Complex Structures of *Thermoactinomyces vulgaris* R-47 α-Amylase 2 with Acarbose and Cyclodextrins Demonstrate the Multiple Substrate Recognition Mechanism*

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‡ The abbreviations used are: CD, cyclodextrin; TVAII, *T. vulgaris* R-47 α-amylase 2; D325N/D421N, mutant TVAII (Asp325→Asn/Asp421→Asn); TVAII-CD (5) and kinetic analysis of mutant TVAII (6), giving new insight into the recognition mechanism of β-CD. Also, the x-ray structures of Thermus. sp. IM6501 maltogenic α-amylase (7), *Bacillus stearothermophilus* Neopullulanase (8), and *Flavobacterium* cyclomaltodextrinase (9), which can hydrolyze CDs as well as TVAII, have been reported. These x-ray structures showed that all of them have an additional domain with a distorted β-barrel structure to three α-amylase-conserved domains (10). Therefore, the identity of these enzyme subclasses has been proposed (11).

The substrate-hydrolyzing mechanisms of α-amylase family enzymes have been extensively studied from various aspects (12, 13). However, the proposed mechanisms are based on the complex structures between α-amylase and substrates/inhibitors with a linear structure like maltopentaose/acarbose, and they are inadequate for CDs with a cyclic structure. Thus, the CD-hydrolyzing mechanisms are still unclear in the above-mentioned enzymes including TVAII.

To obtain new insights into the CD-hydrolyzing mechanism of TVAII, we report here the x-ray structures of the TVAII-α-amylase complex, and complexes of the inactive mutant TVAII (Asp325→Asn/Asp421→Asn; D325N/D421N) with α-CD, β-CD, and γ-CD at a resolution of 2.9, 2.9, 2.8, and 3.1 Å, respectively. D325N/D421N/α-, β-, and γ-CD are the first x-ray structures of complexes between α-amylase and α-, β-, γ-CDs as substrates.

**MATERIALS AND METHODS**

**Site-directed Mutagenesis and Purification of the Enzyme**—The inactive mutant TVAII, D325N/D421N, was prepared from recombinant *Escherichia coli* MV1184 cells. Oligonucleotide-directed mutagenesis was carried out using the plasmid pTNKK (14) with the QuickChange kit (Stratagene) for construction of mutant TVAII (D421N), in which Asp325 was replaced by Asn (15). Because D421N still has hydrolyzing activity for CD, a double mutant D325N/D421N was prepared using the plasmid D421N as a template. The purification of this inactive mutant D325N/D421N was performed in the same way as the wild-type TVAII (4).

**X-ray Structural Analysis**—The purification procedure and crystallization conditions of D325N/D421N were almost the same as those of wild-type TVAII, as reported previously (4). The crystals were grown by a vapor diffusion hanging drop method at 20 °C by using a protein solution (15 mg/ml) and a reservoir solution containing 0.5% (w/v) polyethylene glycol 20,000 and 2.5 mM calcium chloride in 20 mM MES buffer (pH 6.2). A crystal of TVAII-acarbose complex was obtained by a soaking method using the reservoir solution containing 1 mM acarbose;
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Table I

