Biologia, Bratislava, **58**/6: 1127—1132, 2003 **Short Communication**

# The invariant residues in the $\alpha$-amylase family: just the catalytic triad

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Machovič, M. & Janeček, Š., The invariant residues in the $\alpha$-amylase family: just the catalytic triad. Biologia, Bratislava, **58:** 1127—1132, 2003; ISSN 0006-3088. (Biologia). ISSN 1335-6399 (Biologia. Section Cellular and Molecular Biology).

The $\alpha$-amylase family is also known as the glycoside hydrolase clan H (GH-H) consisting of three glycoside hydrolase families GH-13, GH-70, and GH-77. Although the entire $\alpha$-amylase family can be characterised by several well-conserved sequence regions, the number of the amino acid residues conserved totally invariantly throughout the family has been established in the last few years to be only 4. These were the three catalytic residues (two aspartates and one glutamate) plus the arginine in the position *i-2* with respect to the catalytic nucleophile Asp located near the strand $\beta 4$ of the $(\beta/\alpha)_8$-barrel. The present protein bioinformatics study deals with the 4-$\alpha$-glucanotransferase from *Borrelia burgdorferi*, a putative member of the GH-77. The sequence of this hypothetical protein, present in the complete genome sequence of the Lyme disease spirochete, possesses the otherwise invariant $\beta 4$-strand Arg substituted by lysine. It could be the first relevant example of $\alpha$-amylase family member with only 3 invariant residues, i.e. the catalytic triad. The possibility of a sequencing error (Arg→Lys) is disregarded since this protein exhibits substitutions at several other important positions. Its three-dimensional structure was modelled and briefly discussed.

Key words: $\alpha$-amylase family, glycoside hydrolase family 77, invariantly conserved residues, catalytic triad, 4-$\alpha$-glucanotransferase.

## Introduction

The $\alpha$-amylase family (for a review see MacGregor et al., 2001) corresponds to the clan GH-H at present consisting of three families of glycoside hydrolases (GHs), GH-13, GH-70 and GH-77 (Coutinho & Henrissat, 1999). Structurally these enzymes are $(\beta/\alpha)_8$-barrel proteins (Pujadas & Palau, 1999) using a retaining mechanism when acting on glycosidic bonds (McCarter & Withers, 1994). The main family of the clan, GH-13, with more than 25 different enzyme specificities and ∼1400 sequences available, is the largest family of glycoside hydrolases (CAZy web-site: http://afmb.cnrs-mrs.fr/CAZY/GH_13.html). The GH-70 covers the glucan-synthesising glucosyltransferases, such as dextran sucrase and alternan sucrase, that adopt a circularly permuted fold of the $\alpha$-amylase-type $(\beta/\alpha)_8$-barrel (MacGregor et al., 1996). The GH-77 contains only one specificity, the amylomaltase (synonym 4-$\alpha$-glucanotransferase). The main feature discriminating the GH-77 from the GH-13 members is that GH-77 amylomaltases do not possess the domain C succeeding the GH-H catalytic $(\beta/\alpha)_8$-barrel (Przylas et al., 2000b).

Concerning the degree of sequence identity and

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similarity or the number of invariant residues conserved throughout the entire clan GH-H, the situation has been as follows. The sequence similarity was recognised a long time ago to be extremely low (about 10%) for microbial, plant and animal $\alpha$-amylases (Nakajima et al., 1986). Later when the family grew up (i.e. many sequences, sources, and specificities), the number of identical residues in the family decreased to 8-10 amino acids (Janeček, 1994a; Svensson, 1994). At the time of available complete genome sequences there have been only 4 GH-H residues known to be invariantly conserved (Janeček, 2002). These were the 3 catalytic residues corresponding to Asp206, Glu230, and Asp297 (Taka-amylase A numbering; Matsuura et al., 1984) located at the C-terminal ends of the strands $\beta$4, $\beta$5, and $\beta$7, respectively, of the $(\beta/\alpha)_8$-barrel, plus the $\beta$4-strand arginine in position $i$-2 with respect to the catalytic $\beta$4-strand aspartate (equivalent with Arg204 of Taka-amylase A). This arginine is involved in the $Cl^-$-binding site of chloride-dependent $\alpha$-amylases (D'Amico et al., 2000). The amino acid sequences of the enzymes from the $\alpha$-amylase family can be characterised by 4 to 7 short conserved stretches, the so-called conserved sequence regions (Janeček, 2002). The regions I, II, III, IV, VI, and VII cover the strands $\beta$3, $\beta$4, $\beta$5, $\beta$7, $\beta$2, and $\beta$8 of the catalytic $(\beta/\alpha)_8$-barrel, respectively (Nakajima et al., 1986; Janeček, 1994b), while the fifth conserved sequence region (region V) is located near the C-terminal end of domain B that protrudes from the barrel in the place of the loop between the strand $\beta$3 and helix $\alpha$3 (Janeček, 1992, 1995).

