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Thermostable Cyclodextrin Glucanotransferases

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Abstract. Hyperthermophilic microorganisms have developed a variety of molecular strategies in order to survive extremely harsh temperatures like 110 °C. For the utilization of natural polymeric substrates, such as starch, they produce special enzymes. The present review focuses on thermostable cyclodextrin glucanotransferases (CGTases; EC 2.4.1.19), which are responsible for the production of cyclodextrins. Only a limited number of thermostable CGTases have been characterized as yet. The thermostable enzymes characterized so far contain all the four conserved regions found in the family-13 of starch-degrading enzymes. The five domains specific for CGTases (**A** to **E**) have been identified in these enzymes and the effects of C-terminal truncation of thermostable CGTases have been analyzed. Furthermore, results of the construction of a chimeric enzyme have been included and practical advantages of thermostable enzymes are discussed.

Keywords: cyclodextrin, glucanotransferases, CGTases, hyperthermophiles, chimera, thermostable enzymes, starch depolymerization

Introduction. The use of enzymes in organic synthesis is now a routine. Enzymes, native or engineered, provide the selectivity which is desired in a reaction (Hult and Berglund, 2003). Along with several advantages, the use of enzymes for organic synthesis has some constraints in the conditions under which the reaction is performed. The conventional enzymes are irreversibly inactivated by heat while the enzymes from extremophiles show not only great thermostability, but also enhanced activity in the presence of common protein denaturants such as detergents, organic solvents and proteolytic enzymes (Klingeberg *et al*., 1995; Leuschner *et al*., 1995). In terms of stability, the common enzymes are far from ideal catalysts because of their instability. Enzymes with enhanced stability would not only allow prolonged usage, but would also be useful for exploring a broader range of reaction conditions. Indeed, concerted efforts have been made to enhance the stability of enzymes through protein engineering (Burton *et al*., 2002).

A significant advancement that has provided valuable clues for making proteins more thermostable or thermotolerant is the discovery of hyperthermophiles and studies on their proteins. Hyperthermophiles are the organisms that can grow at temperatures above 90 °C (Adams and Kelly, 1998), or optimally grow at 80 °C (Stetter, 1996). Several of these have been reported to grow at temperatures above the boiling point of water (Stetter, 1999). Unlike chemical parameters such as pH, heat cannot be removed or pumped out of the cell, and consequently, all the biomolecules within the hyperthermophilic cell must endure and function at the higher temperature. Therefore, enzymes from hyperthermophiles generally display greater thermostabilities as compared to other microorganisms. Hyperthermophiles have been found to constitute a diverse group of organisms in terms of their energy and carbon metabolism (Amend and Shock, 2001). These organisms have attracted many researchers to study various metabolic aspects of hyperthermophiles due to the possibility that they may represent the most primitive form of present-day life. Hyperthermophilic enzymes have become model systems for the study of enzyme evolution, enzyme stability and activity mechanism, protein structure-function relationships, and biocatalysis under extreme conditions. The present review focuses on thermostable cyclodextrin glucanotransferases (CGTases), which have been characterized with an emphasis on their structural features involved in cyclodextrin product specificity.

Starch-processing enzymes. Starch, a higher molecular weight (as high as 100 million) polymer of glucose, is a ubiquitous and easily accessible source of energy (Bertoldo and Antranikian, 2001). Many microorganisms are able to use starch as their carbon and energy source. Because of the complex structure of starch, microorganisms require an appropriate combination of enzymes for its depolymerization into oligosaccharides and smaller sugars. The enzymes which specifically catalyze the hydrolysis or synthesis of glucosidic linkages of starch are represented by four classes based on the type of reaction they catalyze, which are: (i) α-amylase (EC 3.2.1.1) involved in the hydrolysis of α-1,4-glucosidic linkages; (ii) pullulanase (EC 3.2.1.41) or isoamylase (EC 3.2.1.68) for the hydrolysis of α-1,6-glucosidic linkages; (iii)

