Crystal Structure of the βSer^{178} → Pro Mutant of Tryptophan Synthase

A “KNOCK-OUT” ALLOSTERIC ENZYME*

The catalytic activity of the pyridoxal 5′-phosphate-dependent tryptophan synthase αβ_2 complex is allosterically regulated. The hydrogen bond between the helix βH6 residue βSer^{178} and the loop αL6 residue Gly^{181} was shown to be critical in ligand-induced intersubunit signaling, with the α-β communication being completely lost in the mutant βSer^{178} → Pro (Marabotti, A., De Biasi, D., Tramonti, A., Bettati, S., and Mozzarelli, A. 1999 J. Biol. Chem. 274, 17747–17753). The structural basis of the impaired allosteric regulation was investigated by determining the crystal structures of the mutant βSer^{178} → Pro in the absence and presence of the α-subunit ligands indole-3-acetylglycine and glycerol 3-phosphate. The mutation causes local and distant conformational changes especially in the β-subunit. The ligand-free structure exhibits larger differences at the C-terminal side. In contrast to the wild-type enzyme loop αL6 remains in an open conformation even in the presence of α-ligands. This effects the equilibrium between active and inactive conformations of the α-active site, altering k_{cat} and K_{m}, and forms the structural basis for the missing allosteric communication between the α- and β-subunits.

The αβ_2 complex of tryptophan synthase (TRPS) \(^1\) (EC 4.2.1.20) is a bifunctional enzyme that is considered a para-

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\(^1\) The abbreviations used are: TRPS, tryptophan synthase; IAG, indole-3-acetylglycine; GP, Nα-α-glyceral-3-phosphate; GAPDH, glycerol 3-phosphate; Biicine, N,N-bis(2-hydroxyethyl)glycine; αL2, loop 2 (αPro^{55}→Asp^{60}) of the α-subunit of tryptophan synthase (15), βL6, loop 6 (αArg^{179}→Leu^{183}) of the α-subunit of tryptophan synthase (15); βH6, helix 6 (βThr^{100}→βTrp^{104}) of the β-subunit of tryptophan synthase (15); COMM domain, domain (βGly^{122}→βGly^{138}) for the communication between α- and β-subunit (15); TRPS_{αβ}, tryptophan synthase IAG complex; TRPS_{αβ}, tryptophan synthase GP complex; TRPS_{αβ}, tryptophan synthase IAG complex; βS178P, TRPS Ser → Pro point mutant at βSer^{178}; βS178P_{αβ}, βS178P TRPS mutant IAG complex; βS178P_{αβ}, βS178P TRPS mutant GP complex; r.m.s., root mean square (Å) for Cα-atom pairs of two compared tryptophan synthase structures; bis-Tris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)-1,3-propanediol.

\(βS178P\) TRPS mutant GP complex; r.m.s., root mean square (Å) for Cα-atom pairs of two compared tryptophan synthase structures; bis-Tris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)-1,3-propanediol.

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Crystal Structure of Tryptophan Synthase βS178P Mutant