The main goal of the present study was to demonstrate the fact that the number of residues conserved invariantly throughout the $\alpha$-amylase family had decreased to only three amino acids, i.e. the catalytic triad. The first example, which definitively breaks the invariance of the arginine in the position *i-2* with respect to the $\beta$4 catalytic aspartate, is the 4-$\alpha$-glucanotransferase from *Borrelia burgdorferi*. It belongs to the GH-77 and contains lysine in the position of the otherwise invariant arginine. Although at present this 4-$\alpha$-glucanotransferase is a putative protein only coming from the sequencing the complete genome of the Lyme disease spirochete (Fraser et al., 1997), its sequence features are clearly characteristic of the enzymatically active members of the $\alpha$-amylase family. On the other hand, the Arg→Lys substitution as well as the mutations observed in several other well-conserved positions could make the 4-$\alpha$-glucanotransferase from *B. burgdorferi* a target with great potential for the $\alpha$-amylase family protein engineers and designers.

## Background

The CAZy system (Coutinho & Henrissat, 1999; http://afmb.cnrs-mrs.fr/CAZY/) classifying the $\alpha$-amylase family as the clan GH-H served as the base for studying the sequences and their taxonomical origins. The following amino acid sequences were retrieved from GenBank with GenPept (Benson et al., 2000; http://www.ncbi.nlm.nih.gov/Genbank/) and SwissProt with TrEMBL (Bairoch & Apweiler, 2000; http://www.expasy.org/sprot/): GH-13: $\alpha$-amylase (Toda et al., 1982), cyclodextrin glucanotransferase (Nitschke et al., 1990), oligo-1,6-glucosidase (Watanabe et al., 1990), maltotetraohydrolase (Fujita et al., 1989), isoamylase (Amemura et al., 1988), neopullulanase-like "$\alpha$-amylase" TVA II (Tonozuka et al., 1993), maltogenic amylase (Kim et al., 1999), maltogenic $\alpha$-amylase (Diderichsen & Christiansen, 1988), maltooligosyltrehalose hydrolase (Kobayashi et al., 1996), amylosucrase (Potocki De Montalk et al., 1999), maltosyltransferase (Meissner & Liebl, 1998), neopullulanase-like "$\alpha$-amylase" TVA I (Tonozuka et al., 1995), 4-$\alpha$-glucanotransferase (Heinrich et al., 1994), cyclomaltodextrinase (Kim et al., 1998), branching enzyme (Baecker et al., 1986), neopullulanase (Kuriki & Imanaka, 1989); GH-70: glucosyltransferase (Ferretti et al., 1987); GH-77: amylomaltases (4-$\alpha$-glucanotransferase): *Clostridium butyricum* (Goda et al., 1997), *Chlamydomonas reinhardtii* (Wattebled et al., 2003), *Escherichi coli* (Pugsley & Dubreuil, 1988), *Solanum tuberosum* (Takaha et al., 1993), *Thermus aquaticus* (Terada et al., 1999), *Borrelia burgdorferi* (Fraser et al., 1997).

The three-dimensional structure of the GH-77 *Thermus aquaticus* amylomaltase was retrieved from the Protein Data Bank (Berman et al., 2002;

Fig. 1. (**a**) Selected conserved sequence regions in the $\alpha$-amylase family. The regions I, II, III, IV, and VI (for a review, see Janeček, 2002) correspond to the strands $\beta$3, $\beta$4, $\beta$5, $\beta$7, and $\beta$2, respectively, of the catalytic $(\beta/\alpha)_8$-barrel domain. The clan GH-H representative members are shown for which the three-dimensional structure was solved (except for the family GH-70 with circularly permuted catalytic $(\beta/\alpha)_8$-barrel). (**b**) Corresponding selected conserved sequence regions in the representative amylomaltases (4-$\alpha$-glucanotransferases) of the GH-77. Colour code: catalytic aspartates and glutamate – blue; invariant arginine – yellow; functional histidines – green; non-conserved residues – pink; additional conserved residues in GH-77 – black; substituted residues in the 4-$\alpha$-glucanotransferase from *Borrelia burgdorferi* – red. The three invariant residues of the clan GH-H are signified by asterisk. (**c**) Stereo view of the residues from the active site of the experimentally determined three-dimensional structure of the GH-77 amylomaltase from *Thermus aquaticus* (Przylas et al., 2000b). (**d**) Stereo view of the residues from the active site of the theoretical structural model of the GH-77 4-$\alpha$-glucanotrasferase from *Borrelia burgdorferi*.