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cyclodextrin glucanotransferase (CGTase, EC 2.4.1.19) for transglycosilation to form α-1,4-glucosidic linkages; and (iv) branching enzyme (EC 2.4.1.18) involved in transglycosilation to form α-1,6-glucosidic linkages. The functions of these enzymes in the four reactions mentioned above are well established (Kuriki and Imanaka, 1999). All of the four reactions are representatively catalyzed by four individual types of enzymes. However, exceptionally sometimes α-amylase, for example, weakly catalyzes α-1,4-transglycosylation in addition to the main reaction, i.e., α-1,4-hydrolysis (Hehre *et al.*, 1971). Similarly, CGTases feebly catalyze α-1,4-hydrolysis in addition to the main reaction, α-1,4-transglycosylation (Kitahata and Okada, 1982). Furthermore, some α-amylases are involved in α-1,6-hydrolysis (Sakano *et al.*, 1983; Okada and Mizokami, 1980). Some pullulanases from thermophiles have been reported to hydrolyze not only α-1,6- but also α-1,4-glucosidic linkages (Lee *et al.*, 1994; Mathupala *et al.*, 1993; Melasniemi *et al.*, 1990). However, these observations have not been seriously considered and have been regarded as only trivial side-reactions (Kuriki and Imanaka, 1999).

In general, α-amylases hydrolyze α-1,4-glucosidic bonds, whereas CGTases catalyse transglycosylation reactions to produce cyclodextrins (CDs). The CDs produced by various CGTases mainly consist of six, seven, or eight α-1,4-linked D-glucopyranosyl units named as α, β, and γ-cyclodextrins, respectively (Fig. 1). They are able to form inclusion complexes with several organic and inorganic molecules, thereby changing the physical and chemical properties of the encapsulated compounds. This property makes CDs suitable for numerous applications in the food, cosmetic, and pharmaceutical industries, where they are used for capturing undesirable tastes or odours, stabilize volatile and light- or oxygen-sensitive compounds, increase the water solubility of hydrophobic substances, and protect substances against unwanted modifications (van der Veen *et al.*, 2000a).

Apart from the CDs synthesis, CGTases catalyze three other reactions (Fig. 2), which are: (i) disproportionation, the transfer of a part of a linear oligosaccharide to another oligosaccharide; (ii) coupling, the opening of a CD molecule followed by transfer to a linear oligosaccharide; and (iii) hydrolysis, the transfer of a part of a linear oligosaccharide to a water molecule (van der Veen *et al.*, 2000a).

Most of the amylolytic enzymes belong to three different families of glycosidases: (i) family-13, α-amylases and other enzymes showing amylolytic action (representing about twenty different specificities); (ii) family-14, β-amylases; and (iii) family-15, glucoamylases. Although these three families cleave the α-glycosidic bonds of starch, they are not similar structurally. Cyclodextrin glucanotransferases are members of the family-13 glycosyl hydrolases (Henrissat, 1991). Primary and three-dimensional structural comparisons between CGTases and α-amylases have revealed both common and distinct features among the enzymes. They share three structural domains: **A**, **B**, and **C**. The A domain is the catalytic domain and comprises a $(\beta/\alpha)_8$, or TIM (triosephosphate isomerase) barrel (Janecek, 1994). Domains **B** and **C** are considered to be involved in substrate binding. Cyclodextrin glucanotransferases have two additional domains not found in α-amylases, domains **D** and **E**, with the exception of two CGTases from *Klebsiella pneumoniae* (Binder *et al.*, 1986) and *Nostoc* sp PCC 9229 (Wouters *et al.*, 2003). These two CGTases lack almost the entire typical domain **D**. The function of domain **D** is unknown at present, while domain **E** has been found to be a starch-binding domain (van der Veen *et al.*, 2000a).

Two maltose-binding sites have been identified in the **E** domain of CGTase from *Bacillus circulans* strain 251, and

