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Minireview

An overview of the microbial α-amylase family

N. S. Reddy*, Annapoorna Nimmagadda and K. R. S. Sambasiva Rao

Centre for Biotechnology, Nagarjuna University, Nagarjuna Nagar -522 510, A.P., India.

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Amylases are enzymes which hydrolyze the starch molecules into polymers composed of glucose units. α-Amylases are ubiquitous in distribution, with plants, bacteria and fungi being the predominant sources. Most of the microbial α-amylases belong to the family 13 glycosyl hydrolases, and they share several common properties. But different reaction specificities have been observed across the family members. Structurally α-amylases possess $(\beta/\alpha)_8$ or TIM barrel structures and are responsible for hydrolysis or formation of glycosidic bonds in the α-conformation. Stability of the α-amylases has been widely studied; pH and temperature have very important roles to play. Engineering the enzymes for improved stability enhances their use industrially. This review focuses on the distribution, structural-functional aspects and factors for enhancing the stability of α-amylases.

Key words: α-Amylase, TIM barrel, glycosylhydrolases.

INTRODUCTION

Amylases are enzymes, which hydrolyze starch molecules to give diverse products including dextrins, and progressively smaller polymers composed of glucose units (Windish et al., 1965). The α-amylase family comprises a group of enzymes with a variety of different specificities that all act on one type of substrate being glucose residues linked through an α-1-1, α-1-4, α-1-6, glycosidic bonds. Members of this family share a number of common characteristic properties (van der Maarel et al., 2002). Amylases can be divided into two categories, endoamylases and exoamylases. Endoamylases catalyze hydrolysis in a random manner in the interior of the starch molecule producing linear and branched oligosaccharides of various chain lengths. Exoamylases act from the non-reducing end successively resulting in short end products (Gupta et al., 2003).

Amylases constitute a class of industrial enzymes having approximately 25% of the enzyme market (Sindhu et al., 1997; Rao et al., 1998). It is desirable that α-amylases should be active at the high temperatures of gelatinization (100-110°C) and liquefaction (80-90°C) to economize processes, therefore there has been a need for more thermophilic and thermostable α-amylases (Sindhu et al., 1997). With the availability of thermostable enzymes, a number of new possibilities for industrial processes have emerged (Haki and Rakshit, 2003). While the most widely used thermostable enzymes are the amylases in the starch industry (Poonam and Dalel, 1995; Crab and Mitchinson, 1997; Sarikaya et al., 2000) a number of other applications are in various stages of development. Thermostable enzymes isolated from thermophilic organisms have found a number of commercial applications because of their overall inherent

*Corresponding author. E-mail: naru_sri@yahoo.com.

Table 1. Known activities of Glycoside hydrolase family 13 enzymes.

Enzyme	EC number	Main substrate
Amylosucrase	EC: 2.4.1.4	Sucrose
Sucrose phosphorylase	EC: 2.4.1.7	Sucrose
Glucan branching enzyme	EC: 2.4.1.18	Starch, glycogen
Cyclomaltodextrin glycosyltransferase	EC: 2.4.1.19	Starch
Amylomaltase	EC: 2.4.1.25	Starch, glycogen
Maltopentaose-forming alpha-amylase	EC: 3.2.1.-	Starch
Alpha-amylase	EC: 3.2.1.1	Starch
Oligo-1,6-glucosidase	EC: 3.2.1.10	1,6-alpha-D-glucosidic linkages in some oligosaccharides
Alpha-glucosidase	EC: 3.2.1.20	Starch
Amylopullulanase	EC: 3.2.1.41	Pullulan
Cyclomaltodextrinase	EC: 3.2.1.54	linear and cyclomaltodextrin
Isopullulanase	EC: 3.2.1.57	Pullulan
Isoamylase	EC: 3.2.1.68	Amylopectin
Maltotetraose-forming alpha-amylase	EC: 3.2.1.60	Starch
Glucodextranase	EC: 3.2.1.70	Starch
Trehalose-6-phosphate hydrolase	EC: 3.2.1.93	Trehalose
Maltohexaose-forming alpha-amylase	EC: 3.2.1.98	Starch
Maltogenic amylase	EC: 3.2.1.133	Starch
Neopullulanase	EC: 3.2.1.135	Pullulan
Malto-oligosyl trehalase hydrolase	EC: 3.2.1.141	Trehalose
Malto-oligosyl trehalose synthase	EC: 5.4.99.15	Maltose

stability (Demirijan et al., 2001). The spectrum of amylase application has widened in many other fields, such as clinical, medical, and analytical chemistries, as well as their wide spread application in starch saccharification and in the textile, food, fermentation, paper, brewing and distilling industries.(Pandey et al., 2000).

α-Amylases are universally distributed throughout the animal, plant and microbial kingdoms. However, enzymes from fungal and bacterial sources have dominated applications in industrial sectors (Pandey et al., 2000). Several *Bacillus* sp. and thermostable *Actinomycetes* including *Thermomonospora* and *Thermoactinomyces* are versatile producers of the α-amylases (Ben et al., 1999). The genus *Bacillus* produces a large variety of extracellular enzymes of which amylases and proteases are of significant industrial importance. An extremely thermostable α-amylase is available from the mesophile *B. licheniformis* (Morgan et al., 1981). Alkaliphilic *Bacillus* strains often produce enzymes active at alkaline pH, including alkaline α-amylase, protease and carboxymethylcellulase (Horikoshi, 1996).

