AMYLOLYTIC ENZYMES - FOCUS ON THE ALPHA-AMYLASES FROM ARCHAEA AND PLANTS

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Abstract: Amylolytic enzymes represent a group of starch hydrolases and related enzymes that are active towards the α-glycosidic bonds in starch and related poly- and oligosaccharides. The three best known amylolytic enzymes are α-amylase, β-amylase and glucoamylase that, however, differ from each other by their amino acid sequences, three-dimensional structures, reaction mechanisms and catalytic machineries. In the sequence-based classification of all glycoside hydrolases (GHs) they have therefore been classified into the three independent families: GH13 (α-amylases), GH14 (β-amylases) and GH15 (glucoamylases). Some amylolytic enzymes have been placed to the families GH31 and GH57. The family GH13 together with the families GH70 and GH77 constitutes the clan GH-H, well-known as the α-amylase family. It contains more than 6,000 sequences and covers 30 various enzyme specificities sharing the conserved sequence regions, catalytic TIM-barrel fold, retaining reaction mechanism and catalytic triad. Among the GH13 α-amylases, those produced by plants and archaebacteria exhibit common sequence similarities that distinguish them from the α-amylases of the remaining taxonomic sources. Despite the close evolutionary relatedness between the plant and archaeal α-amylases, there are also specific differences that discriminate them from each other. These specific differences could be used in an effort to reveal the sequence-structural features responsible for the high thermostability of the α-amylases from Archaea.

Key words: α-amylase, glycoside hydrolase families, sequence-structural features, archaebacteria, plants, evolutionary relatedness.

1. Introduction

Starch is an important source of energy for a wide spectrum of animals (including humans), plants and microorganisms. It consists exclusively from glucose monomers that are linked by α-1,4- and α-1,6-glycosidic linkages. Amylose (15-25% of starch) is formed by α-1,4-linearly bound glucoses, whereas amylopectin (75-85% of starch) contains also the branching points with the α-1,6-linked glucoses (LEVEQUE *et al.*, 2000b; BERTOLDO and ANTRANIKIAN, 2002).

Starch industry covers many well-developed and also recently established sophisticated technologies that utilize amylolytic enzymes. These amylases represent approximately 30% of the worldwide industrial enzyme production, the starch hydrolysis being considered to be the main way of their use (VAN DER MAAREL *et al.*, 2002).

2. Amylolytic enzymes

With regard to a complex structure of starch and related oligo- and polysaccharides the starch-degrading organisms have to dispose by relevant

combination of starch hydrolases and related enzymes (LEGIN *et al.*, 1998; BERTOLDO and ANTRANIKIAN, 2002). These enzymes are in general called amylases.

The amylolytic enzymes form a large group of starch hydrolases and related enzymes that are active towards starch, pullulan, glycogen and other related oligo- and polysaccharides (VIHINEN and MANTSALA, 1989; PANDEY *et al.*, 2000; JANECEK, 2009). It is a common way of binding of a glucose residue of the substrate in the enzyme active centre, termed conventionally as a substrate-binding subsite (DAVIES *et al.*, 1997), that is responsible for the activity of amylolytic enzymes. Most of them belong to glycoside hydrolases (GHs) that constitute the individual GH enzyme families without mutual sequence similarities (HENRISSAT, 1991). Now the GH families are part of the CAZy web-server (CANTAREL *et al.*, 2009) that covers also other carbohydrate-active enzymes (Fig. 1).

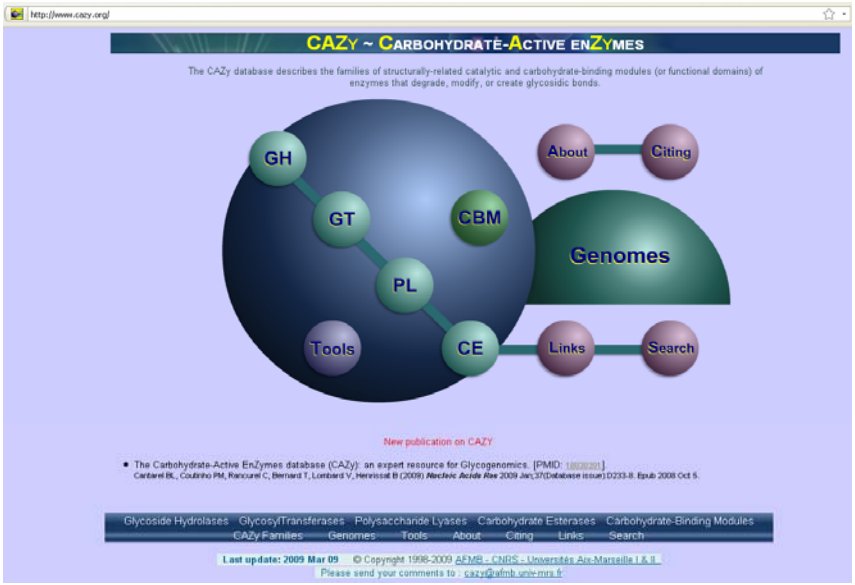

Fig. 1. **C**arbohydrate-**A**ctive en**Zy**me (CAZy) classification (http://www.cazy.org/). The individual proteins and enzymes are within the CAZy server classified into four main groups of sequence-based families: (i) GH, glycoside hydrolases; (ii) GT, glycosyl transferases; (iii) PL, polysaccharide lyases; and (iv) CE, carbohydrate esterases. The CBM stands for the family classification of carbohydrate-binding modules. For details, see CANTAREL *et al.* (2009).

The most known amylolytic enzymes are α-amylase (EC 3.2.1.1), β-amylase (EC 3.2.1.2) and glucoamylase (EC 3.2.1.3) that are, however, quite different from each other. They differ not only in their primary and tertiary structures, but also in their catalytic machineries and reaction mechanisms employed (JANECEK, 1994a; PUJADAS *et al.*, 1996; COUTINHO and REILLY, 1997). They have therefore been classified into different GH families: GH13 - α-amylases, GH14 - β-amylases, and GH15 - glucoamylases (HENRISSAT, 1991).

The enzymatic hydrolysis of a glycosidic bond can be characterized by a general acid catalysis that requires two essential components: a proton donor (an acid) and a nucleophile (a base). According to the anomeric configuration of the resulting hydroxyl group with regard to conformation of the cleaved *O*-glycosidic linkage, two basic mechanisms exist for this hydrolysis (Fig. 2): retaining or inverting (MCCARTER and WITHERS, 1994). Whereas α-amylase employs retaining mechanism (i.e. the products of its action are α-glucans), both β-amylase and glucoamylase are inverting hydrolases (i.e. they produce β-glucans).

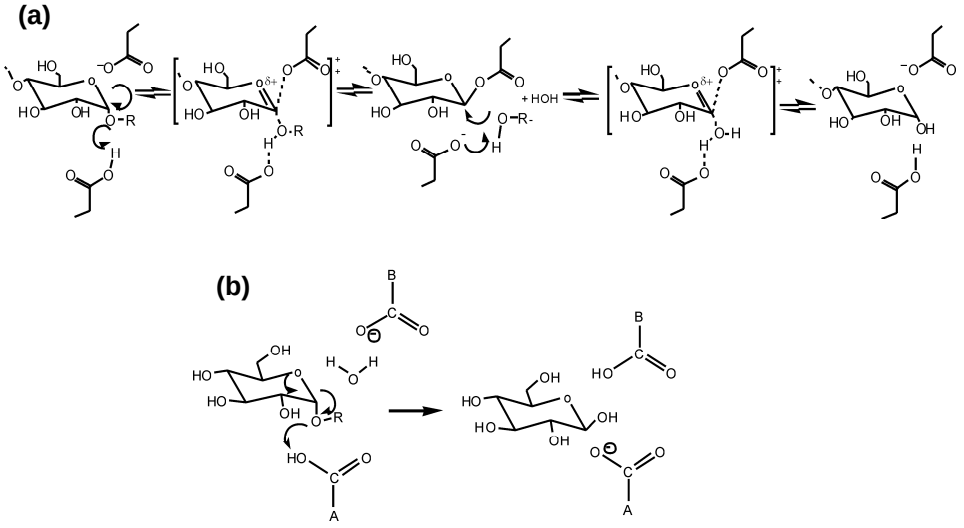

Fig. 2. (a) Retaining reaction mechanism of glycoside hydrolases (MACGREGOR *et al.*, 2001). The proton donor protonates the glycosidic oxygen and the catalytic nucleophile attacks at C1 leading to formation of the first transition state. The catalytic base promotes the attack of the incoming molecule ROH (water in hydrolysis or another sugar molecule in trasnglycosylation) on the formation of the covalent intermediate resulting in a second transition state, leading to hydrolysis or transglycosylation product. (b) Inverting reaction mechanism of glycoside hydrolases (SAUER *et al.*, 2000). The catalytic base (top) and acid (bottom) in the water-assisted hydrolysis of substrate leading to inversion of the configuration of the anomeric carbon.

