Biochemical Properties of Mutant and Wild-type Fructose-1,6-bisphosphatases Are Consistent with the Coupling of Intra- and Intersubunit Conformational Changes in the T- and R-state Transition*

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The significance of interactions between AMP domains in recombinant porcine fructose-1,6-bisphosphatase (FBPase) is explored by site-directed mutagenesis and kinetic characterization of homogeneous preparations of mutant enzymes. Mutations of Lys42, Ile190, and Gly191 do not perturb the circular dichroism spectra, but have significant effects on ligand binding and mechanisms of cooperativity. The $K_m$ for fructose 1,6-bisphosphate and the $K_i$ for the competitive inhibitor, fructose 2,6-bisphosphate, decreased by as much as 4- and 8-fold, respectively, in the Q32L, K42E, K42T, I190T, and G191A mutants relative to the wild-type enzyme. Q32L, unlike the other four mutants, exhibited a 1.7-fold increase in $K_m$. Mg$^{2+}$ binding is sigmoidal for the five mutants as well as for the wild-type enzyme, but the Mg$^{2+}$ affinities were decreased (3-22-fold) in mutant FBPases. With the exception of Q32L (8-fold increase), the 50% inhibiting concentrations of AMP for K42E, K42T, I190T, and G191A were increased over 2,000-fold (>10 mM) relative to the wild-type enzyme. Most importantly, a loss of AMP cooperativity was found with K42E, K42T, I190T, and G191A. In addition, the mechanism of AMP inhibition with respect to Mg$^{2+}$ was changed from competitive to noncompetitive for K42T, I190T, and G191A FBPases. Structural modeling and kinetic studies suggest that Lys42, Ile190, and Gly191 are located at the pivot point of intersubunit conformational changes that energetically couple the Mg$^{2+}$-binding site to the AMP domain of FBPase.

Fructose-1,6-bisphosphatase (d-fructose-1,6-bisphosphate-1-phosphohydrolase, EC 3.1.3.11, FBPase)$^1$ is a key regulatory enzyme in gluconeogenesis. It catalyzes the hydrolysis of fructose 1,6-bisphosphate (Fru-1,6-P$_2$) to fructose 6-phosphate and inorganic phosphate in the presence of required divalent metal ions (1, 2). The enzyme is inhibited synergistically by fructose 2,6-bisphosphate (Fru-2,6-P$_2$) and AMP (3–5). Fru-2,6-P$_2$ inhibits FBPase activity by binding directly to the active site of the enzyme, whereas AMP binds at an allosteric site and exhibits noncompetitive inhibition with respect to the substrate.

FBPase is a tetrameric enzyme consisting of identical subunits of molecular weight 37,000 (6). The activity of FBPase, as a function of Mg$^{2+}$ concentration, is sigmoidal at neutral pH with a Hill coefficient of approximately 2 (7). X-ray diffraction studies showed that there are two divalent metal-binding sites per monomer; however, in the case of Mg$^{2+}$, only one metal ion binds per subunit (8). AMP inhibition of FBPase is cooperative with a Hill coefficient of 2 (9–11). The structure of porcine kidney FBPase, however, reveals one AMP-binding site per subunit (12). The structural basis for the observed cooperativity in AMP and Mg$^{2+}$ interactions is still unclear. AMP and Mg$^{2+}$ are mutually exclusive in their binding to FBPase (13–16); AMP inhibition with respect to Mg$^{2+}$ is competitive at neutral and alkaline pH (13, 17). Crystallographic studies have shown that AMP interactions perturb metal-binding sites (18, 19).

FBPase can exist in at least two forms: (i) the R-state, in which the enzyme is complexed with substrate or product with or without metal ions and (ii) the T-state, in which FBPase is complexed with AMP in the presence or absence of metal ions (12, 20). The structural transition (R- to T-state) involves a 17° rotation of the C1-C2 dimer with respect to the C3-C4 dimer and a 1.9° rotation of the AMP domain (residues 1–200) relative to the FBP domain (residues 200–335) (18). The N-terminal residues, helices H1, H2, and H3, and $\beta$-turn T4, which comprise part of the C1-C4 and symmetry-related C2-C3 interfaces, putatively are involved in the propagation of structural changes throughout the tetramer during the R- to T-state transition (18). Mutation of residues in helices H1 and H3 changed the affinity of AMP or Mg$^{2+}$ (21, 22). Here, we present the effects of mutation of Gly191 (helix H2) and Ile190 (23, 24) and Gly191 (25, 26). The mutations cause a dramatic alteration in Mg$^{2+}$ and AMP affinities. In addition, for some of the mutants, the mechanism of AMP inhibition relative to Mg$^{2+}$ changes from competitive to noncompetitive.

