Review

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Cyclodextrin-preferring glycoside hydrolases: properties and applications

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Abstract: Cyclodextrin-hydrolyzing enzymes are widespread in bacteria and archaea where they play their roles in carbohydrates metabolism. They were previously characterized as cyclodextrinases, neopullulanases and maltogenic amylases. In the Carbohydrate-Active EnZyme (CAZy) database, most of these enzymes are grouped into the GH13_20 subfamily of the α-amylase family GH13. Here, we have summarized the information available on the substrate specificity, structural features, physiological roles and applications of cyclodextrin-preferring glycoside hydrolases. These enzymes form a distinct group in the α-amylase family. Members of this distinct group possess an extra extension at the N-terminus, which causes a modification of the active site geometry thus making these enzymes more specific for smaller molecules like cyclodextrins than for macromolecules such as starches or pullulan. Multi-substrate specificity, hydrolytic as well as transglycosylation activities make these enzymes attractive for applications in the food and pharmaceutical industries. We have tried here to collect information available on their biochemical properties, three-dimensional structures, physiological roles and potential applications.

Keywords: cyclodextrinases; structures; properties; applications.

1 Introduction

Cyclodextrins (CDs) refer to cyclic oligo-glucosides joined via α-1,4-glycosidic linkages. Depending upon the number of D-glucopyranosyl residues, that may be six, seven or eight, CDs are termed as α-, β- or γ-CD, respectively. Most of the CD-hydrolyzing enzymes characterized to date belong to subfamily 20 of the glycoside hydrolase (GH) family 13 [1] (http://www.cazy.org/GH13_20.html). Initially, cyclomaltodextrinases (CDases; EC 3.2.1.54), maltogenic amylases (MAases; EC 3.2.1.133) and neopullulanases (NPases; EC 3.2.1.135) were included in this distinct group [1]. CDases are the enzymes that preferentially hydrolyze CDs, MAases are the enzymes that favor to generate maltose from hydrolysis of starch, and NPases are the enzymes that are capable of hydrolyzing pullulan into panose. Members of this distinct group catalyze all the three reactions, however, they prefer CDs over starch and pullulan, and are involved in intracellular processing of CDs [2,3]. With further advancements in structural biology, it was found that these three classes of enzymes were not very much different from each other. It was hence proposed that CDases, MAases and NPases are nearly the same enzymes in terms of structure and catalytic characteristics, therefore they should be classified under the same name and enzyme code. CDase was proposed as the best name for them [3,4].

The first report on CD-hydrolyzing enzyme was published in 1968 with the identification and pattern of action of a CDase from Bacillus macerans [5,6]. Afterwards, CDases from various sources, such as Bacillus coagulans [7], Clostridium thermohydrodsulfuricum [8], alkalophilic Bacillus sp. [9], Bacillus sphaericus E-244 [10-12], Bacillus sp. I-5 [13], Bacillus subtilis SUH4-2 [14], Flavobacterium sp. [15], Bacillus sphaericus ATCC7055 [16], Bacillus clarkii [17], Bacillus sp. US169 [18], Geobacillus caldolyxooslyticus TK4 [19], Massilia timonae [20], Bacillus subtilis WPD616 [21], Geobacillus thermopakistaniensis [22] and several others were characterized from bacterial sources. Enzymes of archaeal origin were characterized from Thermococcus sp. strain B1001 [23], Thermococcus sp. CL1 [24], Thermococcus
kodakarensis KOD1 [25,26], Thermophilum pendens [27], Pyrococcus furiosus [28], Thermoplasma volcanium GSS1 [29], Staphylothermus marinus [30] and Palaeococcus pacificus [31].

2 Biochemical properties of CDases

2.1 Cyclodextrin preference

Most of the CDases cleave α-1,4-glucosidic linkages, however, a few of them, including the enzyme from Lactobacillus gasseri ATCC 33323, also hydrolyze α-1,6-glucosidic linkages. Ring-opening of CDs is the rate-limiting step, once the ring opens, it is processed, by CDases, similar to linear oligosaccharides [32]. CDases are multi-substrate hydrolyzing enzymes. Usually, they prefer either α-, β- or γ-CD.