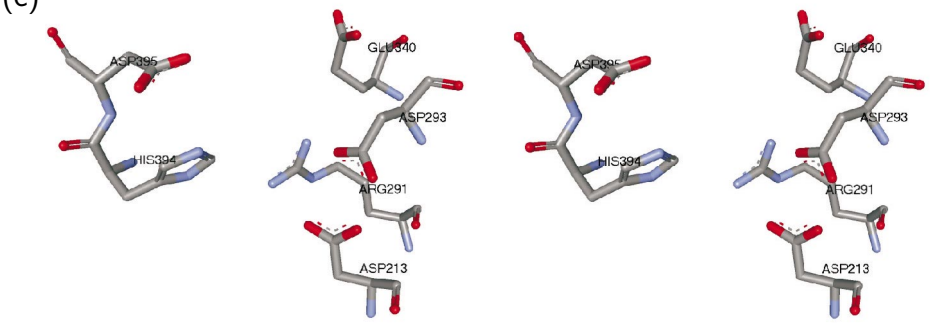
(a)

| Clan GH-H representatives | β2 VI | β3 I | β4 II | β5 III | β7 IV |
|---|---|---|---|---|---|
| ***GH 13:*** | | | * | * | * |
| α-Amylase | *56_GFTAIWITP* | *117_DVVANH* | *202_GLRIDTVKH* | *226_YCIGEVLD* | *292_FVENHD* |
| Cyclodextrin glycosyltransferase | *70_GVTALWISQ* | *135_DFAPNH* | *225_GIRVDAVKH* | *253_FTFGEWFL* | *323_FIDNHD* |
| Oligo-1,6-glucosidase | *44_GIDVIWLSP* | *98_DLVVNH* | *195_GFRMDVINF* | *251_MTVGEMPG* | *324_YWNNHD* |
| Maltotetraohydrolase | *50_GFSAIWMPV* | *112_DVVPNH* | *189_GFRFDFVRG* | *215_FCVGELWK* | *288_FVDNHD* |
| Isoamylase | *218_GVTAVEFLP* | *292_DVVYNH* | *371_GFRFDLASV* | *431_DLFAEPWA* | *505_FIDVHD* |
| Neopullulanase-like (TVA II) | *186_GVTALYFTP* | *239_DAVFNH* | *321_GWRLDVANE* | *350_LIVGEIWH* | *416_LLDSHD* |
| Maltogenic amylase | *189_GITGIYLTP* | *242_DAVFNH* | *324_GWRLDVANE* | *353_YILGEIWH* | *419_LLGSHD* |
| Maltogenic α-amylase | *65_GVTTIWLSP* | *127_DFVPNH* | *221_GLRLDAVKH* | *249_FLVGEWYG* | *321_FIDNHD* |
| Maltooligosyltrehalose hydrolase | *132_GITAIEIMP* | *187_DVVYNH* | *248_GFRLDAVHA* | *279_IVIAESDL* | *372_YIQNHD* |
| Amylosucrase | *134_GLTYLHLMP* | *190_DFIFNH* | *290_ILRMDAVAF* | *332_FFKSEAIV* | *396_YVRSHD* |
| Maltosyltransferase | *133_GADAIYLLP* | *201_DFIPRT* | *381_GARLDMGHA* | *410_VMIAEELD* | *463_SVETED* |
| Neopullulanase-like (TVA I) | *205_GANILYLNP* | *262_DGVFNH* | *352_GWRLDAAQY* | *392_AIIGEYWG* | *467_FLSNHD* |
| 4-α-Glucanotransferase | *36_GIDFVWLMP* | *89_DLPIHH* | *182_GFRFDAAKH* | *212_IFLAEIWA* | *273_FTSNHD* |
| Branching enzyme | *280_GFTHLELLP* | *335_DWVPGH* | *401_ALRVDAVAS* | *454_VTMAEEST* | *521_LPLSHD* |
| Cyclomaltodextrinase | *185_GVNAVYFTP* | *238_DAVFNH* | *321_GWRLDVANE* | *350_YILGEVWH* | *416_LLDSHD* |
| Neopullulanase | *189_GITGIYLTP* | *242_DAVFNH* | *324_GWRLDVANE* | *353_YILGEIWH* | *419_LLGSHD* |
| ***GH 70:*** | | | | | |
| Glucosyltransferase | *828_GITDFEMAP* | *894_DWVPDQ* | *411_SIRVDAVDN* | *449_VSIVEAWS* | *521_FARAHD* |
| ***GH 77:*** | | | | | |
| Amylomaltase | *40_GGRYWQVLP* | *213_DMPIFV* | *289_LVRIDHFRG* | *336_PVLAEDLG* | *390_YTGTHD* |