### TABLE I

| Complex | βS178P | βS178P<sup>AG</sup> | βS178P<sup>GP</sup> |
|---------|--------|----------------------|---------------------|
| Protein Database code | 1KTX | 1K8Z | 1KSY |
| C2 crystal parameters | | | |
| Unit cell (a, b, c) (Å), (β) (degree) | 184.0, 60.0, 67.5, 94.6 | 184.4, 61.0, 67.5, 94.7 | 182.5, 60.0, 67.2, 94.5 |
| Data statistics | | | |
| Resolution (Å) | 45.9–1.7 | 43.4–1.7 | 45.6–1.5 |
| No. of observations | 234,033 | 206,209 | 289,431 |
| No. of unique reflections | 78,258 | 79,508 | 111,900 |
| Compl. (total/high) (Å)<sup>2</sup> | 96.8/95.9 | 96.4/96.1 | 95.1/91.0 |
| R<sub>work</sub> (total/high)<sup>a</sup> | 10.3/36.6 | 6.3/33.4 | 7.8/24.6 |
| R<sub>factor</sub> (total/high)<sup>b</sup> | 9.64/82.7 | 9.29/21.1 | 9.76/93.3 |
| Refinement statistics | | | |
| Resolution range (Å) | 20.0–1.7 | 20.0–1.7 | 20.0–1.5 |
| Included amino acids | α1–α178, α191–α267, β2–β391 | α1–α177, α191–α268, β2–β395 | α1–α178, α193–α268, β2–β391 |
| Non-glycine and non-proline residues | αF212 | αF212 | αF212 |
| No. of protein atoms | 4870 | 4925 | 4877 |
| No. of waters | 437 | 377 | 658 |
| No. of ligand atoms | 15 | 32 | 25 |
| No. of metal ions | 1 | 1 | 1 |
| R<sub>work</sub> (%)<sup>c</sup> | 18.7/23.9 | 21.0/26.5 | 17.1/20.9 |
| R<sub>factor</sub> (%)<sup>d</sup> | 0.059/4.1 | 0.086/3.58 | 0.048/3.64 |
| R<sub>free</sub> (%)<sup>e</sup> | 19.2/36/21/33.5 | 23.1/27.9/28.8/32.9 | 23.1/27.9/31.5/36.6 |
| (B) (mosaic/wig/wat) (Å)<sup>f</sup> | 8.9/23.9/16.0/29.6 | 23.1/27.9/28.8/32.9 | 27.9/28.8/32.9 |
| Alternative side chain α conformations | 5/26/20 | 9.64/82.7 | 9.29/21.1 |

<sup>a</sup> Completeness, R<sub>work</sub>, and (I/σI) are given for all data and for the highest resolution shell: βS178P<sup>AG</sup>, 1.6–1.5 Å; βS178P and βS178P<sup>GP</sup>, 1.8–1.7 Å.

<sup>b</sup> R<sub>work</sub> = Σ|F<sub>obs</sub>−|F<sub>calc</sub>|/Σ|F<sub>obs</sub>|

<sup>c</sup> R<sub>work</sub> = Σ|F<sub>obs</sub>|−|F<sub>calc</sub>|/Σ|F<sub>obs</sub>|

<sup>d</sup> R<sub>free</sub> = Σ|F<sub>obs</sub>|−|F<sub>calc</sub>|/Σ|F<sub>obs</sub>|

<sup>e</sup> 5% of randomly chosen reflections were used for the calculation of R<sub>free</sub>.

<sup>f</sup> mc, main chain; sc, side chain; lig, ligand; wat, water.

### MATERIALS AND METHODS

**Crystalization and Complex Preparation**—IAG and GP were obtained from Sigma and were used without further purification. The βS178P mutant of TRPS was purified as described previously (21). The protein was stored at a concentration of 10 mg/ml in a solution containing 50 mM Na-bicine, pH 7.8, 10 mM Na-EDTA, 1 mM diethylenetriamine, and 20 μM pyridoxal 5-phosphate. Crystals were grown in the dark at room temperature using the hanging drop geometry and mixing equal volumes (2–4 μl) of protein and reservoir solutions. The latter contained 9–12% polyethylene glycol 8000, 1.5 mM spermine, 1 mM EDTA, 100 mM bis-Tris-propane, pH 7.8, 100 mM NaCl, utilizing the difference in absorption between indole and IAG at 290 nm (35, 36). Spectra were taken at 37 °C using 0.2-mm-path-length quartz cuvettes. The forward α-reaction was measured using a protein concentration of 3.61 μM and IGP concentrations between 0.03 and 1.5 mM. The solution was preincubated at 37 °C for 15 min, and the reaction was started with the addition of TRPS. The reverse α-reaction was measured using a protein concentration of 63 nM, an indole concentration of 2 mM, and G3P concentrations between 0.3 and 3.0 mM. The solution was preincubated at 37 °C for 15 min, and the reaction was started with the addition of G3P. The data were fitted to a hyperbolic curve with the program GRAFT.

### RESULTS AND DISCUSSION

Since the influence of the proline mutation in helix βH6 of the COMM domain on the TRPS structure is most pronounced in the ligand-free enzyme, the ligand-free βS178P structure is discussed first in more detail. Then, this structure serves as a basis for the comparison with the IAG and GP complexes.