α-CD-prefering CDases include enzymes from Clostridium thermohydrodursulfuricum 39E [33], Bacillus steaotermophilus HY1 [34], Geobacillus thermopakistaniensis [22], Geobacillus sp. Gh6 [35], Palaeococcus pacificus [31], Pyrococcus furiosus [28], Anoxybacillus flavithermus ZNU-NGA [36] and a CDase expressed from environmental DNA isolated directly from a hot spring in Thailand [37]. CDases characterized from Massilia timonae [20], Paenibacillus sp. A11 [38, 39], Alicyclobacillus acidocaldarius [40], Thermococcus sp. strain B1001 [23], Thermococcus kodakarensis [25], Laceyella sp. [41], and Bacillus sp. [42], and Bacillus sp. ATCC7055 [16] prefer β-CD. The CDases from Thermophila pendens [27] and Bacillus clarkii [17] are among the γ-CD-prefering enzymes.

The CDases that show a slightly higher preference for maltoligosaccharides include enzymes from Flavobacterium sp., Pyrococcus furiosus and Bacillus coagulans [7, 5, 42]. Similarly, a CDase characterized (after heterologous expression of metagenome DNA) from cow rumen showed preference for maltoligosaccharides as compared with CDs [43]. A CDase from Lactobacillus plantarum exhibited almost equal activity against β-CD and starch [44]. Table 1 gives a detailed comparison of CDases with regard to their substrate specificity and other biochemical properties.

2.2 Acarbose hydrolysis

Acarbose is a pseudotetrasaccharide that inhibits the activities of α-amylases and α-glucosidases. Acarbose hydrolysis is the unique feature of CDases, which convert acarbose into a pseudotrisaccharide and glucose [45]. However, in the presence of acarbose, cyclodextrinase activity of CDases may be inhibited possibly due to competition for access to the active site [22]. It has been reported that acarbose-mediated inhibition hinders the ring-opening of CDs [46]. CD-hydrolyzing MAase from Lactobacillus gasseri ATCC 33323 was completely inhibited by acarbose [32], while the MAase from Therms sp. IM6501 (ThMA) and a cyclodextrinase from Streptococcus pyogenes could hydrolyze acarbose to glucose and acarviosine-glucose. ThMA can further hydrolyze acarviosine-glucose into acarviosine and glucose [47, 48]. A CDase from L. plantarum hydrolyzed acarbose via two different modes of action to produce maltose and acarviosine, as well as glucose and acarviosine-glucose [44].

2.2.1 Transglycosylation

CD-hydrolyzing enzymes also exhibit transglycosylation activity with various acceptor molecules mainly by creating different types of glucosidic linkages – α-1,3-, α-1,4- and α-1,6-glucosidic linkages, but α-1,6-linkage is more common as seen in the case of CDases from Lactobacillus gasseri [32] and Streptococcus pyogenes [47]. ThMA transferred pseudotrisaccharide obtained from acarbose hydrolysis to the C-6, C-3 and/or C-4 of the acceptors [45]. Similarly, a MAase from Bacillus thermoalkalophilus exhibited the α-1,3-, α-1,4- and α-1,6-transferase activities [2]. The MAase from B. subtilis showed α-1,3- and α-1,6-transferase activities only [14], whereas the archaeal MAase from Thermoplasma volcanium could form the α-1,4- and α-1,6-linkages by transglycosylation [29]. Transglycosylation property of CDases has successfully been employed to synthesize several valuable compounds, such as branched oligosaccharides [49-51], carbohydrase inhibitors [52] and biologically active compounds, e.g. glycosyl-derivatives of ascorbic acid [4, 53], puerarin [54], genistin [55] and simmondsin [48].

