Structure of Cyclodextrin Glycosyltransferase Complexed with a Derivative of Its Main Product β-Cyclodextrin[†,‡]

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Received December 4, 1997; Revised Manuscript Received January 29, 1998

ABSTRACT: Crystals of the inactive mutant Glu257→Ala of cyclodextrin glycosyltransferase were soaked with the cyclodextrin (CD) derivative *S*-(α-D-glucopyranosyl)-6-thio-β-CD. The structural analysis showed its β-CD moiety with no density indication for the exocyclic glucosyl unit. For steric reasons, however, the position of this unit is restricted to be at only two of the seven glucosyl groups of β-CD. The analysis indicated that the enzyme can cyclize branched α-glucans. The ligated β-CD moiety revealed how the enzyme binds its predominant cyclic product. The conformation of the ligated β-CD was intermediate between the more symmetrical conformation in β-CD dodecahydrate crystals and the conformation of a bound linear α-glucan chain. Its scissile bond was displaced by 2.8 Å from the position in linear α-glucans. Accordingly, the complex represents the situation after the cyclization reaction but before diffusion into the solvent, where a more symmetrical conformation is assumed, or the equivalent state in the reverse reaction. Furthermore, a unifying nomenclature for oligosaccharide-binding subsites in proteins is proposed.

Cyclic maltooligosaccharides consisting of six, seven, or eight α(1→4)-linked D-glucopyranosyl units are termed α-, β-, and γ-cyclodextrin (CD),[1] respectively. They have a hydrophilic outside and a hydrophobic interior and can form inclusion complexes with nonpolar compounds, which finds applications in the pharmaceutical, cosmetics, agricultural, and food industries (*1*−*3*). Cyclodextrins are formed via intramolecular transglycosylation when α(1→4)-glucans such as amylose of starch are processed by cyclodextrin glycosyltransferases (CGTases; EC 2.4.1.19), which are monomeric enzymes of bacterial origin. The reaction is $G_n \rightleftharpoons G_{n-x} + cG_x$, where G_n is the α-glucan ($n \geq 8$), G_x is the transferred chain part ($x \geq 6$), and cG_x, denotes a CD, mostly α-, β-, or γ-CD. Recently, much larger rings have been reported (*4*). The reverse reaction is called coupling, i.e. cleaving and adding of a CD to a linear α-glucan. There exists a competing disproportionation reaction (*5*).

Five closely homologous CGTase structures are presently known (*5*−*9*). The initial structure was for the enzyme from *Bacillus circulans* strain 8 (*10*), which produces predominantly β-CD and is analyzed here. CGTases are structurally homologous to α-amylases (*10*, *11*), various structures of which are known (*12*−*19*). Both groups belong to the glycosyl hydrolase family 13 (*20*). Residues Asp229, Glu257, and Asp328 [CGTase numbers (*11*)] participate in catalysis and are strictly conserved. Their roles had been

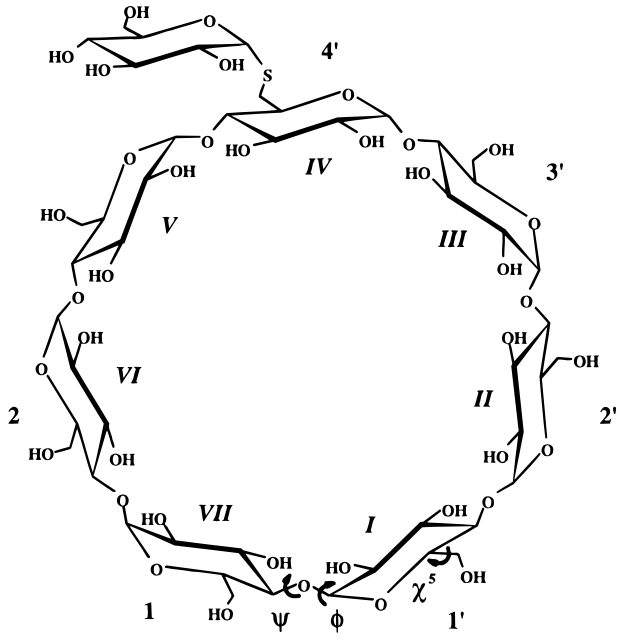

FIGURE 1: Covalent structure of the thioglucosyl-CD (*32*) used in the soaking experiment. The glucosyl units are numbered according to IUPAC−IUBMB recommendations (*38*) starting from the scissile bond and putting the exocyclic unit at the most likely position when ligated to corresponding subsites of CGTase. For reference, the subsite names are shown as they are used in the text; see Figure 2 for the subsite nomenclature. All glucosyl units are shown in the usual $^4C_1$ chair conformation and the torsion angles ϕ, ψ, and χ^5 are indicated.

[†] This work was supported by the European Commission as part of Research Program Bio2-CT943008 and by the Consortium für Elektrochemische Industrie, München, Germany.

[‡] Atomic coordinates and structure factors for the CGTase·β-CD complex have been deposited with the Brookhaven Protein Data Bank under filename 3CGT.

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[1] Abbreviations: CD, cyclodextrin; CGTase, cyclodextrin glycosyltransferase; thioglucosyl-CD, *S*-(α-D-glucopyranosyl)-6-thio-β-cyclodextrin; rms, root-mean-square; σ, standard deviation.