**Ligand-free βS178P Structure**

The crystal structure of the ligand-free βS178P TRPS mutant was solved at 1.7-Å resolution and refined to crystallo-
The overall topology of the mutant structure is the same as for the wild-type (15). Besides the C-terminal residues of both polypeptide chains, weak or no electron density was found for the residues of loop L6 (Leu177–Ala190) and for the side chain of phenylalanine Phe212. The latter residue was modeled as alanine. The missing parts of the protein structure are not due to poor x-ray data quality, since the $\beta$-subunit core residues $\beta$Met$^{187}$, $\beta$Lys$^{219}$, and $\beta$Ser$^{301}$ exhibit clear features for double side chain conformations. The observation of multiple side chains, which also occurs in the $\beta$S178P$^{\text{LAG}}$ and $\beta$S178P$^{\text{GP}}$ structures (see Table 1), indicates high x-ray data quality. Moreover, loop aL6 was also not detectable in other structures of the wild-type and mutant (1, 14, 37–40), confirming that this region of the $\alpha$-subunit is particularly mobile. A close-up of the mutation site (Fig. 1A) shows the interruption of the $\beta$H6 helix caused by the Ser$^{178}$ → Pro substitution. The comparison of the wild-type and mutant structures (Figs. 2 and...
FIG. 2. Cα-RMSD plots of different mutant and wild-type TRPS structures for the α- (left) and β- (right) subunits. A, comparison of ligand-free βS178P mutant and wild-type TRPS structures; B, comparison of βS178P^PAG and wild-type TRPS^PAG structures; C, comparison of ligand-free and βS178P^PAG structures; D, comparison of ligand-free βS178P and βS178P^GP structures.
3) evidences local and distant conformational changes caused by the mutation. Special features of the ligand-free βS178P mutant structure and differences with the wild-type are described and discussed below.

βS178P α-Subunit—The structure of the ligand-free α-subunit is very similar to the wild-type enzyme, indicating that the mutation in the β-subunit does not perturb the α-subunit. Larger structural differences between the βS178P α-subunit and the wild-type were found in loop αL6 at αAsn\(^{157}\), as indicated by a large r.m.s. deviation (Fig. 2A, left panel, peak A). Since this loop region has ambiguous electron density in most structures determined by us (15, 27), the structural difference, indicated by a high r.m.s. deviation for αAsn\(^{157}\), might be caused by distinct interpretations of this part of the electron density map. In one of these possible map interpretations αAsn\(^{157}\) is able to connect the COMM domain to loop αL6, the most mobile region of the TRPS enzyme. On one side, in the mutant structure the side chain of αAsn\(^{157}\) is able to form hydrogen bonds to the hydroxyl group of βTyr\(^{181}\) and to the carbonyl oxygen of βLeu\(^{20}\). On the other side, in the wild-type structure the carbonyl oxygen of the preceding amino acid αPro\(^{156}\) has a distance of approximately 4.5 Å to the amide nitrogen of αLeu\(^{190}\). This distance is too long for a favorable hydrogen bond. However, since this part of the TRPS structure shows the highest flexibility and, therefore, the highest coordinate error, water molecules may be difficult to identify. With the present structural data we cannot exclude the presence of a water molecule mediating the connection between the COMM domain and loop αL6 via αAsn\(^{157}\). Hiraga and Yutani (41) have studied the subunit association of several TRPS interface mutants by titration calorimetry, including two αAsn\(^{157}\) mutants, and found that these mutations did not affect the stimulatory activity, indicating that this subunit interaction is not of crucial importance for the allosteric regulation. This supports our belief that the structural difference at the αAsn\(^{157}\) containing surface loop is of no relevance for the observed different allosteric communication properties of the βS178P mutant.