From the structural point of view (Fig. 3), both α-amylase and β-amylase rank among the TIM-barrel enzymes, i.e. they possess the $(\beta/\alpha)_8$-barrel catalytic domain, while glucoamylase adopts a helical version of catalytic TIM-barrel, the so-called $(\alpha/\alpha)_6$-barrel. Within the CAZy classification the α-amylases from the family GH13 with closely related families GH70 and GH77 constitute the clan GH-H that is well-known as the α-amylase family (MACGREGOR *et al.*, 2001; CANTAREL *et al.* 2009). It is worth mentioning that some α-amylases with sequences and structures different from the main GH13 α-amylases have been placed to the family GH57 (JANECEK, 2005) and some amylolytic enzymes are present also in the family GH31 (NAKAI *et al.* 2005; KANG *et al.*, 2008).

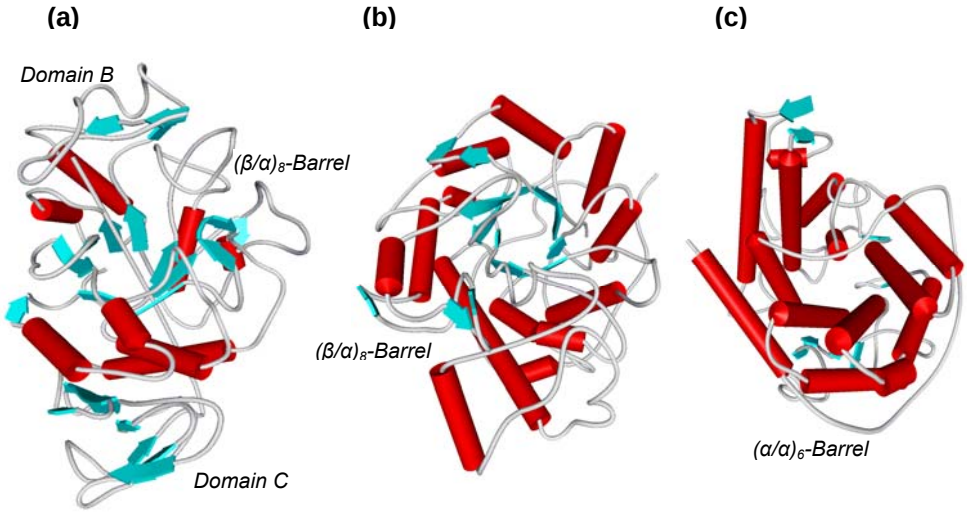

Fig. 3. Three-dimensional structures of amylases. (a) GH13 α-amylase from *Aspergillus oryzae* (PDB code: 2TAA; Matsuura *et al.*, 1984); (b) GH14 β-amylase from soybean (1BYA; Mikami *et al.*, 1993) and (c) GH15 glucoamylase from *Aspergillus awamori* (1AGM; Aleshin *et al.*, 1992).

The catalytic machineries of GH13, GH14 and GH15 α-amylases, β-amylase and glucoamylases, respectively, are also different: whereas the enzymes from the family GH13 possess a catalytic triad formed by two aspartates and one glutamate (UITDEHAAG *et al.*, 1999), both β-amylases (MIKAMI *et al.*, 1993) and glucoamylases (ALESHIN *et al.*, 1992) have their catalytic machineries formed by two glutamic acid residues that are, however, not alignable due to mutual amino acid sequence differences (PUJADAS *et al.*, 1996; COUTINHO and REILLY, 1997).

It thus could be summarised that amylases and related enzymes classified into the families GH13 (forming with GH70 and GH77 the clan GH-H), GH14, GH15 as well as GH31 and GH57 differ from each other by their amino acid sequences, three-dimensional structures, catalytic machineries and reaction mechanism (JANECEK, 2009).

3. α-Amylase enzyme family

Most of amylolytic enzymes are grouped in the α-amylase family (MACGREGOR *et al.*, 2001). It was originally recognised as a group of starch hydrolases and related enzymes (such as α-amylase, cyclodextrin glucanotransferase, neopullulanase, etc.) that exhibited sequence similarities and commonly predicted TIM-barrel fold (MACGREGOR and SVENSSON, 1989; TAKATA *et al.*, 1992). Within the sequence-based classification of GHs, it was originally established as the family GH13 (HENRISSAT, 1991), but later the families GH70 and GH77 were added to form the presently well-accepted GH-H clan (MACGREGOR, 2005; JANECEK, 2009).

3.1. Clan GH-H

The above-mentioned families GH13, GH70 and GH77 form the clan GH-H, i.e. the α-amylase family, which at present consists of 30 various enzyme specificities (Table 1) and contains more than 6,000 sequences (CANTAREL *et al.*, 2009). The members of the α-amylase family are not only hydrolases, but also transferases and isomerases. Based on amino acid sequence similarities, even some heteromeric amino acid transporter proteins may be considered to be the non-amylolytic members of the clan GH-H (JANECEK *et al.*, 1997) (Fig. 4).

Not all family enzymes attack the glycosidic bonds in starch; they are active towards the analogous bonds in glycogen, pullulan and other related poly- and oligosaccharides, like trehalose, sucrose, etc. (MACGREGOR *et al.*, 2001). Whereas the family GH77 is a monospecific family, i.e. it contains only one enzyme specificity - amylomaltase (alternative names 4-α-glucanotransferase or disproportionating enzyme; EC 2.4.1.25), the family GH70 consists of two specificties - glucosyltransferase (glucansucrase; EC 2.4.1.5) and alternansucrase (EC 2.4.1.140), and the family GH13 is formed by all the remaining enzyme specificies (amylomaltase being also present). GH13 is thus taken as the main α-amylase family (MACGREGOR *et al.*, 2001).

Enzymes that are members of the α-amylase family have to obey the following four criteria (KURIKI and IMANAKA, 1999; MACGREGOR *et al.*, 2001; JANECEK, 2002; VAN DER MAAREL *et al.*, 2002): (i) they act on α-glucosidic bonds (not only the α-1,4- and α-1,6-linkages); (ii) they employ the retaining reaction mechanism; (iii) they contain from 4 up to 7 conserved sequence regions; and (iv) they possess the same catalytic machinery within the catalytic TIM-barrel fold consisting of the aspartate residue near the end of the strand β4 (catalytic nucleophile), glutamate residue near the end of the strand β5 (proton donor) and aspartate residue near the end of the strand β7 (transition-state stabiliser).

The conserved sequence regions (Fig. 4) represent the short stretches of amino acid sequence that can be found in every α-amylase family member in equivalent positions and that contain the catalytic triad (Asp206, Glu230 and Asp297; *Aspergillus oryzae* α-amylase numbering; MATSUURA *et al.*, 1984) and other functionally important residues (NAKAJIMA *et al.*, 1986; JANECEK, 2002). These conserved sequence regions - common for the entire clan GH-H - may also be used as the sequence "fingerprints" since they contain amino acid residues exclusively specific for the individual enzyme specificities (JANECEK, 2008).

3.2. Glycoside hydrolase family GH13

α-Amylase is the most known and widely used enzyme of the GH-H clan. In general, α-amylases are endo-enzymes specific towards the α-1,4-glucosidic bonds, but there are also related GH13 exo-amylases, the so-called maltooligosaccharide-producing amylases (maltogenic α-amylase, maltotriohydrolase, maltotetraohydrolase, etc.), preferentially active at one side of the polysaccharide chain producing small oligosaccharides, such as maltose, maltotriose, maltotetraose, etc. (MACGREGOR *et al.*, 2001).

The α-amylase family members are multidomain proteins (Fig. 3a) containing the main catalytic domain in the form of a parallel $(\beta/\alpha)_8$-barrel (domain A) that is interrupted by a usually small domain in the place of the loop 3 connecting the strand β3 with the helix α3 (domain B) and succeeded by the antiparallel β-sandwich domain (domain C). The α-amylase-type of the barrel was confirmed in all members of the α-amylase family whose three-dimensional structure has already been determined (Fig. 4). The $(\beta/\alpha)_8$-barrel of α-amylases was first revealed in the structure of Taka-amylase A (MATSUURA *et al.*, 1984), i.e. in the structure of the α-amylase from *Aspergillus oryzae*. Since this type of fold was first identified in triose-phosphate isomerase (TIM), the $(\beta/\alpha)_8$-barrel is often simply called TIM-barrel (FARBER and PETSKO, 1990). It is a barrel of eight inner parallel β-strands surrounded outside by eight α-helices (Fig. 3a,b).