In the present review an attempt is made to document characteristics of the α-amylase family, structural-functional aspects of α-amylases and some properties of α-amylases that confer stability to these enzymes.

STRUCTURAL AND FUNCTIONAL CHARACTERISTICS

General features

O-Glycosyl hydrolases (EC: 3.2.1.-) are a widespread group of enzymes that hydrolyze the glycosidic bond between two or more carbohydrates, or between a carbohydrate and a non-carbohydrate moiety. A classification system for glycosyl hydrolases, based on sequence similarity, has led to the definition of 85 different families. Most of the starch hydrolyzing enzymes belong to the α-amylase family or family 13 glycosyl hydrolases based on amino acid sequence homology according to the classification of Henrissat (1991).

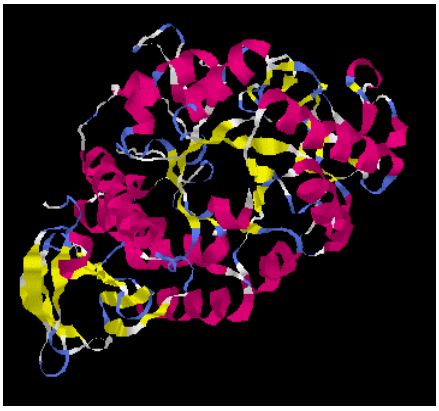
The common features of the α-amylase family

- Hydrolysis activity where the enzymes act on the α-glycosidic bonds and hydrolyse this bond to produce α-anomeric mono- or oligosaccharides or transglycosylation activity where α-1-4 or 1-6 glycosidic linkages are formed or a combination of both activities.
- They possess a $(\beta/\alpha)_8$ or TIM barrel structure containing the catalytic site residues.
- They possess four conserved regions in their primary sequence. Some of these conserved amino acids form the catalytic site and some are involved in the stability of the conserved TIM barrel topology (Kuriki and Imanaka, 1999; van der Maarel et al., 2002). These enzymes are listed in Table 1.

Catalytic residues and domains

The α-glycosidic bond is very stable having a spontaneous rate of hydrolysis at room temperature (Wolfenden et al., 1998). The catalytic mechanism of the

TIM barrel of 2taa (Taka-amylase)



TIM barrel of 2aaa (Acid-amylase)

Figure 1. The three dimensional structures of taka-amylase and acid-amylase from *Aspergillus* sp. Derived from the protein databank (PDB) (Sussman et al., 1998; Abola et al., 1997).

α-amylase family is that of the α- retaining double displacement (van der Maarel et al., 2002).

α-Retaining mechanism is the characteristic feature of the enzymes from the α-amylase family. They vary widely in their reaction specificities. The attachments of different domains to the catalytic site or to extra sugar binding subsites around the catalytic site is the prime reason for these differences (Van der Maarel et al., 2002). The catalytic domain-A is the most conserved domain in the α-amylase family. It consists of an amino terminal $(\beta/\alpha)_8$-barrel structure. The structure of taka-amylase (Matsuura et al., 1984) and acidiamylase (Boel et al., 1990) is shown in Figure 1.

STABILITY OF α-AMYLASES

There is a very huge demand to improve the stability of the enzymes to meet the requirements set by specific applications, especially with respect to temperature and pH.

pH

α-Amylases are generally stable over a wide range of pH from 4 to 11(Fogarty et al., 1979; Vihinen et al., 1989; Hamilton et al., 1999; Saito et al., 1973;Khoo et al., 1994), however, α-amylases with stability in a narrow range have also been reported (Coronado et al., 2000; Robyt et al., 1971).

Temperature

The temperature optimum for the activity of α-amylase is related to the growth of the microorganism (Vihinen et al., 1989). Thermostabilities are affectd by many factors like presence of calcium, substrate and other stabilizers (Vihinen et al., 1989).

Engineering of commercial enzymes for improved stability

Screening for novel microbial strains from extreme environments (Sunna et al., 1997; Niehaus et al., 1999; Veille and Zeikus, 2001) have been reported. Utilization of high concentrations of starch, thermostability, and protein yield are important criteria for commercialization (Schafer et al., 2000).

Engineering of the available commercial enzymes has been advocated. Hybrids of two homologous strains of the *B. licheniformis* and *B. amyloliquefaciens* α-amylases was generated (Suzuki et al., 1989) and two regions that are important for thermostability has been identified. Introduction of disulphide bonds in the enzyme and alteration of the amino acids prone to oxidation by an amino acid that is unaffected by oxidative agents (Barnett et al., 1998) leads to improved stability of the enzyme. Engineering α-amylase for changed pH activity profiles also would add to the stability of the enzymes (Nielsen and Borchert, 2000). van der Maarel et al. (2000) and Gupta et al. (2003) have discussed extensively about the industrial applications of α-amylase family members.

CONCLUSION

Amylases are important in many industrial processes. A number of microbial sources exist for the efficient production of this enzyme, but only a few selected strains of fungi and bacteria meet the criteria for commercial

production. In order to achieve the efficient, large-scale production, the structural and functional relationships of α-amylases have to be known in detail. This will lead to improving the stability of the existing enzymes and discovery of many new ones.

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