Review

Molecular analysis of α-glucosidase belonging to GH-family 31

Hiroyuki Nakai, Masayuki Okuyama, Young-Min Kim, Wataru Saburi, Jintanart Wongchawalit, Haruhide Mori, Seiya Chiba & Atsuo Kimura*

Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan; phone and fax: ++ 81 11 706 2808, e-mail: kimura@abs.agr.hokudai.ac.jp

Abstract: On the basis of amino-acid sequence similarity, α-glucosidases are mainly divided into two groups, GH-family 13 and 31 enzymes. The former enzyme group strongly recognizes the α-glucosyl-moiety in the heterogeneous substrates, such as synthetic α-glucoside and sucrose. The latter family member recognizes the maltosyl-structure of maltooligosaccharide more than the α-glucosyl-structure of heterogeneous substrate, and some enzymes show a higher activity towards polymer-substrate. The hydration of D-glucal as well as the hydrolysis of 2-deoxy-substrate was only catalyzed by the latter group, meaning less recognition of C2 OH-group in the non-reducing terminal glucose unit of the substrate. Inhibitory effects of acarviosine-derivatives, competitive inhibitors, were also different in two enzyme groups. Inhibition of isoacarbose on GH-family 31 α-glucosidases were 20- to 450-times stronger than that on GH-family 13 α-glucosidases, whereas acarviosine-glucose inhibited both groups by almost the same level. Series of one point replacement works of the conserved acidic amino acids, Asp and Glu, of GH-family 31 α-glucosidase from fission yeast revealed that Asp481 and Asp647 were the catalytic residues. Asp481 and Asp647 are observed in the conservative sequences, region-A and region-B, respectively. The D481G mutant catalyzed the "α-glycosynthase-reaction" to form α-configurated products from β-glucosyl fluoride and *p*-nitrophenyl monosaccharides. Plant α-glucosidases showed starch granule-binding and -degrading abilities, in which their C-terminal region was responsible for the binding to raw starch.

Key words: α-Glucosidase, GH-family 31, substrate recognition, isoacarbose, α-glycosynthase.

Abbreviations: AcvGlc, acarviosine-glucose; GH, glycoside hydrolase; IsoAca, isoacarbose; PNP, *p*-nitrophenyl.

Introduction

α-Glucosidase (EC 3.2.1.20) catalyzes the hydrolytic reaction to liberate α-glucose from the non-reducing end of the substrate (Chiba & Shimomura, 1978; Chiba, 1984; 1988; Frandsen & Svensson, 1998; Kimura, 2000). The enzyme also shows the transferring reaction and the condensation as well as the hydration of D-glucal (Fig. 1A,B,D; Chiba & Shimomura, 1979; Chiba et al., 1988; Yamamoto et al., 2004). α-Glucosidases are mainly classified into two groups, GH-family 13 and 31, based on the sequence homology (Henrissat, 1991; Kimura et al., 1992; Henrissat & Bairoch, 1993). Their origins are also different (Chiba & Shimomura, 1978; Chiba, 1988). α-Glucosidases from bacteria, *Saccharomyces cerevisiae*, and insect belong to GH-family 13 (α-amylase family) having four conserved sequences of region 1, 2, 3 and 4, in which the amino acids critical for catalytic reaction are observed (Kuriki & Imanaka, 1999; Kimura, 2000; MacGregor et al., 2001; Janecek, 2002; Matsuura, 2002; Svensson et al., 2002). The overall amino acid sequence similarity between the GH-family 13 α-glucosidase and α-amylase is low, whereas that for dextran-glucosidase (EC 3.2.1.10; Russell & Ferretti, 1990) and trehalose-6-phosphate hydrolase (EC 3.2.1.93; Rimmele & Boos, 1994) are high. But the conserved sequence regions in α-amylase family enzymes were well-discussed by MacGregor et al. (2001) and Janecek (2002). Plant, animal, mold, and bacteria (two species) α-glucosidases as well as α-glucan lyase (EC 4.2.2.13; Bojsen et al., 1999) and α-xylosidase (EC 3.2.1.-; Okuyama et al., 2004) are members of GH-family 31 (Frandsen & Svensson, 1998). Amino acids responsible for catalytic reaction of this enzyme group have not been identified until our research (Okuyama et al., 2001). We are interested in the difference of the reaction catalyzed by the enzymes of the two families, in particular, the molecular recognition of substrate and inhibitor, since the conformations of both families are distinct from each other.

