Exchange of K^+ or Cs^+ for Na^+ Induces Local and Long-Range Changes in the Three-Dimensional Structure of the Tryptophan Synthase $\alpha_2\beta_2$ Complex‡

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ABSTRACT: Monovalent cations activate the pyridoxal phosphate-dependent reactions of tryptophan synthase and affect intersubunit communication in the $\alpha_2\beta_2$ complex. We report refined crystal structures of the tryptophan synthase $\alpha_2\beta_2$ complex from *Salmonella typhimurium* in the presence of K^+ at 2.0 Å and of Cs^+ at 2.3 Å. Comparison of these structures with the recently refined structure in the presence of Na^+ shows that each monovalent cation binds at approximately the same position about 8 Å from the phosphate of pyridoxal phosphate. Na^+ and K^+ are coordinated to the carbonyl oxygens of βPhe-306, βSer-308, and βGly-232 and to two or one water molecule, respectively. Cs^+ is coordinated to the carbonyl oxygens of βPhe-306, βSer-308, βGly-232, βVal-231, βGly-268, and βLeu-304. A second binding site for Cs^+ is located in the β/β interface on the 2-fold axis with four carbonyl oxygens in the coordination sphere. In addition to local changes in structure close to the cation binding site, a number of long-range changes are observed. The K^+ and Cs^+ structures differ from the Na^+ structure with respect to the positions of βAsp-305, βLys-167, and αAsp-56. One unexpected result of this investigation is the movement of the side chains of βPhe-280 and βTyr-279 from a position partially blocking the tunnel in the Na^+ structure to a position lining the surface of the tunnel in the K^+ and Cs^+ structures. The results provide a structural basis for understanding the effects of cations on activity and intersubunit communication.

There has been a recent resurgence of interest in the effects of monovalent cations on enzyme structure and function as the result of X-ray structures which define the monovalent cation binding sites in four enzymes: dialkylglycine decarboxylase (Hohenester et al., 1994; Toney et al., 1993, 1995), tryptophanase (Isupov et al., 1994), tyrosine phenol-lyase (Antson et al., 1994), and pyruvate kinase (Larsen et al., 1994). Monovalent cation activation of *S*-adenosylmethionine synthase has been reported (McQueney & Markham, 1995) and is supported by crystallographic data (Takusagawa et al., 1996). These reports provide the first structural basis for understanding the monovalent cation activation or inhibition that has been shown for a large group of enzymes [for a review see Suelter (1970)]. Dialkylglycine decarboxylase, tryptophanase, and tyrosine phenol-lyase all utilize the coenzyme pyridoxal phosphate (PLP)[1] and belong to a large family of PLP-dependent enzymes, termed the α family (Alexander et al., 1994) or Fold type I (Grishin et al., 1995). [See Woehl and Dunn (1995b) for a recent review of the roles of Na^+ and K^+ in PLP enzyme catalysis.]

The β subunit of tryptophan synthase belongs to the second family of PLP enzymes, termed the β family (Alexander et al., 1994) or Fold type II (Grishin et al., 1995). Monovalent cation activation has been reported for the tryptophan synthase β subunit (Crawford & Ito, 1964; Goldberg et al., 1968; Hatanaka et al., 1962; Miles & McPhie, 1974; Ruvinov et al., 1995a; York, 1972) and for the tryptophan synthase $\alpha_2\beta_2$ complex (Dunn et al., 1994; Peracchi et al., 1994, 1995; Ruvinov et al., 1995a; Schwartz & Bonner, 1964; Woehl & Dunn, 1995a; Woehl & Dunn, 1995b).

The tryptophan synthase (EC 4.2.1.20) $\alpha_2\beta_2$ complex catalyzes the last two reactions in the biosynthesis of L-tryptophan [for reviews see Miles (1979, 1991, 1995), Miles et al. (1994), Swift and Stewart (1991), and Yanofsky and Crawford (1972)]. Early studies (Creighton, 1970; DeMoss, 1962; Matchett, 1974; Yanofsky & Rachmeler, 1958) and recent kinetic investigations (Anderson et al., 1991; Brzović et al., 1992a; Dunn et al., 1990; Lane & Kirschner, 1991) provide evidence that indole is a "channeled" intermediate in the overall $\alpha\beta$ reaction.

α reaction: indole-3-glycerol phosphate ↔ indole + D-glyceraldehyde 3-phosphate

β reaction: L-serine + indole → L-tryptophan + H_2O

$\alpha\beta$ reaction: L-serine + indole-3-glycerol phosphate → L-tryptophan + D-glyceraldehyde 3-phosphate + H_2O

The crystal structure of the tryptophan synthase $\alpha_2\beta_2$ complex from *Salmonella typhimurium* at 2.5 Å resolution (Hyde et al., 1988) revealed that the active sites of the partner

‡ The coordinates of the structures have been deposited in the Brookhaven Protein Data Bank with names 1TTP for the Cs^+ complex, 1TTQ for the K^+ complex, and 1WSY for the Na^+ complex.

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[1] Abbreviations: PEG, poly(ethylene glycol); PLP, pyridoxal 5′-phosphate; rms, a root mean square; the Na^+ (or K^+ or Cs^+) complex, the crystal structure of tryptophan synthase in the presence of Na^+ (or K^+ or Cs^+, respectively).

α and β subunits are about 25 Å apart and are connected by a hydrophobic tunnel. This tunnel provides a plausible structural route for transfer of indole produced at the active site of the α subunit to the active site of the β subunit. Alterations in the geometry of the indole tunnel may be important in controlling passage of indole and in communication between the α and β subunits. Many studies have demonstrated ligand-dependent reciprocal communication between the α and β subunits and have provided evidence for the interconversion of the α and β subunits between "open" and "closed" conformations (Ahmed et al., 1991; Anderson et al., 1991; Brzović et al., 1991, 1992a,b, 1993; Dunn et al., 1991, 1994; Houben & Dunn, 1990; Kawasaki et al., 1987; Kirschner et al., 1991; Lane & Kirschner, 1981, 1983, 1991; Ruvinov et al., 1995b; Yang & Miles, 1993).

Recent investigations in this laboratory have identified a Na^+ binding site in the three-dimensional structure of the wild-type tryptophan synthase $\alpha_2\beta_2$ complex[2] and in a mutant (βK87T) $\alpha_2\beta_2$ complex.[3] We now report crystal structures of tryptophan synthase with bound K^+ and with bound Cs^+ at a resolution of 2.0 and 2.3 Å, respectively, and compare these structures with that of the Na^+ complex.[2] We find that exchange of K^+ or Cs^+ for Na^+ produces local changes near the cation site, affects the interaction site between the α and β subunits, and alters the conformation of the indole tunnel.