2.3 Three dimensional structures of CDases

CDases belong to family GH13 [56], and TAKA-amylase A (the α-amylase from Aspergillus oryzae) was the first enzyme of this family whose three-dimensional structure was solved in 1984 [57]. The enzyme contains a (β/α)_{8}-barrel catalytic domain, which consists of a barrel structure formed by eight α-helices that surround eight parallel β-strands in a highly ordered manner. This (β/α)_{8}-
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| Organism                                      | Temp. | pH  | Substrate preference          | Transglycosylation | AH         | CDH         | Ref.  |
|-----------------------------------------------|-------|-----|-------------------------------|--------------------|------------|-------------|-------|
| Thermus sp. IM6501                            | 60    | 6   | β-CD > Pullulan > Starch      | α-1.3, α-1.4, α-1.6 | Yes        | M1, M2      | [45]  |
| Geobacillus thermapakistaniensis              | 55    | 6   | α-CD > β-CD > γ-CD > Dextrin > Pullulan > Starch | Yes*              | Yes        | M1, M2      | [22]  |
| Geobacillus sp. Gh6                           | 60    | 6   | α-CD > β-CD > γ-CD > Amylose > Amylopectin > Starch > Glycogen | ND                | ND         | ND          | [35]  |
| Geobacillus thermoleovorans                   | 80    | 5-9 | α-CD > β-CD > Starch > Pullulan | Yes*              | Yes        | M2          | [73]  |
| Bacillus thermoalkalophilus ET2               | 70    | 8   | β-CD > Maltotriose > Pullulan > Starch | α-1.6, α-1.4, α-1.3 | Yes        | M1, M2      | [2]   |
| Bacillus steaotermophilus IMA6503             | 60    | 6   | β-CD > Pullulan > Starch      | Yes*              | Yes        | M1, M2      | [108] |
| Bacillus steaotermophilus HY-1                | 55    | 6   | β-CD > Pullulan > Starch      | Yes*              | Yes        | M1, M2      | [34]  |
| Bacillus lehensis G1                          | 40    | 7-9 | β-CD > Starch                 | Yes*              | ND         | M1, M2, MOS | [50]  |
| Bacillus clarkii                              | 30    | 8.5 | γ-CD > Starch > β-CD > α-CD > Pullulan = Glycogen | Yes*              | ND         | M2, MOS     | [17]  |
| Geobacillus caldoxylosilyticus TK4            | 50    | 7   | β-CD > Starch                 | ND                | ND         | M1, M2, MOS | [19]  |
| Bacillus subtilis SUH4-2                      | 40-45 | 7   | β-CD > Starch > Pullulan      | α-1.6, α-1.3       | Yes        | M1, M2      | [14]  |
| Bacillus sp. US149                           | 55    | 6.5 | β-CD > Pullulan > Starch      | ND                | ND         | M1, M2      | [18]  |
| Bacillus sp. WPD616                           | 50    | 6   | β-CD > Pullulan > Starch      | ND                | ND         | ND          | [21]  |
| Anoxybacillus flavithermus ZNU-NGA            | 65    | 7   | α-CD > β-CD > γ-CD > Starch = Glycogen | ND                | ND         | M1, M2      | [36]  |
| Bacillus sphaericus ATCC 7055                 | 40    | 6-6.5 | β-CD > α-CD > γ-CD > Maltodextrins | ND                | ND         | M1, M2      | [16]  |
| Bacillus sphaericus E-244                     | >45   | 8   | β-CD > maltodextrins > α-CD > Starch > Pullulan | ND                | ND         | MOS         | [10]  |
| Lactobacillus plantarum                       | 45    | 5   | β-CD = Starch > Pullulan      | ND                | Yes        | M2          | [44]  |
| Lactobacillus gasseri ATCC 33323              | 55    | 5   | β-CD > Pullulan > Starch      | ND                | Yes        | M1, M2      | [32]  |
| Clostridium thermohydrosulfuricum 39E         | 65    | 5.9 | α-CD > β-CD > γ-CD > Maltodextrins > Pullulan > Starch | ND                | ND         | M1          | [8,33]|
| Bacillus coagulans                            | 50    | 6.2 | Maltodextrins > β-CD > Starch | ND                | ND         | M2          | [7]   |
| Enterococcus faecium K-1                      | 50    | 6   | α-CD > β-CD > Pullulan > Starch | ND                | ND         | M1, M2      | [74]  |
| Massilia timonae CTI–57                       | 40    | 7   | β-CD > Maltodextrins > Starch | ND                | ND         | M1, M2      | [20]  |
| Paenibacillus sp. A11                         | 40    | 7   | β-CD > γ-CD > α-CD > Maltodextrins > Starch > Pullulan | ND                | ND         | M1, M2      | [38,39]|
| Flavobacterium sp. No. 92                     | 30    | 6-7.5 | Maltodextrins > α-CD > β-CD > Starch > Pullulan | Yes*              | ND         | M1, M2, M3  | [15,61]|
| Alicyclobacillus acidocaldarius               | 55    | 5.5 | β-CD > α-CD > Starch > Pullulan | ND                | ND         | ND          | [40]  |
barrel domain was initially identified in triosephosphate isomerase, therefore also referred as TIM barrel domain. Later, this common folding scaffold was found in many enzymes from the GH13 family and now it is considered as a distinguishing feature of this family.