The second structural difference (Fig. 2A, left panel, peak B) within the α-subunit of the βS178P mutant is found at αPhe\(^{212}\), which is modeled as alanine, as this part of the structure also has weak electron density. However, the electron density of the final structure has well defined density for a water molecule, forming hydrogen bonds with the amide nitrogen atoms of αPhe\(^{212}\) and αGly\(^{214}\). βS178P β-Subunit—In the case of the β-subunit, several significant differences between the wild-type and mutant structures are observed. Apart from different conformations in a surface loop (Fig. 2A, right panel, peak A) and the C-terminal end of the β-polypeptide chain (Fig. 2A, right panel, peak D), the βSer\(^{178}\) → Pro mutation introduces a different backbone conformation of the neighboring residues of helix βH6 (Fig. 2A, right panel, peak C). In contrast to the molecular simulation

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**Fig. 3.** A. C-atom trace representation of the superposition of the wild-type and βS178P mutant structures focused on the α-active site, the mutation site and the α/β-subunit interface. The wild-type C-atom trace is colored in gray, and loops αL2 and αL6 of the α-subunit are colored in cyan. The βS178P mutant C-atom trace is colored in red for the α-subunit and in yellow for the β-subunit. The amino acids at position β178 are shown in ball-and-stick representation. B. C-atom trace representation of the superposition of the wild-type TRPS\(^{54\text{MS}}\) and βS178P\(^{54\text{MS}}\) structures focused on the α-active site, the mutation site, and the α/β-subunit interface. The wild-type C-atom trace is colored in gray, and loops αL2 and αL6 of the α-subunit are colored in cyan. The βS178P mutant C-atom trace is colored in red for the α-subunit and in yellow for the β-subunit. The IAG molecule of the βS178P structure and the amino acids at position β178 are shown in ball-and-stick representation. The figure was prepared using MOLSCRIPT (47) and RASTER3D (48, 49).
(21), that indicated the presence of only localized structural changes leading to the loss of the hydrogen bond between the native βSer178 and αGly181, the x-ray structure shows that the change at position β178 disturbs the normal α-helix backbone conformation of helix βH6 (amino acids βThr165–βTyr189 (15)). The normal hydrogen bond between the amide proton of βSer178 and the preceding arginine βArg177 carbonyl oxygen is no longer possible (Fig. 1). The $\psi$ torsion angles for βArg177 changed from $-62.8^\circ$–$-44.4^\circ$ in wild-type TRPS to $-75.7^\circ$–$-36.1^\circ$ in the mutant. By this rotation an unfavorable contact of the new proline ring is avoided. This conformational change is further transmitted to the rest of the N-terminal part of helix βH6. Therefore, compared with the wild-type structure the largest C$_\alpha$-r.m.s. deviation differences are found in this region of the mutant (Fig. 2A, right panel, peak C), whereas the changes are insignificant at the C-terminal end of helix βH6 (Fig. 2A, right panel, and Fig. 3A). A similar conformational change in helix βH6 is observed in all three βS178P complexes: ligand-free, IAG, and GP. The N-terminal part of helix βH6 and the adjacent structural elements of the COMM domain are linked to the metal binding loop (βAsp235/βPro237) via a water-mediated hydrogen bond network and some hydrophobic side chain interactions. In the ligand-free βS178P structure βLeu226 shifted toward the side chain of βPhe106, and the aspartate βAsp235 side chain points toward the α-active side, forming a water-mediated contact to βAsp138, which is in direct neighborhood to the N-terminal part of helix βH6. In case of the ligand-free mutant structure the “disturbance” caused by the mutation is further transmitted by the neighboring secondary structure elements strand βS5 and helix βH5 (Fig. 2A, right panel, peaks B and C, Fig. 3A) to βGlu109, which is believed to play a crucial role in the catalytic activity and the substrate specificity of the TRPS β-reaction (42, 43). This may explain the 2-fold lower β activity of the βS178P mutant compared with the wild-type enzyme (21).