* Corresponding author

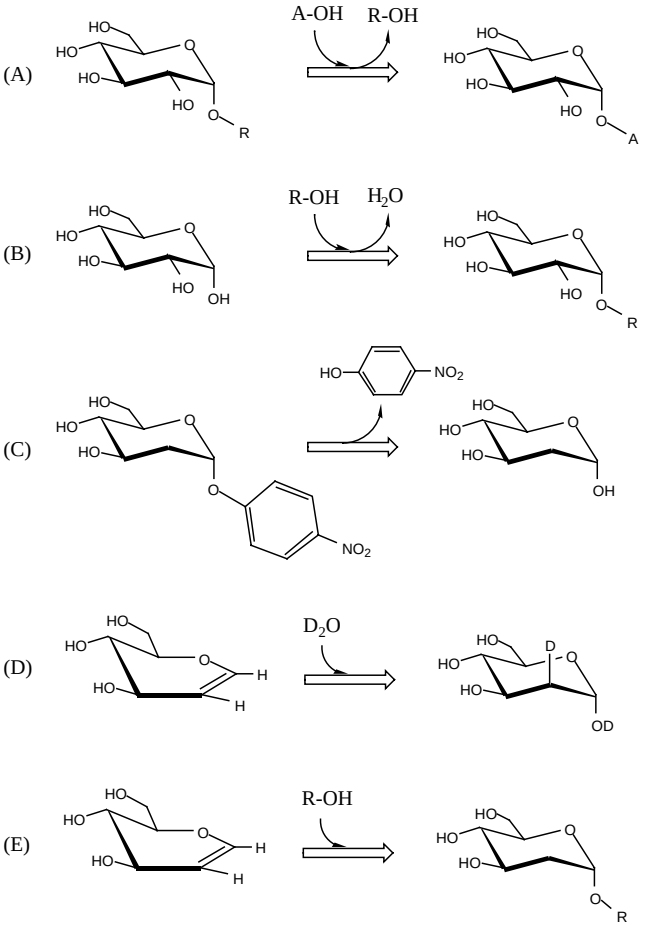

Fig. 1. Reactions catalyzed by α-glucosidases belonging to GH-family 13 (panels A and B) and GH-family 31(panels A to D). (A) Transglucosylation forms products (R-OH and transferred saccharide shown in right) from substrate (left) and acceptor (A-OH). (B) Condensation, a reverse-reaction of hydrolysis, forms product (right) from α-glucose (left) and R-OH by dehydration. (C) 2-Deoxyglucoside substrate (PNP α-2-deoxyglucoside; left) is hydrolyzed to form α-2-deoxyglucose (right). (D) Hydration of D-glucal (left) in D_2O produces 2-deoxy-[2(a)-^{2}H]-2-α-D-glucose (right) through trans-addition. (E) D-Glucal (left) is converted into alkyl α-2-deoxyglucoside (right) in alkyl alcohol (R-OH).

Substrate and inhibitor recognition of α-glucosidases

α-Glucosidases from various origins show the different substrate recognitions, which divide enzymes into three groups of Type-I, -II, and -III (CHIBA & SHIMOMURA, 1978; CHIBA, 1988; 1997). α-Glucosidase Type-I, belonging to GH-family 13, is observed in bacteria, Brewer's yeast (*Saccharomyces cerevisiae*), and insects, which hydrolyzes the heterogeneous substrate, such as sucrose and *p*-nitrophenyl (PNP) α-glucoside of synthetic substrate, more rapidly than the homogeneous substrate of maltooligosaccharide and shows low or no activity towards the polymer substrate (soluble starch, raw starch, and glycogen). This type of α-glucosidase recognizes "α-glucosyl-structure" of substrate molecule. α-Glucosidases Type-II and -III are members of GH-family 31. α-Glucosidase of Type-II, found in mold, prefers homogeneous substrate to heterogeneous and polymer substrates, which means that this group has high recognition of "maltosyl-structure". Type-III α-glucosidase, originated from plant and animal origins, shows almost no activity to heterogeneous substrate, and high activity to homogeneous and polymer substrates. The member of Type-III strongly recognizes "maltosyl-structure" as well as "polymer-structure".