Three dimensional structures of several CDases have been solved. Among them, CDases from bacterial origin include *Thermoactinomyces vulgaris* R-47 [58], *Thermus* sp. IM6501 [59], *Bacillus stearothermophilus* [60] and *Flavobacterium* sp. no. 92 [61], while archaeal counterparts include *Staphylothermus marinus* [62] and *Pyrococcus furiosus* [28]. These structures reveal that, similar to typical α-amylases, CDases are multi-domain enzymes and contain three basic domains (A, B and C). Domain A is the central core domain comprising the common catalytic (β/α)₈-barrel. Domain B contains the most conserved aspartic acid residue and protrudes out of the (β/α)₈-barrel as an insertion between β3-strand and α3-helix. Domain C is positioned after the (β/α)₈-barrel [56,63]. In addition to these common domains, CDases possess one or two N-terminally located exceptional domains, which enable them to hydrolyze CDs (Fig. 1). Bacterial CDase contains a single N-domain, which enables them to adopt a homodimeric configuration through interaction of the N-domain of one subunit with the catalytic barrel domain of the other subunit (Fig. 2). As a result, a narrow and deep active site is generated, which is especially suitable for preferential accommodation of smaller substrates [3,58-60,62]. However, CDases from hyperthermophilic archaea contain an extra domain, the N’-domain, in addition to N-terminal domain, which swaps around its own active site (Fig. 2). In this way, the N’-domain helps the monomeric subunit of archaeal enzymes in creation of a narrow catalytic pocket, similar to the dimeric bacterial counterparts. Consequently, the monomeric CDases from hyperthermophilic archaea are believed to have acquired a novel domain configuration, with all structural components for recognition and cleavage of

| Organism | Temp. | pH  | Substrate preference | Transglycosylation | AH | CDH | Ref. |
|----------|-------|-----|----------------------|--------------------|----|-----|------|
| Anoxybacillus flavithermus | 57.5 | 6.5 | α-CD > β-CD > Soluble Starch > Amylose > Pullulan | ND | ND | ND | [41,69] |
| Laceyella sacchari | 57 | 6 | β-CD > α-CD > Amylose > Starch > Pullulan | ND | ND | ND | [41,69] |
| Streptococcus pyogenes | 45 | 7.5 | β-CD > Maltotriose > Starch > Pullulan | α-1,6 | Yes | M1, M2 | [47] |