MATERIALS AND METHODS

Crystallization, Soaking, and Data Collection. The tryptophan synthase $\alpha_2\beta_2$ complex from *S. typhimurium* was crystallized in a solution containing 50 mM Bicine, 1 mM Na-EDTA, 0.8−1.5 mM spermine, and 12% PEG 8000 adjusted to pH 7.8 with NaOH (Ahmed et al., 1985). Monoclinic crystals grown in the presence of Na^+ ion (typically 0.7 × 0.3 × 0.2 mm) belong to a space group *C*2 with unit cell dimensions $a = 184.5$ Å, $b = 61.1$ Å, $c = 67.7$ Å, and $\beta = 94.7°$. A crystal grown in the presence of Na^+ was first washed several times in a small volume (∼100 μL) of the K^+ or Cs^+ soaking solution (below) and then transferred to a larger volume (1 mL) of the soaking solution for 1−3 days. The soaking solution for K^+ containing 100 mM Bicine, 1 mM EDTA (free acid form), and 15% PEG 8000 was adjusted with KOH to pH 7.8 to give a final concentration of ∼37 mM K^+. The soaking solution for Cs^+ containing 50 mM Bis-Tris propane, 1 mM EDTA (free acid form), 100 mM CsCl, and 15% PEG 8000 was adjusted with HCl to pH 7.8.

Diffraction data were collected at room temperature on a Raxis IIc imaging plate detector mounted on a Rigaku RU-200 rotating anode X-ray generator operating at 50 kV and 100 mA. The crystal to detector distance was 100−120 mm and exposures were 20−25 min per 1.2° oscillation. All diffraction data were integrated with DENZO and scaled with SCALEPACK (Otwinowski, 1993). Table 1 summarizes statistics of the collected data sets.

Structure Determination. Preliminary difference maps were calculated between diffraction data sets from crystals of K^+- or Cs^+-bound enzyme and of Na^+-bound enzyme using phases from the structure of tryptophan synthase,[2] which has been refined to a resolution of 1.9 Å in the presence of Na^+ (concentration of Na^+ is approximately 17 mM). These difference maps were noisy and had many positive and negative difference peaks all over the structure, suggesting that there were structural perturbations in tryptophan synthase as a result of replacing Na^+ with K^+ or Cs^+.

Table 1: Data Collection and Refinement Statistics

	K^+ complex	Cs^+ complex
data statistics		
resolution (Å)	2.0	2.3
no. of measured reflections	322940	179673
no. of unique reflections	39626	25385
R_{merge} (%)[a]	7.9	9.9
completeness (%)	77.8	74.2
	42.4 (2.07−2.00 Å)	43.2 (2.38−2.30 Å)
refinement statistics		
resolution range (Å)	8.0−2.0	8.0−2.3
no. of reflections ($>2\sigma$)	35908	22351
no. of protein atoms	4903	4903
no. of solvent		
water	182	87
cation	1 (K^+)	2 (Cs^+)
R-factor[b]	0.204	0.194
R_{free}	0.289	0.306
rms deviations from ideals		
bond length (Å)	0.014	0.015
bond angle (deg)	3.06	3.19

[a] $R_{merge} = \Sigma|I - \langle I\rangle|/\Sigma I$. [b] *R*-factor $= \Sigma||F_o| - |F_c||/\Sigma|F_o|$.

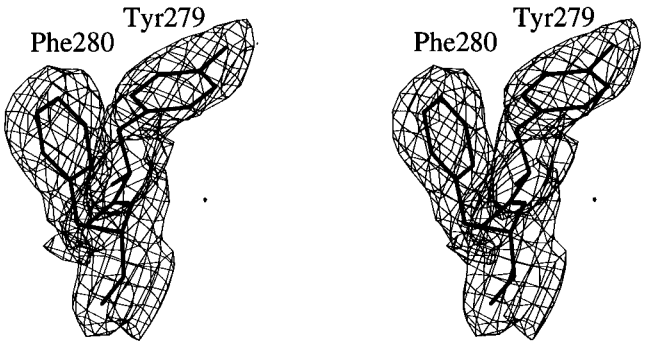


FIGURE 1: The final $2F_o - F_c$ map contoured at 1σ in a region of Tyr-279 and Phe-280 of the β subunit. The map was constructed using the diffraction data from a crystal of the K^+-bound enzyme.

The 1.9 Å resolution Na^+ complex was used as a starting model for further refinement using X-PLOR (Brünger, 1992) against the 2.0 Å data set for the K^+-bound enzyme: coordinates for Na^+ and water molecules were not included in the starting model. Since structural perturbations were observed in the K^+-bound enzyme, a starting model was first subjected to rigid-body refinement. Three substructures were allowed to move independently in a rigid-body motion relative to each other, the whole α subunit (residue numbers 1−268), and the N- and C-terminal domains of the β subunit (residue numbers 3−204 and 205−391, respectively). A simulated annealing (at initial temperature 3000 K) followed by energy minimization and isotropic temperature factor refinement resulted in the model with a crystallographic *R*-value of 0.221 and an R_{free} of 0.290. This refined structure was manually rebuilt using the program O (Jones et al., 1991). At this point, the side chains of Tyr-279 and Phe-280 in the β subunit of the refined model were clearly out of density in a $2F_o - F_c$ Fourier map, and both side chains were repositioned to fit the map (Figure 1). A simulated-annealing map with these residues omitted also indicated that current conformations of βTyr-279 and βPhe-280 are unbiased models. However, due to steric hindrance, fitting the

[2] C. C. Hyde, K. D. Parris, T. N. Bhat, C. Brown, S. A. Ahmed, E. W. Miles, and D. R. Davies, in preparation.

[3] K. D. Parris, C. C. Hyde, S. Rhee, S. A. Ahmed, E. W. Miles, and D. R. Davies, in preparation.

side chain conformation of βTyr-279 into a map also required repositioning of the side chain of Asp-56 in the α subunit, although this side chain is very mobile and has very weak electron density. After the model was rebuilt, water molecules were assigned in a $F_o - F_c$ Fourier map to the strong positive difference peaks with density $>3\sigma$, where σ is the rms deviation in a $F_o - F_c$ Fourier map. At this stage, it was noted that the strongest positive difference peak in a $F_o - F_c$ Fourier map (more than 6σ) was at almost the same position as the Na^+ binding site. Instead of assigning the K^+ ion site, we left this peak as a water molecule and carried out further refinement. Another round of a simulated annealing (at initial temperature 500 K) and energy minimization gave more solid evidence that this strong peak corresponds to a K^+ binding site, because the refined *B*-factor (about 10 Å^2) for this peak is much lower than those (about 26 Å^2) of neighboring atoms within 3.0 Å. The K^+ ion was then included in the model, and in order to estimate accurate distances between K^+ ion and ligand atoms, another round of simulated annealing (at initial temperature 500 K) and energy minimization was performed with metal−ligand distances unrestrained.