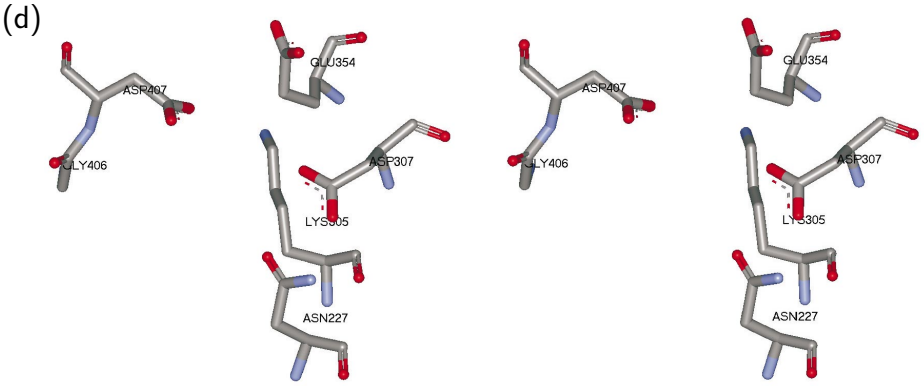
(b)

| Amylomaltases (GH 77) | VI | I | II | III | IV |
|---|---|---|---|---|---|
| *Clostridium butyricum* | *30_GQKYWQILP* | *204_DIPIYI* | *281_ILRIDHFRG* | *328_EIIAEDLG* | *380_YTGTHD* |
| *Chlamydomonas reinhardtii* | *119_GMQCWQLLP* | *297_DMPIYV* | *373_ECRIDHFRG* | *420_PILAEDLG* | *472_YPGTHD* |
| *Escherichi coli* | *178_GGSFIGLNP* | *374_DLAVGV* | *444_ALRIDHVMS* | *492_MVIGEDLG* | *542_VAATHD* |
| *Solanum tuberosum* | *61_GCSLWQVLP* | *241_DMPIYV* | *317_EFRIDHFRG* | *364_NIIAEDLG* | *416_YTGTHD* |
| *Thermus aquaticus* | *40_GGRYWQVLP* | *213_DMPIFV* | *289_LVRIDHFRG* | *336_PVLAEDLG* | *390_YTGTHD* |
| *Borrelia burgdorferi* | *49_SQSYWQMFA* | *227_NVPFFI* | *303_IIKHDHFRG* | *350_KIWVEDFQ* | *402_YTGSGD* |

(c)




(d)



http://www.rcsb.org/pdb/) under the PDB code 1ESW (PRZYLAS et al., 2000b). The three-dimensional structure modelling of the *B. burgdorferi* 4-$\alpha$-glucanotransferase was performed using the SWISS-MODEL automated server (GUEX & PEITSCH, 1997; GUEX et al., 1999; http://swissmodel.expasy.org/). The protein structures were displayed using the program WebLab-ViewerLite (Molecular Simulations, Inc.).

PSI-Blast (ALTSCHUL et al., 1997; http://www.ncbi.nlm.nih.gov/BLAST/) was used for performing the searches in the molecular-biology databases (using the default parameters) in order to retrieve for comparison all the relevant $\alpha$-amylase family enzymes and hypothetical proteins using as the query the entire sequence of the 4-$\alpha$-glucanotransferase from *B. burgdorferi*.