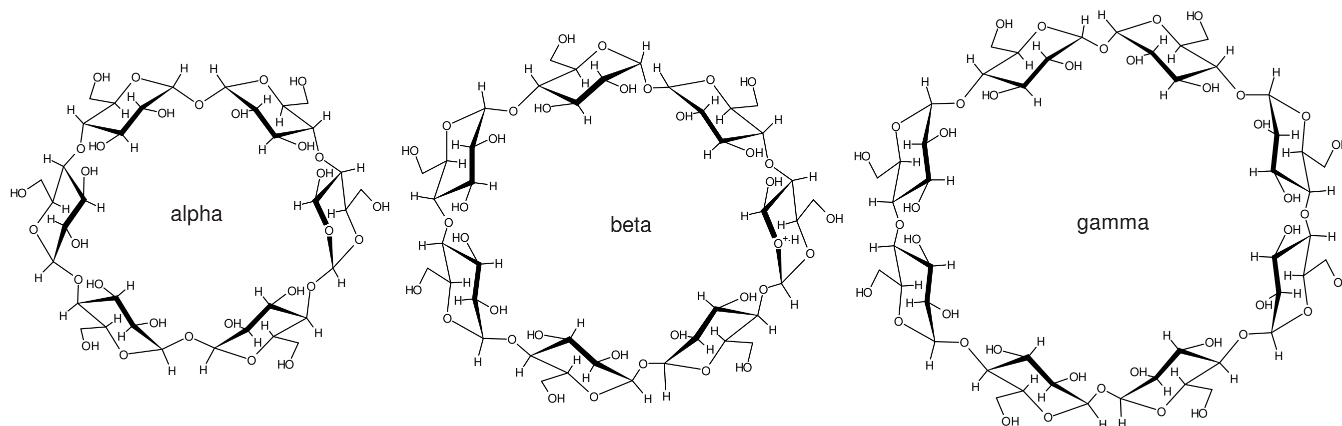

Fig. 1. Structure of α-, β-, and γ-cyclodextrin consisting of six, seven or eight α-1,4-linked D-glucopyranosyl units, respectively.

evidence that this domain contributes to raw-starch binding has been obtained for other enzymes as well (Svensson *et al*., 1989). Although it remains to be clarified whether the effects are direct or a consequence of indirect structural distortion, truncation of the C-terminal region of CGTase from *Bacillus* sp strain 1011 has led to a change in reaction specificities (Kimura *et al*., 1989), suggesting a possible role of the C-terminal region in CGTase activity.

Properties of thermostable CGTases. A number of CGTases have been identified and characterized from mesophilic organisms (Hirano *et al*., 2005; Matioli *et al*., 2002; van der Veen *et al*., 2000a; 2000b). However, thermostable CGTases have only been identified and characterized from a few microorganisms including *Thermoanaerobacterium thermosulfurigenes* EM1 (Knegtel *et al*., 1996), *Thermococcus* sp strain B1001 (Tachibana *et al*., 1999), and *Thermococcus kodakaraensis* KOD1 (Rashid *et al*., 2002). The primary structure of these thermostable CGTases displayed all the four conserved regions (Motif **I** to **IV**) characteristically found in the α-amylase family (Nakajima *et al*., 1986) (Fig. 3). Similarly, the five domains **A** to **E**, characteristically found in CGTases from mesophilic organisms (van der Veen *et al*., 2000a), were also present in these thermostable enzymes (Fig. 4). These three enzymes, from *T. thermosulfurigenes* EM1, *Thermococcus* sp strain B1001 and *T. kodakaraensis* KOD1, displayed significant homology among them in the first four domains (**A** to **D**), whereas domain **E** in the *T. kodakaraensis* KOD1 CGTase displayed a distinct primary structure. The CGTases from