The difference map between the data sets of the Cs^+ and K^+ crystals with phases calculated from the refined K^+ complex described above was much clearer. It shows two very strong positive peaks ($>9\sigma$) for Cs^+ ions: one corresponds to the position of K^+ in the refined K^+ complex and the other is positioned at the $\beta-\beta$ subunit interface that is generated by crystallographic symmetry. The 2.0 Å resolution K^+ complex, excluding K^+ and water molecules, was used as a starting model for further refinement against the 2.3 Å data set of the Cs^+-bound enzyme. The same strategy to refine the K^+ complex as described above was also applied to determine the Cs^+ complex and the distances between Cs^+ and its ligand atoms. Table 1 contains information about the refinement and the quality of the refined final model.

Structure Comparisons. The noisy difference maps between the Na^+ complex and the K^+ or Cs^+ complex suggested that structural changes accompany the replacement of Na^+ by K^+ or Cs^+. We therefore compared the three structures as described below and tried to identify any cation-induced structural changes.

When superpositions were carried out using the main chain atoms between the corresponding subunit of the K^+ or Cs^+ complex and the Na^+ complex, no significant changes were observed in the β subunit (rotation angle of $\sim$0.1°). However, relatively larger movements were noticed in the α subunit (rotation angle of $\sim$1.0°). In order to detect small structural changes in the β subunit, a superposition method developed by Lesk (1991) and Gerstein and Chothia (1991) was used, where iterative superpositions yield a stationary part of the molecule having small rms deviations. This nonmoving part serves as a core for further superpositions. The structural changes in each residue were judged by calculating the rms deviations of the main chain atoms after a superposition. Since the Luzzati plot indicated a coordinate error about 0.30, 0.30, and 0.35 Å for the Na^+, K^+, and Cs^+ complexes, respectively, only those residues with less than 0.4 Å of rms deviation in their main chain atoms were included as core residues for superpositions. The resulting core contained 1360 out of 1556 starting main chain atoms for superposition of the β subunit of the Na^+ and K^+ complexes and 1308 main chain atoms between the β subunits of the Na^+ and Cs^+ complexes.

RESULTS

Monovalent Cation Binding Sites. Cs^+ and K^+ bind to a site in the C-terminal domain of the β subunit (site 1; Figure 2b,c, and Table 2) which had been previously identified as the binding site for Na^+ (Figure 2a,e) in the wild-type $\alpha_2\beta_2$ complex[2] and in the βK87T $\alpha_2\beta_2$ complex.[3] Each site is in approximately the same place and involves mostly the same ligands although the actual site positions differ by 0.5 and 1.1 Å for K^+ and Cs^+, respectively. Each cation is stabilized by interaction with the carbonyl oxygens of three to six enzyme residues and with zero to two water molecules (Table 2). The interacting enzyme residues are located in a long loop (residues 259−310) that connects strand 8 and helix 10 and in a short loop (residues 231−234) between strand 7 and helix 9 (Hyde et al., 1988) (Figure 3). One of these interacting residues, βGly-232, also forms a hydrogen bond with the phosphate group of the coenzyme PLP. The cation binding site is about 8 Å distant from the phosphate of PLP.

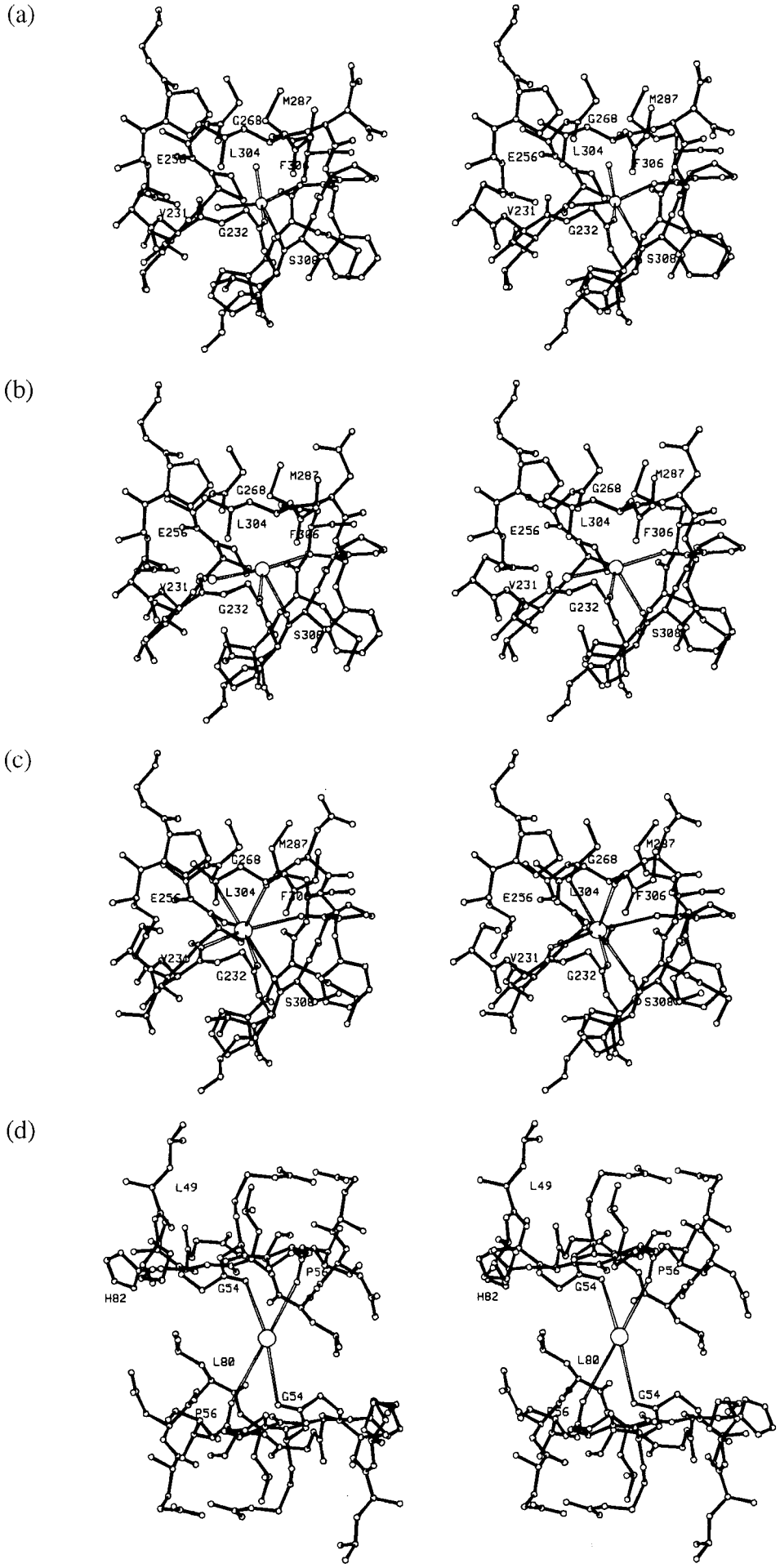
The K^+ binding site (Figure 2b,f, and Table 2) has a geometry which is very similar to that of the Na^+ binding site, except that only one well-defined water molecule (*B*-factor with $\sim$15 Å^2) is in the coordination shell instead of two water molecules (*B*-factor with $\sim$22 Å^2) found in the Na^+ binding site. The main chain carbonyl oxygens of βGly-232, βPhe-306, and βSer-308 and one water molecule form the coordination shell of K^+.

