Extremophiles (2008) 12:61–68
DOI 10.1007/s00792-007-0105-y

REVIEW

The α-L-fucosidase from *Sulfolobus solfataricus*

Beatrice Cobucci-Ponzano · Fiorella Conte · Mosè Rossi · Marco Moracci

Received: 6 November 2006 / Accepted: 17 July 2007 / Published online: 9 August 2007

Abstract Glycoside hydrolases form hyperthermophilic archaea are interesting model systems for the study of catalysis at high temperatures and, at the moment, their detailed enzymological characterization is the only approach to define their role in vivo. Family 29 of glycoside hydrolases classification groups α-L-fucosidases involved in a variety of biological events in *Bacteria* and *Eukarya*. In *Archaea* the first α-L-fucosidase was identified in *Sulfolobus solfataricus* as interrupted gene expressed by programmed −1 frameshifting. In this review, we describe the identification of the catalytic residues of the archaeal enzyme, by means of the chemical rescue strategy. The intrinsic stability of the hyperthermophilic enzyme allowed the use of this method, which resulted of general applicability for β and α glycoside hydrolases. In addition, the presence in the active site of the archaeal enzyme of a triad of catalytic residues is a rather uncommon feature among the glycoside hydrolases and suggested that in family 29 slightly different catalytic machineries coexist.

Keywords Glycoside hydrolase · Chemical rescue · Nucleophile · Acid/base · Catalytic triad

Communicated by D.A. Cowan.

B. Cobucci-Ponzano (✉) · F. Conte · M. Rossi · M. Moracci
Institute of Protein Biochemistry,
Consiglio Nazionale delle Ricerche,
Via P. Castellino 111, 80131 Naples, Italy
e-mail: b.cobucciponzano@ibp.cnr.it

M. Rossi
Dipartimento di Biologia Strutturale e Funzionale,
Università di Napoli "Federico II",
Complesso Universitario di Monte S. Angelo,
Via Cinthia 4, 80126 Naples, Italy

Introduction

Carbohydrates serve as structural components and energy source of the cell and are involved in a variety of molecular recognition processes in intercellular communication (Varki 1993; Sears and Wong 1996). Consequently, glycoside hydrolases play important roles in biological systems ranging from the degradation of polysaccharides as food source through to the modification of glycoconjugates on the surfaces of proteins and cells. Among the available glycoside hydrolases, the enzymes from hyperthermophiles are of particular interest for both basic and applied research. In fact, the function of the glycoconjugates identified in hyperthermophiles and of the enzymes involved in their synthesis and degradation is still largely unknown (Lower and Kennelly 2002). In addition, hyperthermophilic glycosidases are interesting model systems in basic research for the study of protein adaptation to heat and, since they catalyze single substrate reactions by following well-known mechanisms, they are the ideal candidates for the study of catalysis at high temperatures. Furthermore, they are particularly appealing for industrial applications as they show peculiar enzymological properties and can withstand the harsh operational conditions adopted in industrial applications. Beside this, the unique substrate specificities or reduced substrate/product inhibition allow the synthesis of new products that are not produced by their mesophilic counterparts (Fischer et al. 1996). On the other hand, the harsh conditions of growing of these organisms have hindered microbiological and genetic studies in vivo; therefore, the isolation of the genes encoding for hyperthermophilic glycosidases and the detailed enzymological characterization of these enzymes is the only approach to define their role in vivo.

Glycoside hydrolases are classified on the basis of their amino acid sequence similarity in about 100 families (http://www.cazy.org) and 14 clans showing conserved three-dimensional (3D) structures. These enzymes follow two distinct mechanisms which are termed *retaining* or *inverting* if the enzymatic cleavage of the glycosidic bond liberates a sugar hemiacetal with the same or the opposite anomeric configuration of the glycosidic substrate, respectively. *Inverting* glycoside hydrolases operate with a one step, single-displacement mechanism with the assistance of a general acid and a general base group in the active site (McCarter and Withers 1994). Instead, *retaining* enzymes follow a two-step mechanism with formation of a covalent glycosyl-enzyme intermediate (Fig. 1). The carboxyl group in the active centre functions as a general acid/base catalyst, and the carboxylate functions as the nucleophile of the reaction (Koshland 1953). In the first step (glycosylation step), the nucleophile attacks the anomeric group of the substrate, while the acid/base catalyst, acting in this step as a general acid, protonates the glycosidic oxygen, thereby assisting the leaving of the aglycon moiety. The concerted action of the two amino acids leads to the formation of a covalent glycosyl-enzyme intermediate (Sinnot 1990; McCarter and Withers 1994). In the second step (deglycosylation step), the glycosyl-enzyme intermediate is cleaved by a water molecule that acts as nucleophile being polarized by the general base catalyst. The product of the reaction retained the anomeric configuration of the substrate. When an acceptor different from water, such as an alcohol or a sugar, intercepts the reactive glycosyl-enzyme intermediate, *retaining* enzymes work in transglycosylation mode; the interglycosidic linkage of the product maintains the same anomeric configuration of the substrate. This property makes the *retaining* glycosyl hydrolases interesting tools for the synthesis of carbohydrates. Despite the differences, the two mechanisms show significant similarities: they both operate via transition states with substantial oxocarbenium ion character and have a catalytic dyad with a pair of carboxylic acids directly involved in catalysis.