S0006-2960(97)02991-7 CCC: $15.00
Published on Web 04/11/1998

Table 1: Data Collection and Refinement Statistics[a]

data collection, resolution range (Å)	30−2.4
total number of reflections	183 009
number of unique reflections	42 278
redundancy	4.3
R_{sym} (last shell)[b] (%)	9.6 (26.6)
completeness (last shell)[b] (%)	89.1 (71.5)
intensities > 3σ (last shell)[b] (%)	81.3 (46.3)
average I/σ (last shell)[b]	7.2 (2.5)
refinement, number of reflections	41397
R-factor (%)	17.7
R_{free} (3% random subset) (%)	22.8
number of non-hydrogen protein atoms	5263
number of calcium atoms	2
number of carbohydrate atoms	77
number of solvent molecules	205
rms deviations of bond lengths (Å)	0.010
rms deviations of bond angles (deg)	1.6

[a] R_{sym} is defined as $\sum_{i,hkl}|I(i,hkl) - \langle I(hkl)\rangle|/\sum_{i,hkl}I(i,hkl)$, where i runs through the symmetry-related reflections. [b] The last shell has the resolution limits 2.48 and 2.41 Å.

established by mutagenesis in conjunction with crystallographic studies (*12*, *21*−*27*). Glu257 is the general acid/base catalyst (*25*, *27*). A recent study confirmed Asp229 as the catalytic nucleophile (*28*).

Nine subsites for glucosyl units were postulated from kinetic studies with CGTase (*29*). A structure analysis of crystals of the inactive mutant Asp229→Ala soaked in α-glucans showed a maltose bound in the active center and allowed identification of the cleavage point (*25*). Further X-ray structures of other CGTases ligated with linear carbohydrates (*21*, *27*, *30*, *31*) revealed altogether the nine postulated glucosyl subsites. We report here the crystal structure of the inactive CGTase mutant Glu257→Ala complexed with a β-CD derivative showing the predominant product β-CD at a state just after cyclization or before coupling in the reverse reaction.

MATERIALS AND METHODS

Crystallization and Soaking. CGTase mutant Glu257→Ala was crystallized under wild-type conditions (*10*) in space group $P2_12_12_1$ with unit cell axes $a = 94.5$ Å, $b = 105.0$ Å, and $c = 113.8$ Å and one molecule of 684 amino acid residues ($M_r = 74\,416$) per asymmetric unit. Soaking was performed directly in the crystallization drop by adding a 10 mM solution of the β-CD derivative *S*-(α-D-glucopyranosyl)-6-thio-β-CD (thioglucosyl-CD; *32*), depicted in Figure 1, to a final concentration of 1 mM. Since this crystal form is extremely sensitive to the addition of α-glucans (*25*), the soak was monitored and stopped at the first signs of crystal disintegration after about 10 min. Given previous soaking experiences, the observed binding before crystal destruction has to be considered a fortunate event.

Data Collection and Structure Refinement. Intensity data were collected at room temperature using a multiwire area detector (Siemens, model X1000) mounted on a rotating anode generator (Rigaku, model RU200B). Data were processed using the program XDS (*33*). Refinement of the structure was carried out with program X-PLOR using the bulk solvent correction (*34*). The 2.0 Å resolution structure of wild-type CGTase (*5*) including the 171 most strongly

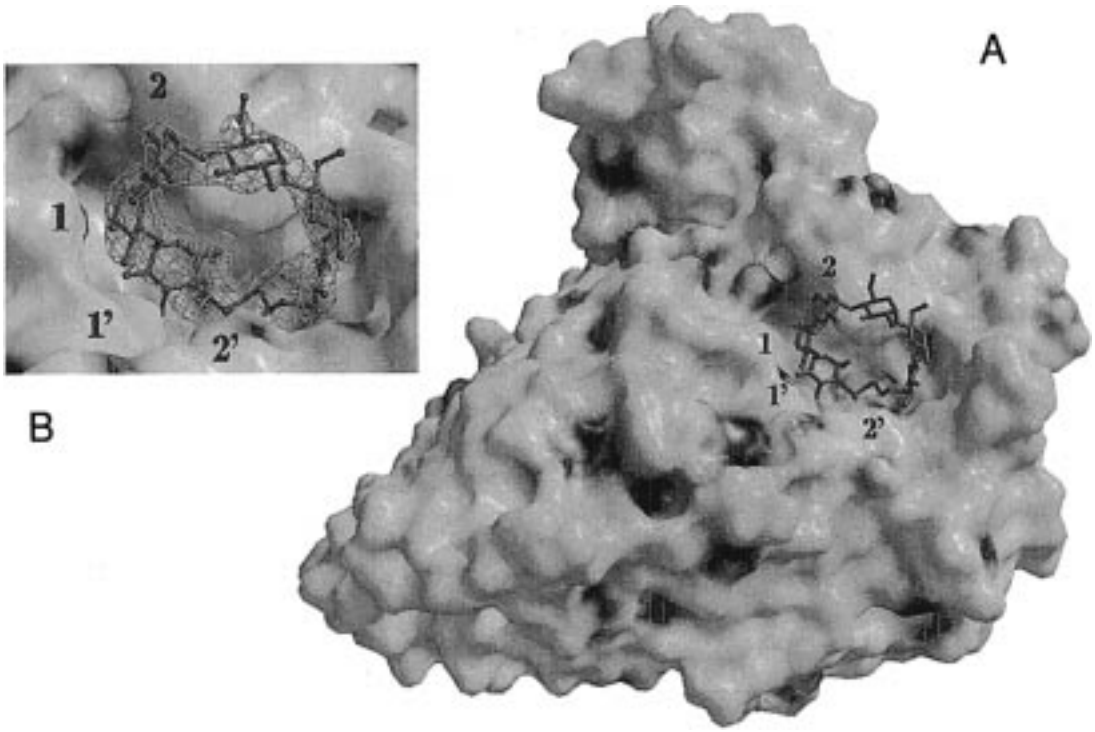

FIGURE 2: Surface representation (*52*−*55*) of CGTase. The electrostatic potentials are represented by red (negative) and blue (positive) coloring. As subsite names we used ...2, 1, 1′, 2′... running from the reducing to the nonreducing end of the oligosaccharide, with the scissile bond between 1 and 1′. This is a newly proposed nomenclature that uses the same names with the same position of the scissile bond as the established nomenclature of protease subsites (*40*). Moreover, it refers to identical directions of the ligated chains, which point from the amino-terminal to the carboxy-terminal end for peptides and from the reducing to the nonreducing end for oligosaccharides according to IUPAC−IUBMB (*38*). The proposal unifies the nomenclatures for two types of biopolymers. It disagrees with the suggestions of Davies et al. (*39*), who use minus signs that could easily be mistaken for hyphens. (A) Wild-type enzyme with ligated β-CD in the active center. (B) Close-up view of the active center of mutant Glu257→Ala with the initial ($F_o - F_c$) electron density at a contour level of 2.7σ together with the final β-CD model.