Furthermore, Type-I, -II, and -III α-glucosidases show different recognition for 2-deoxyglucoside substrate (Fig. 1C; NISHIO et al., 2002). Although Type-I enzyme (GH-family 13 α-glucosidase) hydrolyzes PNP α-glucoside very rapidly, no hydrolytic activity was observed in PNP α-2-deoxyglucoside. The occurrence of 2-OH group in the glycon-side glucose-moiety is essential for the substrate. The reaction of α-glucosidases belonging to Type-II and -III (GH-family 31 α-glucosidases) to PNP α-glucoside is low, but the apparent hydrolytic activity to this 2-deoxyglucoside substrate was observed. These findings indicate that the enzymes of GH-family 13 strictly recognizes 2-OH group of glycon, and the recognition by the enzymes of GH-family 31 is not so tight.

It was reported that α-glucosidase hydrated the double bond of D-glucal to form 2-deoxyglucose of α-anomer (CHIBA et al., 1988). D-Glucal is a prochiral substrate in this reaction, and the hydration using D_2O produces 2-deoxy-[2(a)-^{2}H]-2-α-D-glucose through trans-addition (Fig. 1D). GH-family 13 α-glucosidase cannot hydrate D-glucal, but GH-family 31 enzyme is able to catalyze the hydration. Recently, we have reported that only α-glucosidase of GH-family 31 produced alkyl α-2-deoxyglucoside from D-glucal and alkyl alcohol (Fig. 1E; KIM et al., 2005). Remarkable production yield of 80–93% was performed by alcohol-stable α-glucosidase from *Aspergillus niger* (KITA et al., 1991), where the reaction mixture contained 70% or 90% alcohol.

Two potent inhibitors for α-glucosidase as well as for α-amylase and cyclomaltodextrin glucanotransferase were synthesized by group of Kwan-Hwa Park (CHA et al, 1998; PARK et al., 1998), who has modified acarbose by the hydrolysis and the transglycosylation catalyzed by *Bacillus stearothermophilus* maltogenic amylase to form acarviosine-glucose (AcvGlc, pseudotrisaccharide) and isoacarbose (IsoAca, psuedotetrasaccharide), respectively (Fig. 2). Both compounds, AcvGlc and IsoAca, are the very powerful competitive inhibitors to GH-family 13 and 31 α-glucosidases, but their recognition of two acarbose-analogues is distinct (KIMURA et al., 2004). GH-family 31 α-glucosidase showed almost identical inhibitor constants (K_i) for AcvGlc and IsoAca (K_i = 0.1–8.0 μM; K_i-ratio being 1:2). For GH-family 13 α-glucosidase, the large and small K_i values were obtained in the inhibition of IsoAca (K_i = 7.5–400 μM) and AcvGlc

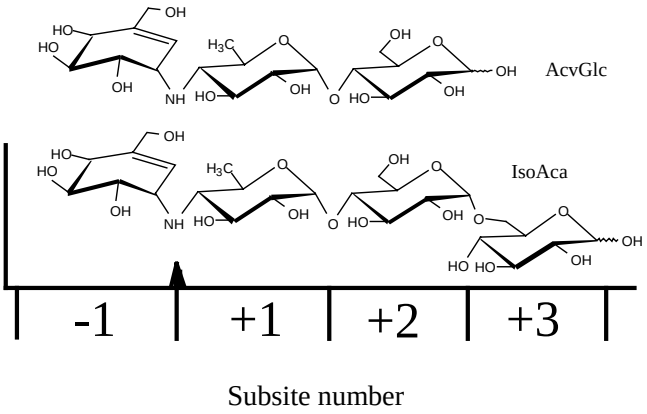

Fig. 2. Subsite structure in the catalytic site of α-glucosidase, and possible binding of acarviosine-glucose (AcvGlc) and isoacarbose (IsoAca). Triangle, cleavage point of substrate.

