Oligosaccharide and Sucrose Complexes of Amylosucrase

STRUCTURAL IMPLICATIONS FOR THE POLYMERASE ACTIVITY*

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The glucosyltransferase amylosucrase is structurally quite similar to the hydrolase α-amylase. How this switch in functionality is achieved is an important and fundamental question. The inactive E328Q amylosucrase variant has been co-crystallized with maltoheptaose, and the structure was determined by x-ray crystallography to 2.2 Å resolution, revealing a maltoheptaose binding site in the B-domain somewhat distant from the active site. Additional soaking of these crystals with maltoheptaose resulted in replacement of Tris in the active site with maltoheptaose, allowing the mapping of the –1 to +5 binding subsites. Crystals of amylosucrase were soaked with sucrose at different concentrations. The structures at ~2.1 Å resolution revealed three new binding sites of different affinity. The highest affinity binding site is close to the active site but is not in the previously identified substrate access channel. Allosteric regulation seems necessary to facilitate access from this binding site. The structures show the pivotal role of the B-domain in the transference reaction. Based on these observations, an extension of the hydrolase reaction mechanism valid for this enzyme can be proposed. In this mechanism, the glycogen-like polymer is bound in the widest access channel to the active site. The polymer binding introduces structural changes that allow sucrose to migrate from its binding site into the active site and displace the polymer.

Amylosucrase (AS) is a bacterial glucosyltransferase (EC 2.4.1.4) that catalyzes the transfer of a β-glucopyranosyl moiety from sucrose onto an acceptor molecule. When the acceptor is a polysaccharide, only α-1,4-linkages are produced. In the presence of glucogen, AS catalyzes the transfer of a β-glucose moiety onto a glycogen branch (1), but in the absence of glucogen the reaction pathway is more complex; here, polymer synthesis, hydrolysis of sucrose, synthesis of smaller maltosaccharides, and synthesis of sucrose isoforms are observed (2). Hydrolysis is always just a minor side reaction. The function of AS in vivo is undoubtedly the extension of glycogen-like oligosaccharides, which is clearly demonstrated by the formidable increase in $k_{cat}$ observed when glucogen is present (1).

Based on sequence similarities, the enzyme has been placed in the retaining α-glycoside hydrolase family 13 according to Henrisat and Davies (3). Family 13 is the largest of all the families and contains mainly enzymes acting on starch, such as α-amylase and cyclodextrin glycosyltransferase (CGTase), but also includes are enzymes specific for the cleavage of other glucosidic linkages such as α-1,6- and α-1,1-bonds. It is widely believed that the catalytic mechanism in family 13 occurs via a double displacement reaction in which a covalent glycosyl-enzyme intermediate is formed (4–7). The existence of such an intermediate has also been found in the retaining β-glycoside hydrolase lysozyme, about which a detailed structural study has recently been published (8). In a subsequent step, this glycosyl moiety is transferred onto a water molecule (main reaction catalyzed by α-amylases) or onto a hydroxyl group from a sugar acceptor in a transglycosylation reaction. Cyclodextrin glycosyltransferases however, react intramolecularly with the non-reducing end of the covalently linked heptasaccharide (6). Both steps are suggested to proceed via oxocarbenium ion transition states.

Full-length AS from Neisseria polysaccharea consists of a single polypeptide chain with 636 amino acid residues, and from sequence alignment the two catalytic acidic residues Asp256 and Glu326 (crystal structure numbering) found in α-amylases were identified (9). The structure of recombinant AS from N. polysaccharea with Tris bound in the active site has been reported recently by Skov et al. (10). Single site mutational studies of residues Asp256 and Glu326 showed in both cases a total inactivation of AS (11). Additionally, the mutation of three other active site residues conserved in family 13 (Asp390, His187, and His189) resulted in a large decrease in activity. From these structural and mutational studies, Asp256 was identified as the nucleophile, and Glu326 was identified as the proton donor. The importance of the conserved residues in the active site was further underlined by the structures of AS in complex with the saccharides glucogen and sucrose (12).