| Data collection and refinement statistics |
|------------------------------------------|
| Data set (complex with)                  | Acarbose | α-CD | β-CD | γ-CD |
| Temperature (K)                          | 100       | 100  | 100  | 100  |
| Resolution (Å)                           | 2.9       | 2.9  | 2.8  | 3.1  |
| No. measured references                  | 184,368   | 89,750 | 174,587 | 68,436 |
| No. unique references                    | 34,431    | 32,497 | 38,612 | 26,454 |
| Completeness (%)                         | 99.5      | 93.3  | 99.8  | 93.7  |
| $R_{merge}$ (a) (%)                      | 8.4 (22.6) | 8.5 (25.8) | 7.6 (28.1) | 9.9 (31.0) |
| $L_i/l_{ref}$                             | 9.2 (3.7) | 4.8 (3.3) | 11.1 (4.0) | 4.1(2.9) |
| Space group                               | $P2_12_1$ | $P2_12_1$ | $P2_12_1$ | $P2_12_1$ |
| Cell dimensions                           |           |       |       |       |
| $a$ (Å)                                   | 114.6     | 115.4 | 115.1 | 113.5 |
| $b$ (Å)                                   | 119.3     | 118.4 | 118.8 | 118.5 |
| $c$ (Å)                                   | 113.0     | 113.7 | 114.3 | 114.1 |
| Structure refinement                      |           |       |       |       |
| Resolution range (Å)                      | 38.9–2.9  | 40.5–2.9 | 40–2.8 | 47.7–3.1 |
| No. references                            | 31,277    | 32,474 | 38,561 | 26,435 |
| Completeness (%)                          | 99.3      | 92.3  | 98.2  | 92.6  |
| $R_{cryst}$                               | 0.179     | 0.193 | 0.200 | 0.208 |
| $R_{merge}$                               | 0.243     | 0.250 | 0.256 | 0.286 |
| r.m.s.d. bond lengths (Å)                 | 0.007     | 0.007 | 0.007 | 0.007 |
| r.m.s.d. bond angles (°)                  | 1.3       | 1.3   | 1.3   | 1.4   |
| No. atoms                                 | 95,550    | 95,550 | 95,550 | 95,550 |
| Protein                                   | 406       | 238   | 474   | 133   |
| Heterogen atoms                           | 110       | 132   | 134   | 176   |
| Solvent molecules                         |           |       |       |       |
| *$R_{merge} = \Sigma |I_I| - |I_F|/|I_F|$, $R_{merge} = \Sigma F_{obs} - F_{ref} / S F_{ref}$
| *r.m.s.d., root mean square deviation.

soaking time was 1 h. Acarbose was kindly provided by Bayer Yakuhin (Japan). Crystals of D325N/D421β-CD and D325N/D421γ-CD were also obtained by a soaking method by using reservoir solution containing 30 mM of α-CD and 20 mM of β-CD. A crystal of D325N/D421γ-CD was obtained by a co-crystallization method, using a protein solution containing 30 mM of γ-CD.

X-ray diffraction data for TVAII/acarbose and D325N/D421β-CD were collected at 100 K using an ADSC/CCD detector system on the BL6A beam line in the Photon Factory (Tukuba, Japan), and data for D325N/D421α-CD and D325N/D421γ-CD were collected at 100 K using an MacScience/DIP2040 imaging plate detector system on the BL4XU beam line in Spring-8 (Hyogo, Japan), using a reservoir solution containing 20% (w/v) 2-methyl-2,4-pentadiol as a cryoprotected solution. Diffraction data were processed using the program MOSFLM (16) and SCALA in the CCP4 program suite (17). The collected data and scaling results are listed in Table I. Initial phases were determined by a molecular replacement method using the structure of TVAII (3, 4) as a probe model with the program CNS (18). Models were corrected on the (2$F_o - F_i$) electron density map using the program Xfit in the XtalView system (19), and structures without solvent molecules were refined using maximum resolution data. Calculations of structural refinements were carried out by the program CNS. Solvent molecules were gradually introduced if peaks above 4.0 σ in the (F$_o$ − F$_i$) electron density map were in the range of a hydrogen bond. To avoid over fitting of diffraction data, a free R-factor with 10% of the test set excluded from the refinement was monitored (20). Refinement statistics are listed in Table I. In a Ramachandran plot, 83.4% (acarbose), 81.7% (α-CD), 81.3% (β-CD), and 79.1% (γ-CD) of residues are shown in the most favored regions as determined by the program PROCHECK (21, 22), and no residue is in the disallowed regions.

The coordinates and structure factors of the acarbose, α-, β-, and γ-CDs complexes were deposited in the Protein Data Bank (10) under the accession codes 1VFK, 1VFM, 1VFO, and 1VFU, respectively.

RESULTS

Overall Structures of Complexes with Acarbose, α-, β-, and γ-CDs—TVAII is composed of four domains as follows: domain N (residues 1–121) with a distorted β-barrel structure; domain A (residues 122–242 and 298–502) with a (βα)n barrel structure; domain B (residues 243–297) protruding from domain A; and domain C (residues 503–585) with a β-sandwich structure. TVAII forms a dimer structure of two molecules, denoted as Mol-1 and Mol-2, with a 2-fold symmetry. Domain N mainly contributes to the molecular surface between Mol-1 and Mol-2, as shown in Fig. 1 (23). In a dimer there are two catalytic sites, formed mainly by the residues from domain A and domain B and partially by domain N of another molecule. In all complex structures, the electron densities of ligands are clearly identified at the catalytic site of Mol-1, but those at the catalytic site of Mol-2 are relatively ambiguous compared with Mol-1. This is because a symmetric molecule partially covers the catalytic site of Mol-2 in the crystalline state, leading to relatively low occupancies for the binding ligands. Therefore, the structural description here concentrates on the catalytic site of Mol-1. The structural models of acarbose and three CDs on F$_o$ – F$_i$ omit maps with the 4 σ contoured level at the catalytic sites in Mol-1 are shown in Fig. 2 (24). Acarbose is a tetrasaccharide, cyclitol-(1,4)-(1,4)-6-deoxyglucose-(1,4)-Glc, but the electron