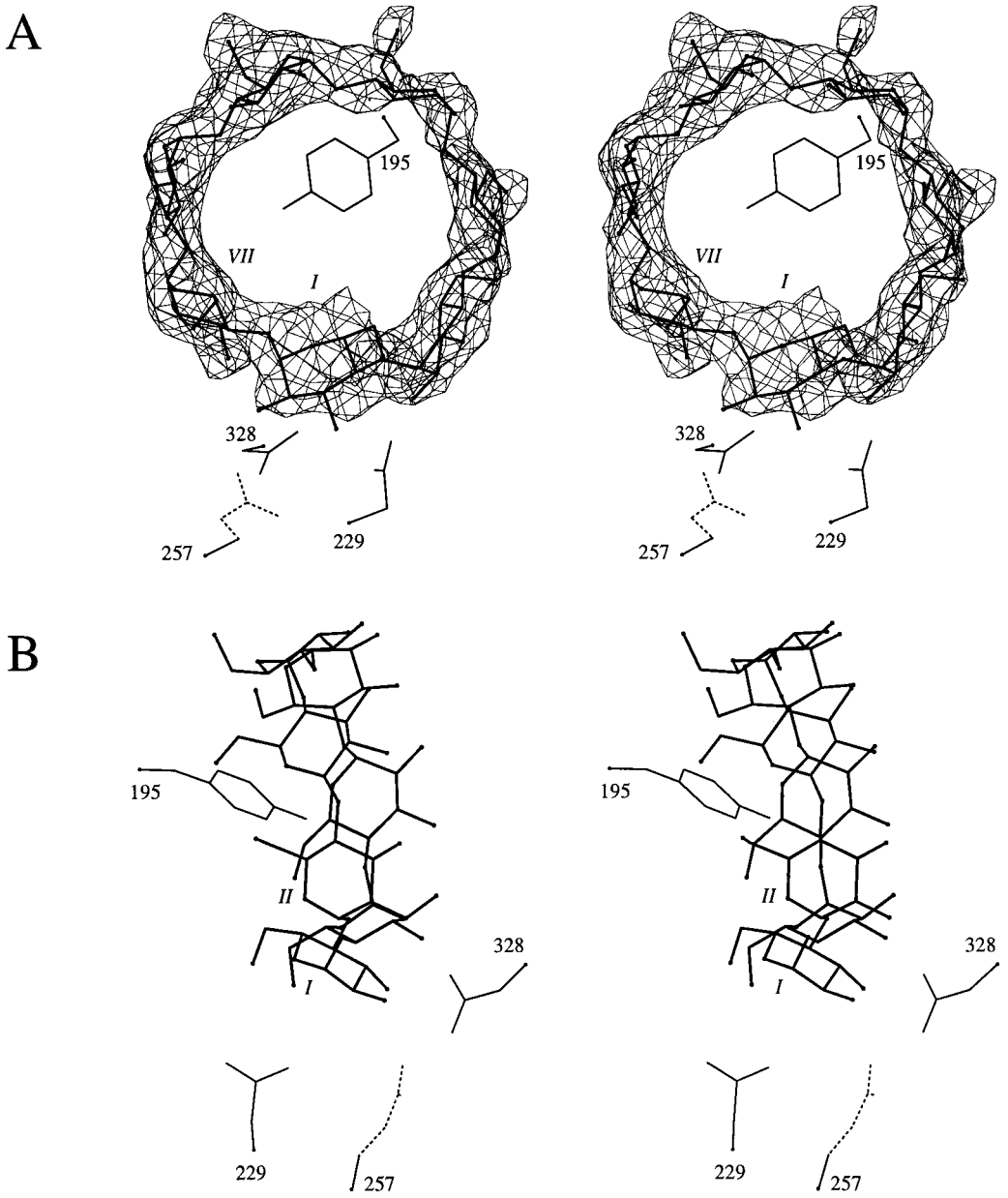

FIGURE 3: Stereoview (*55*) of the refined model of β-CD bound at the active center of CGTase mutant Glu257→Ala. The side chains of the central residue Tyr195 and of the catalytic residues Asp229, Glu257 (wild-type conformation as dashed line, mutant alanine as solid line), and Asp328 are shown. (A) β-CD model together with the final ($2F_o - F_c$) electron density map at a contour level of 1.0σ. (B) Same as in panel A but without density and rotated by 90° to illustrate the distortion of ligated β-CD from a planar ring and its position relative to Tyr195.

bound water molecules was used as a starting model. All water molecules in the active center were removed, and 20 cycles of rigid-body refinement were performed, decreasing the *R*-factor to about 23%. The prerefined wild-type model was used to calculate the initial ($F_{obs} - F_{calc}$) exp($i\alpha_{calc}$) map, which showed positive electron density for a complete β-CD molecule bound to the active center but no density for the exocyclic glucosyl unit. The density could be interpreted unambiguously, and the model was built using program O (*35*). In the further course of the refinement, restrained individual *B*-factors were used for the amino acid residues and for the seven glucosyl units of the β-CD model. Water molecules were edited by accepting new ones at ($F_{obs} - F_{calc}$) electron densities higher than 3.5σ and by deleting existing ones if they formed less than two hydrogen bonds or had ($2F_{obs} - F_{calc}$) densities below 1σ.