A unique feature for AS is an N-terminal domain (termed N) consisting of 90 residues forming five helices. This domain shows no structural similarity to any known protein structure. Apart from the common α-amylase A-, B-, and C-domains (7), it was also observed that AS contained an extra domain (termed...
B') between \(\beta\)-strand 7 and \(\alpha\)-helix 7 of the \(\langle\beta\alpha\rangle\) domain, and the absence of this B' domain in \(\alpha\)-amyloses led to speculations concerning a possible role in the transferase activity. The proposed catalytic residues of AS lie in the bottom of a pocket, a feature seen in other family 13 enzymes such as the hydrodrolase \(\alpha\)-1,6-glucosidase (13). Other exo-acting hydrolases, e.g., \(\beta\)-amylase (14) and glucoamylase (15), also exhibit this pocket architecture.

Because sucrose is a cheap and renewable \(\alpha\)-glucose donor compared with the various nucleotide derivatives, the industrial potential of AS for synthesis of glucan, oligosaccharide, or glucoconjugates is large. For optimal utilization of the enzyme in targeting controlled synthesis of specific \(\alpha\)-glucans starting from sucrose, it is, however, necessary to minimize the side reactions. This requires a thorough analysis of the substrate binding and the specificity determining contacts in the active site.

Recently, we described the active site interactions between AS and a \(\beta\)-\(\alpha\)-glucose molecule (12). We also described the interactions between the inactive active site mutant E328Q and sucrose based on the crystal structure at 2.0 Å resolution. Both complexes gave information on the substrate binding mode. The sucrose complex shows how the substrate binds prior to cleavage of the glucosidic bond, whereas the \(\alpha\)-glucose complex mimics the covalent intermediate. However, to gain insight into the other stages of this complex enzymatic reaction it is necessary to study the binding of the other substrate, the oligosaccharide chain to be elongated, and the product regulation of the enzyme. Such information can be obtained through soaking and co-crystallization experiments. In this paper we describe a number of different complexes such as co-crystallized E328Q-Tris-maltoheptaose before and after soaking with maltoheptaose and AS-Tris-sucrose from soaking AS/Tris crystals with three different concentrations of sucrose.

EXPERIMENTAL PROCEDURES

Expression and Purification—Expression and purification of recombinant wild-type AS from \(N.\) polysaccharae has been described by Potocki de Montalk et al. (1), and described similarly for the E328Q mutant by Sarçaľ et al. (11). AS and E328Q both lack 10 N-terminal residues as compared with the genomic sequence and contain four additional residues originating from the expression vector. Apart from the intended mutation, the E328Q mutant contains another substitution, H314Y, which is evident from the density maps and probably due to a PCR error.

Crystallization, Data Collection, and Refinement—AS crystals were grown as described previously (16). Crystals of the AS variant E328Q with 14 \(\alpha\)-maltose molecules as additive (A crystals) were grown under similar crystallization conditions. These crystals showed a slight difference in morphology (being more cubic) as compared with the thin plate-like crystals obtained previously. Consequently, the diffraction was less anisotropic, and the spots had lower mosaicity. The A crystals were subsequently transferred to a crystallization buffer containing 20 mM Tris, pH 7.5, 200 mM sucrose from soaking AS crystals obtained previously. Consequently, the diffraction was quite similar in the three structures at equivalent binding sites. The B structure has the most extensive carbohydrate-binding network with three maltoheptaose molecules per AS molecule, and a total of 18 glucose units are included in the final model.

**RESULTS**

In three of the structures (A, B, and D) one or more oligosaccharides are identified in specific binding sites. The interactions between AS and carbohydrate are quite similar in the three structures at equivalent binding sites. The B structure has the most extensive carbohydrate-binding network with three maltoheptaose molecules per AS molecule, and a total of 18 glucose units are included in the final model.