The Cs^+ binding site (Figure 2c,g, and Table 2) forms an octahedral coordination shell and has interactions with the main chain carbonyl oxygens of βGly-232, βPhe-306, βSer-308, βVal-231, βGly-268, and βLeu-304 and with no water molecules. The carbonyl oxygen of βLeu-304 forms a longer interaction ($\sim$4.0 Å) with Cs^+ than any of the other interactions which range from 2.9 to 3.4 Å. The average distance between the center of cation density and ligand atoms is 2.23 Å in the Na^+ complex, 2.66 Å in the K^+ complex, and 3.4 Å in the Cs^+ complex. These values agree well with the minimum metal−oxygen distances measured in crystal structures of small molecules: 2.25 Å for Na^+ and 2.46 Å for K^+ (Glusker, 1991).

We observe a second binding site for Cs^+ (site 2) which is located on the molecular 2-fold axis, within the $\beta-\beta$ interface, and is probably the result of the larger ionic radius of Cs^+ ($\sim$1.69 Å) compared with Na^+ ($\sim$0.95 Å) and K^+ ($\sim$1.33 Å). A total of four carbonyl oxygens, two from βGly-54 and βPro-56 and two from their symmetry-related equivalents on the other subunit, assume an approximately tetrahedral geometry with an average O−Cs distance of 3.42 Å (Figure 2d,h).

Structural Changes in the α Subunit. The substitution of K^+ for Na^+ resulted in a small rotation ($\sim$1°) of the α subunit relative to the β subunit. In order to ensure that this small change was not due to a misinterpretation of the data, the coordinates of the fully refined K^+ complex were refined against the diffraction data of the Na^+ complex using X-PLOR. This "back"-refined Na^+ complex was indistinguishable from the original 1.9 Å resolution Na^+ complex, indicating that the small movement of the α subunit in both the K^+ and Cs^+ complexes is reproducible and meaningful.

A structural comparison indicates that the α subunit active site loop containing Asp-60 was displaced more (greater than 1.0 Å rms displacement for the main chain atoms) than the rest of the α subunit (Figure 4A). In addition, a comparison of the refined *B*-factors between the K^+ or Cs^+ complex and


(a)
M287
G268
L304
E256
F306
V231
G232
S308
(b)
M287
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F306
V231
G232
S308
(c)
M287
G268
L304
E256
F306
V231
G232
S308
(d)
L49
P56
G54
H82
L80
G54
P56

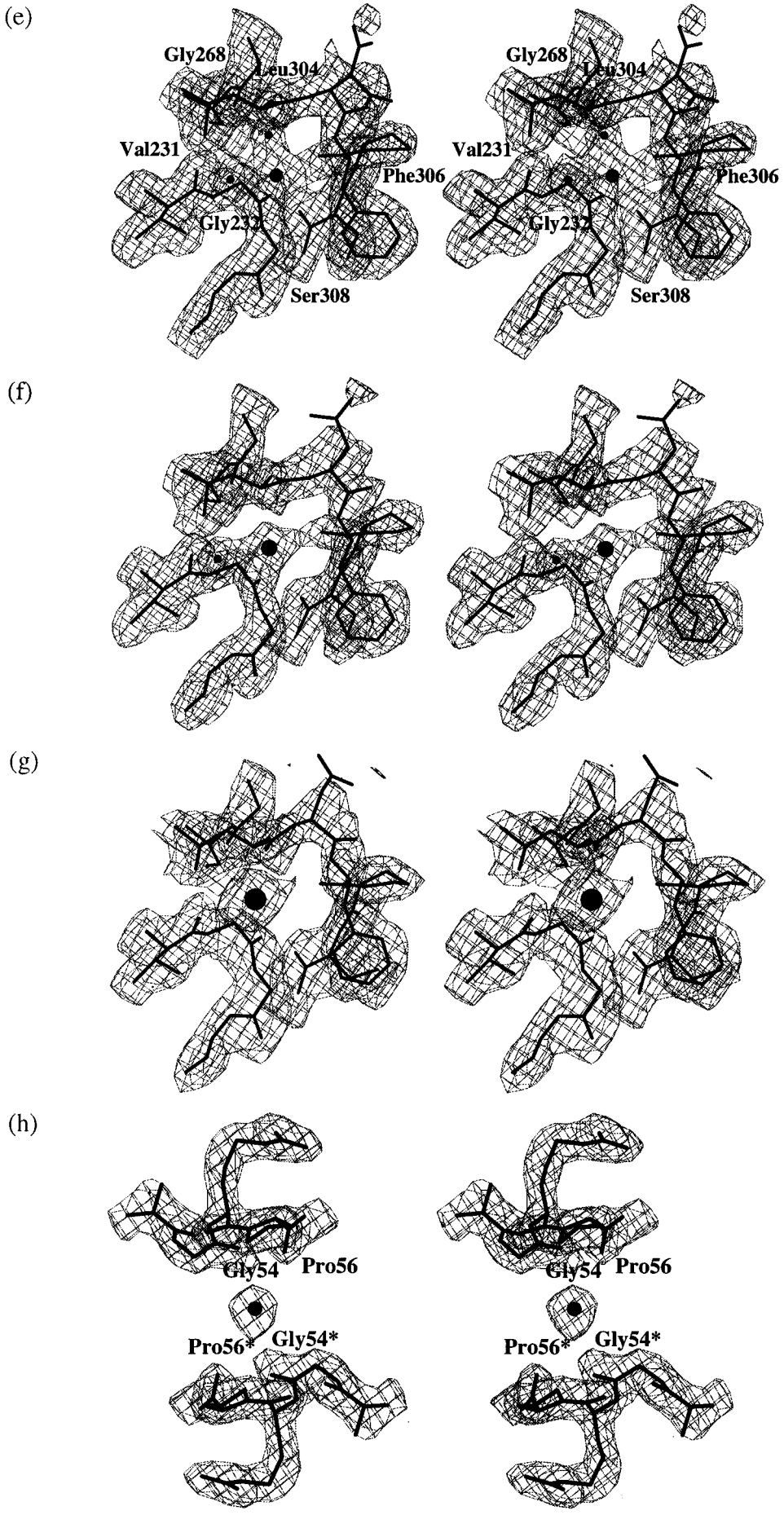

FIGURE 2: Stereoscopic diagrams and electron density of the metal binding site in the β subunit. The binding site (site 1) is shown for (a) Na^+, (b) K^+, and (c) Cs^+. The metal ions and water molecules are represented by large and small circles, respectively. The interacting enzyme residues are labeled. The distances between the metal ions and ligands are listed in Table 2. Site 2 of Cs^+ in the β/β interface is displayed in (d). A final $2F_o - F_c$ map at 1σ is shown for (e) Na^+, (f) K^+, and (g) Cs^+ for site 1 and (h) Cs^+ for site 2.