(K_i = 0.35–3.0 μM), respectively, and their ratios were 20:440. In comparison with conformation of AcvGlc, there is an extra glucose-moiety (Glc-I) in IsoAca, which is considered to bind the subsite +3 in the catalytic site of enzyme (Fig. 2). Glc-I is the sole structural difference in two inhibitors, suggesting that the possible steric hindrance occurred between Glc-I and subsite +3 of GH-family 13 α-glucosidase. In the binding of IsoAca to GH-family 31 α-glucosidase, the steric effect of Glc-I seems to be small. The finding that each of GH-family 13 and 31 α-glucosidases showed the different affinity to IsoAca and AcvGlc implies the conformational variation in close vicinity to the catalytic site, that is, subsite +3 (Kimura et al., 2004).

Catalytic amino acid residues of α-glucosidase belonging to GH-family 31

We have cloned the gene of *Schizosaccharomyces pombe* α-glucosidase, a member of GH-family 31, and succeeded in the gene expression using *S. cerevisiae* system, where the recombinant enzyme produced was secreted to the culture medium (Okuyama et al., 2001). The properties of purified recombinant enzyme are almost identical to those of original enzyme from *S. pombe*. The catalytic residues were investigated by point mutation for Asp and Glu conserved in amino acid sequences of GH-family 31 α-glucosidases, since our previous kinetic studies – measuring (i) the pK_e values of essential ionizable groups; (ii) their heats of ionization; (iii) shifts of pK_e values in the reaction mixture having low dielectric constant; and (iv) analyzing the inactivation mechanism by water-soluble carbodiimide (a specific reagent for carboxyl groups) – indicated that two acidic amino acids were responsible for enzyme reaction (Chiba, 1984; Kimura et al., 1997a). Target acidic amino acids, candidates for catalytic residues, were six Asp (D218, D287, D355, D481, D647, 877D) and two Glu (E484, E714), which were replaced by Asn/Ala/Glu and Gln/Ala/Asp, respectively (Okuyama et al., 2001). D287N and E714Q showed 70% and 26% maltase activities of wild-type enzyme in the culture media, respectively, meaning that both D287 and E714 are not catalytic residues. Mutant enzymes for other 6 amino acids having no or less activities were purified and their kinetic properties were analyzed. The mutants for D355 and D877 isolated showed the small but apparent maltase activities of 0.1 to 0.2%. D218N showed very small maltase activity of 0.06%, but still had 10% PNP α-glucoside-hydrolyzing activity. The E488-mutated enzymes were interesting, since the maltase activities of Q- and A-mutants were almost missing, but D-mutant remained with 9.0% of maltose-hydrolyzing activity, implying that the negative charge is necessary at this position. E488A showed 5.0% of D-glucal hydration-activity. These findings indicate that four Asp (D218, D287, D355, 877D) and two Glu (E484, E714) were not catalytic amino acids. In conclusion, two Asp, i.e. D481 and D647, are the most possible candidates for catalytic residues, since D481N/A and D647/N/A lost the hydrolytic activity to maltose and PNP α-glucoside as well as the D-glucal-hydration activity. It was reported that fluoride substrate (an intermediate trapper, 5-fluoro-α-D-glucopyranosyl fluoride; Lee et al., 2001) and suicide substrate (conduritol B epoxide; Iwanami et al., 1995; Kimura et al., 1997b) modified the Asp corresponding to D481 of *S. pombe* α-glucosidase, which indicates that D481 is the so-called catalytic nucleophile. The "α-glycosynthase" reaction described in the next section also supports the function of D481 (Okuyama et al., 2002). The D647 is a proton donor, which is also supported by three-dimensional structure of α-xylosidase (YicI) from *Escherichia coli*, a GH-family 31 member, published recently (Lovering et al., 2005). As shown in Figure 3, the essential residues D481 and D647 are located in the conservative sequences (designated as "Region A" and "Region B", respectively), which are observed in all the GH-family 31 enzymes (Kimura, 2000; Okuyama et al., 2001). Region A and Region B are present in the central part of amino acid sequence. Between the two catalytic residues, there are about 170 amino acid residues in mold α-glucosidase, about 100 residues in plant and animal α-glucosidases, and about 70 residues in bacterial α-glucosidase.