Co-crystallized E328Q-Tris-Maltoheptaose (A Crystals)—In addition to electron density from the protein, this crystal structure contains electron densities with an elongated shape corresponding to two saccharides on the surface of the enzyme. The two saccharides are probably maltoheptaose molecules, but the precise length could not be determined. Hence, only two and four glucose units, respectively, are included in the final model because of the weak electron density of the other residues. For both oligosaccharide binding (OB) sites, the positions of one of the glucosyl units coincides with the positions of the glucose molecules found in the AS-glucose structure (12). We therefore adopt the same binding site numbering, i.e., the active site is OB1, the first surface binding site is OB2, and the second surface binding site is OB3. Accordingly, the polysaccharide modeled with four glucose units is found in OB2 on the B’ domain, whereas the chain with two units is found in OB3. OB3 is located at the surface of the C-terminal \(\alpha\)-sandwich domain. A Tris molecule is bound in the active site (OB1). The different binding sites are labeled in Fig. 1, and the hydrogen bonding interactions in OB2 are illustrated in Fig. 2. The four glucosyl (Glc) rings modeled in OB2 are Glc724, Glc735, Glc726, and Glc727, where Glc727 is the reducing end of the maltoheptaose molecule. All four glucose moieties are in the most stable \(\text{C}4\) configuration, and the conformation of the sugar chain is very similar to that found in a cyclodextrin glycosyltransferase-maltoheptaose complex (22). The conformation of the polysaccharide can be described by the torsion angles \(\phi\) and \(\psi\) across the glucosidic bond. \(\phi\) is defined with O5 (first unit), C1 (first unit), O1 (bridging atom), C4 (second unit), and \(\psi\) with C1 (first unit), O1 (bridging atom), C4 (second unit), and C5 (second unit). The values being Glc724-Glc725 (73°, −148°), Glc725-Glc726 (82°, −152°), and Glc726-Glc727 (101°, −137°).

In OB3 the \(\phi,\psi\)-value for the Glc733-Glc734 bridge is 115°, −112°.

Maltoheptaose-soaked E328Q-Tris-Maltoheptaose (B Crystals)—Here, three well defined carbohydrate chains are identified in the oligosaccharide binding sites OB1, OB2, and OB3. The three sites are indicated in Fig. 1. In OB2, all seven glucosyl rings of a maltoheptaose molecule could be identified. Detailed OB2 interactions are shown in Fig. 2. The chain has the following \(\phi,\psi\)-values: Glc721-Glc722 (82°, −137°), Glc722-Glc723 (72°, −148°), Glc723-Glc724 (123°, −98°), Glc724-Glc725 (70°, −155°), Glc725-Glc726 (80°, −148°), and Glc726-Glc727 (103°, −132°). The heptasaccharide is bound to the enzyme by 13 direct and 10 water-mediated hydrogen bonds. The binding is further stabilized by hydrophobic interactions between Phe417 and Glc725 and between Phe420 and Glc726. All interactions except three hydrogen bonds are with residues...
Saccharide Complexes of Amylosucrase

Crystal Structure

**Table I**

| Data collection | Crystal Structure |
|-----------------|-------------------|
| Synchrotron beam line  | MAXLAB 711 | ESRF ID29 | ESRF ID14-EH1 | MAXLAB 711 | MAXLAB 711 |
| Wavelength (Å) | 1.016 | 1.008 | 0.934 | 0.983 | 1.021 |
| Unit cell (Å³) | 96.2 x 116.4 x 60.3 | 96.1 x 116.1 x 60.7 | 96.1 x 117.0 x 60.6 | 95.6 x 116.0 x 60.8 | 97.4 x 115.8 x 61.1 |
| Resolution limit (Å) | 2.0 (2.07 - 2.00) | 2.0 (2.07 - 2.00) | 2.1 (2.17 - 2.10) | 2.1 (2.18 - 2.10) | 2.0 (2.07 - 2.00) |
| Observed reflections | 413,822 | 403,150 | 312,396 | 240,573 | 376,535 |
| Unique reflections | 45,487 | 45,913 | 40,291 | 32,986 | 46,322 |
| Completeness (%) | 97.9 (95.5) | 99.7 (99.6) | 99.8 (100) | 81.6 (75.8) | 97.6 (96.5) |
| Rsym (%) | 6.2 (22.0) | 7.1 (30.1) | 10.2 (29.0) | 10.7 (23.3) | 6.1 (17.6) |