**Archaea**

| Organism | Temp. | pH  | Substrate preference | Transglycosylation | AH | CDH | Ref. |
|----------|-------|-----|----------------------|--------------------|----|-----|------|
| Staphylothermus marinus | 100 | 5 | G6 > Cycloamylose > γ-CD | ND | Yes | M1, M2 | [30] |
| Thermoplasma volcanium GSS1 | 80 | 5.5 | Maltodextrins > β-CD > Starch = Pullulan | α-1,4, α-1,6 | Yes | M1, M2 | [29] |
| Pyrococcus furiosus | 90 | 4.5 | α-CD > β-CD > γ-CD > Maltotriose > Pullulan | No | Yes | M7, MOS | [42] |
| Thermofilum pendens | 95 | 5.5 | γ-CD > β-CD > α-CD > MD > PL = SS | ND | Yes | M1, M2 | [27] |
| Thermococcus sp. strain B1001 | 95 | 5.5 | β-CD > α-CD > γ-CD > Dextrin > Starch | ND | ND | M1, M2 | [23] |
| Thermococcus sp. CL1 | 85 | 5 | α-CD > β-CD > γ-CD > Dextrin > Starch > Pullulan | ND | Yes | M1, M2 | [24] |
| Thermococcus kodakarensis | 65 | 7.5 | β-CD > γ-CD > α-CD > Pullulan > Starch | ND | ND | M1 | [25] |
| Palaeococcus pacificus | 95 | 6 | α-CD > β-CD > γ-CD > Starch | ND | ND | M1, M2 | [31] |

* Temp. – temperature (°C); AH – acarbose hydrolysis; CDH - end products of cyclodextrin hydrolysis; Ref. – reference(s); * – transglycosylation activity was detected but the type of newly created glucosidic linkages is not described; ND – not determined; M1 – glucose; M2 – maltose, M3 – maltotriose; M4 – maltotetraose; M6 – maltohexaose; M7 – maltoheptaose; M8 – maltoctaose; MOS – maltooligosaccharides; MD – maltodextrins.  

*Continued Table 1: Biochemical properties and substrate specificity of CDases.*
the smaller substrates [28-30,62]. N-terminal domains of CDases are also known as starch-binding domains (SBD) and classified as carbohydrate binding module (CBM) families [56,64]. Bacterial N-domain is classified as family CBM34, while archaeal N’-domain is classified as CBM48. In the structure of archaeal CDases CBM48 always precedes the CBM34 at the N-terminus [56,64]. Owing to additional N-terminal domains and other similarities, most of the CD-hydrolyzing enzymes are grouped together in subfamily GH13_20 of the α-amylase superfamily GH13. Some of them have also been placed in subfamilies GH13_36, GH13_21 and GH13_2 [64-66]. Subfamily GH13_20 was previously known as neopullulanase subfamily and have extensively been reviewed in terms of domain evolution, sequence and structural similarities [56].

### 2.4 Physiological role

CDases are involved in modulation of carbohydrate metabolism in bacteria [4,67]. Larger saccharides remain unable to cross the bacterial membranes and are converted to smaller subunits (maltose, maltodextrins or CDs) extracellularly with the help of amylases,
pullulanases, cyclodextrin glycosyltransferases or other related enzymes. Maltose, maltodextrins and CDs thus produced are transferred to the cells, through facilitated diffusion, by specific transporters. After entering the cell, these molecules play their role in glucose metabolism or act as osmolytes. Based on previous studies on physiological roles of CDases in bacteria, a schematic illustration of the physiological role of these enzymes is shown in Figure 3.