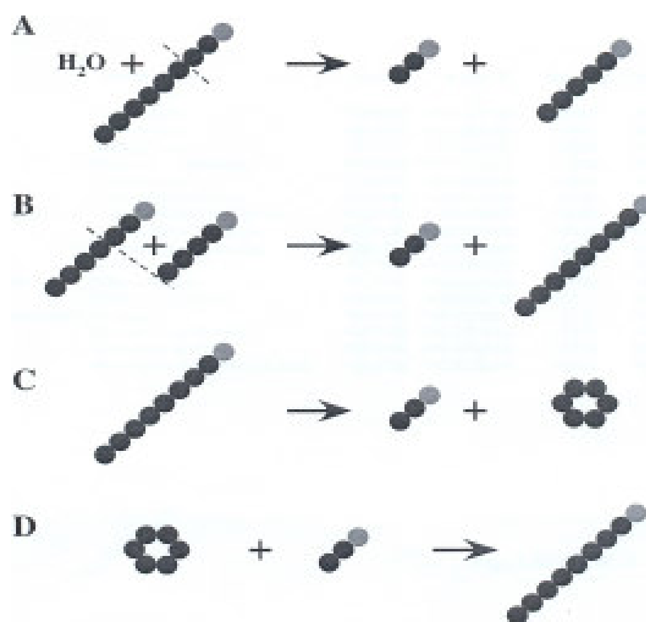

Fig. 2. Schematic representation of the CGTase catalyzed reactions; circles represent glucose residues, the grey circles indicate the reducing end sugars; (**A**) hydrolysis, the transfer of a part of a linear oligosaccharide to a water molecule; (**B**) disproportionation, the transfer of a part of a linear oligosaccharide to another oligosaccharide; (**C**) cyclization, conversion of starch into cyclodextrins; (**D**) coupling, the opening of cyclodextrin molecule followed by transfer to a linear oligosaccharide.

Enzyme	**Source**	**Region I**	**Region II**	**Region III**	**Region IV**
α-Amylase	*Aspergillus oryzae*	117 DVVANH	202 GLRIDTVKH	230 EVLD	292 FVENHD
CGTase	*Bacillus marcerans*	135 DFAPNH	225 GIRMDAVKH	258 EWFL	324 FIDNHD
Pullulanase	*Klebsiella aerogenes*	600 DVVYNH	671 GFRFDLMGY	704 EGWD	827 YVSKHD
Isoamylase	*Pseudomonas amyloderamosa*	292 DVVYNH	371 GFRFDLASV	435 EPWA	506 FIDVHD
Branching enzyme	*Escherichia coli*	335 DWVPGH	401 ALRVDAVAS	458 EEST	521 LPLSHD
Neopullulanase	*Bacillus stearothermophilus*	242 DAVFNH	324 GWRLDVANE	357 EIWH	419 LLGSHD
α-Amylase-pullulanase	*Clostridium thermohydrosulfulocum*	488 DGVFNH	594 GWRLDVANE	627 ENWN	699 LLGSHD
α-Glucosidase	*Saccharomyces carlsbergenesis*	106 DLVINH	210 GFRIDTAGL	276 EVAH	344 YIENHD
Cyclodextrinase	*Thermoanaerobacter ethanolicus*	238 DAVFNH	321 GWRLDVANE	354 EVWH	416 LIGSHD
α-1, 6-Glucasidase	*Bacillus cereus*	98 DLVVNH	195 GFRMDVINF	255 EMPG	324 YWNNHD
Dextran glucasidase	*Streptococcus mutans*	98 DLVVNH	190 GFRMDVIDM	236 ETWG	308 FWNNHD
Amylomatase	*Streptococcus pneumoniae*	224 DMWAND	291 IVRIDHFRG	332 EELG	391 YTGTHD
Glycogen debranching enzyme	*Homo sapiens*	298 DVVYNH	504 GVRLDNCHS	534 ELFT	603 MDITHD
CGTase	*Thermococcus kodakaraensis*	135 DYVPNH	236 GLRIDAVKH	265 EYFT	334 FLDSHD
CGTase	*Thermococcus* sp. B1001	163 DFVPNH	260 GIRIDAVKH	292 EWYQ	359 FVDSHD
CGTase	*Thermoanaerobacter thermosulfurigenes*	161 DFAPNH	251 GIRLDAVKH	283 EWFL	349 FIDNHD

Fig. 3. Enzymes belonging to α-amylase family and their four highly conserved regions; invariable three catalytic sites are indicated by arrow heads; numbering of the amino acid sequences is shown on the left side of the sequence; amino acid residues conserved in all members of family-13 are shown in boxes; thermostable cyclodextrin glucanotransferases are shown in bold.