The determination of the reaction mechanism and the identification of key active-site residues in glycoside hydrolases are crucial issues to allow the classification of these enzymes (Henrissat and Bairoch 1993; Henrissat and Davies 1997), to unravel the catalytic machinery (McCarter and Withers 1994; Zechel and Withers 2000), and to produce enzymes with novel characteristics (Perugino et al. 2004).

Family 29 of glycoside hydrolases (GH29) groups α-L-fucosidases (EC 3.2.1.51) from plants, vertebrates, and pathogenic microbes of plants and humans (Henrissat 1991). α-L-fucosidases are exo-glycosidases capable of cleaving α-linked L-fucose residues from glycoconjugates, in which the most common linkages are α-(1-2) to galactose and α-(1-3), α-(1-4), and α-(1-6) to *N*-acetyl-glucosamine residues. These compounds are involved in a variety of biological events as growth regulators and as the glucidic part of receptors in signal transduction, cell–cell interactions, and antigenic response (Vanhooren and Vandamme 1999). The central role of fucose derivatives in biological processes explains the interest in α-L-fucosidase and fucosyl-transferase activities. α-L-fucosidases in higher plants and in mammals are associated with different mechanisms of cell growth and regulation, since they are involved in the modification of fucosylated glucans (Staudacher et al. 1999). In plants, α-L-fucosylated oligosaccharides derived from xyloglucan have been shown to regulate auxin- and acid pH-induced growth (de La Torre et al. 2002). In mammals, oligosaccharides containing fucose are reported to play important roles in a variety of physiological and pathological events (Xiang and Bernstein 1992; Wiese et al. 1997; Listinsky et al. 1998; Mori et al. 1998; Noda et al. 1998; Russell et al. 1998; Michalski and Klein 1999; Rapoport and Pendu 1999).

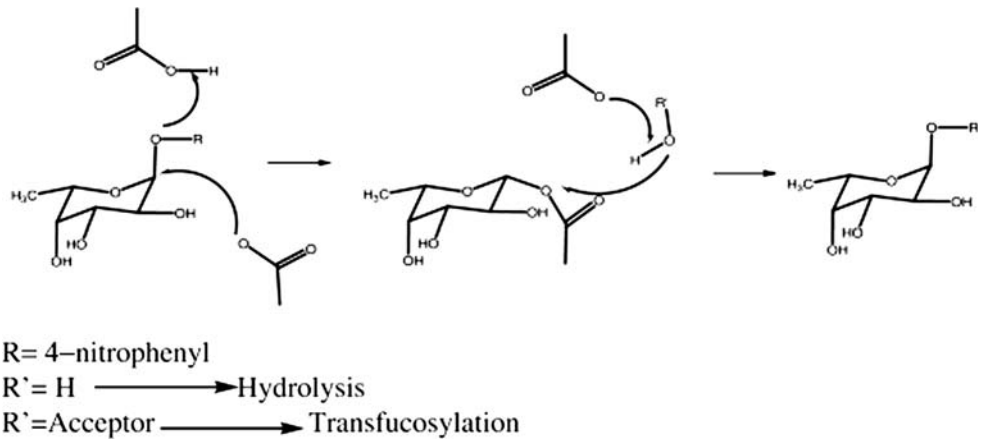

Fig. 1 *Retaining* reaction mechanism of α-L-fucosidases

Here, the characterization of the reaction mechanism and the identification of the catalytic residues of the first archaeal α-L-fucosidase identified in the hyperthermophile *Sulfolobus solfataricus* are briefly reviewed.

General features of the α-L-fucosidase

The first archaeal α-L-fucosidase has been identified and characterized recently (Cobucci-Ponzano et al. 2003a). The analysis of the genome of the hyperthermophilic archaeon *S. solfataricus* (She et al. 2001) revealed the presence of two open reading frames (ORFs), SSO11867 and SSO3060, encoding for 81 and 426 amino acid polypeptides that are homologous to the *N*- and the *C*-terminal parts, respectively, of full-length bacterial and eukaryal GH29 fucosidases (Henrissat 1991). The two ORFs are separated by a −1 frameshift and, to produce a single polypeptide, a single base was inserted by site-directed mutagenesis in the region of overlap between SSO11867 and SSO3060, restoring a single reading frame between the ORFs. The single ORF obtained was used to express the enzyme in *Escherichia coli* (Cobucci-Ponzano et al. 2003a). The recombinant enzyme, Ssα-fuc, is a nonamer of 57 kDa molecular mass subunits in solution and is highly active and specific for 4NP-α-L-fucoside (4-NP-α-L-Fuc) at 65°C (Cobucci-Ponzano et al. 2003a; Rosano et al. 2004). Moreover, Ssα-fuc is thermoactive and thermostable, as expected for an enzyme from a hyperthermophilic microorganism. The optimal temperature of Ssα-fuc is 95°C and the enzyme displayed high stability maintaining 60% of the residual activity after 2 h at 80°C (Cobucci-Ponzano et al. 2003a). It is worth noting that the mutation inserted to obtain the recombinant Ssα-fuc was designed on the basis of a mechanism of regulation of gene expression known as programmed −1 frameshifting (Farabaugh 1996). Very recently it was found that the two ORFs express in vivo a full length protein by programmed −1 frameshifting, demonstrating, for the first time, that this mechanism of gene expression, known so far only in *Eukarya* and *Bacteria* (Baranov et al. 2001) is used to regulate the expression of this gene in *S. solfataricus* (Cobucci-Ponzano et al. 2006).