## Results and discussion

The selected conserved sequence regions of the $\alpha$-amylase family members whose three-dimensional structures have already been solved are shown in Fig. 1a. The regions of the GH-70 glucan-synthesising glucosyltransferase were extracted based on the prediction study (MACGREGOR et al., 1996) and site-directed mutagenesis (DEVULAPALLE et al., 1997), since no three-dimensional structure is available for GH-70 at present (COUTINHO & HENRISSAT, 1999). It is clear that the clan GH-H contains the invariant catalytic triad consisting of two aspartates (at strands $\beta$4 and $\beta$7) and one glutamate (at strand $\beta$5). The two functionally important histidines (at strands $\beta$3 and $\beta$7), although strongly conserved and apparently essential for several specificities (MACGREGOR et al., 2001), are not present in GH-13 maltosyltransferase (both His) and the members of both GH-70 and GH-77 (the $\beta$3 His) (Fig. 1a). The histidines have nevertheless been demonstrated to be critical in transition-state stabilisation (SØGAARD et al., 1993). The fourth invariant residue of the $\alpha$-amylase family had seemed to be the arginine in the position *i-2* with respect to the catalytic $\beta$4-strand aspartate (JANEČEK, 2002). This is no longer true because the sequence of GH-77 4-$\alpha$-glucanotransferase from *Borrelia burgdorferi* has the arginine substituted by a lysine (Fig. 1b). This substitution is not a general feature characteristic of the GH-77 since it was not possible to detect more examples with such Arg→Lys substitution in the sequence databases by PSI-Blast. Moreover, the *B. burgdorferi* 4-$\alpha$-glucanotransferase exhibits several remarkable sequence features that discriminate it slightly from the rest of the GH-77. These are (Fig. 1b): Pro→Ala in region VI ($\beta$2), Asp→Asn in region I ($\beta$3), Ile(Leu)→Trp and Leu-Gly→Phe-Gln in region III ($\beta$5), and His→Gly in region IV ($\beta$7).

With regard to the eventual protein function, catalytic activity, and enzyme specificity of the *B. burgdorferi* 4-$\alpha$-glucanotransferase, it is worth mentioning that this amino acid sequence is deduced from the nucleotide sequence of the Lyme disease spirochete genome (FRASER et al., 1997), i.e. it is only a translated ORF. The 4-$\alpha$-glucanotransferase specificity was thus assigned due to sequence similarity with other GH-77 4-$\alpha$-glucanotransferases/amylomaltases. The conserved catalytic triad, however, supports the possibility that the function has been saved. For example, the Arg→Lys mutant of *Bacillus stearothermophillus* $\alpha$-amylase had 12% of the specific activity of the parental enzyme (VIHINEN et al., 1990) and the same mutant of the maize branching enzyme retained also some residual activity (LIBESSART & PREISS, 1998). The eventuality of a sequencing error (Arg→Lys exchange) should be disregarded because the *B. burgdorferi* 4-$\alpha$-glucanotransferase contains several unusual substitutions in positions characteristic of clan GH-H (Fig. 1b).

To shed more light on these theoretical observations the three-dimensional structure of the putative 4-$\alpha$-glucanotransferase from *B. burgdorferi* was modelled using the X-ray structure of *Thermus aquaticus* amylomaltase (PRZYLAS et al., 2000b) as the template. *B. burgdorferi* hypothetical protein exhibits all structural features typical for the amylomaltase from *T. aquaticus*, e.g. also the helical region succeeding the strand $\beta$2 of the catalytic $(\beta/\alpha)_8$-barrel that is unique for the GH-77 members (STRÄTER et al., 2002). Although it is not possible to draw the unambiguous conclusions from the modelled structure, the exchanged important active-site residues probably resulting in different side-chain orientations (Fig. 1c,d) could cause some problems in achieving the predicted 4-$\alpha$-glucanotransferase activity. The catalytic nucleophile Asp293 in *T. aquaticus* amylomaltase is hydrogen bonded just to Arg291 (PRZYLAS et al., 2000a), the residue that is substituted by lysine in the *B. burgdorferi* protein. Any $\alpha$-amylase family member with Arg→Lys substitution in the position *i-2* with respect to the catalytic nucleophile may be lacking in the strongly basic $\delta$-guanido group of the arginine (VIHINEN et al., 1990).

Taking all this into account it is clear that the putative 4-$\alpha$-glucanotransferase from *B. burgdorferi* can become an attractive model for experimental studies within the entire clan GH-H. If it is a functional amylolytic enzyme, it would mean that even the catalytic triad alone is enough for the activity in general and the other active-site residues can thus be suitable substituted. This would open the door for novel ideas in the $\alpha$-amylase family protein engineering and design. Simultaneously, it would be of special interest to confirm, revise and/or find out the exact enzyme specificity of the hypothetical 4-$\alpha$-glucanotransferase from *B. burgdorferi*. In the future work we would like therefore to focus on the cloning, expression and biochemical characterisation of this protein.

## Acknowledgements

This work was financially supported in part by the VEGA grant no. 2/2057/23.

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Received July 11, 2003
Accepted August 19, 2003