![Fig. 1. Overall structure of the TVAII dimer (D325N/D421β-CD) illustrated by the program MOLSCRIPT (23). Mol-1, Mol-2, and domain N are shown by different gray scales. Binding β-CDs are drawn by a wire model, and the loop regions of 42–50°, 198–205, and 283–290 are indicated by thick black lines (see text and Fig. 4).](http://www.jbc.org/Downloaded from)
density strongly showed a binding oligosaccharide composed of five saccharide units, Glc-α-(1,4)-Glc-α-(1,4)-cyclitol-α-(1,4)-6-deoxyglucose-α-(1,4)-Glc. This is because TVAII has activity for both hydrolysis and transglycosylation, and the transglycosylation of acarbose is frequently found in acarbose complexes of the glycoside hydrolase family 13 (25–30). For discussion, saccharide units of acarbose are designated Glc-3, Glc-2, Cyt-1, Glc-1, and Glc-2 from the nonreducing to reducing end. The numbers in parentheses in Fig. 2 indicate the subsite. The catalytic site is between subsites 1 and 1. In the α-CD (Fig. 1b), β-CD (Fig. 2c), and γ-CD (Fig. 2d) complexes, saccharide units are designed in the same manner as shown in Fig. 2.

Acarbose Binding to the Catalytic Site—Fig. 3a shows the interaction between TVAII and acarbose. The torsion angles of glycoside bonds of acarbose at the hydrolyzing site, ϕ (O-5—C-1—N-4—C-4′) = 32° and ψ (C-1—N-4—C-4′—C-5′) = −151°, are largely deviated from those of the regular helical structure of amylose, ϕ = 104° and ψ = −121° (31). The cyclitol unit binding to subsite −1 adopts a half-chair conformation, which is a mimic of the transition state. Three catalytic residues, Asp325, Glu354, and Asp421, which are strictly conserved in α-amylases, make many hydrogen bonds with Glc-1 and Cyt-1 at the hydrolyzing site. The O-1 atom of Glu354 forms a hydrogen bond with the N-4 atom between Cyt-1 and Glc-1. The O-6 atom of Asp325 forms hydrogen bonds with the O-6 atom of Cyt-1, causing the O-62 atom to be in direct contact with the C-1 atom of cyclitol with a distance of 2.9 Å. The O-61 and O-82 atoms of Asp421 form hydrogen bonds with the O-3 and O-2 atoms of Cyt-1 to fix its orientation. Additional hydrogen bonds were made between Arg469 and Glc-2. Three planar residues nicely make stacking interactions with saccharide units; they are Phe356 with Glc-1, Tyr204 with Glc-2, and His202 with Glc-2. The stacking interaction by Trp356 was induced by acarbose binding, because Trp356 in unliganded TVAII adopts a different side chain conformation, as shown in Fig. 3a. Phe296, Val326, and Tyr458 make van der Waals contacts with the

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**Diagram Notes**: The diagrams illustrate the binding of acarbose to TVAII, showing the interaction of saccharide units Glc-1 through Glc-2 with the enzyme's catalytic site. The maps (a), (b), (c), and (d) correspond to α-CD, β-CD, and γ-CD complexes, respectively.
Acarbose at the opposite side of the three catalytic residues, helping to fix the substrate in a proper position. Interactions between acarbose and the enzyme found in this study are very similar to those in other α-amylase-acarbose complex structures (25–30).