Table 1. The members of the α-amylase family (clan GH-H).

Enzyme class	Enzyme	EC	GH
Hydrolases	α-Amylase	3.2.1.1	13
	Oligo-1,6-glucosidase	3.2.1.10	13
	α-Glucosidase	3.2.1.20	13
	Pullulanase	3.2.1.41	13
	Amylopullulanase	3.2.1.1/41	13
	Cyclomaltodextrinase	3.2.1.54	13
	Maltotetraohydrolase	3.2.1.60	13
	Isoamylase	3.2.1.68	13
	Dextran glucosidase	3.2.1.70	13
	Trehalose-6-phosphate hydrolase	3.2.1.93	13
	Maltohexaohydrolase	3.2.1.98	13
	Maltotriohydrolase	3.2.1.116	13
	Maltogenic α-amylase	3.2.1.133	13
	Maltogenic amylase	3.2.1.133	13
	Neopullulanase	3.2.1.135	13
	Maltooligosyltrehalose hydrolase	3.2.1.141	13
	Maltopentaohydrolase	3.2.1.-	13
	Sucrose hydrolase	3.2.1.-	13
Transferases	Amylosucrase	2.4.1.4	13
	Glucansucrase	2.4.1.5	70
	Sucrose phosphorylase	2.4.1.7	13
	Glucan branching enzyme	2.4.1.18	13
	Cyclodextrin glucanotransferase	2.4.1.19	13
	4-α-Glucanotransferase (Amylomaltase)	2.4.1.25	13, 77
	Glucan debranching enzyme	2.4.1.25/3.2.1.33	13
	Alternansucrase	2.4.1.140	70
	Maltosyltransferase	2.4.1.-	13
Isomerases	Isomaltulose synthase	5.4.99.11	13
	Maltooligosyltrehalose synthase	5.4.99.15	13
	Trehalose synthase	5.4.99.16	13
HATs[a]	rBAT protein	---	13
	4F2hc antigen	---	13

[a] HATs means the heteromeric amino acid transporter proteins. Adapted from JANECEK (2009).

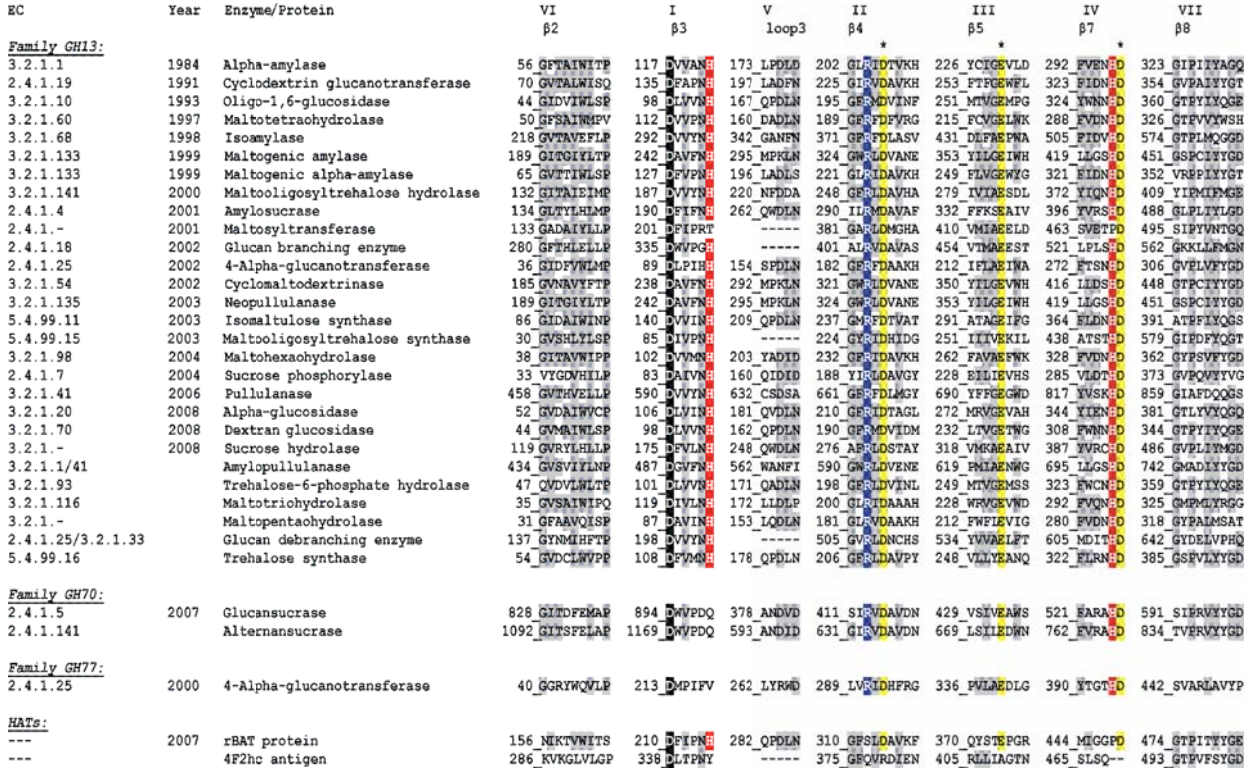

Fig. 4. Sequence fingerprints of the α-amylase family members. One representative of each enzyme specificity is presented. The catalytic triad is highlighted in yellow and signified by asterisks. The other functionally important residues corresponding with His122, Arg204, and His296 of α-amylase are also coloured. The well-conserved aspartate (beginning of the strand β3) is signified by black-and-white inversion. The residues conserved in at least 50% of sequences are coloured with grey background. The representatives of heteromeric amino acid transporter proteins (HATs) are also shown. The 'Year' denotes the year of three-dimensional structure determination (if any). Adapted from JANECEK (2002).

The active site of these enzymes is localised at the C-terminal end of the TIM-barrel (MATSUURA *et al.*, 1984, QIAN *et al.*, 1993; KADZIOLA *et al.*, 1994; LINDEN *et al.*, 2003). Comparison of known tertiary structures of various α-amylase family members with sequence alignments have shown that differences in specificity result from different variation of substrate binding at the β->α loops (SVENSSON, 1994; JANECEK, 1997). Also the active-site cleft is not of the same shape in each case (KAMITORI *et al.*, 1999; PRZYLAS *et al.*, 2000), despite the fact it always contains the same catalytic triad accompanied, however, by several additional residues depending on a given enzyme specificity (MATSUURA, 2002). Differences especially in the length, sequence and secondary structure have also been seen within the domain B protruding out of the catalytic TIM-barrel in the place of the loop 3 (JESPERSEN *et al.*, 1991, 1993). It was pointed out that these differences may be directly related to enzyme specificity (JANECEK *et al.*, 1997). With regard to domain C succeeding the catalytic TIM-barrel, this domain could contribute to the overall catalytic domain stability by shielding the hydrophobic residues of the barrel (KATSUYA *et al.*, 1998).

As far as the conserved sequence regions of the α-amylase family are concerned (Fig. 4), four of them (the regions I, II, III and IV) belong to the best known regions established more than 20 years ago, whereas the three additional ones (the regions V, VI and VII) were identified more recently. The former regions (FRIEDBERG, 1983; NAKAJIMA *et al.*, 1986; MACGREGOR *et al.*, 2001), positioned near the C-termini of the β-strands β3, β4, β5 and β7 of the catalytic TIM-barrel, contain most of the functionally important residues including the catalytic triad (Fig. 4). The latter regions (JANECEK, 1992, 1994a,b, 1995, 2002), located near the C-terminal end of domain B and of β-strands β2 and β8, cover the features distinguishing the individual enzyme specificities from each other. Even the absence of the fifth conserved sequence region, for example, may be used as a feature characteristic of a given specificity (JANECEK, 2000).