**2.5 Oligomeric state**

Dimerization or oligomerization is a common physical property of proteins. It provides structural stability, increases enzyme activity by concentrating the active site, facilitates thermostability and helps to minimize genome size [68]. Oligomerization plays a unique role in the active-site formation of certain structurally known CDases [59]. In addition to stability, oligomeric state
of CDases plays role in substrate preference [41,69,70]. Despite these facts, there are CDases that are monomeric in nature. The monomeric CDases include the enzymes from Paenibacillus sp. A11 [39] and Thermococcus sp. CL1 [24]. The CD-preferring hydrolase from Staphylothermus marinus is active in monomeric form, yet it forms a dimeric structure when studied using the X-ray crystallography [62]. The protein concentration may play a role in monomer to dimer formation. Most of the bacterial CDases exist in a dimeric or an oligomeric form. CDases from Bacillus licheniformis [71], Bacillus steaothermophilus ET1 [72], Thermus sp. IM6501 [45], Geobacillus thermoleovorans [73], Enterococcus faecium K-1 [74], Bacillus halodurans C-125 [75] and Pyrococcus furiosus [42] exist in dimeric form. CDases isolated from Massilia timonae, Lactobacillus gasseri ATCC 33323 and cow rumen metagenome showed tetrameric structures [20,32,43]. Similarly, CDases from alkalophilic Bacillus sp. I-5, Bacillus clarkii and Lactobacillus plantarum showed dodecameric assembly [13,17 44]. CDase from Thermoplasma volcanium GSS1 existed in a higher oligomeric state, which dissociated into monomers and dimers in the presence of 1 M NaCl. Moreover, the dissociated forms of this enzyme were less stable as compared to its native higher oligomeric form [29]. In a similar study, the ThMA dimeric molecule was found dissociating into monomeric one in the presence of 1 M KCl [76]. ThMA, in the dimeric form, was more active for hydrolysis of β-CD, whereas the monomeric form preferred relatively large substrates, such as starch, although its overall activity was lower than that of the dimeric form [29,76]. In addition to enzyme activity, thermostability studies on CDase from Bacillus sp. I-5 revealed that oligomeric form was more stable than dimeric one [70].

### 3 Applications of CDases

#### 3.1 Production of branched oligosaccharides

Branched maltooligosaccharides have importance in the food industry due to their properties of mild sweetness and low viscosity [49,77,78]. Isomaltooligosaccharides are beneficial for optimized intestinal and colon function. These are also known for antidiabetic effects and their role in improvement of lipid metabolism as well as obesity management [79,80]. Production of branched oligosaccharides/isomaltooligosaccharides can be achieved using coupled hydrolytic and transglycosylation activities exhibited by MAases/CDases (Fig. 4). A CDase from Bacillus lehensis proved to be a good candidate for the production of oligosaccharides by virtue of its

![Figure 4: Schematic diagram showing the production of branched oligosaccharides by the action of CDases. Adapted and modified from Lee et al. [81] and Lee et al. [83]. Glucose residues are represented with filled hexagons, while crossed hexagons with overhangs are representing the reducing ends.](image-url)
high transglycosylation activity [50]. In addition to this enzyme, MAases from Lactobacillus gasseri [51], Bacillus licheniformis [81], Bacillus steareothermophilus [82, 83] and Bacillus subtilis [84] have also been employed for the production of branched oligosaccharides.

3.2 Production of maltose syrup

Maltose syrup finds wide applications in food industry as sweetener, humectant and preservative. High maltose syrups are categorized as a mixture of saccharides (glucose, maltose and oligosaccharides) having more than 70% maltose content. These syrups are used as substitute to glucose syrups in canning, bakery and confectionary, especially in the production of hard candies to manage stickiness, crystallization and glossiness [85]. These syrups are obtained from the enzymatic hydrolysis of starch. The traditional process involves starch liquefaction using thermostable α-amylases, and saccharification using exo-amylases (β-amylases) and debranching enzymes (pullulanases). The use of MAases showed superiority over β-amylases, resulting in enhanced yield of maltose content [86]. Maltose syrups with more than 90% maltose contents have successfully been produced employing variants of MAases from Bacillus steareothermophilus [87,88].