### Crystal Structure of Tryptophan Synthase βS178P Mutant

The structure was solved at a resolution of 1.7 Å. As in the uncomplexed structure, the amino acids of loop αL6 (αLeu178–αAla180) are not detectable. In addition to the α- and β-subunits, the final model contains 377 water molecules, a sodium ion, and an indole-3-acetylglycine bound to the active site. This model was refined to R-factors of $R_{work} = 21.0\%$ and $R_{free} = 26.5\%$, respectively (Table I). Although all three x-ray data sets are of the same quality (Table I), a B-factor analysis of the structures (data not shown) shows that the sodium binding loop βL8, loop αL2, and the C-terminal part of the COMM domain (loop βL3, strand βS4, helix βH5, strand βS5, and helix βH6) have higher mobility in βS178P$^{\beta\alpha\gamma}$ (24). Furthermore, critical inspection of the final SigmaA-weighted (44) (2mFo – DFc and mFo – DF) electron density maps indicates a second backbone conformation (which is not included in the final model) for these regions and the loop between βLeu208 and βSer209 that is consistent with the ligand-free βS178P structure. For the following reasons we believe that it is a reduced IAG and not a reduced sodium occupancy that causes the conformational changes within the sodium binding loop. A series of sodium titrations (data not shown) with the wild-type and the βS178P mutant in the absence and presence of IAG showed no influence of the α-ligand on the sodium affinity, and in contrast to the findings of Marabotti et al. (21), the βS178P mutant has the same sodium affinity as the wild-type. Moreover, a cross-check for the ligand occupancy showed a slightly lower IAG occupancy (data not shown), but the resolution of the x-ray data does not allow further refinement.

IAG binds to the mutant in the same manner as to the wild-type (24), but loop αL6 is not closed. The indole nitrogen forms a hydrogen bond with aspartate αAsp60 and the acetyl carboxylate group mimics the IGP/IPP/GP phosphate group. The second catalytically important amino acid αGlu109 is modeled in two conformations and forms a hydrogen bond with the IAG acetyl oxygen atom. The implications of this interaction for the α-reaction are discussed in the accompanying paper (24). The hydrogen bonding patterns and distances are shown in Fig. 1B and in Table II, respectively.

### Table II

| Protein | Amino acid/water molecule | Atom | Atom | GP Distance | IAG Distance |
|---------|--------------------------|------|------|-------------|-------------|
| Asp$^{\beta\alpha\gamma}$ | O$_\alpha$ | O$_\alpha$ | 01 | 2.8 | 2.9 |
| Tyr175 | O$_\alpha$ | O$_\alpha$ | 01 | 2.7 | 02 |
| Glu$^{\beta\alpha\gamma}$ | O$_\alpha$ | O$_\alpha$ | 01 | 3.0 | O3P |
| Trp | O | O | 02P | 2.7 | 2.9 |
| Ser135 | O$_\alpha$ | O$_\alpha$ | 02P | 2.6 | 02 |
| Ser138 | O$_\alpha$ | O$_\alpha$ | 03P | 2.6 | 02 |
| Gly124 | O$_\alpha$ | O$_\alpha$ | 03P | 2.9 | 2.7 |
| Gly139/Trp138 | O | O | 03P | 2.6 | O2 |
| Gly141 | O$_\alpha$ | O$_\alpha$ | 04P | 2.7 | 02 |
| Phe144 | O$_\alpha$ | O$_\alpha$ | 02 | 3.0 | O3P |

βS178P/βS178P$^{\beta\alpha\gamma}$ Comparison—The C$_\alpha$-r.m.s. deviation structure comparison of the ligand-free βS178P and the βS178P$^{\beta\alpha\gamma}$ structure shows, apart from a large difference in the phosphate binding loop αL8 at the α-active site (Fig. 2C, left panel, peak A), which is also observed in case of the analogous wild-type structures (data not shown), only minor differences in the α-subunit and also in the COMM domain (Fig. 2C). However, some larger deviations are found at the metal binding site within βL8 (Fig. 2C, right panel, peak A). Although the water network at the sodium binding site is similar in both structures, in the βS178P mutant structure the βAsp205 side chain points toward the β-active site, a conformation also found in the wild-type IAG complex (24). In contrast, in the βS178P$^{\beta\alpha\gamma}$ structure the aspartate side chain is pointing away from the β-active side, forming a new hydrogen bond with the carbonyl oxygen of βSer207. The origin of the different conformation of the metal binding loop can be traced back to IAG bound at the α-active site by the following hydrogen bonding network: the βPro307 carbonyl oxygen atom, which is also involved in the metal binding, is linked to the βMet387 amide proton. The neighboring glutamine βGlu388 side chain forms an intersubunit hydrogen bond to αAsn104 and the close by αTyr105 side chain interacts with the aspartate αAsp100 side chain. Besides this intersubunit pathway starting from glutamine βGlu388, a second one exists via the βGlu388 carbonyl oxygen atom, which is linked to the amide proton of βGlu295. The conformational changes are further transmitted by a back-
bone shift of the adjacent residues βGln181 and βHe203, which form hydrogen bonds to the loop αL2 amino acid αSer56 and finally via a backbone shift of loop αL2 residues with aspartate αAsp70. The latter forms a hydrogen bond to the indole nitrogen of IAG.