RESULTS AND DISCUSSION

Structure Analysis. In contrast to preceding short-time soaking experiments with α-glucans, the crystals were remarkably stable in the X-ray beam. The data collection statistics are given in Table 1. The structure of the soaked crystals was refined at a resolution of 2.4 Å to an *R*-factor of 17.7% (R_{free} = 22.8%). The initial difference Fourier map revealed positive electron density for a complete β-CD molecule bound in the active site as shown in Figure 2B but no density indication for the position of the exocyclic α-(1→6)-linked glucosyl unit. This result indicates either a high mobility of the exocyclic glucosyl unit or multiple binding positions or a combination of both. In fact, it has been shown that thio analogues are more mobile than natural oligosaccharides (*36*). A remainder of the thioglucosyl-CD

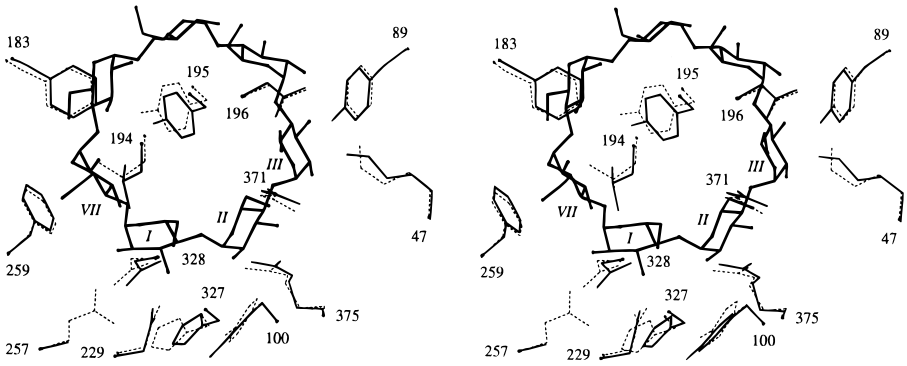

FIGURE 4: Stereoview (*55*) of the conformational changes on binding of thioglucosyl-CD to CGTase mutant Glu257→Ala. The ligated structure is depicted with solid lines and the wild-type structure with dashed lines. They have been superimposed on all backbone atoms.