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Tt   MKKTFKLILVLMLSLTLVFGLTAPIQAASDTAVSNVVNYSTDVIYQIVTDRFVDGNTSNN 60
Tb   -MRSRKIYMLVVLLLFLGFSEQISTVAAGVSPSYPAGDPQTWVIYQIVIDRFYDGNTSNN 59
Tk   --------MGRLVSVVLIVLLLTPIASAWEVPER-------GIIYQVMVDRFYDGNTSNN 45
                       *                      *             ***   *** *******

Tt   P---TGDLYDPTHTSLKKYFGGDWQGIINKINDGYLTGMGVTAIWISQPVENIYAVLPDS 117
Tb   DPLKSPGLYDPNKENWKLYWGGDLEGIIAKLP--YLYELGVSAIWISPVFDNIDVPING- 115
Tk   E-----PFYDPTHSNYRLYWGGDLEGLIEKLD--YIKSLGVSMIWVSPLNDNINSLAYG- 97
            ***        * ***   * * *     *     **  ** *   **
                                                      A1 <---|---> B
Tt   TFGGSTSYHGYWARDFKRTNPYFGSFTDFQNLINTAHAHNIKVIIDFAPNHTSPASETDP 177
Tb   SNGLEAGYHGYWPKDFKVIEEHFGTWEIFRRLSQEAAKYNITIIIDFVPNHSNPN----- 170
Tk   ----SAPYHGYWTRDYKRIEEHFGGWEDFRRLVKEAKKRGICIIVDYVPNHSNPVN---- 149
         *****  * *     **    *  *   *    *  *  * *  *** *

Tt   TYAENGRLYDNGTLLGGYTNDTN-------------GYFHHYGGTDFSSYEDGIYRNLFD 224
Tb   DAGEYGALYDNGTFVIDYPTDANYATVHPITKSLSYIYNHNGGITNWNDRWEVRYKNLFN 230
Tk   -YGEYGALYDNGTFLTDYFKDTKNAEVNPITGIRENVYHHNGNIYTWSG-IPLKYANLYG 207
       * * ******    *  *                  * *                * **
B <---|---> A2
Tt   LADLNQQNSTIDSYLKSAIKVWLDMGIDGIRLDAVKHMPFGWQKNFMDSILSYRPVFTFG 284
Tb   LADLNQLNPWVDNYLKESTVSYLEAGIGGIRIDAVKHLEPGWLKTYADYVYARKNVFMFG 290
Tk   LADFNQLNPWVDSYLTEGAMLFVDSGACGLRIDAVKHMELGWLETFYLRLYSKGPLFIYG 267
     *** ** *    * **           *  * * *****   **                * *

Tt   EWFLGTN-EIDVNNTYFANESGMSLLDFRFSQKVRQVFRDNTDTMYGLDSMIQSTASDYN 343
Tb   EWYQGFNDEMYWDMVKFANYTGIGLINIPLQQVLVDVFAYDTKTMWDLESAVNKYTIDFM 350
Tk   EYFTPSLQKGDDLYEFYRYSNVSPVLSIPIREDIVRIFAFFGG-LDKLSEELGDYYSHFV 326
     *                                      *         *

Tt   FINDMVTFIDNHDMDRFYNGG----STRPVEQALAFTLTSRGVPAIYYGTEQYMTGN--- 396
Tb   WQNKLTIFVDSHDVPRFLSLRN---DLIRFHQALAFVLTAPGIPIIYYGDEQYLHNDTVN 407
Tk   YPTKAVNFLDSHDLVRFLNAGDRKDEIQRFHMALALTLTLPGIPVIYYGDESYLVSKDGK 386
            * *  **   **                 ***  **   * *  **** * *
                                                   A2 <---|---> C
Tt   ------GDPYNRAMMTSFNTSTTAYNVIKKLAPLRKSNPAIAYGTTQQRWINNDVYIYER 450
Tb   DFGQVGGDPYNRPMMKTWNTSTTAFKLIKTLASLRRYNPALAYGLIATRYVSDDIYIFER 467
Tk   ------GDPYNRPTMVFDNT-TEASRIIRTLGGLRKTNDALVFGDFMTVTASYETWAFER 439
           ******  *   ** * *   *   *  **  * *^  *                **