In the framework of our mechanistic studies on glycoside hydrolases, the reaction mechanism of Ssα-Fuc was studied in detail and the residues directly involved in catalysis were identified. The *retaining* reaction mechanism was demonstrated, for the first time in GH29, by using Ssα-fuc. In fact, the enzyme is able to function in transfucosylation mode as reported for several mesophilic α-fucosidases (Murata et al. 1999; Farkas et al. 2000); its synthetic ability was demonstrated by using 4-NP-α-L-Fuc and 4-NP-α-D-glucoside (4-NP-α-D-Glc) as donor and acceptor, respectively. The fucosylated products were disaccharides of the acceptor in which the α-L-fucose moiety of the donor is attached at positions 2 and 3 of Glc (α-L-Fuc*p*-(1-2)-α-D-Glc-*O*-4-NP and α-L-Fuc*p*-(1-3)-α-D-Glc-*O*-4-NP) (Cobucci-Ponzano et al. 2003a). The α-anomeric configuration of the interglycosidic linkages in the products demonstrated that GH29 α-fucosidases follow a *retaining* reaction mechanism (Cobucci-Ponzano et al. 2003a). The hydrolytic activity of Ssα-fuc on the disaccharide α-L-Fuc-(1-3)-α-L-Fuc-*O*-4-NP revealed that the enzyme is an exo-glycosyl hydrolase that attacks the substrates from their non-reducing end (Cobucci-Ponzano et al. 2003a).

Identification of the nucleophile of the reaction

The active-site residues of retaining α- and β-glycosidases have been identified with a variety of methods, including mechanism-based inhibitors labelling the catalytic nucleophile and inspection of X-ray structures (McCarter and Withers 1996; Vocadlo et al. 2000, 2001; Tarling et al. 2003). An alternative approach often exploited for retaining glycoside hydrolases consists in the mutation of aspartic/glutamic acid residues identified by sequence analysis and conserved in the family of interest. Mutations of the catalytic residues with non-nucleophilic amino acids lead to the strong reduction or even abolition of the enzymatic activity (Ly and Withers 1999). However, these mutants can be reactivated in the presence of external nucleophiles such as sodium azide. The isolation of glycosyl-azide products with an anomeric configuration opposite to that of the substrate allows the identification of the catalytic nucleophile of the reaction (Fig. 2) (Ly and Withers 1999). This methodology is termed chemical rescue. Once the reaction mechanism and the active-site residues of a particular enzyme have been experimentally determined, they can be easily extended to all the homologous enzymes by following the classification in families.

The nucleophile of GH29 α-L-fucosidases was identified, for the first time, by reactivating the Ssα-fuc D242G inactive mutant in the presence of sodium azide and by analyzing the anomeric configuration of the fucosyl-azide product (Cobucci-Ponzano et al. 2003b). The D242G mutant showed a turnover number (k_{cat}) of 0.24 s^{-1} on 4-NP-α-L-Fuc, which is 1.2×10^{-3} times that of the wild type activity (287 s^{-1}), indicating that the D242G mutation affected a residue involved in catalysis in Ssα-fuc. In the presence of 2 M sodium azide the mutant revealed a k_{cat} value of 9.66 s^{-1}, indicating a 40-fold reactivation by azide (Table 1). The fucosyl-azide product obtained by the D242G mutant was found in the inverted

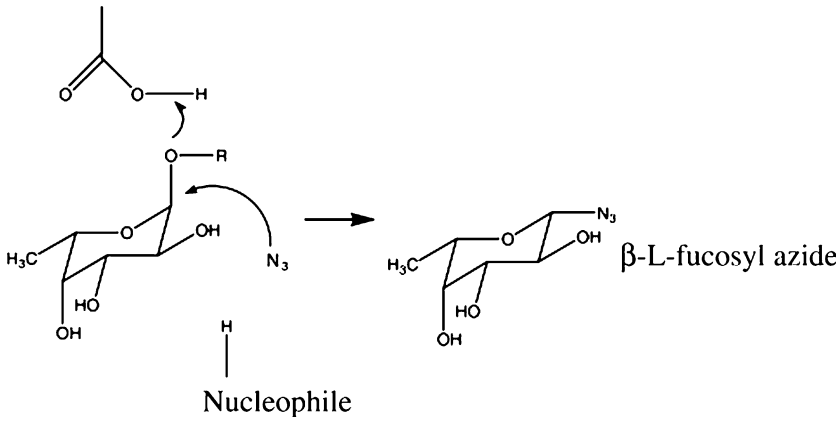

Fig. 2 Identification, by chemical rescue, of the nucleophile residue in *retaining* α-fucosidases. The α-L-fucoside substrate is converted in β-L-fucosyl-azide