**Fig. 1. Stereo picture illustrating the various sucrose- and maltoheptaose binding sites on amylosucrase.** The three oligosaccharide binding sites are labeled OB1, OB2, and OB3. Sucrose at the surface site is colored magenta and labeled SB2. The B-domain is colored yellow.

from the B’ domain. Glc722 and Glc723 are involved in two hydrogen bonds to Arg425 from a symmetry-related molecule. Only Glu465 changes conformation upon binding of the heptasaccharide, enabling it to make a hydrogen bond with Glc725.

Under these conditions, the Tris molecule has been displaced from the active site (OB1), and a well defined maltoseptaose molecule is now found here. OB1 includes the α-amylase family −1 and +1 binding sites, which were mapped out previously in the E328Q-sucrose complex (12). A composite omit map of the electron density observed in OB1 is shown in Fig. 3. The electron density is very clear, and five additional connected glucosyl binding sites are determined. Specific interactions with AS amino acids are shown in Fig. 4. The maltoseptaose chain has a very distinct kink around the −1,+1 glucosidic bond with a φ-angle of 33° and a ψ-angle of −155°. The rest of the chain has the following φ,ψ-values: Glc712-Glc713 (67°, −148°), Glc713-Glc714 (72°, −170°), Glc714-Glc715 (104°, −129°), Glc715-Glc716 (80°, −152°), and Glc716-Glc717 (102°, −140°). At the non-reducing end of the molecule (in the active site) there are many contacts to the enzyme and only few water molecules. At the other end of the molecule, water contacts dominate. However, the last glucosyl unit (Glc717) has three hydrogen bonding contacts to amino acid side chains from a symmetry-related molecule.

In OB3, the electron density from four of the glucosyl rings of the maltoseptaose molecule could be seen and modeled. The chain has the following φ,ψ-values: Glc731-Glc732 (1°, −134°), Glc732-Glc733 (103°, −143°), and Glc733-Glc734 (114°, −116°). AS-Tris-Sucrose (C-E Crystals), 14 mM Complex (C Crystals)—A single sucrose molecule is found in the structure located on the surface of AS. This is referred to as the second sucrose binding site (SB2) because it was shown that the AS mutant E328Q binds sucrose at the active site (12). The binding position is shown on Fig. 1 labeled SB2. The residues involved in hydrogen bonding are shown on Fig. 5. No water-mediated hydrogen bonds are observed. Additional interactions include hydrophobic contacts to residues Phe430, Trp499, and Asp506. The pyranose ring of the sucrose moiety is in the most stable chair form, the 4C1 configuration, whereas the furanose

Fig. 1. **Stereo picture illustrating the various sucrose- and maltoheptaose binding sites on amylosucrase.** The three oligosaccharide binding sites are labeled OB1, OB2, and OB3. Sucrose at the surface site is colored magenta and labeled SB2. The B’ domain is colored yellow.
is in the 4E envelope form. High B-factors for the sucrose molecule revealed that the binding site was not fully occupied. Further fitting of the sucrose molecule gave an occupancy of 0.77 with a B-factor of 23 Å². The average B factor for the amino acid residues in AS was 13.9 Å². A Tris molecule is found in the active site as in the previously published structure (10) and in the D and E structures described below.

