenylethyl chloromethyl ketone

Introduction

α-Amylase (1,4-α-D-glucan glucanohydrolase, EC 3.2.1.1) hydrolyzes 1,4-α-glycosidic bonds in starch predominantly in a random manner, resulting in oligosaccharides with reducing glycosidic groups in α-configuration. The oligosaccharides are further hydrolyzed by glucoamylase to simple sugars [1]. Therefore, α-amylases play a key role in industries that involve starch conversion, for example, in the fructose and glucose syrup production, brewing, baking, and paper industry [2]. The enzyme is also used in the desizing step in textile production and as an additive in detergents [3]. Recently, the enzyme has been assessed to be used for development of renewable energy sources [4] and prevention of coronary heart diseases and of certain cancers [5].

In syrup production, for example, gelatinization and liquefaction require high temperature (80–110 °C); thus, a thermally stable α-amylase is required [6]. The use of α-amylase in ethanol production for a renewable energy source requires capability of degrading raw starch [4]. Therefore, α-amylase with high thermal stability and/or capability to degrade raw starch is highly desired.

α-Amylase from *Saccharomycopsis fibuligera* R64 (Sfamy) has an optimum working temperature of 50 °C and is active at a broad pH range with an optimum at pH5.0 [7]. The enzyme retains its activity up to 3 months of storage at room temperature (unpublished result). Like many α-amylases, the enzymatic activity is affected by chelating agents, such as ethylenediaminetetraacetate (EDTA). Chelating agents extract calcium ion, which is essential for activity and integrity [1]. Interestingly, the calcium ion of Sfamy can be exchanged with magnesium or zinc. Moreover, a structure–function study revealed that