Table 1 Steady-state kinetic constants of wild type and D242G mutant at 65°C

	k_{cat} (s^{-1})	K_M (mM)	k_{cat}/K_M (s^{-1} mM^{-1})
Wild type	287 ± 11	0.028 ± 0.004	10,250
D242G	NA[a]	NA[a]	–
+NaN_3 2M	9.7 ± 0.3	0.19 ± 0.02	51.5
+NaCOOH 0.1 M	5.9 ± 0.2	1.0 ± 0.1	5.8

[a] *NA* (not measurable activity) means that, using 20 μg of enzyme in the assay, the rates of change in absorbance at substrate concentration below 0.1 mM did not vary in the experimental conditions

(β-L) configuration when compared with the substrate (Fig. 2). This finding allowed, for the first time, the unambiguous assignment of Asp242 and its homologous residues as the nucleophilic catalytic residues of GH29 α-L-fucosidases (Cobucci-Ponzano et al. 2003b). The activity of the mutant D242G was also rescued on 4-NP-α-L-Fuc in the presence of sodium formate. The steady-state kinetic constants of D242G, determined in the presence of external nucleophiles, revealed that sodium azide and sodium formate produced about 0.5 and 0.056% of reactivation of the mutant, respectively, which was calculated by taking as 100% the k_{cat}/K_M of the wild type (Table 1) (Cobucci-Ponzano et al. 2003b). The higher nucleophilicity of sodium azide, when compared with formate, explains the higher reactivation produced by the former. The specific activity of the mutant increases with temperature up to 80°C, indicating that, despite the mutation, the reactivated enzyme maintains its thermophilicity.

This was the first example of the application of the chemical rescue method to α-(D/L)-glycosidases as it has been used so far only for β-D-glycosidases. Later, by following a similar approach, the corresponding residue was also identified in the α-fucosidase from *Thermotoga maritima* (Tmα-fuc) (Tarling et al. 2003). These results on GH29 enzymes demonstrated that chemical rescue could be of general applicability for retaining enzymes.

Identification of the acid/base catalyst of the reaction

The approach utilized for the identification of the acid/base catalyst of retaining glycosyl hydrolases is less straightforward if compared to the nucleophile. In fact, the use of specific inhibitors for the acid/base catalyst is still elusive and successful results are less common (Tull et al. 1996; Vocadlo et al. 2002). For these reasons, the acid/base catalyst of several retaining glycosidases was identified through 3D structure inspection and detailed characterization of mutants in which conserved aspartic and glutamic acid residues have been replaced by isosteric and non-ionizable amino acids as asparagine, glutamine, alanine, or glycine (Ly and Withers 1999). Replacing the acid/base catalyst with the small non-ionizable glycine residue generally reduces dramatically the activity of the mutant and modifies its pH profile. In fact, when the acid/base is removed, the basic limb of the typical bell-shaped pH dependence curve is severely affected (Ly and Withers 1999). The chemical rescue of the activity of the inactive mutant is also a useful tool. In fact, as described above for the mutant in the residue acting as the nucleophile of the reaction, the presence of the glycine generates a room in the active site allowing the access of a small nucleophilic ion. However, this time, the external nucleophile (i.e. azide) occupies the cavity formed by mutation after the formation of the glycosyl-enzyme intermediate. In these cases, the rate enhancement and the isolation of a glycosyl-azide product with the same anomeric configuration of the substrate resulted in the most effective method to unequivocally identify the acid/base catalyst (MacLeod et al. 1996; Viladot et al. 1998; Ly and Withers 1999; Vallmitjana et al. 2001; Debeche et al. 2002; Li et al. 2002; Rydberg et al. 2002; Shallom et al. 2002; Vocadlo et al. 2002; Bravman et al. 2003; Zechel et al. 2003; Paal et al. 2004; Sulzenbacher et al. 2004).

By following this line of approach, in Ssα-fuc several aminoacids among highly conserved histidine, aspartic,

and glutamic acid residues were picked and mutated into a glycine; the mutants H46G, E58G H123G, D124G, D146G were characterized in detail. Furthermore, the mutant E292G was added to this collection since the corresponding residue was identified as the acid/base catalyst in Tmα-fuc. Surprisingly, this residue falls in a region of alignment scarcely conserved in GH29 (Sulzenbacher et al. 2004). The preliminary kinetic characterization of the Ssα-fuc mutants on 4-NP-α-L-Fuc, reported in Table 2, revealed that D124 and D146 were not directly involved in catalysis since the activity was not significantly affected by the mutations. Instead, the affinity for the substrate of H46G and H123G was remarkably different from that of the wild type: the mutation of His46 produced a 607-fold increase in the K_M, while no saturation was observed with the mutant H123G. Also the mutation of the residues Glu58 and Glu292 affected catalysis severely (Table 2); again, no saturation could be observed with the former residue, while E292G showed unchanged affinity for 4-NP-α-L-Fuc but a 154-fold reduction in the turnover number (Cobucci-Ponzano et al. 2005).