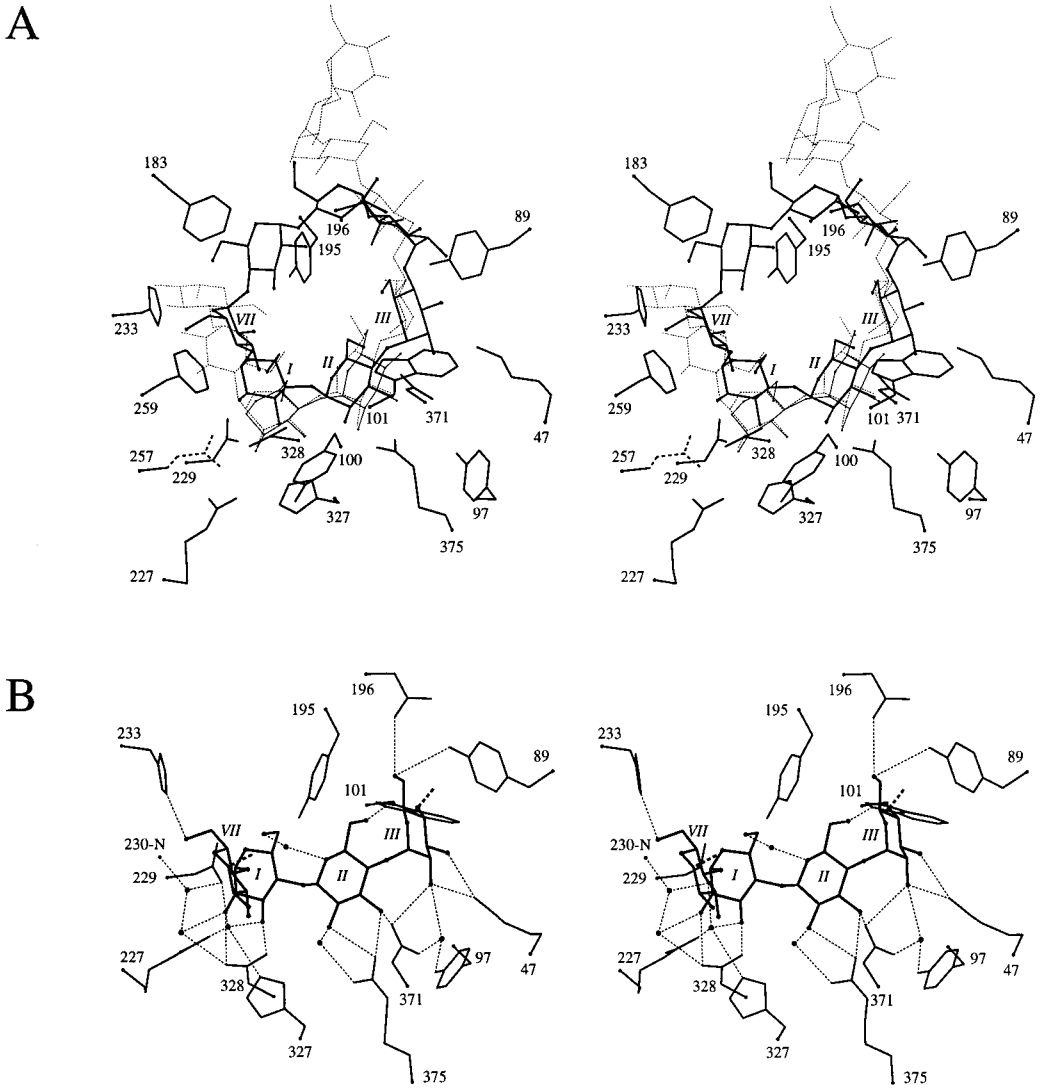

FIGURE 5: Stereoview (*55*) of the β-CD moiety of thioglucosyl-CD ligated to the active center of CGTase mutant Glu257→Ala. The glucosyl units Glc^{VII}, Glc^{I}, Glc^{II}, and Glc^{III} occupy subsites 1, 1′, 2′, and 3′, respectively, with the scissile glycosidic bond between subsites 1 and 1′. Note that Ala230 interacts via its main-chain N atom. Tyr195 and Ala257 (mutated glutamate) do not make any interactions to the ligand. (A) β-CD with surrounding residues (solid lines; Glu257 from the wild type with dashed line), ligated maltose (thin solid lines) (*25*), and a ligated "maltononaose" (dotted lines) consisting of an acarbose at subsites 2, 1, 1′, and 2′ extended by five glucosyl units occupying subsites 3′, 4′, etc. (*30*). The three structures were superimposed on the backbone atoms of 45 conserved residues around the active center. (B) Hydrogen bonding to β-CD; only Glc^{VII}, Glc^{I}, Glc^{II}, and Glc^{III} are shown.

sample used for soaking was checked by HPLC shortly after the experiment, showing a pure compound quite different from β-CD and thus confirming that no decomposition had occurred. An enzymatic removal of the exocyclic glucosyl unit in the crystal is highly unlikely, because CGTase lacks hydrolytic activity for $\alpha(1\rightarrow6)$ bonds and thiooligosaccha-

rides are generally resistant to glucanase hydrolysis (*37*).

The structure was refined using a model of unsubstituted β-CD in the active center and interpreted as such, assuming that the exocyclic unit exerts no influence. In the refined model, all seven glucosyl units of the β-CD have well-defined electron density in the ($2F_{obs} - F_{calc}$) map (Figure 3A). The mutation Glu257→Ala was confirmed by strong negative difference density representing the removed glutamate side chain except for the CB atom. The refinement statistics are given in Table 1. The glucosyl units are numbered according to IUPAC and IUBMB (*38*). The subsite names are defined in Figure 2, taking previous literature into consideration (*39*, *40*).

The structure of the ligated CGTase mutant Glu257→Ala is essentially identical to that of the wild type, and the rms deviation for all non-hydrogen protein atoms is 0.3 Å, which is just slightly above the limits of error. The largest differences occur at the active center where most side chains have moved toward the ligand in the complex as shown in Figure 4. A similar observation had been made for the CGTase complex with maltononaose (*30*).

Binding of β-CD in the Active Center. The orientation of the ligated β-CD is identical to that of the maltose molecule found in the soak of CGTase mutant Asp229→Ala (*25*); i.e., the 6-hydroxyls point to the protein interior. The glucosyl units of the ligand are labeled such that Glc^{VII}, Glc^{I}, and Glc^{II} occupy subsites 1, 1′, and 2′, respectively (*38*). The scissile glycosidic bond lies between subsites 1 and 1′ close to the catalytically competent carboxylates. The enzyme cuts an incoming α-glucan chain such that it keeps a maltooligosaccharide bound to subsites 1′, 2′, etc. and then catalyzes the transfer of the remaining oligosaccharide onto an acceptor entering subsite 1.

Of particular interest is the spatial relation between β-CD and residue 195, where CGTases contain a tyrosine or a phenylalanine and α-amylases have a glycine, serine, or valine instead (*41*). This suggested that Tyr195 forms a nonpolar core around which the α-glucan could wrap when forming the ring in CGTases. Various mutants at this position have been produced and shown to affect the cyclization reaction (*41*−*44*). As illustrated in Figure 3, the plane defined by the bound β-CD ring is nearly perpendicular to the tyrosine ring plane, but the side chain of Tyr195 does not protrude into the nonpolar cavity of β-CD and is not hydrogen-bonded to β-CD. This arrangement does not contradict the "wrapping-around" hypothesis, however, because the α-glucan chain could still wrap around Tyr195 in order to occupy subsite 1 for cyclization and could afterward be displaced toward the solvent (at the right-hand side of Figure 3B).

An analysis of the bound ligand revealed that only the portion of β-CD that neighbors the scissile bond is involved in polar contacts to the protein (Figure 5B). Only the four glucosyl units occupying subsites 1, 1′, 2′, and 3′ form direct or water-mediated hydrogen bonds to the protein as listed in Table 2. This was expected because tight contacts of the other glucosyl units would interfere with ring formation and product release. All hydroxyl groups of Glc^{I}, Glc^{II}, and Glc^{III} at subsites 1′, 2′, and 3′, respectively, but only one of Glc^{VII} at subsite 1 are employed for interactions with the protein. Significantly different from previous studies is the binding mode at subsite 1, where His233-NE2 forms a hydrogen bond to Glc^{VII}-O6 of ligated β-CD, whereas this bond involves the O2 atom in linear α-glucans (*21*, *27*, *30*). This is a consequence of the shift after cyclization.