βS178PΔAG/WTΔAG Comparison—The comparison of mutant and wild-type IAG complexes shows large Cα r.m.s. deviation within helix βH6 and also in the sodium binding loop at βAsp50 (Fig. 2B, right panel, peak C, and Fig. 3B), as described above. Surprisingly, the major differences occur at the C-terminal side of helix βH6 (Fig. 2B, right panel, peak B), whereas they occur at the N-terminal side in the ligand free structures, taking the mutation site as origin. This difference is caused by the α-ligand modifying the conformation of loop αL2 via the interaction between the nitrogen atom of the indole ring of IAG and the αAsp60 carboxylate. In this conformation loop αL2 residues interact with the N-terminal part of helix βH6 (15), and the helix adopts in the βS178PΔAG structure the same conformation as in TRPSΔAG. The proline substitution at β178 prevents the formation of a hydrogen bond with the αGlu181 carbonyl oxygen atom, thereby interrupting the α → βα-subunit communication upon IAG binding (see Fig. 3B). On the C-terminal side of the mutation the βS178PΔAG structure adopts a different conformation than the wild-type, since the prolyl ring causes a slight backbone shift. It is transmitted via residues of the following strand βS6 to the surface residue lysine βLeu103 (Fig. 2B, right panel, peak A). The remaining part of the β-subunit, especially the β-active site, is not altered with respect to the wild-type complex.

The comparison of the mutant βS178PΔAG and wild-type TRPSΔAG structures shows no large differences in the core of the α-subunit (Fig. 2B, left panel), indicating that the βSer178 → Pro mutation does not affect the α-subunit grossly. However, although the IAG molecule binds to the mutant in the same manner as to the wild-type enzyme, loop αL6 is not closed in the mutant. The most obvious reason for the open loop is the point mutation at serine βSer178. As the structure superposition shows no steric hindrance by the introduced βPro178, removal of the single hydrogen bond between βSer178 and αGlu181 prevents stabilization of the closed αL6 conformation, which is linked via αThr183 to the α-active site residue αAsp60. As a result, allosteric signals are no longer able to travel from the α- to the β-active site, and the intersubunit regulation is lost (21).

In the context of an open αL6 in βS178PΔAG it is interesting to note that ligand binding to the α-subunit appears to be a two-step process consisting of an initial binding step and a subsequent isomerization to an activated form $E^*$ (8)

$$E + \alpha\text{-ligand} \rightleftharpoons E - \alpha\text{-ligand} \rightleftharpoons E^* - \alpha\text{-ligand}$$

with $E$ representing an open conformation of the α-subunit, while $E^*$ represents the closed conformation (20).