"α-Glycosynthase" reaction catalyzed by D481G of *S. pombe* α-glucosidase

A glycosynthase is a glycoside hydrolase where the catalytic nucleophile has been mutated, the first example of which was the E358A variant of *Agrobacterium* β-glucosidase (Wang et al., 1994; MacKenzie et al., 1998). Mutant enzyme catalyzed the formation of oligosaccharide(s) from α-glucosyl fluoride and PNP-monosaccharide(s), in which the oligosaccharide was just accumulated with no further degradation, since the catalytic nucleophile-replaced enzyme

Origin	Region A	Region B
α-Glucosidase		
Schizosaccharomyces pombe	476 SGIWTDMNEPSSF	641 GAHWLGDNHSLW
Aspergillus niger (P2 subunit)	219 DGVWYDMSEVSSF	388 AGHWGGDNYSKW
Schwanniomyces occidentalis	465 DGIWADMNEVSSF	632 TGHWGGDNTADW
Sugar beet	464 DGIWIDMNEASNF	562 TAHWTGDNAATW
Barley	432 DGLWIDMNEISNF	528 TAYWTGDNAATW
Spinach	460 DGLWIDMNEISNF	558 TAHWTGDNAATW
Human	513 DGMWIDMNEPSNF	610 AGHWTGDVWSSW
Rabbit (sucrase)	1389 DGLWIDMNEPSSF	1494 AGHWLGDNYARW
Rabbit (isomaltase)	500 DGLWIDMNEVSSF	598 AAHWLGDNTATW
Bacillus thermoamyloliquefaciens	402 EGIWNDMNEPSVF	478 AAVWTGDNRSFW
Escherichia coli (YihQ)	400 GGWMADFGEYLPT	466 TMMWAGDQNVDW
α-Xylosidase		
Escherichia coli (YicI)	411 DCFKTDFGERIPT	476 PVHWGGDCYANY
α-1,4-Glucan lyase		
Gracilariopsis lemaneiformis	598 DFVWQDMTVPAMM	709 GGMWVGDNSTTS
Morchella costata	543 EFVWQDMTTPAIH	629 AGLWTGDNASTW
Isomaltosyltransferase		
Sporosarcina globispora	840 DGFKTDGGEFVFG	905 PMHWAGDERSTF
6-Glucosyltransferase		
Sporosarcina globispora	561 DGFKTDGGEMVWG	615 QIFWSGDQESTF

Fig. 3. Region A and region B responsible for catalytic reactions of GH-family 31 enzymes. Underlined D (Asp) is the catalytic residue analyzed experimentally (KIMURA, 2000; LEE et al., 2002; LOVERING et al., 2005).

(E358A) lost the hydrolytic activity. The same type of reaction was observed in D481G of *S. pombe* α-glucosidase (OKUYAMA et al., 2002). D481G did not show any hydrolytic reaction, but catalyzed the production of PNP disaccharide(s) from β-glucosyl fluoride and PNP-monosaccharide(s). For example, PNP α-isomaltoside and PNP α-maltoside were synthesized from β-glucosyl fluoride and PNP α-glucoside with the high productivity of 70% (41% for the former product and 29% for the latter product). D481G also accepted the second substrate of PNP α-xyloside (yield of PNP disaccharide, 82%), PNP α-mannoside (10%), or PNP β-glucoside (10%). In all cases, the linkage formed was α-configuration. This is the first observation on synthesizing the α-glycosyl bond by glycosynthase. Therefore, D481G of *S. pombe* α-glucosidase was designated as "α-glycosynthase" (OKUYAMA et al., 2002).

Plant α-glucosidases show starch granule (so-called raw starch) binding ability

Recently, we found that rice α-glucosidase showed starch granule-degrading and -binding abilities (NAKAI et al., 2004a). Furthermore, the same phenomena were observed in all plant α-glucosidases tested. α-Glucosidases from mold (yeast and fungi) did not have any degrading activity or binding ability towards raw starch. Plant and mold α-glucosidases are members of GH-family 31 enzymes. Therefore, it could be concluded that there was a structural difference in α-glucosidases from plant and mold. We concentrated on their C-terminal structures, since the low homology of about 10% between the two enzyme groups was recorded. A chimeric enzyme was constructed in order to have: (i) the N-terminal to central-region derived from yeast enzyme; and (ii) the C-terminal region from rice enzyme. Chimera was found to acquire the binding ability and degradation activity to raw starch, indicating that C-terminal region of plant enzymes is directly responsible for both phenomena. Site-directed mutation approach revealed that two aromatic residues (Trp and Phe) in the C-terminal region were crucial for starch granule-binding and -degrading abilities (NAKAI et al., 2004b). Three-dimensional conformation of YicI (LOVERING et al., 2005) predicts that an α-glucosidase belonging to GH-family 31 has five-domains structure, in which the C-terminal region analyzed in this study forms a single domain.