Table 2: Effects of Monovalent Cations on the Structure of Tryptophan Synthase

	Na^+ complex	K^+ complex	Cs^+ complex
cation binding site			
cation	Na^+	K^+	Cs^+
ionic radius (Å)	0.95	1.33	1.69
coordination no.	5	4	6
ligands[a]			
			βVal-231 (3.4)
	βGly-232(2.3)	βGly-232(2.2)	βGly-232(2.9)
			βGly-268(3.4)
			βLeu-304(4.0)
	βPhe-306(2.3)	βPhe-306(2.7)	βPhe-306(3.3)
	βSer-308(2.4)	βSer-308(3.1)	βSer-308(3.4)
	H_2O (2.0)	H_2O (2.7)	
	H_2O (2.3)		
structural changes			
changes in α subunit		1° rotation	1° rotation
α loop-2		more flexible	more flexible
possible ionic interaction			
αAsp-56		βLys-167	βLys-167
βAsp-305	βLys-167		
side chain movement of tunnel-forming residues			
βTyr-279		tunnel wall	tunnel wall
βPhe-280	in tunnel	tunnel wall	tunnel wall

[a] Ligands are the carbonyl oxygens or water, and the distance between metal ion and ligands is given in parentheses (Å).

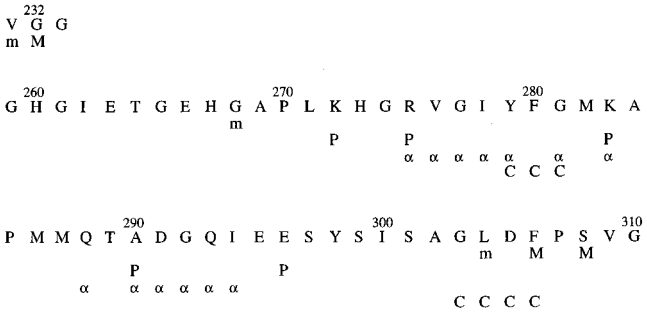

FIGURE 3: Regions in the β subunit (residues 231−233 and residues 259−310) that contain residues that bind Na^+, K^+, or Cs^+ (M) or Cs^+ alone (m) are susceptible to limited proteolysis (P), interact with the α subunit (α), or line the channel wall (C). The assignment of α and C is taken from Figure 3 in Zhao and Somerville (1992) and C. Hyde (personal communication).

the Na^+ complex (Figure 5) shows that residues Ser-55 to Gln-80 in the α subunit, which include the active site loop-2 and many of the α−β interface residues, are significantly more flexible in the K^+ and Cs^+ complexes, whereas the *B*-factors for the β subunit residues do not change significantly. Consequently, there is no electron density for the side chain of αAsp-56 in the K^+ and Cs^+ complexes (see next section for details). These differences in *B*-factors might have resulted from the different resolutions of the complexes (1.9, 2.0, and 2.3 Å for the Na^+, K^+, and Cs^+ complex, respectively), but the almost identical *B*-factors between the K^+ and Cs^+ complexes suggest that they represent intrinsic properties of the enzyme with different cations.

Structural Changes in the β Subunit. Replacement of Na^+ with K^+ or Cs^+ slightly displaces the cation binding loop residues in order to accommodate binding of the larger cations. Table 3 shows the changes in position of the liganding residues and their neighbors; the largest movements occur for βPhe-306 and βAsp-305.

βAsp-305, a residue that does not interact directly with the cation, exhibits positional shifts for the main chain atoms similar to those of βPhe-306 (0.40 Å in the K^+ complex

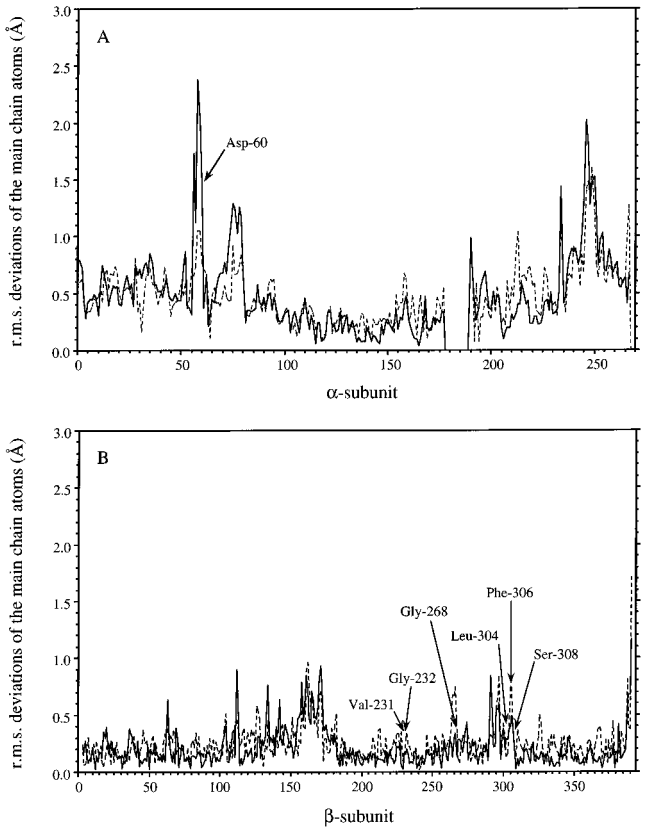

FIGURE 4: Positional differences in the main chain atoms of the α and β subunits. Positional differences were calculated from rms deviations of the main chain atoms for corresponding residues after superposition of the core residues between the K^+ or Cs^+ complex and the Na^+ complex. The solid line represents differences of the Na^+ complex versus the K^+ complex and the dashed line differences of the Na^+ complex versus the Cs^+ complex. Asp-60, an active site residue in the α subunit, and the metal ion interacting residues in the β subunit are indicated. (A) α subunit; (B) β subunit.

and 0.75 Å in the Cs^+ complex). In the crystal structure of the Na^+ complex, two distinct side chain conformations of βAsp-305 have been modeled to fit the electron density.[2] In one conformation, termed the "swing-in position", the carboxylate of βAsp-305 is oriented toward the active site of the β subunit, whereas in the other conformation, the "swing-out position", the carboxylate is oriented away from the active site. The negative charge of βAsp-305 in the swing-in position is neutralized by an ionic interaction with the ϵ-amino group of βLys-167, which is 2.8 Å away (Figures 6a and 7a). In the swing-out orientation βAsp-305 can no longer interact with βLys-167 (the distance between the opposing charged atoms is more than 7 Å) but instead makes hydrogen bonds to other groups. In the crystals of the Na^+ complex the swing-in position is favored, although the electron density is indicative of some disorder.