This preliminary characterization indicated that the residues His46, His123, Glu58, and Glu292 are involved in substrate binding or in catalysis; however, experiments of chemical rescue of the enzymatic activity on the mutants H46G and H123G allowed us to exclude their involvement in catalysis (Cobucci-Ponzano et al. 2005). Furthermore, the inspection of the crystal structure of Tmα-fuc suggested that His46 and His122, which correspond to His34 and His128 in Tmα-fuc, respectively, stabilize the 4-hydroxyl group of fucose.

The characterization of the mutants E58G and E292G, compared to the data collected on the corresponding residues in Tmα-fuc (Glu66 and Glu262, respectively), gave unexpected results, suggesting that in GH29 two catalytic machineries coexist. The analysis of the 3D structure of the Tmα-fuc and the kinetic characterization of the mutants clearly indicated that, in this enzyme, Glu66 and Glu266 were involved in substrate binding and in the acid/base catalysis, respectively (Sulzenbacher et al. 2004). In particular, the mutation in the residue Glu66 produced a tenfold drop of activity while the mutation in Glu266 determined the absence of saturation by the substrate. Intriguingly, in Ssα-fuc the mutant E58G mirrored the behaviour of the Tmα-fuc mutant in the Glu266 residue (lack of saturation) while the Ssα-fuc mutant E292G showed a marked inactivation as observed for the Tmα-fuc mutant in Glu66 (Table 2). These results suggested that in Ssα-fuc Glu58 is the acid/base catalyst. This conclusion was further supported by the analysis of the pH dependence of wild type and mutants Ssα-fuc. Wild type enzyme has a peculiar pH dependence, which is not bell-shaped, but shows a reproducible increase of activity at pH 8.6 (Fig. 3), suggesting that more than two ionizable groups are involved in catalysis (Debeche et al. 2002). The pH dependence of the mutant E292G is similar to that of the wild type enzyme; instead, this pH profile was dramatically changed in the E58G mutant, producing a typical bell-shaped curve with a pH optimum at 4.6 sharper than that of the wild type (3.0–5.0) (Fig. 3). These data suggested that the removal of Glu58 unmasked the ionization of a group responsible for the basic limb (p*K*a 5.3) and possibly increased the p*K*a of the nucleophile of the reaction mainly determining the acidic limb. Unfortunately, the pH dependence of the wild type and mutant Tmα-fuc was not reported, precluding a detailed comparison.

To try to better define the nature of the acid/base catalyst in Ssα-fuc the chemical rescue, which, as described above, is one of the most definite tools to assign this role in glycosidases, was exploited. Remarkably, E58G was activated by more than 70-fold in the presence of sodium azide, formate and acetate (Table 3). In addition, it was observed

Table 2 Steady-state kinetic constants of wild type and mutants at 65°C

	k_{cat} (s^{-1})	K_M (mM)	k_{cat}/K_M (s^{-1} mM^{-1})
Wild type	287 ± 11	0.028 ± 0.004	10,250
D124G	240 ± 7	0.09 ± 0.01	2,740
D146G	224 ± 5	0.033 ± 0.003	6,791
H46G	419 ± 99	17.0 ± 5.7	25
H123G	ND[a]	ND[a]	3.5
E58G	ND[a]	ND[a]	1.9
E292G	1.86 ± 0.09	0.06 ± 0.01	33

The specificity constants were calculated by using 4-NP-α-L-Fuc in the range 1–7 mM and by plotting (velocity) vs. (S)

[a] *ND* not determined. No saturation was observed on up to 25 mM 4-NP-α-L-Fuc

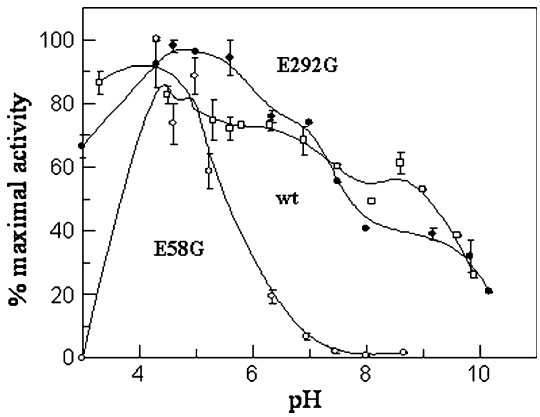

Fig. 3 pH Dependence of wild type, E58G and E292G. Data are reported as percent of maximal activity on 4-NP-α-L-Fuc at 65°C

Table 3 Steady-state kinetic constants of wild type, E58G and E292G mutants in different reaction conditions

	k_{cat} (s^{-1})	K_M (mM)	k_{cat}/K_M (s^{-1} mM^{-1})	pH	Reaction condition[a]
Wild type	287 ± 11	0.028 ± 0.004	10,250	6.3	Sodium phosphate
	430 ± 49	0.26 ± 0.09	1,624	5.0	Sodium citrate
E58G	ND[b]	ND[b]	1.9	6.3	Sodium phosphate
	143 ± 8	1.6 ± 0.3	89	4.6	Sodium citrate
	586 ± 43	0.6 ± 0.2	950	4.6	Sodium acetate
	846 ± 46	1.1 ± 0.2	790	4.6	Sodium formate
	679 ± 82	2.9 ± 0.4	229	6.3	Sodium phosphate + NaN_3[c]
E292G	1.86 ± 0.09	0.06 ± 0.01	33	6.3	Sodium phosphate
	1.86 ± 0.17	0.09 ± 0.02	21	4.6	Sodium acetate