EXPERIMENTAL PROCEDURES

Materials—Fru-1,6-P$_2$, Fru-2,6-P$_2$, NADP, MgCl$_2$, AMP, and IPTG were purchased from Sigma. DNA-modifying and restriction enzymes were from Promega and Clontech Laboratories, Inc. Glucose-6-phosphate dehydrogenase and phosphoglucose isomerase were obtained from Boehringer Mannheim. Other chemicals were of reagent grade or equivalent.

Mutagenesis of FBPase—Mutations were obtained by the introduction of specific base changes into a double-stranded plasmid (23). Five mutagenic primers, 5’-GAGATGACCCAGTCTGCTCAAC-3’, 5’-ACCGCGGTAGCCGCATTCT-3’, 5’-CGCAGGCTCAAGGCGCATCCTC-3’, 5’-ACCCGGCCAGCGAGAGTT-3’, and 5’-CGCAGGCTACGAGAGTTCAT-3’ were used to mutate Q32L, K42E, K42T, I190T, and G191A. The mismatched bases are in boldface. A selective primer, 5’-CAGCTCGCCCTCGGAACAGCCA-3’, which exchanged the original NruI site for a unique XhoI site on the pET-11a vector, was used. The double-stranded

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**TABLE I**

| Enzyme    | Specific activity | pH 7.5/9.5 | $K_{cat}$ | Fru-1,6-P$_2$, $K_m$ | Fru-2,6-P$_2$, $K_i$ | $I_{0.5}$ (AMP) | $Mg^{2+}$ activity |
|-----------|------------------|------------|-----------|---------------------|----------------------|-----------------|------------------|
| Wild-type | 30.9 ± 2.80      | 3.0        | 18.0 ± 1.70 | 3.51 ± 0.24         | 0.24 ± 0.02          | 4.52 ± 1.26     | 0.49 ± 0.22      |
| Q32L      | 52.7 ± 1.63      | 3.2        | 32.1 ± 0.99 | 1.42 ± 0.20         | 0.32 ± 0.02          | 37.9 ± 6.99     | 1.43 ± 0.07      |
| K42E      | 31.6 ± 3.13      | 2.0        | 19.3 ± 1.90 | 1.67 ± 0.16         | 0.07 ± 0.00          | 17.7 ± 3.35     | 1.70 ± 0.13      |
| K42T      | 30.0 ± 1.20      | 2.0        | 18.3 ± 0.73 | 1.53 ± 0.23         | 0.08 ± 0.00          | 12.1 ± 2.23     | 1.91 ± 0.11      |
| I190T     | 34.6 ± 0.90      | 2.0        | 21.1 ± 0.55 | 1.81 ± 0.19         | 0.03 ± 0.00          | 10.0 ± 1.16     | 4.37 ± 0.20      |
| G191A     | 38.9 ± 3.26      | 2.3        | 23.7 ± 1.98 | 0.84 ± 0.07         | 0.03 ± 0.00          | 14.7 ± 3.73     | 10.7 ± 0.89      |

The half-inhibiting concentration of AMP ($I_{0.5}$) was obtained from plots of $1/velocity$ versus $1/Fru-1,6-P_2$ at 5–12 mM Mg$^{2+}$. The $I_{0.5}$ of AMP for the wild-type and Q32L mutant are in μM; all others are in mM.

The Hill coefficient for AMP inhibition is 1.0.

The p values represent a comparison with the wild-type enzyme.

FBPase expression plasmid (pET-FBP) (24) and mutagenic and selective primers were denatured, annealed, and polymerized as described by Deng and Nickoloff (23).

Mutations were confirmed by NruI/Xhol digestion and by fluorescent dideoxy chain-termination sequencing at the Nucleic Acid Facility at Iowa State University. The sequencing primer, 5'-GTCATTGGAGAGACATC-3', was used to confirm the mutation of Q32L, K42E, K42T, and 5'-TCTGAGAAGGACGCACTG-3' was used to confirm the mutation of I190T, and G191A. The mutagenesis plasmid was finally transformed into *Escherichia coli* DL657, a strain deficient in the FBPase gene.