In the K^+ and Cs^+ complexes (Figures 6b and 7b) the structures differ from that observed for the Na^+ complex. First, the side chain of βAsp-305 becomes even more disordered, and although there is some very weak electron density corresponding to the swing-out position, neither position can be directly modeled from the density. Second, the side chain of the α−β interface residue, βLys-167, now occupies a different position. There is clear electron density in both the K^+ and Cs^+ crystals for the side chain of βLys-167 that now points in the direction of an α subunit residue, probably to the side chain carboxylic group of αAsp-56, a nearby negatively charged residue. Although electron den-

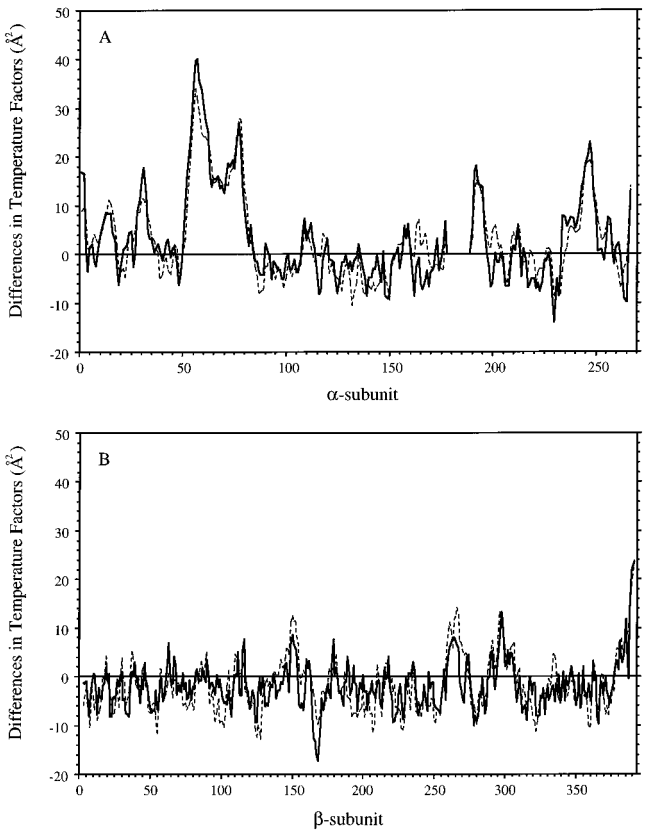

FIGURE 5: Plots showing differences in *B*-factors between the K^+ or Cs^+ complex and the Na^+ complex. The differences are obtained from subtracting *B*-factors of the Na^+ complex from those of the K^+ or Cs^+ complex, and are represented with a solid or dashed line, respectively. (A) α subunit; (B) β subunit.

Table 3: Positional Changes in the Main Chain Atoms of the Liganding Residues and Their Neighbors by Replacing Na^+ with K^+ or Cs^+

	rms deviations (Å)	
residues	K^+ complex	Cs^+ complex
βVal-231	0.16	0.28
βGly-232	0.20	0.33
βLeu-304	0.36	0.33
βGly-268	0.28	0.12
βAsp-305	0.40	0.75
βPhe-306	0.49	0.74
βSer-308	0.24	0.25

sity for the side chain of αAsp-56 in these crystals is absent, in other structures such as the βK87T mutant with bound ligand there is clear observation of this ionic interaction with similar density for βLys-167 and clear density for the side chain of αAsp-56.[3]

Unlike the small and disordered structural perturbations of some of the residues described above, the displacement of the side chains of βTyr-279 and βPhe-280 was large and unambiguous in the presence of K^+ and Cs^+. Those movements are accompanied by changes in the side chain dihedral angle (χ1) by 70° for βTyr-279 and 100° for βPhe-280 (Figures 6 and 7). The movements of the side chains of βTyr-279 and βPhe-280 in the presence of K^+ and Cs^+ require the concurrent movement of αAsp-56 side chain (Figures 6 and 7).

DISCUSSION

In the present paper and elsewhere[2,3] we have demonstrated that K^+, Cs^+, and Na^+ bind at the same general position (site 1) in the β subunit. This is the first monovalent cation site to be determined for a PLP-dependent enzyme in the β family (Alexander et al., 1994) or Fold type II (Grishin et al., 1995). Since the tryptophan synthase β subunit is the only member of this family that has been solved crystallographically (PDB entry 1WSY), the β subunit and its monovalent cation site serve as prototypes for other members of this family (cysteine synthase, cystathionine β synthase, L-serine and D-serine dehydratases, threonine dehydratase, threonine synthase, 1-aminocyclopropane-1-carboxylate deaminase, and alliin lyase) that exhibit similarities in sequence, secondary structure, hydrophobicity profiles, and reaction specificity (Alexander et al., 1994; Grishin et al., 1995) although the binding specificities of these enzymes for monovalent cations are not known. However, it has been shown that K^+ ions can specifically lower the apparent K_d value of L-serine dehydratase (Pestaña, 1971) and of D-serine dehydratase (Federiuk & Shafer, 1981) for pyridoxal phosphate. Studies of the effects of K^+ and of Na^+ on the ^{31}P NMR spectrum of the pyridoxal phosphate cofactor of D-serine dehydratase have suggested that these two cations stabilize conformational states that differ with respect to O−P−O bond angle, conformation, and/or hydrogen bonding of the phosphate group of the enzyme-bound cofactor (Kojiro et al., 1989). Thus, other members of the β family may also have cation binding sites.

Cation Binding Site. Na^+, K^+, and Cs^+ bind to a site in the β subunit (site 1) about 8 Å from the phosphate of PLP and too distant from the active site to play a direct role in catalysis. The cation site involves residues from two different regions of the same subunit (Figures 2 and 3). One of the regions (β subunit residues 259−310) folds in a complicated way and lacks well-defined secondary structural elements (Hyde et al., 1988). Residues in this region make several contacts with the α subunit and contribute to the wall that lines the indole tunnel. This region has been termed a "hinge region" since it contains five sites that in the separate β subunit are susceptible to proteolysis by trypsin (Lys-272, Arg-275, and Lys-283) (Ahmed et al., 1986; Högberg-Raibaud & Goldberg, 1977) or by endopeptidase Glu-C (Glu-290 and Glu-296) (Friguet et al., 1989; Kaufmann et al., 1991; Linkens et al., 1993, 1994). We speculate that the bound cation may stabilize the structure of the β subunit by bridging these two regions of the molecule.

Comparison with Other PLP Enzymes. The monovalent cation binding sites in three members of the α family of PLP enzymes [dialkylglycine decarboxylase (Hohenester et al., 1994; Toney et al., 1993, 1995), tryptophanase (Isupov et al., 1994), and tyrosine phenol-lyase (Antson et al., 1994)] are also distinctly separate from the active site but occur in different protein folds and have different ligands in the coordination spheres. The cation site in these enzymes occurs in the interaction site between two different subunits and may be important for subunit interaction and for PLP binding at the subunit interface.