3.3 Antistaling agents for bakery products

CD-preferring hydrolases, which produce maltose, maltotriose and maltotetraose, are reported to increase the shelf-life of bakery products by delaying retrogradation of starch [89,90]. Retrogradation occurs due to re-crystallization of amylose and amylopectin from gelatinized starch, and results in the hardness of bread crumb commonly known as bread staling. A thermostable maltogenic α-amylase (Novamyl®) from Bacillus sp. TS-25, formerly Bacillus steareothermophilus, is widely employed as an antistaling agent in the baking industry. Although this enzyme possesses a little endo-activity, it does act as an exo-enzyme during baking. It modifies starch at a temperature when it starts to gelatinize [91]. Novamyl® was initially of limited use as it was ineffective at lower pH. Mutants of this enzyme with improved performance at low pH found applications in the production of sourdough and rye bread [92]. A few other MAases have also been employed recently to decrease the retrogradation and hardness of bread [91].

3.4 Modification of rice starch

CD-preferring hydrolases are being used to modify starch structure in order to produce low-amylose starch products. Rice starch incubated with alkaliophile Bacillus sp. I-5 CDase showed a decrease in amylose content from 28.5 to 9%, while the amylopectin content remained almost unchanged. As a result, the modified rice starch showed a decreased rate of retrogradation when stored at 4 °C for 7 days [93]. Similarly, a thermostable MAase from Thermofilum pendens has been used to reduce the retrogradation of rice foods [94]. In addition to these enzymes, a NPase/CDase from Bacillus stearethermophilus TRS40 was able to selectively hydrolyze relatively small amylose molecules in the presence of large amylopectin molecules in starch. Such enzymes can be used to produce low-amylose starch [95].

3.5 Production of carbohydase inhibitors

During the past couple of decades, carbohydase inhibitors have gained the attention of physicians to manage disorders of carbohydrate metabolism, such as type 2 diabetes and ruminal acidosis [96]. In addition, the carbohydase inhibitors can also be employed for uncovering the physiological roles of various glycans [97]. CDases have been utilized for the production and modification of these inhibitors. For example, acarbose, an inhibitor of several carbohydrases, has been modified by transglycosylation capability of CDases to give acarviosine-glucose (4-α-acarviosine-D-glucopyranose), isocarbose (4n-α-acarviosine isomaltose), 4-α-acarviosine-6n-α-glucopyranosyl cellobiose and 4-α-acarviosine-6α-D-glucopyranosyl lactose. These analogues are found to be very potent and specific inhibitors of α-amylases [52,98].

3.6 Amylolytically-resistant starch

Amylolytically-resistant starches are digested slowly in the small intestine, thereby consumed by beneficial gut microbiota in the colon. Due to this property, these resistant starches are often termed as prebiotics since they promote the growth of probiotics. Resistant starches are produced by modification of native starches through hydrolysis, transglycosylation and rearrangement of chain lengths and branch points [99]. Tapioca starch, extracted from roots of the cassava plant, is one of the most important carbohydrate food sources in many regions of the tropics. It has been modified using a branching enzyme and a
MAase to produce highly branched and low molecular weight starch [100]. The resultant modified starch had decreased molecular weight, increased proportion of shorter branch chains, decreased proportion of longer branch chains, and thus decreased amylose content. Modified tapioca starch showed a slow hydrolysis rate by α-amylases and glucoamylases. Such properties of modified starch ensure slow digestion, which is desirable during prolonged periods of exercise [99]. Enzymatic strategies to modify the structures of starch are expected to take over chemical methods soon [99], providing new routes towards healthy starches. Similar to these studies, treatment of pea starch with MAase and pullulanase resulted in decreased chain length and increased branch density of modified starch [101].

3.7 Transglycosylation of ascorbic acid

Ascorbic acid, a natural antioxidant, has been modified using transglycosylation activity of CDase from *Bacillus stearothermophilus*. Using maltotriose as donor molecule, 6-O-R-D-glucosyl- and 6-O-R-D-maltosyl-ascorbic acids have been synthesized from reaction of maltotriose and ascorbic acid (Fig. 5A). Similarly, 6-O-R-α-carboxysine-D-glucosyl- and 2-O-R-α-carboxysine-D-glucosyl ascorbic acids have been synthesized in the transglycosylation reaction of ascorbic acid (as acceptor) and acarbose (as donor) [53]. Concerning oxidative stability, the 6-O-R-D-maltosyl-ascorbic acid was found to be more stable than the ascorbic acid, while antioxidant effects of glycosyl-derivatives of ascorbic acid are similar to that of ascorbic acid [53].