A shift in the equilibrium between $E$ and $E^*$, e.g. by a mutation, would effect $k_{cat}$ correspondingly. A reduction of the occupancy of αL6 by a factor of two to three would be enough for it not to be visible in the electron density. To gain more insight in the influence of the βS178P mutation on the equilibrium distribution at the α-active site, we analyzed the steady state kinetics of the forward and reverse α-reactions of the βS178P mutant. As can be seen in Table III, there are differences in $K_m$ and $k_{cat}$ between mutant and wild-type enzymes, the latter being faster in both reactions. There is no difference in $K_m$ for G3P and IGP in the reverse and forward reactions for the mutant, whereas they differ by a factor of 6 in the wild-type. In the latter case the binding energy of the indolyl ring is used for enzymatic rate acceleration (Table III). The rate-limiting step in the forward α-reaction is the isomerization from the catalytically inactive ($E^*$IGP) to the active IGP complex ($E$IGP) (8). This step is slowed by a factor of two in the βS178P mutant, correspondingly the equilibrium between inactive (open) and active (closed) conformations shifted by a factor of two, which explains the lack of electron density for αL6 in the βS178PΔAG complex. These findings agree with the hypothesis that an open αL6 corresponds to the inactive conformation of the α-active site (e.g. TRPSΔIGP), whereas the closed αL6 corresponds to the active conformation (e.g. TRPSΔPP) (20, 27). Thus, the combination and comparison of the structural information of TRPSΔAG and βS178PΔAG and the kinetic data allows the direct correlation of the equilibrium distribution between open and closed conformations of αL6 with $k_{cat}$.

### βS178PΔGP Structure

The mutant GP structure was solved at a resolution of 1.5 Å. As in the βS178PΔAG structure the amino acids of loop αL6 (αArg195–αPro196) are missing in the βS178PΔGP structure. Besides protein atoms, the final model contains 658 water molecules, a sodium ion, and a glycerol 3-phosphate molecule bound to the α-active site. This model was refined to R-factors of Rwork = 17.1% and Rfree = 20.9%, respectively (Table I). The βS178PΔGP structure (Fig. 1C) shows how GP binds to the α-active site. The orientation of the phosphate moiety is similar to that of the IAG carboxylate group, pointing toward αS235. The hydrogen bond network between GP and α-active site residues is shown in Fig. 1C. The comparison of the distances between selected atoms of GP or IAG and amino acid residues (Table II) indicates a close agreement of the binding modes of both allosteric effectors, despite their chemical differences. Another common feature with the TRPSΔAG structure is the lack of loop αL6 closure, indicating again the structural basis for the absence of an allosteric effect of GP on the β-subunit activity (21). Since a wild-type TRPSΔGP structure is not available, the βS178PΔGP structure was only compared with the ligand-free mutant structure (Fig. 2D). The Cα r.m.s. deviation plots show that larger differences occur at the α-active site at loop αL7 (Fig. 2D, left panel, peak A) and loop αL8 (Fig. 2D, right panel, peak B). Amino acids of both loops are involved in the binding of the GP phosphate moiety. In case of the β-subunit, the Cα r.m.s. deviation plot of the ligand-free and GP mutant structure (Fig. 2D, right panel) shows only minor differences. Thus, the conforma-
tation of the mutant β-subunit seems to be independent of GP binding to the α-active site, confirming the kinetic finding that the βS178P mutant has lost the signaling capability between the α- and β-subunits (21). It is interesting that loop αL6 is in the closed conformation in the βS87T mutant complexed with GP (α-site) and serine (external aldimine, β-site) (Protein Data Bank code 2TSY (14)). The βS87T mutant has no measurable activity in the β-subunit but retains α-subunit activity (45).

CONCLUSIONS

The correlation of the three-dimensional structure of the βSer178 → Pro mutant of tryptophan synthase, reported herein, with the functional and regulatory properties described previously (21) allows to unequivocally attribute a key role in the transmission of ligand binding information between α- and β-sites to the hydrogen bond between βSer178 and αGly181. This is supported by equilibrium studies of a mutant in which αGly181 was mutated to proline. Also in this case, the allosteric properties of the enzyme are knocked out.2 The structural element that mediates the communication is loop αL6 that, in the wild-type enzyme, closes on the α-active site residues upon α-ligand binding, whereas it remains open in the βS178P mutant. This not only affects the allosteric α-β interactions but also shifts the equilibrium between active and inactive conformations of the α-active site, resulting in different kcat and Km values. In tryptophan synthase, the data presented here indicate that the intersubunit hydrogen bond between the αGly181 amide proton and the βSer178 carbonyl oxygen atom represents an “informational pivot point” for α → β communication, and a loss of this functionality is enough to breakdown allosteric regulation. The importance of this interaction is also reflected in it being mediated by backbone atoms, since these are invariant toward mutations.

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