Monovalent Cation Selectivity. Peracchi et al. have reported that the tryptophan synthase $\alpha_2\beta_2$ complex exhibits low selectivity for cations and suggest that this low selectivity may be a consequence of the coordination with the oxygen of carbonyl groups and waters reported herein (Peracchi et al., 1995). The authors quote Eisenman as suggesting that cation binding sites containing water are not very selective and that sites with few dipole interactions between the cation and the protein might allow ions of different size to bind

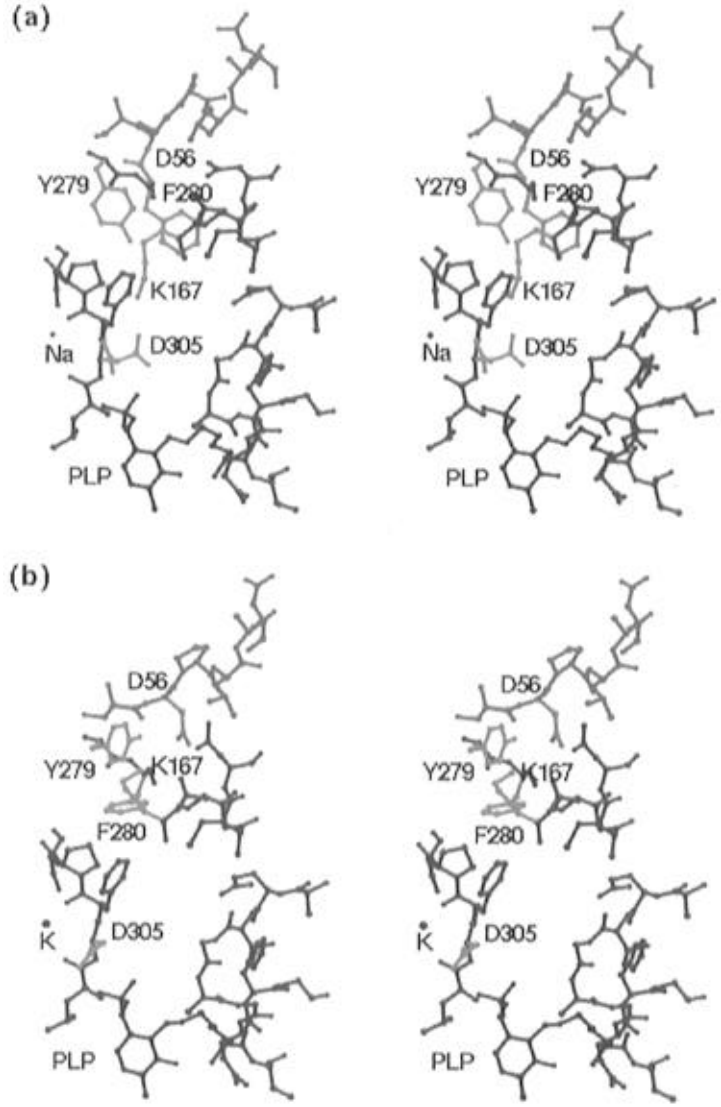

FIGURE 6: Model illustrating the locations of amino acid residues that change conformation, showing their relation to the $\alpha\beta$ interface, the channeling tunnel, and the pyridoxal phosphate for (a) the Na^+ complex and (b) the K^+ complex. Color codes are as follows: red for α subunit residues 55−60; green for the side chain of αD56; blue for β subunit residues 87−88, 108−116, 167−171, 279−280, and 304−308; magenta for the side chains of β subunit residues K167, Y279, F280, and D305; black for pyridoxal phosphate (internal aldimine) and bound cation. Note that the side chain of βD305 is modeled as two alternative conformations in (a).

without large conformational changes (Eisenman & Dani, 1987). In contrast, dialkylglycine decarboxylase (Hohenester et al., 1994; Toney et al., 1993, 1995), tryptophanase (Isupov et al., 1994), and tyrosine phenol-lyase (Antson et al., 1994) exhibit more selective cation binding than tryptophan synthase. For example, dialkylglycine decarboxylase requires a monovalent cation with an ionic radius of 1.3−1.5 Å (K^+, Rb^+, or NH_4^+) for full catalytic activity (Hohenester et al., 1994). Only weak activation is observed with the smaller Na^+ and the larger Cs^+. The cation binding sites in these enzymes are formed by more strongly interacting residues including the carboxylate of an Asp or Glu and one serine hydroxyl (Woehl & Dunn, 1995b).

Structural Changes at the Cation Binding Site. Exchange of K^+ or Cs^+ for Na^+ results in some displacement of the contacting side chains from the cation site in order to accommodate the larger cation. Accompanying these movements is a significant increase in the disorder of the side chain of βAsp-305 and a displacement of βLys-167. The side chain of βLys-167 is well-defined and now appears to point in the direction of αAsp-56; because αAsp-56 is disordered, the details of the interaction cannot be determined. However, it is interesting to note the striking change in the position of βLys-167 in which the lysine side chain swings from pointing in the general direction of the β subunit active site to a position in which it points toward the $\alpha-\beta$ interface (Figures 6 and 7 and Table 2).

Mutagenesis studies indicate that αAsp-56 (Lim et al., 1991) and βLys-167 (Yang & Miles, 1993) play roles in intersubunit communication. The mutant D56G shows normal α subunit activity but very low activity in the β and the $\alpha\beta$ reactions. The mutant K167T shows a 25-fold reduction in the rate of the β reaction which can be restored by the presence of α subunit ligands and also notably by high concentrations of potassium phosphate, possibly by stabilizing a more active conformation of the mutant β subunit.

The studies of the wild-type enzyme reported here showing structural changes in residues αAsp-56, βLys-167, and βAsp-305 produced by monovalent cations provide a partial basis for understanding the effects of cations on the activity of PLP-dependent reactions of tryptophan synthase and on intersubunit communication (Crawford & Ito, 1964; Dunn et al., 1994; Goldberg et al., 1968; Hatanaka et al., 1962;