3.8 Glycosylation of biologically active compounds

CDases have been employed for modification of several biologically active compounds to improve their properties. A few examples are described here.

Naringin, a flavonone glycoside, is one of the predominant bitter components present in grapefruit. In order to reduce bitterness and enhance solubility, narinigin has been modified using a CDase from *Bacillus stearothermophilus*. The resulting compound, maltosylnaringin (Fig. 5B), was 250-times more soluble in water and 10-times less bitter than the parent compound naringin [102].

Puerarin (daidzein 8-C-glucoside), an isoflavone is prescribed for coronary heart diseases, cardiac infarction, problems in ocular blood flow, sudden deafness, and alcoholism. However, puerarin cannot be given by injection due to its low solubility in water. In order to increase solubility, puerarin was transglycosylated (Fig. 5C). Glycosylation of puerarin using *Bacillus stearothermophilus* MAase enhanced the solubility up to 168 times as compared to that of unglycosylated puerarin [103]. Similarly, an archaeal CDase from *Thermofilum pendens* was employed for glycosylation of puerarin at high temperatures to ensure lower viscosity of solution and avoid contamination (Fig. 5D) [54]. In another study, a puerarin inclusion complex with cycloamylose was synthesized enzymatically using the MAase from *Bacillus stearothermophilus* and 4-α-glucanotransferase from *Thermus scotoductus*. The encapsulated glycosylated puerarin was presumed to be widely applicable due to its improved water solubility and stability. Carbohydrate-active enzymes in the human intestine may readily digest the inclusion complex thus improving the bioavailability of puerarin [104].

Genistin, an isoflavone, with proven bioactive role within the neonatal intestine potentially reduces the severity of rotavirus infections. The CD-preferring hydrolase from archaeon *Staphylothermus marinus* has been employed to catalyze the genistin glycosylation using γ-CD as glucosyl donor (Fig. 5E). The glycosylated genistins exhibited enhanced free radical scavenging capacity, indicating its increased antioxidant potential [55].

Erythritol, a four-carbon sugar alcohol, has been transglycosylated by using the MAase from *Bacillus stearothermophilus* (Fig. 5F). The resulting compound, maltosyl-erythritol, displayed quite less negative sensory properties and its sweetness was 40% to that of sugar [105].

3.9 Production of maltodextrins

CDases can be employed for the production of maltodextrins. The CDase from *Pyrococcus furiosus* has been reported to produce maltodextrins from β-CD. The enzyme immobilized in a packed-bed reactor produced maltohexaose and maltooctaose with 92% and 97% purity, respectively [106]. Another archaeal CDase from *Palaeococcus pacificus* was reported to produce high purity maltoheptaose from β-CD [31].
Figure 5: Transglycosylation reactions catalysed by CDases for modification of biologically active compounds.
3.10 Recovery of cholesterol

Nowadays, a rise in cardiac disorders attributed to high cholesterol levels is of significant clinical concern. Efforts have been made to reduce the cholesterol content of dairy products, such as cheese, butter and milk by using β-CD forming inclusion complexes. The resulting β-CD-cholesterol complex can be treated with CDases to recover cholesterol [107].

4 Conclusion

In the present review we have tried to summarize the information available on the substrate specificity, structural features, physiological roles and applications of CD-prefering GHs. These enzymes form a distinct group in the α-amylase family based on their structural features and substrate preferences. Multi-substrate specificity along with coupled hydrolytic and transglycosylation activities make these enzymes potential candidates for food and health sectors.

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