**Purification of Wild-type and Mutant FBPase—**Wild-type, Q32L, K42E, K42T, I190T, and G191A FBPases were purified by using 30–70% (NH$_4$)$_2$SO$_4$ precipitation, Sephadex G-100 column chromatography, and CM-Sephadex C-50 column chromatography. The experimental details for these procedures are described elsewhere (24, 25).

Protein concentration was assayed as described by Bradford (26) with bovine serum albumin (from Sigma) as the standard. The protein purity was determined by using 12% SDS-polyacrylamide gel electrophoresis according to Laemmli (27).

**Kinetic Studies—**Specific activity during purification was determined by the phosphoglucoisomerase and glucose-6-phosphate dehydrogenase coupled spectrophotometric assay at pH 7.5 and 9.6 (13, 28). All other kinetic experiments were done at pH 7.5 (Hepes buffer) and 24 °C by using a coupled spectrofluorometric assay (13). Initial-rate data were analyzed by using a computer program written in MINITAB with an α value of 2.0 (13, 29). Cooperativity was evaluated by using either the ENZFITTER program (30) or the MINITAB program.

**Circular Dichroism Spectrometry—**CD spectrometry was used to analyze the secondary structure of the mutant FBPases and the wild-type enzyme. The CD spectra of the six enzymes are superimposable from 200 to 260 nm (data not shown). These results indicated that no major conformational changes occurred in the mutant FBPases with use of CD as a criterion of secondary structure of the proteins.

**Kinetic Properties of FBPase Mutants—**To evaluate the effects of mutations on residues at the C1-C4 and C2-C3 interface of FBPase, initial-rate kinetic studies were undertaken with the wild-type and mutant forms of the enzyme at a concentration of Fru-1,6-P$_2$ or Mg$^{2+}$ that does not cause substrate inhibition. The kinetic parameters are summarized in Table I. A 1.7-fold increase in $K_{cat}$ compared with that of wild-type enzyme was found for Q32L, suggesting that the catalytic efficiency is slightly enhanced in this mutant. Small decreases (2-fold) in Fru-1,6-P$_2$ $K_m$ were found for Q32L, K42E, K42T, and I190T, and a 4-fold decrease was noted for G191A relative to wild-type FBPase.

**Mg$^{2+}$ Activation—**The Hill coefficient for Mg$^{2+}$ and the $K_m$ for Mg$^{3+}$ of the wild-type and mutant forms of FBPase were determined at saturating Fru-1,6-P$_2$ concentrations (10–15 μM). 3-, 9-, 15-, 17-, and 22-fold increases in $K_m$ values for Mg$^{2+}$ were found for Q32L, K42E, K42T, I190T, and G191A, respectively, relative to the wild-type enzyme (Table I). The five mutants as well as the wild-type enzyme showed sigmoidal kinetics for Mg$^{2+}$. Different $K_m$ values for Mg$^{3+}$ relative to that of the wild-type enzyme were observed for other mutants of the subunit interface of FBPase (21, 22).

**Fru-2,6-P$_2$ Inhibition—**Table I shows that $K_i$ for Fru-2,6-P$_2$ was decreased 3-fold for K42E and K42T and 8-fold for I190T and G191A relative to the wild-type enzyme. Fru-2,6-P$_2$ competes with Fru-1,6-P$_2$ for the active site of wild-type FBPase (31, 32) and the six mutants of the present study. The mutations at interface residues located in the AMP domain resulted in changes in Fru-1,6-P$_2$ and Fru-2,6-P$_2$ affinity, suggesting that these residues may be involved in communication between the Fru-1,6-P$_2$ and AMP domains.

**Kinetics of AMP Inhibition—**AMP is an allosteric inhibitor of FBPase, exhibiting sigmoidal binding with a Hill coefficient of 2.0 (9–11). The pattern of AMP inhibition is nonlinear, non-