Table 2: Hydrogen Bonds of Ligated β-CD in CGTase Mutant Glu257→Ala[a]

β-CD atom	protein atom	water molecule	secondary atom[b]
Glc^{VII}-O6	His233-NE2 (3.0)		
Glc^{I}-O2	Asp328-OD1 (3.1)		
Glc^{I}-O2	Asp328-OD2 (2.9)	190 (2.7)	Asp328-OD2 (3.0)
Glc^{I}-O3	His327-NE2 (3.4)		
Glc^{I}-O3	Asp328-OD1 (2.5)		
Glc^{I}-O3	Asp328-OD2 (3.4)	121 (2.7)	Asp229-OD1 (2.9)
Glc^{I}-O6		196 (2.5)	Glc^{II}-O5 (3.2)
Glc^{II}-O2	Arg375-NH1 (3.4)		
Glc^{II}-O2	Arg375-NH2 (3.0)	169 (2.8)	Asp328-OD1 (2.9)
Glc^{II}-O3	Asp371-OD2 (2.9)		
Glc^{II}-O3	Arg375-NH2 (2.6)	52 (3.6)	Tyr97-OH (2.5)
Glc^{II}-O5		196 (3.2)	Glc^{I}-O6 (2.5)
Glc^{II}-O6	Trp101-NE1 (2.7)		
Glc^{III}-O2	Lys47-NZ (2.6)	52 (3.3)	Tyr97-OH (2.5)
Glc^{III}-O2	Asp371-OD2 (3.4)		
Glc^{III}-O3	Lys47-NZ (3.1)		
Glc^{III}-O6	Tyr89-OH (3.3)		
Glc^{III}-O6	Asp196-OD1 (3.1)		

[a] The distances are in parentheses and are given in angstroms. [b] The secondary atom supports the water molecule.

This shift is also visible in the interactions of the catalytic nucleophile Asp229, which forms a hydrogen bond to the O6 atom of the glucosyl unit at subsite 1′ in the maltotetraose complex (*21*) but is too far away (3.8 Å) for such a bond to ligated β-CD. The hydrogen-bonding pattern of the glucosyl unit at subsite 2′ is similar in all three complexes depicted in Figure 5A, demonstrating the importance of this subsite for α-glucan binding. The polar contacts of Glc^{III} resemble those for linear α-glucans with respect to Asp196 and Asp371 but differ with respect to Lys47 (Arg47 in the CGTase from *B. circulans* strain 251) and Tyr89. Accordingly, Glc^{III} is at a point where the linear α-glucan chain deviates from the cyclic product. The distances between the pyranose centers of β-CD and the linear α-glucans are listed in Table 3; the minimum is at subsite 2′. As expected, the subsites beyond 3′ are much less defined than those around the scissile bond. The exocyclic glucosyl unit of the applied compound must be at Glc^{IV} or at Glc^{V} for steric reasons.

In addition to polar contacts, there are also stacking interactions between glucosyl units and aromatic side chains, which is a typical feature of carbohydrate-binding proteins (*45*−*47*). Within the geometric limits defined in ref *47*, such stacking occurs between Glc^{VII} and Phe259 and between Glc^{III} and Tyr89. Slightly exceeding the limits are interactions with Tyr100, Phe183, and Tyr195. Phe183 forces the 6-hydroxymethyl group of Glc^{VI} at subsite 2 to adopt an unusual negative χ^5 torsion angle and form a weak hydrogen bond to Glc^{VII}-O5 (Figure 5A, Table 3).

The linear α-glucans behave somewhat differently. Within the limits of ref *47*, there occurs stacking between Glc^{I} at subsite 1′ and Tyr100 (*21*, *25*, *30*) as well as between Glc^{VI} at subsite 2 and both Phe183 and Phe259 (*21*, *30*). Stacking between the glucosyl unit at subsite 4′ and Phe195 is observed in the maltononaose case (*30*). Taken together, these interactions point to an essential role of the aromatic side chains. Moreover, Tyr89 and Tyr195 are likely to form the end of a guideway for the bound linear α-glucan, after

Table 3: Conformational Analysis of Ligated β-CD[a]

	glycosidic bond angle[b] τ (deg)	torsion angle[c] χ^5 (deg)	torsion angle[c] ϕ (deg)	torsion angle[c] ψ (deg)	O2′−O3 distance (Å)	average *B*-factor (Å^2)	distance to tetraose (*21*)[d] (Å)	distance to nonaose (*30*)[d] (Å)
Glc^{I}	117 (119)	59 (−174)	141 (111)	−106 (−121)	3.6 (3.0)	68	2.0 [2.8]	2.4
Glc^{II}	117 (116)	45 (59)	87 (103)	−109 (−109)	3.3 (2.9)	61	1.2 [0.8]	0.8
Glc^{III}	118 (118)	150 (57)	93 (108)	−115 (−114)	2.6 (2.9)	67		1.2
Glc^{IV}	119 (117)	72 (51)	125 (111)	−117 (−110)	2.8 (2.8)	77		1.4
Glc^{V}	119 (118)	72 (−175)	112 (120)	−129 (−126)	2.6 (2.8)	73		6.8
Glc^{VI}	119 (119)	−66 (52)	117 (103)	−117 (−96)	2.7 (2.9)	71	4.7	4.4
Glc^{VII}	120 (121)	67 (−169)	119 (119)	−97 (−107)	2.9 (2.9)	63	2.8	3.0
average	118 (118)		113 (111)	−113 (−112)	2.9 (2.9)	69		
σ	1 (1)		19 (6)	10 (9)	0.4 (0.1)			

[a] Data for the small-molecule crystal structure of β-CD dodecahydrate (*48*) are given in parentheses. The unprimed atom names belong to the denoted glucosyl unit and the primed ones to its neighbor (Figure 1). [b] Defined by C1−O4′−C4′. [c] Torsion angles χ^5, ϕ, and ψ are defined by C4−C5−C6−O6, O5−C1−O4′−C4′, and C1′−O4−C4−C5, respectively. [d] The distance is between the centers of corresponding pyranose rings. The distances to ligated maltose (*25*) are given in brackets.

which it can curl back to occupy subsites 2 and 1 for cyclization. The hydrophobic nature of subsites 1 and 2 diminishes hydrolysis.