Although the basic arrangement of the α-amylase family members is the same counting the three domains A, B and C (Fig. 3a), it should be taken into account that there are some family members that contain additional C- and/or N-terminal domains, for example cyclodextrin glucanotransferase (KLEIN and SCHULZ, 1991) and neopullulanase (HONDOH *et al.*, 2003). They may play various and still not completely recognised functions, but most of them have been anticipated to be involved in binding starch (glycogen, pullulan) and related substrate analogues. These non-catalytic domains were in many cases confirmed to have this property and thus have been called starch-binding domains (PENNINGA *et al.*, 1995; SORIMACHI *et al.*, 1997). It was found that starch-binding domain disrupts the starch surface and thus increases the effect of the amylolytic hydrolysis (SOUTHALL *et al.*, 1999). Within the CAZy server (Fig. 1), these motifs have been classified into the CBM (carbohydrate-binding module) families (CANTAREL *et al.*, 2009). At present, nine families of starch-binding domains are known: CBM20, CBM21, CBM25, CBM26, CBM34, CBM41, CBM45, CBM48, and CBM53. The motifs from the family CBM20 belong to most intensively studied starch-binding domains (SVENSSON *et al.*, 1989; JANECEK and SEVCIK, 1999; RODRIGUEZ-SANOJA *et al.*, 2005; MACHOVIC and JANECEK, 2006a). Based on a detailed bioinformatics analysis it was suggested

to establish a common CBM clan from the families CBM20 and CBM21 (MACHOVIC *et al.*, 2005) and the motifs classified recently into the families CBM48 and CBM53 could also join the proposed CBM clan (MACHOVIC and JANECEK, 2006b, 2008).

3.3. Glycoside hydrolase families GH70 and GH77

The family GH70 contains the sucrose-utilising glucosyltransferases (glucansucrase and alternansucrase) that possess a circularly permuted version of the α-amylase-type catalytic TIM-barrel (MACGREGOR *et al.*, 1996). The first element of the GH70-type barrel is the α-helix equivalent to helix α3 of the α-amylase-type TIM-barrel, whereas the last element is the β-strand equivalent to strand β3 of α-amylases (Fig. 5). This means that instead of E1-H1-E2-H2….E8-H8 present in α-amylases (and overall in both the families GH13 and GH77), in GH70 glucosyltransferases there is H3-E4-H4-E5….H2-E3, where E and H stand for β-strand and α-helix, respectively (MACGREGOR *et al.*, 1996). The glucansucrases are usually large multidomain proteins occurring exclusively in lactic acid bacteria (VAN HIJUM *et al.*, 2006).

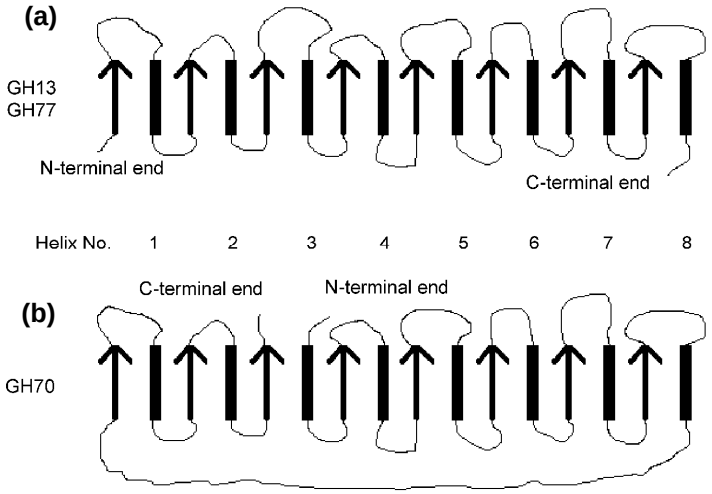

Fig. 5. The arrangement of the secondary structure elements in GH70 with respect to GH13 α-amylase type TIM-barrel. (a) Typical "ordinary" TIM-barrel present in the members of the family GH13 (and also GH77); (b) circularly permuted version of the family GH70. The helices are represented by black rectangles and the strands are shown as arrows. The order of the helices in the GH13 (and GH77) is 12345678 from the N-terminal end of the protein, whereas in the GH70 the order is 34567812. Adapted from MACGREGOR (2005).

The structure/function relationships within the family GH70 and its relatedness to the main α-amylase family GH13 were recently elucidated by determining the tertiary structure of the GH70 glucansucrase from *Leuconostoc mesenteroides* (PIJNING *et*

al., 2008) that has confirmed the previous predictions concerning the circular permutation (Fig. 5). The solved structure interestingly revealed that the enzyme adopts the so-called "U-fold" domain arrangement so that 4 of the 5 domains are formed by combining an N- and a C-terminal part of the polypeptide chain (DIJKSTRA *et al.*, 2007).

The family GH77 contains only one enzyme specificity, the amylomaltase (Table 1), known also as 4-α-glucanotransferase in bacteria (TERADA *et al.*, 1999) and archaeons (KAPER *et al.*, 2005) or disproportionating enzyme (D-enzyme) in plants (TAKAHA *et al.*, 1993). They exhibit a lower degree of sequence similarity to the family GH13 (Fig. 4) and the main feature characteristic for the GH77 members is the lack of domain C (PRZYLAS *et al.*, 2000) succeeding typically the catalytic TIM-barrel in GH13 (Fig. 3). The GH77 structure contains several, mainly α-helical insertions that can be divided into three subdomains (Fig. 6): (i) subdomain B1 corresponds with GH13 domain B; (ii) subdomain B2 is unique for the GH77 amylomaltases; and (iii) subdomain B3 is equivalent to GH13 domain C (STRATER *et al.*, 2002).

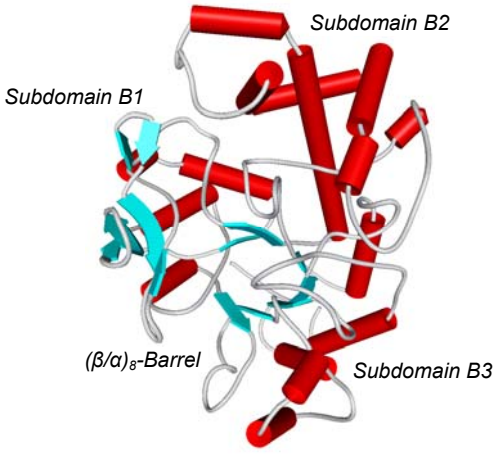

Fig. 6. Three-dimensional structure of GH77 amylomaltase from *Thermus aquaticus* (1CWY; Przylas *et al.*, 2000).

The interest in the family GH77 was recently increased by revealing the putative amylomaltases from a few borreliae that exhibited in their amino acid sequences the non-GH77 features (GODANY *et al.*, 2008). It was especially the arginine positioned two residues before the catalytic nucleophile in the conserved sequence region II (Fig. 4) that was recognized to be replaced naturally by a lysine in the GH77 amylomaltase-like protein from *Borrelia burgdorferi* (MACHOVIC and JANECEK, 2003). This arginine was otherwise considered to belong to the four residues conserved invariantly throughout the α-amylase family, i.e. the entire clan GH-H (JANECEK, 2002). The exclusive (i.e. the non-GH77) sequence features present in GH77-like proteins from borreliae have already been confirmed as well as it was determined that the *B.*

burgdorferi GH77 amylomaltase-like protein exhibits a typical amylomaltase activity, i.e. the enzyme catalyzes both the hydrolysis of maltooligosaccharides and formation of their transglycosylation products (GODANY *et al.*, 2008). Based on the bioinformatics analysis of various GH77 real and hypothetical amylomaltases, some of the borrelial GH77-like proteins were suggested to exhibit an intermediary character within this family (JANECEK, 2008).

3.4. Glycoside hydrolase families GH31 and GH57

The families GH31 and GH57 are not the members of the clan GH-H, i.e. they do not belong to the α-amylase family in terms as it is widely accepted (MACGREGOR *et al.*, 2001), but they both deserve some attention here since they contain similar enzyme specificities (α-amylase, α-glucosidase, amylopullulanase, 4-α-glucanotransferase, branching enzyme, etc.).