The specificity constants were calculated by using 4-NP-α-L-Fuc in the range 1–7 mM and by plotting (velocity) vs. (S)

[a] Assays were performed at 65°C in the reported buffers in 0.05 M concentration on 4-NP-α-L-Fuc

[b] Not determined; no saturation was observed with up to 25 mM 4-NP-α-L-Fuc

[c] Sodium azide was used at the concentration of 0.15 M

that the sodium azide anion activated E58G only in the presence of larger ions (phosphate and citrate) while this effect was much reduced in acetate and formate, which already activate the mutant. Presumably, in phosphate and citrate buffers, azide has full access to the small cavity created by the mutation in the active site. Instead, acetate and formate, occupying this space, could activate the reaction hampering the access of azide. In striking contrast, the activity of E292G could not be rescued by any of the nucleophiles used. These results made it very unlikely that Glu292 is the acid/base catalyst of Ssα-fuc, and allowed to assign this role to Glu58.

These data demonstrated that the Glu58 is the acid-base catalyst and suggested that the Glu292 has a relevant role in catalysis presumably modulating the p*K*a of the latter, thereby affecting the pH optimum of the enzyme. Intriguingly, the behaviour of the catalytic residues of Ssα-fuc is different from that of Tmα-fuc (Sulzenbacher et al. 2004). Nevertheless, considering that among the amino acid sequences of GH29 the predicted acid/base residues are not invariant, it would not be surprising that the enzymes show structural differences in the active site explaining the different catalytic machineries.

Conclusions

The first archaeal α-L-fucosidase was identified in *S. solfataricus* and is encoded by an interrupted gene. The recombinant enzyme Ssα-fuc, obtained by site directed mutagenesis, is fully active and thermostable and allowed the first detailed study on the *retaining* reaction mechanism of GH29 glycoside hydrolases. Interestingly, the inspection of the catalytic machinery of Ssα-fuc revealed the presence in the active site of a triad of catalytic residues, namely Asp242, Glu58, and Glu292. This is a rather uncommon feature among the glycoside hydrolases and the comparison with the Tmα-fuc suggested that in GH29 slightly different catalytic machineries coexist. It is worth noting that the use of the chemical rescue method at harsh pHs and ionic strengths was possible because of the intrinsic stability of Ssα-fuc and resulted of general applicability for β and α glycoside hydrolases.

The body of this work demonstrates that the α-L-fucosidase from *S. solfataricus* is an interesting model system to uncover new mechanisms of gene expression in *Archaea* and to study the reaction mechanisms of glycoside hydrolases. In addition, the transfucosylating activity of Ssα-fuc and the availability of several mutants in the active site could be the starting points for the biotechnological exploitation of this enzyme in the synthesis of fucosylated oligosaccharides.

Acknowledgments This work was supported by MIUR project "Folding di proteine: l'altra metà del codice genetico" RBAU015B47_006. The IBP-CNR belongs to the Centro Regionale di Competenza in Applicazioni Tecnologico-Industriali di Biomolecole e Biosistemi.

References

Baranov PV, Gurvich OL, Fayet O, Prere MF, Miller WA, Gesteland RF, Atkins JF, Giddings MC (2001) RECODE: a database of frameshifting, bypassing and codon redefinition utilized for gene expression. Nucleic Acids Res 29:264–267

Bravman T, Belakhov V, Solomon D, Shoham G, Henrissat B, Baasov T, Shoham Y (2003) Identification of the catalytic residues in family 52 glycoside hydrolase, a beta-xylosidase from *Geobacillus stearothermophilus* T-6. J Biol Chem 278:26742–26749

Cobucci-Ponzano B, Trincone A, Giordano A, Rossi M, Moracci M (2003a) Identification of an archaeal alpha-L-fucosidase encoded by an interrupted gene. Production of a functional enzyme by mutations mimicking programmed −1 frameshifting. J Biol Chem 278:14622–14631

Cobucci-Ponzano B, Trincone A, Giordano A, Rossi M, Moracci M (2003b) Identification of the catalytic nucleophile of the family 29 alpha-L-fucosidase from *Sulfolobus solfataricus* via chemical rescue of an inactive mutant. Biochemistry 42:9525–9531

Cobucci-Ponzano B, Mazzone M, Rossi M, Moracci M (2005) Probing the catalytically essential residues of the alpha-L-fucosidase from the hyperthermophilic archaeon *Sulfolobus solfataricus*. Biochemistry 44:6331–6342

Cobucci-Ponzano B, Conte F, Benelli D, Londei P, Flagiello A, Monti M, Pucci P, Rossi M, Moracci M (2006) The gene of an archaeal alpha-L-fucosidase is expressed by translational frameshifting. Nucleic Acids Res 34:4258–4268