Cyclodextrin Binding to the Catalytic Site—The saccharide units in the binding β-CD occupy six subsites, −3 to +3 (Fig. 3b), with a distorted structure at the hydrolyzing site between subsites −1 and +1 (φ = 36° and ψ = −143°), as found in the TVAII/acarbose. At subsites −3, −2, −1, and +1, the conformation of saccharide units and interactions between saccharide units and the enzyme are similar to those found in TVAII/acarbose. Also, three catalytic residues, Asn421, Glu354, and Asn325, make hydrogen bonds with β-CD in a similar manner to acarbose, despite the replacement of amino acids (Asn421 from Asp and Asn325 from Asp). However, because of the cyclic structure of α-CD, interactions between α-CD and the enzyme at − subsites are different from those in TVAII/acarbose. Glc−2 does not make stacking interactions with Trp356 as found in TVAII/acarbose, because of the different locations of Glc−2 from that of acarbose. If Trp356 adopts the same side chain conformation as found in TVAII/acarbose, unusual short contacts between Trp356 and Glc−2 occur. The O-6 atom of Glc−2 forms a hydrogen bond with Arg446, and Glc+2 and Glc+3 make van der Waals contacts with Tyr454*, showing that residues from domain N are very important to recognize Glc+2 and Glc+3 of β-CD.

The saccharide units in the binding β-CD occupy seven subsites, −3 to +4 (Fig. 3c). Most interesting, the β-CD retains regular conical shape, different from TVAII/acarbose and D325N/D421N/α-CD. The glycoside linkage at the hydrolyzing site is not changed (φ = 119° and ψ = −116°). Such a regular conical structure of β-CD is also found in β-CD complex structures with other α-amylase family enzymes (5, 11, 32). Glc−1, Glc−2, and Glc−3 interact with the enzyme in a similar manner to that found in TVAII/acarbose and D325N/D421N/α-CD, but unique locations of Glc−1, Glc−2, and Glc−3 give structural change to the catalytic site. Glu354 forms a hydrogen bond not with the O-4 atoms but with the O-3 atoms of Glc−1, because the lone pair of electrons for the O-4 atoms of Glc−1 moves to an

**FIG. 3.** Stereo views of interactions between catalytic site residues (light gray) and acarbose (gray) (a), α-CD (gray) (b), β-CD (gray) (c), and γ-CD (gray) (d), illustrated by the program MOLSCRIPT (23). Trp356 and Tyr374 in the unliganded TVAII are superimposed (white). The selected hydrogen bonds are shown with dotted lines.
opposite side to Glu^{354} because of the regular conical structure of β-CD. The conformational change of Trp^{356} was induced to make a stacking interaction with Glc^{−2}, but it occurs in a different manner from the acarbose complex. Glc^{−3} makes van der Waals contacts with Tyr^{45*}, and Glc^{−4} shows no efficient interaction with the catalytic site residues. The electron density of the binding γ-CD also shows a conical structure of γ-CD (Fig. 2d), but it is significantly bent at glucoside linkages of Glc^{−1−2} and Glc^{−4−5}, with the angle of 36°, giving the intermediate conformation of Glc^{−1} between those of the binding α- and β-CDs. Glc^{−3}, Glc^{−2}, and Glc^{−1} adopt a similar structure to other complexes, and interactions of them with the enzyme are also almost conserved. Although the electron density of saccharide units at + subsites of γ-CD is not enough to determine the precise conformations as shown in Fig. 2d, the quality of electron density for catalytic site residues allows us to deduce the interactions between γ-CD and the enzymes. Trp^{356} adopts a completely different conformation from those in other complex structures to avoid steric hindrance with γ-CD. It makes van der Waals contacts with O-2 and O-3 atoms of Glc^{−1} and Glc^{−2}, giving a bent structure to γ-CD. Ala^{44*} and Arg^{46*} possibly make hydrogen bonds with Glc^{−3} and Glc^{−2}, respectively. Glc^{−4} and Glc^{−5} make no direct interactions with the catalytic site residues.

**Induced Fit Movement of Main Chain by Ligand Binding**—
Through ligand binding, the induced fit movements were observed at the catalytic site. Fig. 4 shows the superimposed catalytic site structures of unliganded TVAII (white), TVAII/ acarbose (black), and D325N/D421N/β-CD (gray) complexes. Three regions of the catalytic site significantly move through the ligand binding; they are the loops of 198–205 and 283–293 from Mol-1 and 42*–50* from Mol-2. When ligands bind to the catalytic site, the loop of 198–205 moves close to the ligand to make a stacking interaction between His^{202} and Glc^{−2}. The movements of this region are almost equivalent between acarbose and CD complexes. The loops of 283–293 and 42*–50* recognize the ligands at the opposite side of loop 198–205, and the movements of these loops vary depending on the ligands. In acarbose binding, the loop of 42*–50* largely moves to make a favorable contact between Tyr^{45*} and Glc^{−1}, whereas in α-, β-, and γ-CD bindings, Tyr^{45*} makes a favorable contact not with Glc^{−1} but with Glc^{−2}, giving a small movement of this loop.
the side chain conformations of Trp356 and Tyr374. In unliganded Trp356 and Tyr374 are not observed, and they are almost the
stabilizing the ligand conformation for the catalytic reaction.