The family GH31 contains, in addition to the above-mentioned α-glucosidases (EC 3.2.1.20 similar to GH13), also α-xylosidases and α-glucan lyases (FRANDSEN and SVENSSON, 1998; LEE *et al.*, 2005; KANG *et al.*, 2008). Although it employs the retaining mechanism (Fig. 2a) and its members adopt the catalytic TIM-barrel domain (Fig. 7a) similar to that adopting in the α-amylase family (LOVERING *et al.*, 2005;

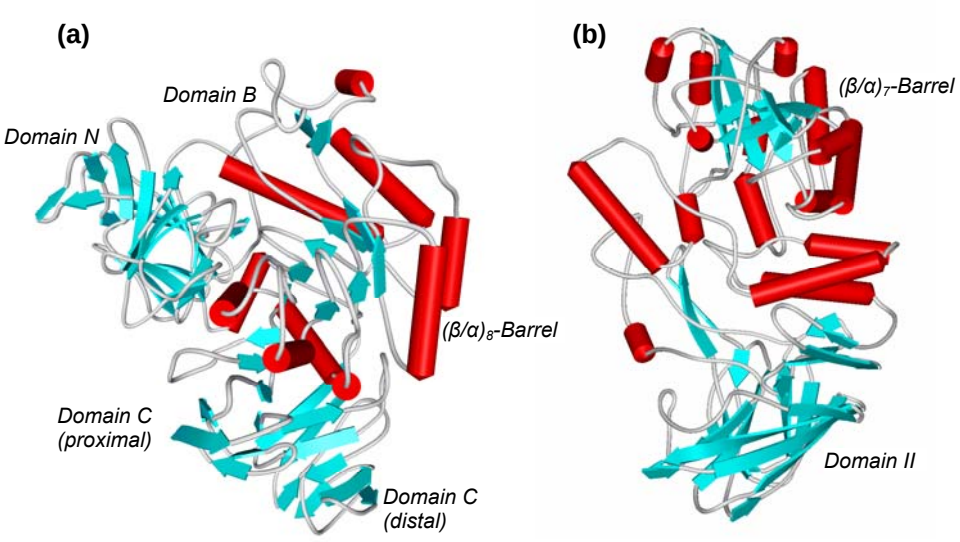

Fig. 7. Three-dimensional structure of (a) GH31 α-xylosidase from *Escherichia coli* (1XSI; Lovering *et al.*, 2005) and (b) GH57 4-α-glucanotransferase from *Thermococcus litoralis* (1K1W; Imamura *et al.*, 2003).

ERNST *et al.*, 2006; SIM *et al.*, 2008) with even the corresponding catalytic nucleophile (RIGDEN, 2002), the family GH31 has not joined the clan GH-H. One of the reasons is the difference in the proton donors used in GH31 and GH-H

(MATSUURA *et al.*, 1984; UITDEHAAG *et al.*, 1999; LOVERING *et al.*, 2005). Based on a detailed bioinformatics study, an idea on the so-called remote homologies between the family GH31 and clan GH-H was proposed recently (JANECEK *et al.*, 2007) indicating a possibility to create a level of evolutionary hierarchy higher than a clan.

As far as the family GH57 is concerned, it contains several enzyme specificities that are also members of the main α-amylase family, only the α-galactosidase (EC 3.2.1.22) being different (JANECEK, 2005; MURAKAMI *et al.*, 2006). It also employs the retaining mechanism, but due to a different catalytic domain - an incomplete version of a TIM-barrel, i.e. a $(\beta/\alpha)_7$-barrel (Fig. 7b) and catalytic machinery (IMAMURA *et al.*, 2003; DICKMANNS *et al.*, 2006) - it should be evolutionarily more distantly related to GH13 than is the family GH31 (JANECEK, 1998). Moreover, GH57 exhibits its own conserved sequence regions (ZONA *et al.*, 2004) that are different from those characteristic for the clan GH-H (JANECEK, 2002).

4. α-Amylases from archaebacteria and plants

At present it is well-known and accepted that plant and archaeal α-amylases from the family GH13 are sequentially similar and evolutionarily related. This remarkable finding was first observed ten years ago (JANECEK *et al.*, 1999; JONES *et al.*, 1999). Before the first GH13 α-amylases from Archaea became available, the plant α-amylases were positioned in the evolutionary tree (Fig. 8) on a branch next to the cluster of bacterial liquefying and intracellular α-amylases represented by bacilli and enterobacteria, respectively (JANECEK, 1994b).

4.1. Similarities and differences

The first detailed bioinformatics study focused on the archaeal α-amylases and their counterparts from a wide spectrum of remaining living organisms from Bacteria and Eucarya revealed (JANECEK *et al.*, 1999) that the sequence features exclusive for the α-amylases from hyperthermophilic archaeons are present also and almost only in the plant α-amylases (Fig. 9). These features are as follows (JANECEK *et al.*, 1999): (i) Ile107 (*Thermococcus hydrothermalis* α-amylase numbering; LEVEQUE *et al.*, 2000a) succeeding the conserved aspartate in the conserved sequence region region I (strand β3); (ii) (Ala194)-Trp195 at the beginning, Tyr199 in the middle and Gly202 at the end of the region II (strand β4); (iii) Ala219 succeeding the conserved tryptophane and Tyr223-Trp224 succeeding the catalytic proton donor (Glu222) in the region III (strand β5); (iv) Ala286 in the region IV (strand β7); (v) Ile196 in the region V (located within the loop3, i.e. domain B); (vi) Ile42 succeeding the conserved glycine at the beginning and dipeptide Pro48-Pro49 at the end of the region VI (strand β2); and (vii) Gln309 succeeding the conserved glycine at the beginning, tripeptide Ile312-Phe313-Tyr314 in the middle and Asp316 at the end of the region VII (strand β8). It is worth mentioning that some of the above-mentioned residues have already been recognised as functionally important residues (KADZIOLA *et al.*, 1998; LINDEN *et*

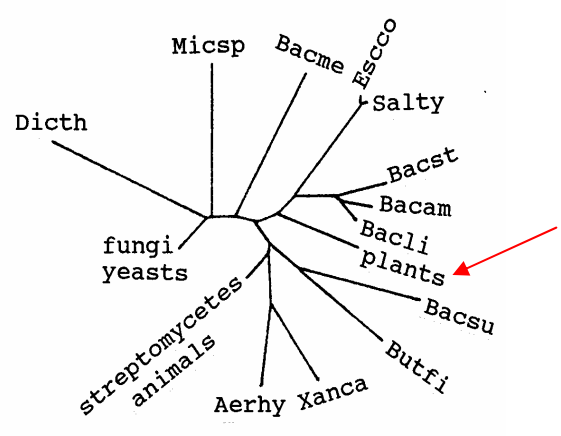

Fig. 8. The evolutionary tree of microbial (including fungi and yeasts), plant and animal α-amylases. The bacterial sources are abbreviated as follows: Dicth, *Dictyoglomus thermophilum*; Micsp, *Micrococcus* sp.; Bacme, *Bacillus megaterium*; Escco, *Escherichia coli*; Salty, *Salmonella typhimurium*; Bacst, *Bacillus stearothermophilus*; Bacam, *Bacillus amyloliquefaciens*; Bacli, *Bacillus licheniformis*; Bacsu, *Bacillus subtilis*; Butfi, *Butyrivibrio fibrisolvens*, Xanca, *Xanthomonas campestris*; Aerhy, *Aeromonas hydrophila*. The tree does not contain any archaeal α-amylase since at the beginning of 90s of the previous century no sequence of an archaeal α-amylase was available. The red arrow indicates the the cluster of plant α-amylases. Adapted from JANECEK (1994b).

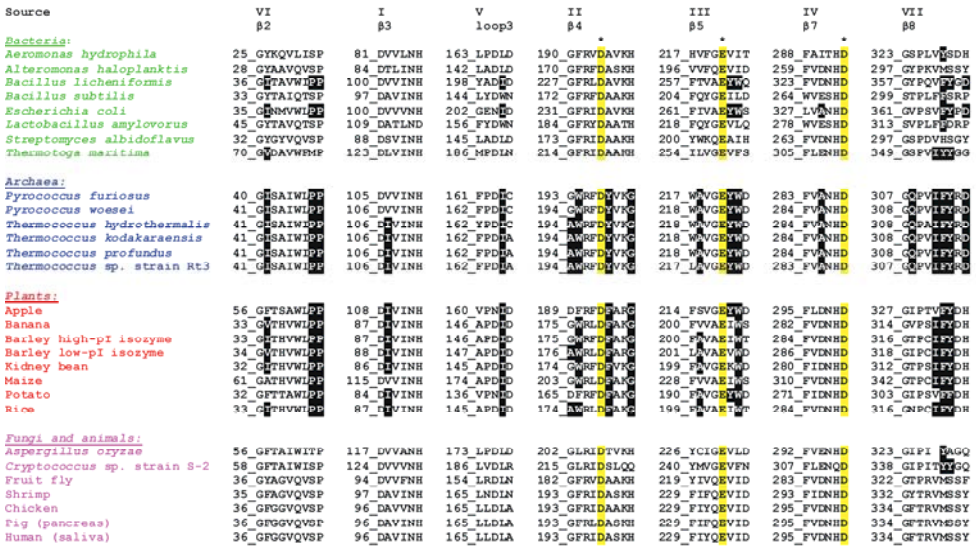

Fig. 9. Sequence fingerprints of α-amylases. The enzymes represent the individual taxonomic sources with focus on Archaea and plants. The sequence features characteristic of the archaeal α-amylases are highlighted in black-and-white inversion. The catalytic triad is signified by asterisks and yellow highlighting. Adapted from JANECEK (2008).

al., 2003). Thus for example the glycine from the region II (Gly202 of the archaeal α-amylase) serves as a specific ligand for calcium ion and the tryptophane from the region III (Trp224 of the archaeal α-amylase) forms a stacking interaction with one of the acarbose rings bound in the active site in the complex structure of barley α-amylase with acarbose (KADZIOLA *et al.*, 1998). These residues should play the same roles in the structure of the archaeal α-amylase from *Pyrococcus woesei* (LINDEN *et al.*, 2003).