Debeche T, Bliard C, Debeire P, O'Donohue MJ (2002) Probing the catalytically essential residues of the alpha-L-arabinofuranosidase from *Thermobacillus xylanilyticus*. Protein Eng 1:21–28

de La Torre F, Sampedro J, Zarra I, Revilla G (2002) AtFXG1, an *Arabidopsis* gene encoding alpha-L-fucosidase active against fucosylated xyloglucan oligosaccharides. Plant Physiol 128:247–255

Farabaugh PJ (1996) Programmed translational frameshifting. Microbiol Rev 60:103–134

Farkas E, Thiem J, Ajisaka K (2000) Enzymatic synthesis of fucose-containing disaccharides employing the partially purified alpha-L-fucosidase from *Penicillium multicolor*. Carbohydr Res 328:293–299

Fischer L, Bromann R, Kengen SWM, de Vos WM, Wagner F (1996) Catalytical potency of beta-glucosidase from the extremophile *Pyrococcus furiosus* in glucoconjugate. Biotechnology 14:88–91

Henrissat B (1991) A classification of glycosyl hydrolases based on amino acid sequence similarities. Biochem J 280:309–316

Henrissat B, Bairoch A (1993) New families in the classification of glycosyl hydrolases based on amino acid sequence similarities. Biochem J 293:781–788

Henrissat B, Davies G (1997) Structural and sequence-based classification of glycoside hydrolases. Curr Opin Struct Biol 7:637–644

Koshland DE (1953) Stereochemistry and the mechanism of enzymatic reactions. Biol Rev Camb Philos Soc 28:416–436

Li YK, Chir J, Tanaka S, Chen FY (2002) Identification of the general acid/base catalyst of a family 3 beta-glucosidase from *Flvobacterium meningosepticum*. Biochemistry 41:2751–2759

Listinsky JJ, Siegal GP, Listinsky CM (1998) Alpha-L-fucose: a potentially critical molecule in pathologic processes including neoplasia. Am J Clin Pathol 110:425–440

Lower BH, Kennelly PJ (2002) The membrane-associated protein-serine/threonine kinase from *Sulfolobus solfataricus* is a glycoprotein. J Bacteriol 184:2614–2619

Ly HD, Withers SG (1999) Mutagenesis of glycosidases. Annu Rev Biochem 68:487–522

MacLeod AM, Tull D, Rupitz K, Warren RA, Withers SG (1996) Mechanistic consequences of mutation of active site carboxylates in a retaining beta-1,4-glycanase from *Cellulomonas fimi*. Biochemistry 35:13165–13172

McCarter JD, Withers SG (1994) Mechanisms of enzymatic glycoside hydrolysis. Curr Opin Struct Biol 4:885–892

McCarter JD, Withers SG (1996) Unequivocal identification of Asp-214 as the catalytic nucleophile of *Saccharomyces cerevisiae* alpha-glucosidase using 5-fluoro glycosyl fluorides. J Biol Chem 271:6889–6894

Michalski JC, Klein A (1999) Glycoprotein lysosomal storage disorders: alpha- and beta-mannosidosis, fucosidosis and alpha-*N*-acetylgalactosaminidase deficiency. Biochim Biophys Acta 1455:69–84

Mori E, Hedrick JL, Wardrip NJ, Mori T, Takasaki S (1998) Occurrence of reducing terminal *N*-acetylglucosamine 3-sulfate and fucosylated outer chains in acidic *N*-glycans of porcine zona pellucida glycoproteins. Glycoconj J 15:447–456

Murata T, Morimoto S, Zeng X, Watanabe S, Usui T (1999) Enzymatic synthesis of alpha-L-fucosyl-*N*-acetyllactosamines and 3′-*O*-alpha-L-fucosyllactose utilizing alpha-L-fucosidases. Carbohydr Res 320:192–199

Noda K, Miyoshi E, Uozumi N, Gao CX, Suzuki K, Hayashi N, Hori M, Taniguchi N (1998) High expression of alpha-1-6 fucosyltransferase during rat hepatocarcinogenesis. Int J Cancer 75:444–450

Paal K, Ito M, Withers SG (2004) *Paenibacillus* sp. TS12 glucosylceramidase Kinetic studies of a novel sub-family of family 3 glycosidases and identification of the catalytic residues. Biochem J 378:141–149

Perugino G, Trincone A, Rossi M, Moracci M (2004) Oligosaccharide synthesis by glycosynthases. Trends Biotechnol 1:31–37

Rapoport E, Pendu JL (1999) Glycosylation alterations of cells in late phase apoptosis from colon carcinomas. Glycobiology 9:1337–1345

Rosano C, Zuccotti S, Cobucci-Ponzano B, Mazzone M, Rossi M, Moracci M, Petoukhov MV, Svergun DI, Bolognesi M (2004) Structural characterization of the nonameric assembly of an Archaeal alpha-L-fucosidase by synchrotron small angle X-ray scattering. Biochem Biophys Res Commun 320:176–182

Russell L, Waring P, Beaver JP (1998) Increased cell surface exposure of fucose residues is a late event in apoptosis. Biochem Biophys Res Commun 250:449–453

Rydberg EH, Li C, Maurus R, Overall CM, Brayer GD, Withers SG (2002) Mechanistic analyses of catalysis in human pancreatic alpha-amylase: detailed kinetic and structural studies of mutants of three conserved carboxylic acids. Biochemistry 41:4492–4502

