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Does high pressure have any effect on the structure of alpha amylase and its ability to binding to the oligosaccharides having 3–7 residues? Molecular dynamics study

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ABSTRACT

Studies have shown that deletion of amino acids from the C-terminus of amylase do not alter its amylolytic activity. Although high pressure is used to modify the structure and function of this enzyme, the effects of high pressures on the structures of the wild-type and truncated amylases have not yet been understood at the molecular level. Using molecular dynamic simulations and docking, we studied the structures of wild-type and truncated Taka-amylases at high pressures (1000–4000 bar). To construct the truncated Taka-amylase, 50 and 100 C-terminal residues were removed in two separate steps. Results of simulation showed that, although the overall shape partly agglomerates with rise in pressure, high pressure fails to modify the structure of the barrel-like region of the β-sheet in the wild-type and truncated enzymes. A comparison of contact graphs revealed that the changes at the N-terminus were less extensive than those at the C-terminus. Further analysis showed that 10 regions of the secondary structures changed due to pressure change in wild-type amylase, of which 6 regions were associated with the loops and 4 with helix, while the structure of β-sheets remained unchanged. The docking of maltotriose, maltotetraose, maltopentaose, maltohexaose, and maltoheptaose with the averaged structures obtained from different simulations was conducted to characterize the influence of pressure on the activities of the wild-type and truncated enzymes. The results showed that maltoheptaose made hydrophobic contacts with residues Tyr238-Asp117-Tyr82-Leu166-Leu232-Tyr155 and hydrogen contacts with residues Asp233-Gly234-Asp206-Arg204-His296-Glu230. Similar results were obtained for other malto-oligosaccharides.

1. Introduction

Amylases are very important biocatalysts used for various industrial applications [1]. The Taka-amylase (1, 4-α-D-glucanglucanohydrolase, EC 3.2.1.1) has been engineered [2] and used for improving the bread past (for reducing the past viscosity), accelerating the fermentation process, and for better bread taste and durability. It is an alpha-amylase produced by *Aspergillus oryzae* and catalyzes the endoamylolytic destruction of starch. Kaneko et al. demonstrated that the catalytic activity of alpha-amylase family follows an SN1 reaction mechanism [3]. This model explains the regioselectivity of the enzymatic reactions and is in agreement with experimental observations. Docking of the maltotriose and maltoheptaose to Taka-amylase revealed that the interatomic distances in these molecules are compatible with the spectroscopic results obtained for hen egg white lysozyme (HEWL) and CGTase. Before binding maltoheptose, the non-ionized Glu230 of Taka-amylase A retains its hydrogen bonding by donating a proton to Asp297 [4]. The alpha-amylase, pullulanase/iso-amylase, and the CGTase show similar structures and catalytic mechanisms [5]. The primary structure of Taka-amylase A is smaller than that of other starch-hydrolyzing enzymes such as αSLi, cyclodextrine glucanotransferase (CGTase, E.C.2.4.1.19), branched enzymes (E.C.2.4.1.18), and neopullulanase (E.C.3.2.1.135) [6–10]. The Taka-amylase has 478 residues in two domains (A and B) that are linked by a hydrophobic polypeptide chain. The A domain consists of a supersecondary structure containing the first 380 N-terminal residues, whereas domain B comprises eight anti-parallel β-sheet structures. There is a large cleft in the β-barrel region of the C-terminal A domain [11]. The alpha-amylases catalyze the hydrolysis of 1,4 glycosidic linkages in starch [12]. Based on the present criteria, the reduced Taka-amylase A molecule in 8 M urea or 6 M

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guanidine hydrochloride is fully denatured to a random coil structure [13].

Miyagawa et al. studied the activity of Taka-amylase A at high pressure [14–17]. These authors showed that the enzyme could be inactivated under a pressure of ~6000 kg/cm^2. The inactivation was mildly influenced by the initial concentration of the enzyme (inactivation above concentration of about 0.006 mg N/mL). At lower concentrations, the enzyme was more resistant to pressure. The process of the inactivation followed a first-order kinetics [15]. In 1964, these authors reported that enzyme concentration, pH, temperature, and ionic strength influenced the velocity of the recovery of activity. Moderate pressure (600–4000 kg/cm2) was found to reactivate Taka-amylase A. The recovery of the partially inactive Taka-amylase A is greater than that of the inactive enzyme [14]. Further, these authors studied the effect of pressure denaturation by physicochemical methods and reported that the mobility of the pressure-denatured Taka-amylase A was lower than that of the native enzyme and that the isoelectric point of the pressure-denatured Taka-amylase A shifted slightly toward higher pH. The optical rotatory dispersion constant λc was found to be 228 mμ (279 mμ for native enzyme) and the right helical content was found to be 10.8% (15.9% for native enzyme). Further, these authors concluded that Taka-amylase A is not greatly altered by pressure and that the inactivation and recovery were accompanied by changes in conformation [16,17].