Conformational Analysis of the β-CD Ligand. The conformational properties of the ligated β-CD were compared with those in β-CD dodecahydrate crystals (*48*). Because β-CD is cyclic, an initial assignment of corresponding glucosyl units is arbitrary. Both rings are not circular, however, such that superpositions with one ring rotated in seven steps against the other ring yield rms deviations ranging from 0.5 to 1.0 Å (omitting all O4 and O6 atoms). The best superposition was used to compare the conformational angles as given in Table 3.

The β-CD dodecahydrate conformation is more symmetrical than that of β-CD ligated to CGTase. The glycosidic bond angles, the torsion angles (ϕ, ψ), and the O3−O2′ interglucose distances (Figure 1) of the two β-CD molecules are similar and the averages are virtually identical (Table 3). The variations around the averages, however, are appreciably larger in the ligated β-CD. The extreme values $\phi = 141°$ and $\psi = -97°$ are observed at the linkage between Glc^{VII} and Glc^{I}, which is the scissile glycosidic bond. Obviously the glucosyl units at the cleavage point are forced into a distorted unfavorable conformation, as is observed with linear α-glucans (*21*, *25*, *30*). The distortion of ligated β-CD is intermediate between that of ligated linear α-glucans and an almost symmetrical β-CD in solution. This distortion is not influenced by packing because the β-CD together with the whole active center is not involved in a crystal contact (*5*). The exocyclic torsion angle χ^5 has of course fewer restraints, resulting in large variations within and between both structures (Table 3).

While the reported β-CD complex concerns an enzymatic reaction product bound to the respective active center, four more structures have been published in which cyclodextrins were located either at general saccharide-binding sites or as inhibitors at or close to the active center of a different enzyme (*21*, *49*−*51*). In all these cases, the cyclodextrins were more symmetrical than the β-CD reported here, the distortion of which is caused by the functional requirement at the scissile bond.

Conclusion. The analysis showed that β-CD ligated to CGTase is much more distorted from the symmetrical conformation than crystalline β-CD dodecahydrate (*48*). The electron density (Figure 3A) and the *B*-factors (Table 3) are not uniform along the ring, indicating that the most mobile units are Glc^{IV} and Glc^{V}, at which point a bound linear α-glucan chain curls back around Tyr195 to reach subsites 2 and 1 for cyclization. The largest deviation from a symmetrical β-CD occurs at the scissile bond. Still, the bridging oxygen atom of this bond in the ligated β-CD is not available for protonation, showing that the local α-glucan conformation has already changed after the cyclization. There are appreciable displacements at subsites 1′, 1, and 2 (Table 3, Figure 5A), and the best conserved binding mode occurs at subsite 2′, which acts like a fix point for α-glucan binding. As usually observed (*47*), there are numerous aromatic side chains participating in glucosyl binding. For steric reasons, the invisible exocyclic glucosyl unit of thioglucosyl-CD (Figure 1) could only be located at Glc^{IV} or at Glc^{V}. This suggests that the enzyme may process singly α(1→6)-branched starch fragments, giving rise to α-, β-, and γ-cyclodextrins that carry exocyclic α-glucan chains of various lengths.

ACKNOWLEDGMENT

The crystals of CGTase mutant Glu257→Ala were from the diploma thesis of C. Wiesmann (Albert-Ludwigs-Universität, 1992). We thank J. E. W. Meyer for help in the structure refinement, C. Vonrhein for help in manuscript preparation, and C. Wiesmann for discussions.

REFERENCES

1. Saenger, W. (1980) *Angew. Chem., Int. Ed. Engl. 19*, 344−362.
2. Szejtli, J. (1988) *Cyclodextrin Technology*, Kluwer Academic Publishers, Dordrecht, The Netherlands.
3. Wenz, G. (1994) *Angew. Chem., Int. Ed. Engl. 33*, 803−822.
4. Terada, Y., Yanase, M., Takata, H., Takaha, T., and Okada, S. (1997) *J. Biol. Chem. 272*, 15729−15733.
5. Klein, C., and Schulz, G. E. (1991) *J. Mol. Biol. 217*, 737−750.
6. Kubota, M., Matsuura, Y., Sakai, S., and Katsube, Y. (1991) *Denpun Kagaku 38*, 141−146.
7. Lawson, C. L., van Montfort, R., Strokopytov, B., Rozeboom, H. J., Kalk, K. H., de Vries, G. E., Penninga, D., Dijkhuizen, L., and Dijkstra, B. W. (1994) *J. Mol. Biol. 236*, 590−600.
8. Knegtel, R. M. A., Wind, R. D., Rozeboom, H. J., Kalk, K. H., Buitelaar, R. M., Dijkhuizen, L., and Dijkstra, B. W. (1996) *J. Mol. Biol. 256*, 611−622.
9. Harata, K., Haga, K., Nakamura, A., Aoyagi, M., and Yamane, K. (1996) *Acta Crystallogr. D52*, 1136−1145.