The close sequence similarity between the α-amylases from Archaea and plants has evoked the idea on a possibility to reveal the factors responsible for the high thermostability of the archaeal α-amylases that exhibit the temperature optima around and above 80 °C (LEVEQUE *et al.*, 2000b; BERTOLDO and ANTRANIKIAN, 2002). The plant enzymes are generally substantially less thermostable. It is worth mentioning that on the one side the archaeal and plant α-amylases contain the common sequence features that discriminate them from the remaining sources, but on the other side they have to possess the additional sequence features that should enable one to distinguish them from each other, e.g., the alanine from the region IV (Ala286 of the archaeal α-amylase) that has no correspondence in the plant counterparts (Fig. 4). Such specific differences could be utilized in an effort to identify the molecular basis of high thermostability of the archaeal α-amylases via the approaches of site-directed mutagenesis and protein design.

Fig. 10. The evolutionary tree of α-amylases. The tree reflects the conserved sequence fingerprints of α-amylases (Fig. 9). Adapted from JANECEK (2008).

4.2. Evolutionary relatedness

The close evolutionary relatedness of the α-amylases from Archaea and plants from the family GH13 is shown in Figure 10. The GH13 as one of the largest GH families (CANTAREL *et al.*, 2009) has recently been divided into the subfamilies (STAM *et al.*, 2006), the plant and archaeal α-amylases being placed into the subfamilies GH13_6 and GH13_7, respectively. With regard to the α-amylases most closely related to those from plants and Archaea (Fig. 10), these are the bacterial enzymes from *Bacillus licheniformis* (YUUKI *et al.*, 1985) and *Escherichia coli* (RAHA *et al.*, 1992) that represent the liquefying and intracellular α-amylases, respectively, as observed originally (JANECEK, 1994b). It should be noted, however, that the close evolutionary relationships between the α-amylases from Archaea and plants illustrated here only for a limited sample of living organisms (Fig. 10) has been confirmed also in the more recent evolutionary trees comparing a wider spectrum of taxonomic sources including novel groups of α-amylases from bacteria (DA LAGE *et al.*, 2004) and fungi (VAN DER KAAIJ *et al.*, 2007).

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References

ALESHIN, A.E., GOLUBEV, A., FIRSOV, L.M., HONZATKO, R.B.: Crystal structure of glucoamylase from *Aspergillus awamori* var. X100 to 2.2 Å resolution. J. Biol. Chem., 267, 1992, 19291-19298.

BERTOLDO, C., ANTRANIKIAN, G.: Starch-hydrolyzing enzymes from thermophilic archaea and bacteria. Curr. Opin. Chem. Biol., 6, 2002, 151-160.

CANTAREL, B.L., COUTINHO, P.M., RANCUREL, C., BERNARD, T., LOMBARD, V., HENRISSAT, B.: The Carbohydrate-Active enZymes database (CAZy): an expert resource for glycogenomics. Nucleic Acids Res., 37, 2009, 233-238.

COUTINHO, P.M., REILLY, P.J.: Glucoamylase, structural, functional, and evolutionary relationships. Proteins, 29, 1997, 334-347.

DA LAGE, J.L., FELLER, G., JANECEK, S.: Horizontal gene transfer from Eukarya to bacteria and domain shuffing: the α-amylase model. Cell. Mol. Life Sci., 61, 2004, 97-109.

DAVIES, G.J., WILSON, K.S., HENRISSAT, B.: Nomenclature for sugar-binding subsites in glycosyl hydrolases. Biochem. J., 321, 1997, 557-559.

DICKMANNS, A., BALLSCHMITER, M., LIEBL, W., FICNER, R.: Structure of the novel α-amylase AmyC from *Thermotoga maritima*. Acta Crystallogr. D Biol. Crystallogr., 62, 2006, 262-270.

DIJKSTRA, B.W., VUJICIC-ZAGAR, A., PIJNING, T., KRALJ, S., DIJKHUIZEN, L.: Structural and functional characterization of a glucansucrase (ΔN-GTF180) from *Lactobacillus reuteri* 180, a GH family 70 enzyme. Abstract Book of the 3rd Symposium on the Alpha-Amylase Family. Smolenice Castle, Slovakia, Sep 23-27, 2007, 15.

ERNST, H.A., LO LEGGIO, L., WILLEMOES, M., LEONARD, G., BLUM, P., LARSEN, S.: Structure of the *Sulfolobus solfataricus* α-glucosidase: implications for domain conservation and substrate recognition in GH31. J. Mol. Biol., 358, 2006, 1106-1124.

FARBER, G.K., PETSKO, G.A.: The evolution of α/β barrel enzymes. Trends Biochem. Sci., 15, 1990, 228-234.

FRANDSEN, T.P., SVENSSON, B.: Plant α-glucosidases of the glycoside hydrolase family 31. Molecular properties, substrate specificity, reaction mechanism, and comparison with family members of different origin. Plant Mol. Biol., 37, 1998, 1-13.

FRIEDBERG, F.: On the primary structure of amylases. FEBS Lett., 152, 1983, 139-140.

GODANY, A., VIDOVA, B., JANECEK, S.: The unique glycoside hydrolase family 77 amylomaltase from *Borrelia burgdorferi* with only catalytic triad conserved. FEMS Microbiol. Lett., 284, 2008, 84-91.

HENRISSAT, B.: A classification of glycosyl hydrolases based on amino acid sequence similarities. Biochem. J., 280, 1991, 309-316.

HONDOH, H., KURIKI, T., MATSUURA, Y.: Three-dimensional structure and substrate binding of *Bacillus stearothermophilus* neopullulanase. J. Mol. Biol., 326, 2003, 177-188.

IMAMURA, H., FUSHINOBU, S., YAMAMOTO, M., KUMASAKA, T., JEON, B.S., WAKAGI, T., MATSUZAWA, H.: Crystal structures of 4-α-glucanotransferase from *Thermococcus litoralis* and its complex with an inhibitor. J. Biol. Chem., 278, 2003, 19378-19386.

JANECEK, S.: New conserved amino acid region of α-amylases in the third loop of their $(\beta/\alpha)_8$-barrel domains. Biochem. J., 288, 1992, 1069-1070.

JANECEK, S.: Parallel β/α-barrels of α-amylase, cyclodextrin glycosyltransferase and oligo-1,6-glucosidase versus the barrel of β-amylase: evolutionary distance is a reflection of unrelated sequences. FEBS Lett., 353, 1994a, 119-123.

JANECEK, S.: Sequence similarities and evolutionary relationships of microbial, plant and animal α-amylases. Eur. J. Biochem., 224, 1994b, 519-524.

JANECEK, S.: Close evolutionary relatedness among functionally distantly related members of the $(\alpha/\beta)_8$-barrel glycosyl hydrolases suggested by the similarity of their fifth conserved sequence region. FEBS Lett., 377, 1995, 6-8.

JANECEK, S.: α-Amylase family: molecular biology and evolution. Progr. Biophys. Mol. Biol., 67, 1997, 67-97.

JANECEK, S.: Sequence of archaeal *Methanococcus jannaschii* α-amylase contains features of families 13 and 57 of glycosyl hydrolases: a trace of their common ancestor? Folia Microbiol., 43, 1998, 123-128.

JANECEK, S.: Structural features and evolutionary relationships in the α-amylase family. *In*: M. OHNISHI, T. HAYASHI, S. ISHIJIMA, T. KURIKI (Eds.) Glycoenzymes. Japan Scientific Societies Press, Tokyo, 2000, 19-54.