Tt   KFGNNVALVAINRNLSTSYNITGLYTALPAGTYTDVLGGLLNGNSISVASDGSVTPFTLS 510
Tb   KFFDNVVLVAINRNLNSPYVVSNVYTSLPDGSYNDYLGGLLNGVGITVSSG--TFSVELP 525
Tk   TFGNHSLLVVMNKGPAVNLTFS---VDWPDGNYRDALY----GGEMVVSGG--KASVYLP 490
      *  **  *             * * * * *     *    *             *
     C <---|---> D
Tt   AGEVAVWQYVSSSNSPLIGHVGPTMTKAGQTITIDGRGFGTTSGQVLFG----STAGTIV 566
Tb   AGSVSVWQYKATPTDPWVGAIDPVMGRAGNIVTVSGEGFGDVPGRVLITNGQDYWTAEVT 585
Tk   RDSVYVFHIEGEQKKPLIGSITPYAARPGQEIVIGGAGFGKGGKVIIGG-----REAKVL 545
       * *          *  *   *     *     * ***
                                                D <---|---> E
Tt   SWDDTEVKVKVPS-----VTPGKYNISLKTSSGATSNTYNNINILTGNQICVRFVVN--- 618
Tb   YWSDKSVEFIVPSGITTQLNENHVEVRIERADGATSNGIAFEYLTNKQIPAIFEVRNTQG 645
Tk   SWEDGKIVVEVPR-----LETSAAWVNVTVVSDGGRSPPRPLRYYSGNDVPALIALN--- 597
      * *     **                                               *

Tt   -NASTVYGENVYLTGNVAELGNWDTSKAIGPMFNQVVYQYPTWYYDVSVPAGTTIQFKFI 677
Tb   TNLETQVGEFLWLTGSVPELSYWSP-ETIKAVGPMLCPGWPDWFVVASVPADTYIEFKFL 704
Tk   ASLVGEVSGTLWLSGDLPELGEPRPLLKSSMGYYFTVAPLPEGVPFSVRLYEGKAWGALR 657
               * *   **                         *

Tt   KK-NGNTITWEGGSNHTYTVPSSSTG--------------------TVIVNWQQ--     710
Tb   KAPLGGTGIWEVGSNHAYLTPSSGIG--------------------EVSVEANR--     739
Tk   PLNLTLYGVGNRTVTLTEKPPGVSEGQKAGQKDVALYALSVVMIAALIAVVWKRKG     713
                    *    *                        *
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Fig. 4. Alignment of thermostable cyclodextrin glucanotransferases from *Thermococcus kodakaraensis* KODI (**Tk**), *Thermococcus* sp B1001 (**Tb**), and *Thermoanaerobacterium thermosulfurigenes* EM1 (**Tt**); asterisks indicate conserved regions in the three thermostable cyclodextrin glucanotransferases; four conserved motifs are shaded, and five domains are indicated by letters **A** to **E**; construction site of a chimera is shown by an arrow head.

Thermococcus sp strain B1001 and *T. kodakaraensis* KOD1 display a limited homology to the known CGTases, whereas CGTase from *T. thermosulfurigenes* EM1 exhibits significant homology. The CGTase from *Thermococcus* sp strain B1001 mainly produce α-cyclodextrin along with minor quantities of β- and γ-cyclodextrins (Tachibana *et al*., 1999), while the major product of the enzyme from *T. kodakaraensis* KOD1 was β-cyclodextrin (Rashid *et al*., 2002). On the other hand, CGTase from *T. thermosulfurigenes* EM1 has been reported to produce almost equal amounts of α- (46%) and β-cyclodextrin (43%) (Wind *et al*., 1995). All the three enzymes can efficiently degrade starch. The CGTase from *Thermococcus* sp strain B1001 displayed a higher optimal temperature (110 °C) for starch degrading activity, as compared to the enzyme from *T. kodakaraensis* KOD1 and *T. thermosulfurigenes* EM1 (80 to 90 °C).