**AS-Tris-Sucrose (C–E Crystals), 100 mM Complex (D Crystals)—**A sucrose molecule is located at the same position as in the 14 mM complex with a similar hydrogen bonding pattern and similar hydrophobic interactions to the enzyme. In addition to the residues involved in hydrophobic contacts in the 14 mM complex, there are additional interactions to Val134 and Asp207. The occupancy was found to 0.81 with a B-factor of 20 Å², indicating a higher occupancy of the sucrose-binding site compared with the 14 mM complex. The average B factor for the amino acid residues in AS was 14.2 Å² here.

A large electron density corresponding to at least a maltotriose molecule is observed in OB2 in this structure. It is not possible to determine the precise length of the bound oligosaccharide. The carbohydrate-enzyme interactions are very similar to those found in the A-structure.

**AS-Tris-Sucrose (C–E Crystals), 1 mM Complex (E Crystals)—**Three molecules of sucrose are found on the surface of AS in this structure. A sucrose molecule very similar to those observed in the C and D crystals is seen here with almost identical enzyme interactions (Figs. 1 and 5). Two additional water-mediated hydrogen bonds are seen. Bave for this sucrose molecule is 17 Å², indicating full occupation of the site. The average B factor for the amino acid residues in AS was 14.1 Å². The second sucrose molecule is located near the N-domain of AS. Residues Tyr145 and Arg205 are involved in hydrogen bonding, and two additional water-mediated hydrogen bonds are observed. Apparently, no strong hydrophobic interactions exist in this binding site. The occupancy was found to 0.90 with a B-factor of 21 Å². The third sucrose found on the AS surface is positioned in the interface between two symmetry-related molecules with interactions to Asp207, Thr596, and Phe559. The occupancy was found to 0.92 with a B-factor of 23 Å². There is no oligosaccharide observed in this structure.

**DISCUSSION**

**Maltoheptaose Complexes—**The result of co-crystallizing E328Q with maltoheptaose (crystal A structure) is partial occupation of the surface oligosaccharide-binding site (OB2). In a previous soaking experiment with maltotriose (data not shown), the trisaccharide was also found at this binding site with the glucosyl moieties occupying position similar to Glc724, Glc725, and Glc726 in the B crystals. It is also at this position that an oligosaccharide (probably a reaction product) is found in the D structure (see below). This emphasizes that OB2 most probably is a genuine oligosaccharide binding site in vivo and not a crystallization artifact.

The active site is not occupied by an oligosaccharide, but, as in the native structure (10), a Tris molecule is bound here. This is not very surprising, because Tris was present in the crystallization medium and has been found in the active site of a number of other glycoside hydrolases (24–26). However, as seen in the B crystal structure, further soaking of these crystals with maltoheptaose (but without Tris) has two effects. The OB2 site now has all seven subsites occupied (Fig. 2), and, in addition, maltoheptaose is now able to exclude Tris from the active site and map out OB1 (Figs. 1, 3, and 4). Apart from the OB2 hydrogen-bonding interactions shown in Fig. 2, a number of hydrophobic interactions are observed, but only one obvious stacking interaction between the glucosyl ring of Glc726 and Phe417 is seen. The detailed interactions between the maltoheptaose and the active site access channel (OB1) are shown in Fig. 4. Using α-amylase nomenclature, these would be the subsites −1 to +6. In contrast to the α-amylases, the active site architecture is of the pocket type in which further negative subsites are blocked by the Asp144, Arg508 salt bridge. The electron density clearly shows that the non-reducing end of the maltoheptaose is at the −1 position. This is one of the few structures wherein a substrate/product spans the active site. It can be seen (Fig. 4) that the conserved residues in the active site are involved in hydrophobic bonding with the maltoheptaose molecule. At the reducing end of the maltoheptaose molecule the glucosyl ring of Glc717 has contacts to a symmetry-related...
E328Q molecule, so only subsites \(-1\) to \(+5\) are expected to be functionally relevant. OB1 lacks aromatic stacking interactions outside the active site (subsites \(-1, +1\)), but Arg\(_{415}\) seems to provide a hydrophobic platform for the rings at subsites \(+4\) and \(+5\).