The findings indicate two important points: the substrate recognition of α-glucosidases and the metabolism of starch granule in the plant tissues (in particular, the germination-stage of seeds). The first point is that the difference in substrate specificity between Type-II and -III α-glucosidases (i.e. the activity to polymer substrates) can be explained through the starch granule-binding and -degrading abilities, which are lacking in the C-terminal region of mold α-glucosidases (Type-II group) and present in the C-terminal region of plant α-glucosidases (Type-III group). Enzyme groups of Type-II and -III, belonging to even the same GH-family 31, have distinct conformations in their C-terminal regions, which contribute to the individual substrate specificities of both enzyme groups for the polysaccharides (soluble starch and starch granule) of very important substrates for α-glucosidase. Based on the distinction of substrate recognitions as well as the protein structures, the classification of Type-I, -II and -III is converted to that of α-glucosidase family-I, -II and -III, respectively, since the former classification was derived from only the difference in substrate recognition of α-glucosidases. The second is the discovery of alternative route in the starch granule degradation system in plant tissues. Plant biochemistry textbooks describe that the degradation of starch granule is initiated by α-amylase, since the α-amylase is the sole enzyme to have the raw starch-binding ability and -degrading ac-

tivity. Glucose-oligomer and -polymer produced are hydrolyzed by the further attacks of debranching-enzyme and β-amylase as well as α-amylase to form maltose and short glucose-oligomers of final products, which are converted into glucose by α-glucosidase. However, it was found that α-glucosidase itself could bind and degrade the starch granule directly, meaning that α-glucosidase and α-amylase are key enzymes in the starch granule degradation system in plant tissues (Nakai et al., 2004b).

References

Bojsen, K., Yu, S.K. & Marcussen, J. 1999. Plant Mol. Biol. **40:** 445–454.

Cha, H.J., Yoo, H.G., Kim, Y.W., Lee, H.S., Kim, J.W., Kweon, K.S., Oh, B.H. & Park, K.H. 1998. Eur. J. Biochem. **253:** 251–262.

Chiba, S. 1984. J. Jpn. Soc. Starch Sci. **31:** 55–66.

Chiba, S. 1988. In: The Amylase Research Society of Japan (ed.) Handbook of Amylase and Related Enzymes, Pergamon Press, Oxford, pp. 104–105; pp. 109–116.

Chiba, S. 1997. Biosci. Biotechnol. Biochem. **61:** 1233–1239.

Chiba, S., Brewer, C.F., Okada, G., Matsui, H. & Hehre, E.J. 1988. Biochemistry **27:** 1564–1569.

Chiba, S. & Shimomura, T. 1978. J. Jap. Soc. Starch Sci. **25:** 105–112.

Chiba, S. & Shimomura, T. 1979. J. Jap. Soc. Starch Sci. **26:** 59–67.

Frandsen, T.P. & Svensson, B. 1998. Plant Mol. Biol. **37:** 1–13.

Henrissat, B. 1991. Biochem. J. **280:** 309–316.

Henrissat, B. & Bairoch, A. 1993. Biochem. J. **293:** 781–788.

Iwanami, S., Matsui, H., Kimura, A., Ito, H., Mori, H., Honma, M. & Chiba, S. 1995. Biosci. Biotechnol. Biochem. **59:** 459–463.

Janecek, S. 2002. Biologia, Bratislava **57 (Suppl. 11):** 29–41.

Kim, Y.M., Okuyama, M., Mori, H., Nakai, H., Saburi, W., Chiba, S. & Kimura, A. 2005. Tetrahedron: Asymmetry **16:** 403–409.

Kimura, A. 2000. Trends Glycosci. Glycotechnol. **12:** 373–380.

Kimura, A., Lee, J.-H., Lee, I.-S., Park, K.-H., Chiba S. & Kim, D. 2004. Carbohydr. Res. **339:** 1035–1040.