(N-CA-CB-CG)

same as those of unliganded TVAII (23). The
unliganded TVAII, TVAII/acarbose, and D325N/D421N/β-CD structures are shown in white, black, and gray, respectively.

Because Phe236 and Tyr45 are important residues for recognizing the hydrophobic cavities of CDs (5, 6), these regions need to move flexibly to make favorable interactions with various sized ligands. The relatively high temperature factors of these loops suggest that these loops are inherently flexible and dynamically moving through the ligand binding.

**DISCUSSION**

Multiple Substrate Recognition Mechanism of TVAII—In all complexes, the relative positions and orientations of saccharide units at subsites −1, −2, and −3 to the enzymes are almost the same, and the interactions of these saccharide units with the enzymes are equivalent to others. Striking differences in the catalytic site structures are found at + subsites, especially the side chain conformations of Trp356 and Tyr374. In unliganded TVAII, the side chain conformations of these residues are θ1 (N-CA-CB-CG) = −55°, θ2 (CA-CB-CG-CD1) = 134° for Trp356, and θ1 = 73° and θ2 = 95° for Tyr374, and there is no stacking interaction between Trp356 and Tyr374, as shown in Fig. 3. The acarbose binding causes a drastic change in the side chain conformations of Trp356 to make a strong stacking interaction with Glc1. This stacking interaction is reinforced by the stacking interaction with Tyr374, whose side chain conformations also change with the acarbose binding. The conformational changes of Trp356 and Tyr374 are expected to play an important role in stabilizing the ligand conformation for the catalytic reaction.

In the α-CD complex, significant conformational changes of Trp356 and Tyr374 are not observed, and they are almost the same as those of unliganded TVAII (θ1 = −52° and θ2 = 140° for Trp356, and θ1 = 69° and θ2 = 106° for Tyr374). When α-CD with a conical structure approaches the catalytic site of TVAII, the N-e and C-δ1 atoms of Trp356 should first make contact with Glc−1 of α-CD, and possibly distort the conical structure of α-CD by pushing Glc−1 from the inside of the cavity like a wedge, giving the same conformation of the glycoside linkage between Glc−1 and Glc−2 as that of acarbose. Because Trp356 cannot form a stacking interaction with Glc−2 because of the cyclic structure of α-CD, Trp356 and Tyr374 maintain their inherent conformations, fixing the glucose linkage suitable for the catalytic reaction. Therefore, the inherent conformations of Trp356 found in unliganded TVAII are thought to be important to change the glycoside linkage at the hydrolyzing site of α-CD.

In the β-CD complex, Trp356 rotates its side chain (θ1 = −159° and θ2 = 55°) to make a nicely stacking interaction with Glc−2 from the outside of β-CD, and significant conformational change of Tyr374 is not found (θ1 = 75° and θ2 = 93°). The location of Trp356 is extensively fitted to the regular conical structure of β-CD, which is unfavorable to the proposed hydrolyzing mechanism, as described later. Generally, CDs have a truncated conical structure with the O-6 atoms at the narrow side and the O-2 and O-3 atoms at the wide side, and the O-2 atom forms an intramolecular hydrogen bond with the O-3 atoms of the neighboring glucose unit. The glucose units of β-CD are arranged in a symmetrical structure, and the O-2—O-3 intramolecular hydrogen bonds strongly contribute to maintaining the round shape of the CD cavity. Therefore, the regular conical structure is thought to be one of the most stable structures for β-CD, and this is a reason why β-CD maintains its regular conical structure through binding to the enzyme. On the other hand, the cavity of α-CD is too small to form a symmetrical structure, and α-CD has a more inclined conical structure lacking a few intramolecular hydrogen bonds of O-2—O-3 (33). The distorted conical structure of α-CD found in D325N/D421N/α-CD is expected to be more likely than β-CD.