Studies have shown that artificial and naturally occurring C-terminal truncations of alpha-amylase do not alter the amylolytic activity of the enzyme [18–21]. Deletion of 90 amino acid residues from the C-terminus of the enzyme found in *Klebsiella pneumoniae* yields a truncated enzyme with an activity comparable to that of the wild-type enzyme [22]. Hofmannet al. reported the presence of a repetitive sequence close to the starch-binding site at the N-terminal region of the amylase superfamily [8]. Sanoja et al. demonstrated that the first 410 amino acids of the enzyme from *Lactobacillus amylovorus* were sufficient for its catalytic activity. These authors also showed that the wild-type and truncated alpha-amylases had similar activities toward amylose, amylopectine, alpha cyclodextrine, and starch [23]. Ohdan et al. found that truncation of 186 C-terminal amino acids of the *B. subtilis* X-23 alpha-amylase did not alter the molar catalytic activity, amylolytic pattern, transglycosylation ability, pH effect on the activity and stability, optimum temperature, and the ability to bind starch [24]. In some cases, the removal of C-terminal amino acids has resulted in a loss of enzyme activity. For instance, deletion of 125 or 225 C-terminal amino acids of *Bacillus circulans* var. alkalophilus CGTase inactivated the enzyme. However, indicative of a protective role of

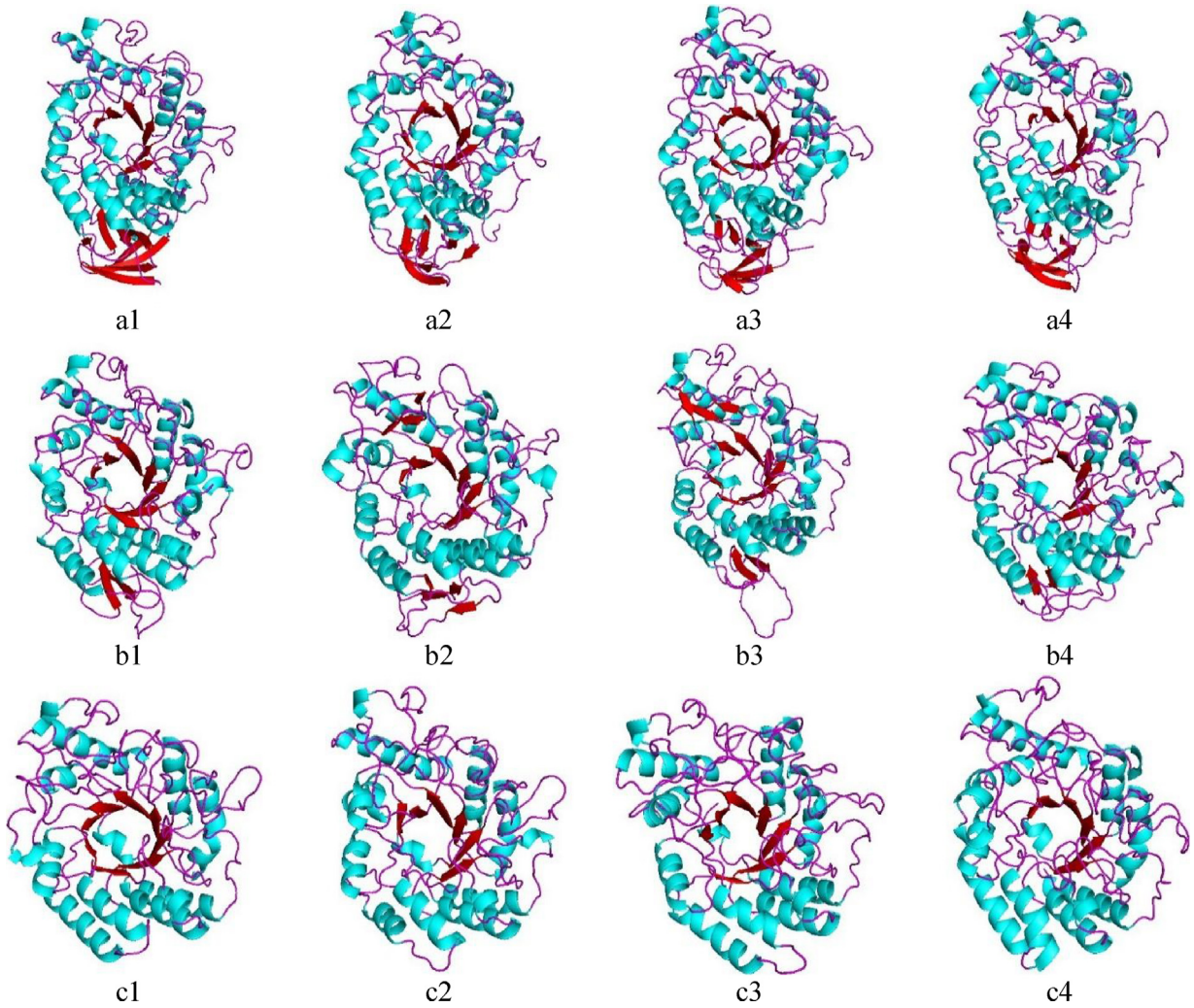

Fig. 1. Graphical representation of (a) wild-type Taka amylase (Amyl-C) under four different pressure conditions (P1, P2, P3, P4), (b) truncated Taka amylase generated by eliminating 50 amino acids from Amyl-C (Amyl-S1) under four different pressure conditions (P1, P2, P3, P4), and (c) truncated Taka amylase generated by eliminating 100 amino acids from Amyl-C (Amyl-S2) under four different pressure conditions (P1, P2, P3, P4).