JANECEK, S.: How many conserved sequence regions are there in the α-amylase family? Biologia, 57 (Suppl. 11), 2002, 29-41.

JANECEK, S.: Amylolytic families of glycoside hydrolases: focus on the family GH-57. Biologia, 60 (Suppl. 16), 2005, 177-184.

JANECEK, S.: Sequence fingerprints in the evolution of the α-amylase family. *In*: K.H. PARK (Ed.) Carbohydrate-active enzymes: structure, function and applications. Woodhead Publishing, Ltd., Cambridge, 2008, 45-63.

JANECEK, S.: Bioinformatics of the α-amylase family. *In*: A. PANDEY, C. LARROCHE, C.R. SOCCOL, C.G. DUSSAP (Eds.) New horizons in biotechnology. Asiatech Publishers, Inc., New Delhi, 2009, 37-52.

JANECEK, S., LEVEQUE, E., BELARBI, A., HAYE, B.: Close evolutionary relatedness of α-amylases from Archaea and plants. J. Mol. Evol., 48, 1999, 421-426.

JANECEK, S., SEVCIK, J.: The evolution of starch-binding domain. FEBS Lett., 456, 1999, 119-125.

JANECEK, S., SVENSSON, B., HENRISSAT, B.: Domain evolution in the α-amylase family. J. Mol. Evol., 45, 1997, 322-331.

JANECEK, S., SVENSSON, B., MACGREGOR, E.A.: A remote but significant sequence homology between glycoside hydrolase clan GH-H and family GH31. FEBS Lett., 581, 2007, 1261-1268.

JESPERSEN, H.M., MACGREGOR, E.A., HENRISSAT, B., SIERKS, M.R., SVENSSON, B.: Starch- and glycogen-debranching and branching enzymes: prediction of structural features of the catalytic $(\beta/\alpha)_8$-barrel domain and evolutionary relationship to other amylolytic enzymes. J. Protein Chem., 12, 1993, 791-805.

JESPERSEN, H., MACGREGOR, E.A., SIERKS. M.R., SVENSSON, B.: Comparison of the domain-level organization of starch hydrolases and related enzymes. Biochem. J., 280, 1991, 51-55.

JONES, R.A., JERMIIN, L.S., EASTEAL, S., PATEL, B.K., BEACHAM, I.R.: Amylase and 16S rRNA genes from a hyperthermophilic archaebacterium. J. Appl. Microbiol., 86, 1999, 93-107.

KADZIOLA, A., ABE, J., SVENSSON, B., HASER, R.: Crystal and molecular structure of barley α-amylase. J. Mol. Biol., 1994, 239, 104-121.

KADZIOLA, A., SOGAARD, M., SVENSSON, B., HASER, R.: Molecular structure of a barley α-amylase-inhibitor complex: implications for starch binding and catalysis. J. Mol. Biol., 278, 1998, 205-217.

KAMITORI, S., KONDO, S., OKUYAMA, K., YOKOTA, T., SHIMURA, Y., TONOZUKA, T., SAKANO, Y.: Crystal structure of *Thermoactinomyces vulgaris* R-47 α-amylase II (TVA II) hydrolyzing cyclodextrins and pullulan at 2.6 Å resolution. J. Mol. Biol., 287, 1999, 907-921.

KANG, M.S., OKUYAMA, M., YAOI, K., MITSUISHI, Y., KIM, Y.M., MORI, H., KIMURA, A.: Glycoside hydrolase family 31 *Escherichia coli* α-xylosidase. Biocatal. Biotransform., 26, 2008, 96-103.

KAPER. T., TALIK, B., ETTEMA, T.J., BOS, H., VAN DER MAAREL, M.J., DIJKHUIZEN, L.: Amylomaltase of *Pyrobaculum aerophilum* IM2 produces thermoreversible starch gels. Appl. Environ. Microbiol., 71, 2005, 5098-5106.

KATSUYA, Y., MEZAKI, Y., KUBOTA, M., MATSUURA, Y.: Three-dimensional structure of *Pseudomonas* isoamylase at 2.2 Å resolution. J. Mol. Biol., 281, 1998, 885-897.

KLEIN, C., SCHULZ, G.E.: Structure of cyclodextrin glycosyltransferase refined at 2.0 Å resolution. J. Mol. Biol., 217, 1991, 737-750.

KURIKI, T., IMANAKA, T.: The concept of the α-amylase family: structural similarity and common catalytic mechanism. J. Biosci. Bioeng., 87, 1999, 557-565.

LEE, S.S., YU, S., WITHERS, S.G.: Mechanism of action of exo-acting α-1,4-glucan lyase: a glycoside hydrolase family 31 enzyme. Biologia, 60 (Suppl. 16), 2005, 137-148.

LEGIN, E., COPINET, A., DUCHIRON, F.: Production of thermostable amylolytic enzymes by *Thermococcus hydrothermalis*. Biotechnol. Lett., 20, 1998, 363-367.

LEVEQUE, E., HAYE, B., BELARBI, A.: Cloning and expression of an α-amylase encoding gene from the hyperthermophilic archaebacterium *Thermococcus hydrothermalis* and biochemical characterisation of the recombinant enzyme. FEMS Microbiol. Lett., 186, 2000a, 67-71.

LEVEQUE, E., JANECEK, S., BELARBI, A., HAYE, B.: Thermophilic archaeal amylolytic enzymes. Enzyme Microb. Technol., 26, 2000b, 2-13.

LINDEN, A., MAYANS, O., MEYER-KLAUCKE, W., ANTRANIKIAN, G., WILMANNS, M.: Differential regulation of a hyperthermophilic α-amylase with a novel (Ca,Zn) two-metal center by zinc. J. Biol. Chem., 278, 2003, 9875-9884.

LOVERING, A.L., LEE, S.S., KIM, Y.W., WITHERS, S.G., STRYNADKA, N.C.J.: Mechanistic and structural analysis of a family 31 α-glycosidase and its glycosyl-enzyme intermediate. J. Biol. Chem., 280, 2005, 2105-2115.

MACGREGOR, E.A.: An overview of clan GH-H and distantly related families. Biologia, 60 (Suppl. 16), 2005, 5-12.

MACGREGOR, E.A., JANECEK, S., SVENSSON, B.: Relationship of sequence and structure to specificity in the α-amylase family of enzymes. Biochim. Biophys. Acta, 1546, 2001, 1-20.

MACGREGOR, E.A., JESPERSEN, H.M., SVENSSON, B.: A circularly permuted α-amylase-type α/β-barrel structure in glucan-synthesizing glucosyltransferases. FEBS Lett., 378, 1996, 263-266.

MACGREGOR, E.A., SVENSSON, B.: A super-secondary structure predicted to be common to several α-1,4-D-glucan-cleaving enzymes. Biochem. J., 259, 1989, 145-152.

MACHOVIC, M., JANECEK, S.: The invariant residues in the α-amylase family: just the catalytic triad. Biologia, 58, 2003, 1127-1132.

MACHOVIC, M., JANECEK, S.: Starch-binding domains in the post-genome era. Cell. Mol. Life Sci., 63, 2006a, 2710-2724.

MACHOVIC, M., JANECEK, S.: The evolution of putative starch-binding domains. FEBS Lett., 580, 2006b, 6349-6356.

MACHOVIC, M., JANECEK, S.: Domain evolution in the GH13 pullulanase subfamily with focus on the carbohydrate-binding module family 48. Biologia, 63, 2008, 1053-1064.

MACHOVIC, M., SVENSSON, B. MACGREGOR, E.A., JANECEK, S.: A new clan of CBM families based on bioinformatics of starch-binding domains from families CBM20 and CBM21. FEBS J., 272, 2005, 5497-5513.

MATSUURA, Y.: A possible mechanism of catalysis involving three essential residues in the enzymes of α-amylase family. Biologia, 57 (Suppl. 11), 2002, 21-27.

MATSUURA, Y., KUSUNOKI, M., HARADA, W., KAKUDO, M.: Structure and possible catalytic residues of Taka-amylase A. J. Biochem., 95, 1984, 697-702.

MCCARTER, J.D., WITHERS, S.G.: Mechanisms of enzymatic glycoside hydrolysis. Curr. Opin. Struct. Biol., 4, 1994, 885-892.

MIKAMI, B., HEHRE, E.J., SATO, M., KATSUBE, Y., HIROSE, M., MORITA, Y., SACCHETTINI J.C.: The 2.0 Å resolution structure of soybean β-amylase complexed with α-cyclodextrin. Biochemistry, 32, 1993, 6836-6845.