Sears P, Wong CH (1996) Intervention of carbohydrate recognition by proteins and nucleic acids. Proc Natl Acad Sci USA 93:12086–12093

Shallom D, Belakhov V, Solomon D, Gilead-Gropper S, Baasov T, Shoham G, Shoham Y (2002) The identification of the acid-base catalyst of alpha-arabinofuranosidase from *Geobacillus stearothermophilus* T-6, a family 51 glycoside hydrolase. FEBS Lett 514:163–167

She Q, Singh RK, Confalonieri F, Zivanovic Y, Allard G, Awayez MJ, Chan-Weiher CC, Clausen IG, Curtis BA, De Moors A, Erauso G, Fletcher C, Gordon PM, Heikamp-de Jong I, Jeffries AC, Kozera CJ, Medina N, Peng X, Thi-Ngoc HP, Redder P, Schenk ME, Theriault C, Tolstrup N, Charlebois RL, Doolittle WF, Duguet M, Gaasterland T, Garrett RA, Ragan MA, Sensen CW, Van der Oost J (2001) The complete genome of the crenarchaeon *Sulfolobus solfataricus* P2. Proc Natl Acad Sci USA 98:7835–7840

Sinnot ML (1990) Catalytic mechanism of enzymic glycosyl transfer. Chem Rev 90:1171–1202

Staudacher E, Altmann F, Wilson IB, Marz L (1999) Fucose in *N*-glycans: from plant to man. Biochim Biophys Acta 1473:216–236

Sulzenbacher G, Bignon C, Nishimura T, Tarling CA, Withers SG, Henrissat B, Bourne Y (2004) Crystal structure of *Thermotoga maritima* α-L-fucosidase. Insights into the catalytic mechanism and the molecular basis for fucosidosis. J Biol Chem 279:13119–13128

Tarling CA, He S, Sulzenbacher G, Bignon C, Bourne Y, Henrissat B, Withers SG (2003) Identification of the catalytic nucleophile of the family 29 α-L-fucosidase from *Thermotoga maritima* through trapping of a covalent glycosylenzyme intermediate and mutagenesis. J Biol Chem 278:47394–47399

Tull D, Burgoyne DL, Chow DT, Withers SG, Aebersold R (1996) A mass spectrometry-based approach for probing enzyme active sites: Identification of Glu 127 in *Cellulomonas fimi* exoglycanase as the residue modified by *N*-bromoacetyl cellobiosylamine. Anal Biochem 234:119–125

Vallmitjana M, Ferrer-Navarro M, Planell R, Abel M, Ausin C, Querol E, Planas A, Perez-Pons JA (2001) Mechanism of the family 1 beta-glucosidase from *Streptomyces* sp: catalytic residues and kinetic studies. Biochemistry 40:5975–5982

Vanhooren PT, Vandamme EJ (1999) L-fucose: occurrence, physiological role, chemical, enzymatic and microbial synthesis. J Chem Technol Biotechnol 74:479–497

Varki A (1993) Biological roles of oligosaccharides: all of the theories are correct. Glycobiology 2:97–130

Viladot JL, de Ramon E, Durany O, Planas A (1998) Probing the mechanism of Bacillus 1,3-1,4-beta-D-glucan 4-glucanohydrolases by chemical rescue of inactive mutants at catalytically essential residues. Biochemistry 37:11332–11342

Vocadlo DJ, Mayer C, He S, Withers SG (2000) Mechanism of action and identification of Asp242 as the catalytic nucleophile of *Vibrio furnisii N*-acetyl-beta-D-glucosaminidase using 2-acetamido-2-deoxy-5-fluoro-alpha-L-idopyranosyl fluoride. Biochemistry 1:117–126

Vocadlo DJ, Davies GJ, Laine R, Withers SG (2001) Catalysis by hen egg-white lysozyme proceeds via a covalent intermediate. Nature 412:835–838

Vocadlo DJ, Wicki J, Rupitz K, Withers SG (2002) A case for reverse protonation: identification of Glu160 as an acid/base catalyst in *Thermoanaerobacterium saccharolyticum* beta-xylosidase and detailed kinetic analysis of a site-directed mutant. Biochemistry 41:9736–9746

Wiese TJ, Dunlap JA, Yorek MA (1997) Effect of L-fucose and D-glucose concentration on L-fucoprotein metabolism in human Hep G2 cells and changes in fucosyltransferase and alpha-L-fucosidase activity in liver of diabetic rats. Biochim Biophys Acta 1335:61–72

Xiang J, Bernstein IA (1992) Differentiative changes in fucosyltransferase activity in newborn rat epidermal cells. Biochem Biophys Res Commun 189:27–32

Zechel DL, Withers SG (2000) Glycosidase mechanisms: anatomy of a finely tuned catalyst. Acc Chem Res 33:11–18

Zechel DL, Reid SP, Stoll D, Nashiru O, Warren RA, Withers SG (2003) Mechanism, mutagenesis, and chemical rescue of a beta-mannosidase from *Cellulomonas fimi*. Biochemistry 42:7195–7204