Also in the γ-CD complex, the conformational change is found only at Trp356, and it occurs in a different manner from that found in acarbose and β-CD complexes (θ1 = −55° and θ2 = −124° for Trp356, and θ1 = 70° and θ2 = 107° for Tyr374). Trp356 does not strongly interact with γ-CD, and it occupies the space between Tyr374 and Glu354 in order to avoid any steric hindrances with Glc−2 and Glc−3 of γ-CD. If Trp356 adopts other side chain conformations, there are many unusual contacts between γ-CD and the enzyme. This side chain conformation of Trp356 is indispensable for the recognition of γ-CD having a large cavity.

Trp356 and Tyr374 can change their conformations of side chain depending on the structure and size of substrates to stabilize the enzyme-substrate complex. Accompanied with the flexible loops of 198–205, 283–293, and 428–505°, mentioned above, Trp356 and Tyr374 are responsible for the multiple substrate-recognition mechanism of TVAII, performing the unique substrate specificity that hydrolyzes both starch and CDs efficiently.

Possible Hydrolyzing Mechanism of TVAII for CDs—Aasp325, Glu354, and Asp421 are catalytic residues in TVAII. At the first step of the proposed hydrolyzing mechanism shown in Fig. 5a, the O-atom of Glu354 protonates the O-4 atom (glucoside oxygen) at a hydrolyzing site (12, 13), and subsequently the O-δ atom of Aasp325 attacks the C-1 atom of the saccharide at subsite −1 (34). Asp421 has been suggested to be involved in fixing the substrate and in stabilizing the transition state. In both acarbose and α-CD complexes, the Glu354 makes a hydrogen bond with the N-4 atom of acarbose or O-4 atom of α-CD at the hydrolyzing site, and Asp421 fixes the orientation of Cyt−1/Glc−1 by making hydrogen bonds with O-2 and O-3 atoms, leading to the short distance between the O-6 atom of Aasp325 and C-1 atom of Cyt−1/Glc−1. These interactions between the binding acarbose and α-CD and the three catalytic residues, Aasp325, Glu354, and Asp421, found in this study strongly support the proposed hydrolyzing mechanism for α-amylase.

However, in the β-CD complex, the binding CDs maintain a regular conical structure. Because both torsion angles (θ1 and θ2) of Trp356 are completely different from those found in α-CD complex, the resultant structure of the β-CD complex is difficult to be the state prior to the structure of α-CD complex. The lone pair of O-4 atoms at the hydrolyzing site moves to the inside of the CD cavity, and Glu354 does not form a hydrogen bond with the O-4 atom at the hydrolyzing site. It is very difficult for Glu354 to protonate the O-4 atom of β-CD, suggest-
ing that the protonation mechanism for hydrolyzing \( \beta \)-CD is different from that for \( \alpha \)-CD and starch. In \( \beta \)-CD complexes, the hydrogen bond between the Glu\(^{354} \) and the O-3 atom of Glc\(^{-1} \) is observed, where a proton may be donated from Glu\(^{354} \). In wild-type TVAII, the carboxyl group of Asp\(^{421} \) should form hydrogen bonds with O-2 and O-3 atoms of Glc\(^{-1} \), acting as a proton acceptor. Therefore, in the O-2-O-3 hydrogen bond between Glc\(^{-1} \) and Glc\(^{+1} \), a proton should be donated from the O-3 atom of Glc\(^{-1} \) as shown in Fig. 5b; the distance between the proton and glycosidic oxygen is about 2.5 Å. It could be proposed that the protonation of glycosidic oxygen at the hydrolyzing site is possibly carried out via a proton between O-2 and O-3 atoms, as shown in Fig. 5b.

In the \( \gamma \)-CD complex, Glc\(^{+1} \) adopts the intermediate conformation between those found in \( \alpha \)-CD and \( \beta \)-CD complexes, and the side chain of Trp\(^{356} \) also adopts the intermediate conformation, where \( \psi1 \) is almost the same as that found in the \( \alpha \)-CD complex. Therefore, the resultant structure of the \( \gamma \)-CD complex is possibly the previous state of the structure of \( \alpha \)-CD complex, from which the rotation of the indole ring of Trp\(^{356} \) about \( \psi2 \) could lead to a similar structure of \( \alpha \)-CD complex. This is supported by the fact that the \( \gamma \)-CD binding to cyclodextrin glycosyltransferase can change the conformation of the glucosidic bond at the hydrolyzing site like acarbose because of the eight glucose units making the cyclic structure loose (35).

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Complex Structures of *Thermoactinomyces vulgaris* R-47 α-Amylase 2 with Acarbose and Cyclodextrins Demonstrate the Multiple Substrate Recognition Mechanism
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