10. Hofmann, B. E., Bender, H., and Schulz, G. E. (1989) *J. Mol. Biol. 209*, 793−800.
11. Nitschke, L., Heeger, K., Bender, H., and Schulz, G. E. (1990) *Appl. Microbiol. Biotechnol. 33*, 542−546.
12. Matsuura, Y., Kusunoki, M., Harada, W., and Kakudo, M. (1984) *J. Biochem. (Tokyo) 95*, 697−702.
13. Boel, E., Brady, L., Brzozowski, A. M., Derewenda, Z., Dodson, G. G., Jensen, V. J., Petersen, S. B., Swift, H., Thim, L., and Woldike, H. F. (1990) *Biochemistry 29*, 6244−6249.
14. Qian, M., Haser, R., and Payan, F. (1993) *J. Mol. Biol. 231*, 785−799.
15. Kadziola, A., Abe, J., Svensson, B., and Haser, R. (1994) *J. Mol. Biol. 239*, 104−121.
16. Machius, M., Wiegand, G., and Huber, R. (1995) *J. Mol. Biol. 246*, 545−559.
17. Brayer, G. D., Luo, Y., and Withers, S. G. (1995) *Protein Sci. 4*, 1730−1742.
18. Ramasubbu, N., Paloth, V., Luo, Y., Brayer, G. D., and Levine, M. J. (1996) *Acta Crystallogr. D52*, 435−446.
19. Morishita, Y., Hasegawa, K., Matsuura, Y., Katsube, Y., Kubota, M., and Sakai, S. (1997) *J. Mol. Biol. 267*, 661−672.
20. Henrissat, B. (1991) *Biochem. J. 280*, 309−316.
21. Knegtel, R. M. A., Strokopytov, B., Penninga, D., Faber, O. G., Rozeboom, H. J., Kalk, K. H., Dijkhuizen, L., and Dijkstra, B. W. (1995) *J. Biol. Chem. 270*, 29256−29264.
22. Buisson, G., Duée, E., Haser, R., and Payan, F. (1987) *EMBO J. 6*, 3909−3916.
23. Holm, L., Koivula, A. K., Lehtovaara, P. M., Hemminki, A., and Knowles, J. K. C. (1990) *Protein Eng. 3*, 181−191.
24. Takase, K., Matsumoto, T., Mizuno, H., and Yamane, K. (1992) *Biochim. Biophys. Acta 1120*, 281−288.
25. Klein, C., Hollender, J., Bender, H., and Schulz, G. E. (1992) *Biochemistry 31*, 8740−8746.
26. Nakamura, A., Haga, K., Ogawa, S., Kuwano, K., Kimura, K., and Yamane, K. (1992) *FEBS Lett. 296*, 37−40.
27. Strokopytov, B., Penninga, D., Rozeboom, H. J., Kalk, K. H., Dijkhuizen, L., and Dijkstra, B. W. (1995) *Biochemistry 34*, 2234−2240.
28. Mosi, R., He, S., Uitdehaag, J., Dijkstra, B. W., and Withers, S. G. (1997) *Biochemistry 36*, 9927−9934.
29. Bender, H. (1990) *Carbohydr. Res. 206*, 257−267.
30. Strokopytov, B., Knegtel, R. M. A., Penninga, D., Rozeboom, H. J., Kalk, K. H., Dijkhuizen, L., and Dijkstra, B. W. (1996) *Biochemistry 35*, 4241−4249.
31. Kubota, M., Matsuura, Y., Sakai, S., and Katsube, Y. (1994) *Oyo Toshitsu Kagaku 41*, 245−253.
32. Cottaz, S., and Driguez, H. (1989) *Synthesis*, 755−758.
33. Kabsch, W. (1988) *J. Appl. Crystallogr. 21*, 67−71.
34. Jiang, J.-S., and Brünger, A. T. (1994) *J. Mol. Biol. 243*, 100−115.
35. Jones, T. A., Zou, J.-Y., Cowan, S. W., and Kjeldgaard, M. (1991) *Acta Crystallogr. A47*, 110−119.
36. Bock, K., Duus, J. O., Refn, S. (1994) *Carbohydr. Res. 253*, 51−67.
37. Driguez, H. (1997) in *Glycoscience* (Driguez, H., and Thiem, J., Eds.), Vol. 187, pp 85−116, Springer-Verlag, Berlin.
38. IUPAC and IUBMB, Joint Commission, Nomenclature for carbohydrates, 2-Carb-37, oligosaccharides (1996) World Wide Web http://www.chem.qmw.ac.uk/iupac/2carb/.
39. Davies, G. J., Wilson, K. S., and Henrissat, B. (1997) *Biochem. J. 321*, 557−559.
40. Schechter, I., and Berger, A. (1967) *Biochem. Biophys. Res. Commun. 27*, 157−162.
41. Penninga, D., Strokopytov, B., Rozeboom, H. J., Lawson, C. L., Dijkstra, B. W., Bergsma, J., and Dijkhuizen, L. (1995) *Biochemistry 34*, 3368−3376.
42. Fujiwara, S., Kakihara, H., Sakaguchi, K., and Imanaka, T. (1992) *J. Bacteriol. 174*, 7478−7481.
43. Nakamura, A., Haga, K., and Yamane, K. (1994) *Biochemistry 33*, 9929−9936.
44. Sin, K. A., Nakamura, A., Masaki, H., Matsuura, Y., and Uozumi, T. (1994) *J. Biotechnol. 32*, 283−288.
45. Vyas, N. K. (1991) *Curr. Opin. Struct. Biol. 1*, 732−740.
46. Quiocho, F. A., Spurlino, J. C., and Rodseth, L. E. (1997) *Structure 5*, 997−1015.
47. Meyer, J. E. W., and Schulz, G. E. (1997) *Protein Sci. 6*, 1084−1091.
48. Lindner, K., and Saenger, W. (1982) *Carbohydr. Res. 99*, 103−115.
49. Mikami, B., Hehre, E. J., Sato, M., Katsube, Y., Hirose, M., Morita, Y., and Sacchettini, J. C. (1993) *Biochemistry 32*, 6836−6845.
50. Sharff, A. J., Rodseth, L. E., and Quiocho, F. A. (1993) *Biochemistry 32*, 10553−10559.
51. Larson, S. B., Greenwood, A., Cascio, D., Day, J., McPherson, A. (1994) *J. Mol. Biol. 235*, 1560−1584.
52. Merritt, E. A., and Murphy, M. E. P. (1994) *Acta Crystallogr. D50*, 869−873.
53. Sharp, K., and Honig, B. (1990) *Annu. Rev. Biophys. Biophys. Chem. 19*, 301−332.
54. Connolly, M. L. (1993) *J. Mol. Graphics 11*, 139−141.
55. Kraulis, P. J. (1991) *J. Appl. Crystallogr. 24*, 946−950.

BI9729918