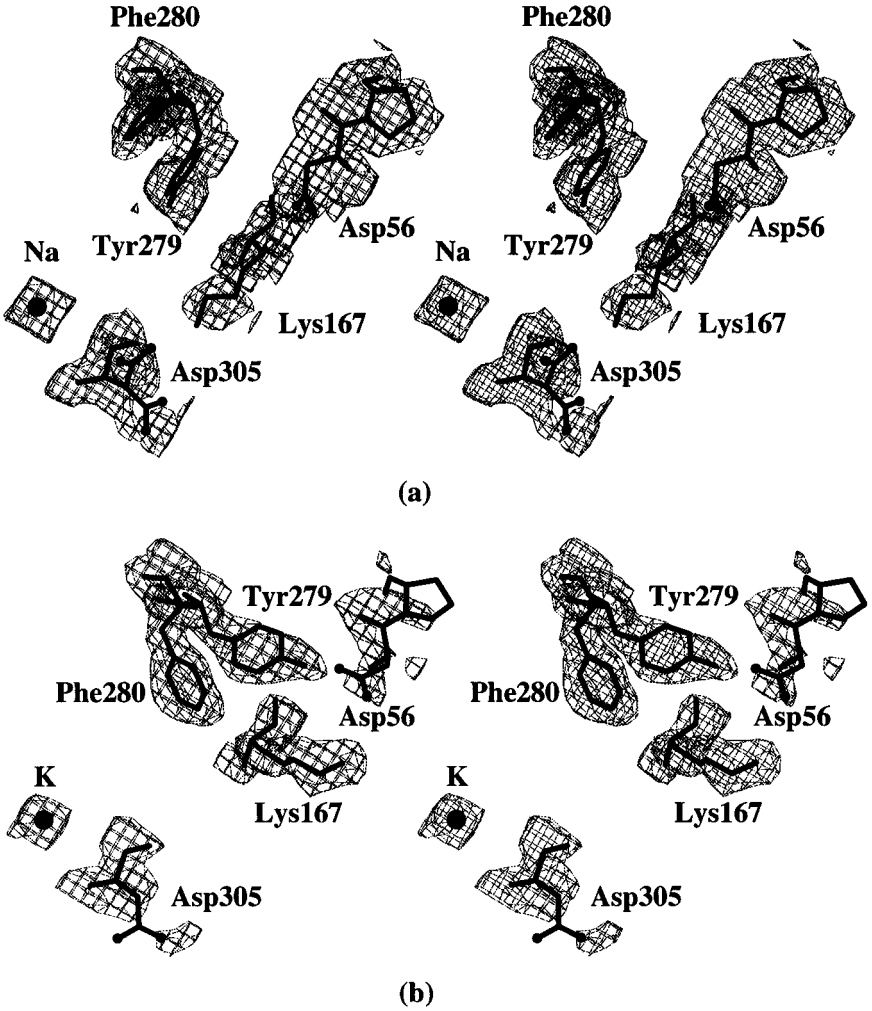

FIGURE 7: $2F_o - F_c$ maps of (a) the Na^+ complex and (b) the K^+ complex, illustrating the major changes in density corresponding to movements in position of αAsp-56, βLys-167, βTyr-279, βPhe-280, and βAsp-305. The models correspond to our interpretations of the side chain movements of these residues. The carboxy oxygens in the side chain of αAsp-56 and βAsp-305 are represented with filled small circles. Note that we cannot be certain of the position of αAsp-56 in the K^+ complex because the density is extremely weak. The side chain of βAsp-305 is modeled as two alternative conformations in (a).

Peracchi et al., 1994, 1995; Ruvinov et al., 1995a; Schwartz & Bonner, 1964; Woehl & Dunn, 1995a,b; York, 1972). On the whole we see only small changes in the β active site. However, we do observe changes in the density of βAsp-305, a residue which interacts with the hydroxyl of serine in the external aldimine of the βK87T mutant structure.[3]

Effects of Monovalent Cations on the Indole Tunnel. In the course of the 1.9 Å refinement of the crystal structure of the $\alpha_2\beta_2$ complex in the presence of Na^+, it has been noticed that the side chains of βPhe-280 and αLeu-58 are located within the tunnel that connects the α and β sites.[2] These side chains have low electron density and high *B*-factors, suggesting either that they are mobile or that there may be static disorder involving two or more positions. In the refined K^+ and Cs^+ structures, the side chain of βPhe-280 moves out of the tunnel and is clearly located within the surface of the tunnel where it replaces the side chain of βTyr-279 which in turn moves toward the α subunit and interacts with part of the flexible loop-2 residues (54−61) of the α subunit (Figures 6b and 7b).

Our results lead us to speculate that the following chain of events accompanies the exchange of K^+ or Cs^+ for Na^+. Binding of the larger cation near the side chain of βAsp-305 will perturb the interaction between the side chains of βAsp-305 and βLys-167 which in turn allows βLys-167 to interact with Asp-56 in the α subunit loop-2. This interaction may then allow the side chains of βTyr-279 and βPhe-280 to orient toward the α subunit and out of the tunnel. The interaction with αAsp-56 in the K^+ and Cs^+ structures could cause the observed structural perturbations of the flexible active site loop-2 in the α subunit and the small rotation of the α subunit relative to the β subunit.

Comparison of Crystallographic and Solution Studies. Solution studies of the tryptophan synthase $\alpha_2\beta_2$ complex have determined the effects of different monovalent cations on the equilibrium distribution of enzyme−substrate intermediates, on the rates of individual steps in the reaction of L-serine, and on steady-state kinetic constants (Peracchi et al., 1995; Ruvinov et al., 1995a; Woehl & Dunn, 1995a,b). Because the binding of Na^+ gives significantly more of the external aldimine of L-serine and less of the external aldimine of aminoacrylate than does K^+, Na^+ appears to be more effective than K^+ in stabilizing the conformation of the L-serine intermediate (Peracchi et al., 1995; Woehl & Dunn, 1995a,b). Our crystallographic results show that Na^+ and K^+ stabilize different conformational states of the internal aldimine and are thus consistent with the solution studies which show that Na^+ and K^+ stabilize different conformational states of the external aldimines. Microspectrophotometric studies of single crystals of the $\alpha_2\beta_2$ complex in the

presence of L-serine show that monovalent cations also affect the equilibrium distribution of enzyme−substrate intermediates in the crystalline state (Peracchi et al., 1995). Thus studies of the enzyme in both crystalline state and in solution indicate that cations play a structural role, stabilizing alternative enzyme conformations and enzyme−substrate intermediates.

Early studies showed that some monovalent cations (K^+, Li^+, and NH_4^+, but not Na^+) activate the β_2 subunit (Crawford & Ito, 1964; Hatanaka et al., 1962). Studies of the effects of Cs^+ on the β_2 subunit and a mutant $\alpha_2\beta_2$ complex (βE109A) also support the hypothesis that Cs^+ stabilizes an alternative conformation of the β_2 subunit and the mutant $\alpha_2\beta_2$ complex which is more similar to that of the wild-type $\alpha_2\beta_2$ complex (Ruvinov et al., 1995a).

The crystallographic results which show that Na^+ and K^+ affect residues in the interaction site between the α and β subunits also support the solution studies which suggest that monovalent cations play a role in allosteric linkage between the α and β subunits (Woehl & Dunn, 1995a,b).

CONCLUSION

The results presented here complement the spectroscopic and kinetic data of Peracchi et al. (1995) and Woehl and Dunn (1995a) and provide a structural basis for understanding the role of cations in regulating the activity and intersite communication of tryptophan synthase. A surprising result from this investigation is the opening of the tunnel connecting the α and β active sites by the movement of βPhe-280 out of the tunnel in the presence of K^+ and Cs^+. Although we cannot directly use this effect to explain the spectroscopic results, they provide a basis for the design of further experiments to relate the effect of cations with the other allosteric properties of this bifunctional enzyme system.

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