competitive relative to Fru-1,6-P₂, but nonlinear, competitive relative to Mg²⁺ for wild-type FBPase at either neutral or alkaline pH (13). The expected competitive inhibition pattern of AMP relative to Mg²⁺ was found in wild-type, Q32L and K42E FBPases; however, the kinetics were changed to noncompetitive inhibition in the case of mutants K42T, I190T, and G191A. Figs. 1 and 2 show double-reciprocal plots of 1/velocity versus 1/(Mg²⁺)² at various fixed concentrations of AMP for the Q32L and K42E mutants of FBPase, respectively. The data in Fig. 1 gave excellent fits to Equation 1, which is consistent with a steady-state random mechanism, when \( n = 2 \). The Goodness of Fit was 6% when \( n = 2 \) and 16% when \( n = 1 \). The data in Fig. 2, however, fit better to Equation 1 when \( n = 1 \). The Goodness of Fit was 5% when \( n = 1 \) and 17% when \( n = 2 \). The form of Equation 1 is as follows,

\[
\frac{1}{v} = \frac{1}{V_m} \left[ 1 + \frac{K_a}{A} \left( 1 + \frac{I}{K_a} + \frac{I^2}{K_a^2} \right) - \frac{K_a}{A} \left( 1 + \frac{I}{K_a} \right) \right]^{-1}
\]  

(Eq. 1)

where \( v, V_m, A, I, K_a, K_i, K_{ii}, K_{ii}, \) and \( K_{ii} \) represent initial velocity, maximal velocity, the concentration of Mg²⁺, Fru-1,6-P₂, AMP, the Michaelis constants for Mg²⁺ and Fru-1,6-P₂, the dissociation constants for Mg²⁺ and the dissociation constants for AMP from the enzyme-AMP, the enzyme-AMP-AMP, and the enzyme-Fru-1,6-P₂-AMP-AMP complexes, respectively. \( n \) represents the Hill coefficient for AMP with FBPase. When \( n = 2 \), the binding of AMP to FBPase exhibits cooperativity, on the other hand, there is no cooperativity when \( n = 1 \). In other words, mutation of Gln⁴² to Leu results in no alteration in AMP cooperativity for FBPase; however, the K42E mutation causes loss of AMP cooperativity.

Fig. 3 illustrates double-reciprocal plots of 1/velocity versus 1/(Mg²⁺)² at various fixed concentrations of AMP for the K42T mutants. Unexpectedly, a family of lines was obtained intersecting to the left of 1/v axis. A similar result was found with the I190T and G191A mutants (data not shown). These findings indicate that the inhibitor, AMP, exhibited noncompetitive inhibition relative to Mg²⁺ with these mutants. The data fit best to Equation 2, the form of which is as follows,

\[
\frac{1}{v} = \frac{1}{V_m} \left[ 1 + \frac{I}{K_i} + \frac{K_i}{K_{ii}} \left( 1 + \frac{I}{K_i} \right) \right]^{-1}
\]

(Eq. 2)

where \( v, V_m, A, I, K_i, K_{ii} \) and \( K_{ii} \) represent initial velocity, maximal velocity, the concentration of Mg²⁺, the dissociation constants for Mg²⁺, the Michaelis constants for Mg²⁺-AMP complexes, respectively. \( n \) represents the Hill coefficient for AMP with FBPase. When \( n = 2 \), the binding of AMP to FBPase exhibits cooperativity, on the other hand, there is no cooperativity when \( n = 1 \). The data for K42T, I190T, and G191A all fit best to Equation 2 when \( n = 1 \). The Goodness of Fit is 4%, 5%, and 5% when \( n = 1 \) in K42T, I190T, and G191A, respectively.
respectively. Goodness of Fit values are all more than 12% in all instances when $n = 2$. The proposed scheme from which Equation 2 was derived is as follows.

\[
\text{E-AMP} \rightarrow \text{E-Mg}^{2+} \rightarrow \text{E-Fru-1,6-P}_2 \rightarrow \text{product}
\]

All five mutants investigated, as well as wild-type FBPase, exhibited noncompetitive inhibition by AMP relative to Fru-1,6-P$_2$ (data not shown). The $K_i$ value (I$_{0.5}$) for AMP for Q32L was increased 8-fold; however, mutations at Lys42, Ile190, and Gly191 caused dramatic effects on AMP affinity (2,000–4,000-fold decreases). These findings indicate that Lys42, Ile190, and Gly191 play important roles in AMP affinity, cooperativity, and the mechanism of AMP binding.