MURAKAMI, T., KANAI, T., TAKATA, H., KURIKI, T., IMANAKA, T.: A novel branching enzyme of the GH-57 family in the hyperthermophilic archaeon *Thermococcus kodakaraensis* KOD1. J. Bacteriol., 188, 2006, 5915-5924.

NAKAI, H., OKUYAMA, M., KIM, Y.M., SABURI, W., WONGCHAWALIT, J., MORI, H., CHIBA, S., KIMURA, A.: Molecular analysis of α-glucosidase belonging to GH-family 31. Biologia, 60 (Suppl. 16), 2005, 131-135.

NAKAJIMA, R., IMANAKA, T., AIBA, S.: Comparison of amino-acid-sequences of 11 different α-amylases. Appl. Microbiol. Biotechnol., 23, 1986, 355-360.

PANDEY, A., NIGAM, P., SOCCOL, C.R., SOCCOL, V.T., SINGH, D., MOHAN, R.: Advances in microbial amylases. Biotechnol. Appl. Biochem., 31, 2000, 135-152.

PENNINGA, D., VAN DER VEEN, B.A., KNEGTEL, M.R., VAN HIJUM, S.A., ROZEBOOM, H.J., KALK, K.H., DIJKSTRA, B.W., DIJKHUIZEN, L.: The raw starch-binding domain of cyklodextrin glycosyltransferase from *Bacillus circulans* strain 251. J. Biol. Chem., 271, 1996, 32777-32784.

PIJNING, T., VUJICIC-ZAGAR, A., KRALJ, S., EEUWEMA, W., DIJKHUIZEN, L., DIJKSTRA, B.: Biochemical and crystallographic characterization of a glucansucrase from *Lactobacillus reuteri* 180. Biocatal. Biotransform., 26, 2008, 12-17.

PRZYLAS, I., TOMOO, K., TERADA, Y., TAKAHA, T., FUJII, K., SAENGER, W., STRATER, N.: Crystal structure of amylomaltase from *Thermus aquaticus*, a glycosyltransferase catalysing the production of large cyclic glucans. J. Mol. Biol., 296, 2000, 873-886.

PUJADAS, G., RAMIREZ, F.M., VALERO, R., PALAU, J.: Evolution of β-amylase: patterns of variation and conservation in subfamily sequences in relation to parsimony mechanisms. Proteins, 25, 1996, 456-472.

QIAN, M., HASER, R., PAYAN, F.: Structure and molecular model refinement of pig pancreatic α-amylase at 2.1 Å resolution. J. Mol. Biol., 231, 1993, 785-799.RAHA, M., KAWAGISHI, I., MUELLER, V., KIHARA, M., MACNAB, R.M.: *Escherichia coli* produces a cytoplasmic α-amylase, *AmyA*. J. Bacteriol., 174, 1992, 6644-6652.

RIGDEN D.J.: Iterative database searches demonstrate that glycoside hydrolase families 27, 31, 36 and 66 share a common evolutionary origin with family 13. FEBS Lett., 53, 2002, 17-22.

RODRIGUEZ-SANOJA, R., OVIEDO, N., SANCHEZ, S.: Microbial starch-binding domain. Curr. Opin. Microbiol., 8, 2005, 260-267.

SAUER, J., SIGURSKJOLD, B.W., CHRISTENSEN, U., FRANDSEN, T.P., MIRGORODSKAYA, E., HARRISON, M., ROEPSTORFF, P., SVENSSON, B.: Glucoamylase: structure/function relationships, and protein engineering. Biochim. Biophys. Acta, 1543, 2000, 275-293.

SIM, L., QUEZADA-CALVILLO, R., STERCHI, E.E., NICHOLS, B.L., ROSE, D.R.: Human intestinal maltase-glucoamylase: crystal structure of the N-terminal catalytic subunit and basis of inhibition and substrate specificity. J. Mol. Biol., 375, 2008, 782-792.

SORIMACHI, K., LE GAL-COEFFET, M.F., WILLIAMSON, G., ARCHER, D.B., WILLIAMSON, M.P.: Solution structure of the granular starch binding domain of *Aspergillus niger* glucoamylase bound to β-cyclodextrin. Structure, 15, 1997, 647-661.

SOUTHALL, S.M., SIMPSON, P.J., GILBERT, H.J., WILLIAMSON, G., WILLIAMSON, M.P.: The starch-binding domain from glucoamylase disrupts the structure of starch. FEBS Lett., 447, 1999, 58-60.

STAM, M.R., DANCHIN, E.G., RANCUREL, C., COUTINHO, P.M., HENRISSAT, B.: Dividing the large glycoside hydrolase family 13 into subfamilies: towards improved functional annotations of α-amylase-related proteins. Protein Eng. Des. Sel., 19, 2006, 555-562.

STRATER, N., PRZYLAS, I., SAENGER W., TERADA Y., FUJI, K., TAKAHA, T.: Structural basis of the synthesis of large cycloamyloses by amylomaltase. Biologia, 57 (Suppl. 11), 2002, 93-99**.**

SVENSSON, B.: Protein engineering in the α-amylase family: catalytic mechanism, substrate specificity, and stability. Plant Mol. Biol., 25, 1994, 141-157.

SVENSSON, B., JESPERSEN, H., SIERKS, M., MACGREGOR, E.A.: Sequence homology between putative raw starch-binding domains from different starch degrading enzymes. Biochem. J., 264, 1989, 309-311.

TAKAHA, T., YANASE, M., OKADA, S., SMITH, S.M.: Disproportionating enzyme (4-α-glucanotransferase; EC 2.4.1.25) of potato. Purification, molecular cloning, and potential role in starch metabolism. J. Biol. Chem., 268, 1993, 1391-1396.

TAKATA, H., KURIKI, T., OKADA, S., TAKESADA, Y., IIZUKA, M., MINAMIURA, N., IMANAKA, T.: Action of neopullulanase. Neopullulanase catalyzes both hydrolysis and transglycosyltaion at α-(1->4)- and α-(1->6)-glucosidic linkages. J. Biol. Chem., 267, 1992, 18447-18452.

TERADA, Y., FUJII, K., TAKAHA, T., OKADA, S.: *Thermus aquaticus* ATCC 33923 amylomaltase gene cloning and expression and enzyme characterization: production of cycloamylose. Appl. Environ. Microbiol., 65, 1999, 910-915.

UITDEHAAG, J.C.M., MOSI, R., KALK, K.H., VAN DER VEEN, B.A., DIJKHUIZEN, L., WITHERS, S.G., DIJKSTRA, B.W.: X-ray structures along the reaction pathway of cyclodextrin glycosyltransferase elucidate catalysis in the α-amylase family. Nat. Struct. Biol., 6, 1999, 432-436.

VAN DER KAAIJ, R.M., JANECEK, S., VAN DER MAAREL, M.J., DIJKHUIZEN, L.: Phylogenetic and biochemical characterization of a novel cluster of intracellular fungal α-amylase enzymes. Microbiology, 153, 2007, 4003-4015.

VAN DER MAAREL, M.J.E.C., VAN DER VEEN, B., UITDEHAAG, J.C.M., LEEMHUIS, H., DIJKHUIZEN, L.: Properties and applications of starch-converting enzymes of the α-amylase family. J. Biotechnol., 94, 2002, 137-155.

VAN HIJUM, S.A., KRALJ, S., OZIMEK, L.K., DIJKHUIZEN, L., VAN GEEL-SCHUTTEN, I.G.: Structure-function relationships of glucansucrase and fructansucrase enzymes from lactic acid bacteria. Microbiol. Mol. Biol. Rev., 70, 2006, 157-176.

VIHINEN, M., MANTSALA, P.: Microbial amylolytic enzymes. Crit. Rev. Biochem. Mol. Biol., 24, 1989, 329-418.

YUUKI, T., NOMURA, T., TEZUKA, H., TSUBOI, A., YAMAGATA, H., TSUKAGOSHI, N., UDAKA, S.: Complete nucleotide sequence of a gene coding for heat- and pH-stable α-amylase of *Bacillus licheniformis*: comparison of the amino acid sequences of three bacterial liquefying α-amylases deduced from the DNA sequences. J. Biochem., 98, 1985, 1147-1156.

ZONA, R., CHANG-PI-HIN, F., O'DONOHUE, M.J., JANECEK, S.: Bioinformatics of the family 57 glycoside hydrolases and identification of catalytic residues in amylopullulanase from *Thermococcus hydrothermalis*. Eur. J. Biochem., 271, 2004, 2863-2872.