The role of the C-terminal region has been a focus of research on CGTases, and many studies have reported various effects of C-terminal deletions (Bender *et al*., 1990; Hellman *et al*., 1990; Kimura *et al*., 1989). We have shown that a truncation of 23 amino acids at the extreme C-terminus of CGTase from *T. kodakaraensis* KOD1 resulted in an increase in starch- degrading activity, as compared to the parental enzyme. Though cyclization activity was abolished, other properties of the enzyme such as secondary structure, thermostability, and starch-binding activity remained unaltered (Rashid *et al*., 2002). These observations indicate a direct function of the C-terminal region in cyclization activity. As the C-terminal region in *T. kodakaraensis* KOD1 is quite distinct, as compared to the other characterized CGTases, a chimeric protein consisting of domain **A**, **B**, and **C** [amino acids 1 to 414 (KOD1 numbering)] from *T. kodakaraensis* KOD1 (β-cyclodextin producer), and domain **D** and **E** [amino acids 443 to 739 (B1001 numbering)] from *Thermococcus* sp strain B1001 (α-cyclodextrin producer) was constructed, which aimed at chan-ging the reaction product specificity of the chimeric protein. However, the resulting chimeric protein failed to produce any cyclodextrin, indicating that the CGTase activity was completely abolished. In fact, the chimeric enzyme even failed to degrade starch (unpublished data), suggesting a distortion in the tertiary structure of the chimeric protein. When retrieving CGTase amino acid sequence from the SWISS-PORT/EMBL protein database the following description of CGTases is provided: "CGTase may consist of two protein domains; the one in the amino-terminal side cleaves the α-1,4-glucosidic bond in starch, and the other in the C-terminal side catalyses other activities, including the reconstitution of an α-1,4-glucosidic linkage for cyclizing the malto-oligosaccharides produced. The construction of the above mentioned chimeric protein and its inability to degrade starch suggests that the overall structure of the protein determines the product specificity instead of the individual domains. Modern techniques, such as genomics, proteomics, DNA shuffling, gene evolution, and protein engineering are likely to help generate new tailor-made enzymes with subtle changes in order to get desired properties.

Practical advantages. Thermostable enzymes exhibit several practical advantages, other than their thermostability, as compared to their thermolabile counterparts. An extremely valuable advantage of conducting biotechnological processes at higher temperatures is reducing the risk of contamination by common mesophiles. A higher reaction rate due to decrease in substrate viscosity and higher process yield due to increased solubility of substrates and products can be achieved at elevated temperatures. Another important fact is that thermostable proteins have less hydrophobic patches at the surface, and as a result the hydrophilicity of the solvent accessible surface of a thermostable protein is generally higher than that of a thermolabile protein (Atomi *et al*., 2000). This allows use of these enzymes at higher concentrations in aqueous environments, and may also increase the chances of obtaining crystals for structural studies. A further advantage can be easily envisaged considering the high stability of the structure, mutations should be possible without fatally disturbing the protein fold itself.

After gene expression in a mesophilic host such as *Eschericia coli*, purification of the expressed product can be performed to a relatively high degree simply by heating the cell free extract at 80 to 90 °C for 10 to 20 min. Almost all the proteins derived from the host cell precipitate after this heat treatment, while the recombinant thermostable protein remains in the soluble form. In most of the cases, the enzymes from hyperthermophiles are activated during this heat treatment to an optimal protein conformation (Imanaka and Atomi, 2002). Another practical advantage is that chromatographic steps required for the purification of the protein can be performed at ambient temperatures in contrast to thermolabile proteins where these are to be performed at 4 °C.

Conclusion

A number of CGTases have been identified from mesophilic organisms and are being used as the starting materials for further improvement in their performance (Kometani *et al*., 1996a; 1996b). The number of CGTases from hyperthermophiles that can synthesize CD is still limi-ted (Rashid *et al*., 2002). Cyclodextrin production involves α-amylase-catalyzed starch liquefaction followed by CD formation using a mesophilic CGTase. Cyclodextrin glucano-transferases characterized from

hyperthermophiles are highly stable at 100 °C and optimally active around 90 °C under acidic pH (Rashid *et al*., 2002; Tachibana *et al*., 1999; Knegtel *et al*., 1996). They also exhibit high starch-degrading (α-amylase) activity. These enzymes, therefore, may probably be used to develop a one-step CD production in which they would replace α-amylase for starch liquefaction. A major disadvantage of CD production by CGTases is that all known wild type CGTases produce a mixture of α-, β-, and γ-cyclo-dextrin and are sensitive to product inhibition by these cyclic compounds (Hirano *et al*., 2005; Matioli *et al*., 2002; van der Veen *et al*., 2000b). There is, therefore, a need to search for novel CGTases that can produce a specific CD and are not sensi-tive to product inhibition.

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