The \(-1\) glucosyl moiety of OB1 is bound in a conformation identical to the conformation observed in the previously described AS-D-glucose and E328Q-sucrose complexes (12). Furthermore, it superimposes perfectly on the sucrose pyranosyl moiety when the E328Q-sucrose complex and the B structure are compared. It differs, however, from the D-glucose complex when the E328Q sucrose complex and the B structure are compared. The glucosidic bond between the \(-1\) and \(+1\) glucosyls is oriented as in the E328Q sucrose complex so that the oxygen lone pair would be aligned almost linearly with the H-OE1 of the wild-type Glu\(_{228}\). This complex thus resembles the product complex with maltopentaose (29). Comparing the interactions seen (number of hydrogen bonds) in the \(+1, +2, +3\) subsites of the Bacillus and Aspergillus amyloses to amylosucrase (Glc712-Glc714) suggests that the binding is significantly stronger in amylosucrase. It should also be noted that seven of the nine hydrogen bonds observed are with residues from the B’-domain. Comparisons of the B-structure with the Tris, D-glucose, and E328Q-sucrose complexes show significant movements of the residues Arg\(_{226}\) and Thr\(_{398}\). Upon binding of maltobetaeose in OB1, the Thr\(_{398}\) backbone conformation changes with a \(\psi\)-angle shift of 90°, probably due to a change of the Thr side-chain conformation, which enables hydrogen bonding to the \(+3\) glucosyl moiety. This results in slight backbone movement of the residues 397–409. The position of Arg\(_{226}\) is changed so that it is possible to make two hydrogen bonds to the \(+2\) glucosyl. This, in turn, results in a backbone movement of residues 224–229. Probably as a consequence, the positions of the Phe residues 229, 250, and 436 are affected. Phe\(_{229}\) and Phe\(_{436}\), which are both partially solvent-accessible and located on the interface between the B and B’-domain, show the largest deviations with changes in the \(\gamma1\) dihedral angle for both residues and also a change in the \(\gamma2\) dihedral angle for Phe\(_{229}\).

The OB1 and OB2 sites are only separated by the B’-helix 410–422 (Fig. 1), giving a possibility of allostery between the two binding sites. The position of residue Phe\(_{417}\) is related to the position of Arg\(_{415}\) via the helix hydrogen bonding. This helix has crystal contacts to a symmetry-related molecule mentioned above, thus unfortunately blocking any connecting subsites. Furthermore, the glucosyl rings that are closest together are both identified as the reducing ends of the two maltopentaose molecules. To connect the two subsites by a linear \(\alpha\)-1,4-linked oligoglucosyl chain, this chain would have to bulge away from the enzyme, making a helical turn. Detailed experimentally based knowledge on how (if at all) the two subsites can be connected can only be obtained from a co-crystallization of AS and an oligosaccharide with a length of \(\leq10\) glucosyl units in another crystal form. This could perhaps also reveal whether the third observed binding site, OB3, could be connected to OB1 and/or OB2.

**Sucrose Complexes**—The crystal structures of native AS soaked with different concentrations of sucrose (structures C–E) reveal that AS has an additional sucrose-binding site (SB2) close to the active site (Fig. 1). SB2 is positioned just outside where the \(\alpha\)-amylase active site cleft would be if the Asp\(_{344}\)-Arg\(_{359}\) salt bridge (10) was open. The direct AS-sucrose hydrogen bonding contacts shown in Fig. 5 reveal interactions (including some hydrophobic interactions not shown on Fig. 5) with the B’-domain (residues 394–450). As can be imagined...
(Fig. 1), sucrose bound at this position can enter the active site only if a large amplitude movement of the B'-domain take place. This suggests the need for allosteric regulation.