**Temperature Sensitivity of Wild-type and Mutant Forms of FBPases**—To evaluate the influence of mutations on the C1-C4 (C2-C3) subunit interface, the thermostability of wild-type and mutant FBPases were studied. The wild-type enzyme retains its activity after incubation at 57° for 10 min (Fig. 4); however, the relative activity was decreased 70% at 67° after a 10-min incubation. At 47°, K42E and K42T exhibited a decreased activity of approximately of 50%, and I190T and G191A activities were decreased 73 and 84%, respectively. The four mutants were all inactivated at 57°. Compared with wild-type enzyme, K42E, K42T, I190T, and G191A exhibited increased thermostability. Subtle changes in the enzyme structure 17° in the C1-C4/C2-C3 interface may cause the observed thermosensitivity in the FBPase mutants. The CD spectra of mutants and wild-type FBPases are superimposable, ruling out significant changes in secondary structure. The Q32L mutation shows a slight change in activity at 57° but retains 70% of its activity when incubated at 67°, which is about 40% higher than that of wild-type enzyme. The elevated thermostability of Q32L may be a consequence of enhanced hydrophobic interactions in the mutant.

**DISCUSSION**

The allosteric transformation of FBPase is initiated putatively by structural perturbations in the AMP domain upon the binding of AMP (18). Conformational changes in the AMP domain lead to a 1.9° rotation of this domain relative to the FBP domain and to a 17° twist about a 2-fold axis of the tetramer (Fig. 5). The 1.9° rotation of the AMP domain relative to the FBP domain affects the Mg$^{2+}$-binding site and may be the basis for allosteric regulation of activity. The model above
has pedagogic value, but does not provide a framework sufficiently precise for the understanding of biochemical properties of mutant FBPases reported here and elsewhere (21, 22). Several issues are difficult to address within the framework of the simplified description of the allosteric transition. First, can the T-state of FBPase exist in equilibrium with the R-state and in the absence of AMP? Second, how is it possible for AMP to bind near the C1-C4/C2-C3 interface and drive the change in conformational relationships of the AMP and FBP domains? AMP could more effectively leverage a conformational change by binding directly to the interface between the AMP and FBP domains. Finally, is the 1.9° rotation, mentioned above, coupled to the 17° rotation? Allosteric signals may pass directly from the AMP to the FBP domain within a subunit (18), or conversely, allosteric signals might also pass indirectly from the AMP to the FBP domain, by way of conformational changes in the C1-C4/C2-C3 interface.

The more detailed description of the allosteric transition, given below, is motivated by observations reported by Zhang et al. (18). The axis of 17° rotation of the AMP domains does not coincide with a molecular 2-fold axis of the tetramer (18). Instead, that rotation axis is parallel to the molecular twofold axis, but passes through approximately NZ of Lys (18). Thus, the intersubunit conformational change is due to 17° rotations about two axes displaced from each other by approximately 20 Å (Fig. 6). The 17° rotation about axes so displaced from each other will produce domain reorientations not only in the C1-C4/C2-C3 interface but also in the C1-C2 and C3-C4 interfaces. Reorientations are observed in the C1-C2 and C3-C4 interfaces between AMP domains (18). The interactions between the FBP

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**Fig. 3.** Plot of reciprocal of initial velocity in arbitrary fluorescent units versus reciprocal of [Mg²⁺]² for K42T FBPase. The concentrations of AMP are 0 (■), 2 μM (+), 10 μM (▲), and 20 μM (□). The coupled spectrofluorescence assay was used at 25 °C in 50 mM Hepes buffer (pH 7.5) containing 0.1 M KCl and 10 mM Fru-1,6-P₂. The lines are theoretical based upon Equation 2 when n = 1, and the points were experimentally determined. The inset shows a plot of the slope of the family of curves in Fig. 3 versus [AMP].

**Fig. 4.** Temperature sensitivity of wild-type and mutant forms of FBPases. The purified wild-type (■), Q32L (▲), K42E (▲), K42T (□), I190T (▲), and G191A (▲) were incubated 10 min at 30, 37, 47, 57, and 67 °C, respectively. The enzyme activity was assayed at 25 °C by spectrophotometry (as described under “Experimental Procedures”) immediately after the incubation, which is expressed as a percentage of the relative activity at 30 °C. The protein concentration was 0.12 mg in each assay. Each reaction was either in duplicate or triplicate.
domains of the C1-C2 and C3-C4 interfaces, however, are unchanged. As demonstrated in Fig. 6, the combination of reorientated AMP domains and static FBP domains in the C1-C2 and C3-C4 interfaces requires the reorientation of AMP domains relative to FBP domains within a subunit. The direction of rotation of the AMP domain relative to the FBP domain reproduces the observed 1.9° rotation. Thus the small reorientation of the AMP domain relative to the FBP domain within a subunit is coupled to the 17° rotations about offset axes. Rather than directly effecting the relative orientation of the AMP and FBP domains, AMP may exert its influence indirectly by acting on the C1-C4/C2-C3 interface.