Kimura, A., Somoto, A., Mori, H., Sakai, O., Matsui, H. & Chiba, S. 1997a. Biosci. Biotechnol. Biochem. **61:** 475–479.

Kimura, A., Takata, M., Fukushi, Y., Mori, H., Matsui, H. & Chiba, S. 1997b. Biosci. Biotechnol. Biochem. **61:** 1091–1098.

Kimura, A., Takata, M., Sakai, O., Matsui, H., Takai, N., Takayanagi, T., Nishimura, I., Uozumi, T. & Chiba, S. 1992. Biosci. Biotechnol. Biochem. **56:** 1368–1370.

Kita, A., Matsui, H., Somoto, A., Kimura, A., Takata, M. & Chiba, S. 1991. Agric. Biol. Chem. **55:** 2327–2335.

Kuriki, T. & Imanaka, T. 1999. J. Biosci. Bioeng. **87:** 557–565.

Lee, S. S., He, S. M. & Withers, S. G. 2001. Biochem. J. **359:** 381–386.

Lee, S. S., Yu, S. & Withers, S. G. 2002. J. Am. Chem. Soc. **124:** 4948–4949.

Lovering, A.L., Lee, S.L., Kim, Y.W., Withers, S.G. & Strynadka, N.C.J. 2005. J. Biol. Chem. **280:** 2105–2115.

MacGregor, E.A., Janecek, S. & Svensson, B. 2001. Biochim. Biophys. Acta **1546:** 1–20.

MacKenzie, L.F., Wang, Q., Warren, R.A.J. & Withers S.G. 1998. J. Am. Chem. Soc. **120:** 5583–5584.

Matsuura, Y. 2002. Biologia, Bratislava **57 (Suppl. 11):** 21–27.

Nakai, H., Tanizawa, S., Saburi, W., Son, M., Ito, T., Matsubara, K., Yamamoto, T., Okuyama, M., Mori, H., Chiba, S., Sano, Y. & Kimura, A. 2004a. Plant Polysaccharide Workshop, July 21–23, 2004, York (UK).

Nakai, H., Tanizawa, Ito, T., Matsubara, K., Yamamoto, T., Okuyama, M., Mori, H., Chiba, S., Sano, Y. & Kimura, A. 2004b. 22nd International Carbohydrate Symposium, July 23–27, 2004, Glasgow (UK).

Nishio, T., Hakamada, W., Kimura, A., Chiba, S., Takatsuki, A., Kawachi, R. & Oku, T. 2002. Carbohydr. Res. **337:** 629–634.

Okuyama, M., Mori, H., Chiba, S. & Kimura, A. 2004. Protein Expres. Purif. **37:** 170–179.

Okuyama, M., Mori, H., Watanabe, K., Kimura, A. & Chiba, S. 2002. Biosci. Biotechnol. Biochem. **66:** 928–933.

Okuyama, M., Okuno, A., Shimizu, N., Mori, H., Kimura, A. & Chiba, S. 2001. Eur. J. Biochem. **268:** 2270–2280.

Park, K.H., Kim, M.J., Lee, H.S., Han, N.S., Kim, D. & Robyt, J.F. 1998. Carbohydr. Res. **313:** 235–246.

Rimmele, M. & Boos, W. 1994. J. Bacteriol. **176:** 5654–5664.

Russell, R.R.B. & Ferretti, J.J. 1990. J. Gen. Microbiol. **136:** 803–810.

Svensson, B., Jensen, M.T., Mori, H., Bark-Jensen, K.S., Bonsager, B., Nielsen, P.K., Kramhoft, B., Praetorius-Ibba, M., Nohr, J., Juge, N., Greffe, L., Williamson, G. & Driguez, H. 2002. Biologia, Bratislava **57 (Suppl. 11):** 5–19.

Yamamoto, T., Unno, T., Watanabe, Y., Yamamoto, M., Okuyama, M., Mori, H., Chiba, S. & Kimura, A. 2004. Biochim. Biophys. Acta **1700:** 189–198.

Wang, Q., Graham, R.W., Trimbur, D., Warren, R.A.J. & Withers S.G. 1994. J. Am. Chem. Soc. **116:** 11594–11595.

Received December 29, 2004
Accepted May 16, 2005