SB2 was not occupied in the E328Q-sucrose structure in which the sucrose concentration involved was comparable with that used in the crystal C experiment. However, the experimental conditions were different. The E328Q-sucrose complex was the result of a co-crystallization, whereas soaking experiments were used in crystal structures C–E. Also, in the C–E structures native AS was used in contrast to the inactive mutant used for E328Q-sucrose structure. We have not been able to displace Tris from the active site of the wild type enzyme with sucrose or an oligosaccharide, stressing that the substrate binding properties of the two proteins certainly are different. This difference probably reflects that AS can compensate the positive charge of Tris at neutral pH better than the E328Q variant. The changes in occupancy and B-factors for the sucrose molecule observed from crystals C to E suggests that the affinity of sucrose for the SB2 site is weak to moderate, and it is likely that the SB2 site would also be observed in the E328Q structure at higher concentrations. This observed weak binding is also in agreement with a subsequent transfer of the sucrose molecule away from SB2 into the active site.

In crystal structure D an oligosaccharide is observed at the OB2 site. This suggests that the enzyme has been active during the experiment, either in the crystalline state or from residual enzyme in solution. It is remarkable that the site is occupied in the D crystals but not in C or E crystals. However, 100 mM has been shown to be an optimal sucrose concentration for the enzyme; at higher sucrose concentrations the reaction rate is saturated (1, 11). The additional sucrose binding sites observed when AS crystals were soaked with 1 M sucrose (E structure) are not expected to be of functional relevance.

Implications for Polymer Synthesis—Fig. 1 clearly shows that the interactions between AS and its substrates, sucrose and a α-1,4-linked glucosyl chain, are closely related to the B'-domain (residues 394–450). Of the 57 amino acid residues, six had contacts to OB2, eight had contacts to OB1, and three had contacts to SB2. We have previously shown that it is likely that AS uses the well established double displacement reaction mechanism characteristic for the glycoside hydrolase family 13, but many details of the catalysis need to be clarified. One important question that arose from the E328Q-sucrose and AS-glucose structures was how the enzyme controlled hydrolysis with only one access channel to the active site. The structures described here suggest that there could be two entrances to the active site, i.e. one that is occupied by an oligosaccharide and one that can be in an open and in a shut state. Because AS is expected to be a chain elongator of already existing oligosaccharides in vivo, it seems reasonable to assume that the large substrate access channel is occupied by the polymer already at the start of the elongation reaction. The superposition of structures D and B excluding the B'-domain (Fig. 6) shows that, even in the crystalline environment, sucrose binding does introduce domain movement. It can be hypothesized that, in solution, this movement is sufficient to allow sucrose to enter the active site. The domain movement relative to the AS-Tris complex is smaller than the one shown in Fig. 6, indicating an allosteric mechanism wherein oligosaccharide binding on the B'-domain enhances domain mobility upon sucrose binding. If this access was closed when sucrose moves to the active site, water access would be prohibited. Binding of sucrose in the active site will displace the oligosaccharide at least two sub-sites in the positive direction, probably still shielding for water access through this active site substrate access channel. How fructose can exit the active site after the sucrose molecule has been split is not clear. If this happens before transglycosylation, the architecture suggests that it can only occur through the oligosaccharide access channel. It should be remembered that it is only the glucosyl-enzyme intermediate that has to be protected from water to avoid hydrolysis. AS seems to do this very effectively, because hydrolysis is always a minor side reaction (2).

The structures presented show that the B'-domain is designed for alteration of a hydrodase into a polymerase. The B'-domain has two (probably connected) oligosaccharide binding sites and also defines a low affinity sucrose-binding site. The domain seems to become more mobile upon oligosaccharide binding, so allosteric regulation of the sucrose access is suggested.

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