The strong coupling of intra- and intersubunit transitions, proposed above, necessarily requires that FBPase be a two-state system. Conceivably, a dynamic equilibrium exists between the R- and T-states with AMP influencing the equilibrium in favor of the T-state. Such a hypothesis is consistent with the observed binding of AMP near the C1-C4/C2-C3 interface, where it can directly stabilize the T-state conformation of that interface. Furthermore, given the pairwise juxtaposition of AMP-binding sites across the C1-C4/C2-C3 interface, one can intuitively understand the observed Hill coefficient of 2 for AMP cooperativity.

Within the framework of the above model mutations will fall within three categories: (i) those which influence the R- and T-state equilibrium, (ii) those which affect the putative coupling of intra- and intersubunit conformational changes, and (iii) those which directly affect the relationship of the AMP and FBP domains within a subunit. Mutations presented here fall into the first two categories.

Mutations of Lys42, Ile190, and Gly191 lie near the 17° rotation axes. Here, Lys42 and a symmetry-related mate participate in a complex network of hydrogen bonds, which is conserved in both the T- and R-states (Fig. 7). NZ of Lys interacts with backbone carbonyls 190 and 191 and the carboxyl group of Glu192. Glu192 makes an additional hydrogen bond with symmetry-related Thr39. All of the mutants near the rotation axes disturb intersubunit hydrogen bonds. The addition of two methyl groups between loops T4 in G191A causes steric repulsion and presumably the weakening of the hydrogen bond network around Lys42. I190T introduces an oxygen atom, which can make a hydrogen bond with either the carboxylate of Asp187 or Glu192. This interaction may influence the hydrogen bonding networks near the rotation axes. The mutation of Lys42 to Thr disrupts hydrogen bonds, whereas the Glu42 mutant introduces an opposite electrostatic charge. All mutants of this area change the AMP and Mg\(^{2+}\) affinities by similar amounts. All mutants lack AMP cooperativity and for three of the four mutants AMP inhibition with respect to Mg\(^{2+}\) is non-competitive. AMP inhibition with respect to Mg\(^{2+}\) is competitive for wild-type FBPase. These significant effects caused by mutations near the 17° rotation axes and approximately 25 Å away from both the AMP and Mg\(^{2+}\)-binding sites are consistent with the putative coupling of inter- and intersubunit confor-
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result from a shift in equilibrium toward the T-state. Gln32
protein Data Bank entries 1fbc and 1fpe) by using MOLSCRIPT (33).
produced from Protein Data Bank coordinates of FBPase (8, 19) (Pro-
reduced Mg$_2^+$ enhances hydrophobic interactions in the T-state (Fig. 8). The
diagram was
interface are shown as sticks. The
bond interactions of Gln32 in the T-state of FBPase. The diagram was
views orientation is similar to that of Fig. 7. The backbone part of the
protein is shown as smoothed coils, and the side chains forming the
interface as a group affect either AMP or Mg$_2^+$
stabilization.
affinity exhibited by the M18I mutant could
1

taminewithhydrophobicleucineshoulddestabilizetheT-state
seems to be more hydrophilic. Substitution of hydrophilic glu-
The R-state (Fig. 8). In addition, its environment in the T-state
T-state, but is not involved in intersubunit hydrogen bonds in
participates in hydrogen bonds between helix H1 and helix H2
of a symmetry related subunit when the enzyme exists in the
parameters relative to the R-state. As a result, changes in kinetic param-
initial interface may influence the equilibrium between R- and T-
states and thereby alter the observed kinetic properties of the
system.

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Fig. 8. Stereoview of the peripheral area of the C1-C4/C2-C3

interface in the T-state (upper) and in the R-state (lower). The view orientation is similar to that of Fig. 7. The backbone part of the
protein is shown as smoothed coils, and the side chains forming the
interface as shown as sticks. The dotted lines indicate the hydrogen
bond interactions of Gln32 in the T-state of FBPase. The diagram was
produced from Protein Data Bank coordinates of FBPase (8, 19) (Pro-
tein Data Bank entries 1fbc and 1fpe) by using MOLSCRIPT (33).