A steady state kinetic investigation of the Pi activation of 5-phospho-\(\alpha\)-1-diphosphate synthase from *Escherichia coli* suggests that Pi can bind randomly to the enzyme either before or after an ordered addition of free Mg\(^{2+}\) and substrates. Unsaturation with ribose 5-phosphate increased the apparent cooperativity of Pi activation. At unsaturating Pi concentrations partial substrate inhibition by ribose 5-phosphate was observed. Together these results suggest that saturation of the enzyme with Pi directs the subsequent ordered binding of Mg\(^{2+}\) and substrates via a fast pathway, whereas saturation with ribose 5-phosphate leads to the binding of Mg\(^{2+}\) and substrates via a slow pathway where Pi binds to the enzyme last. The random mechanism for Pi binding was further supported by studies with competitive inhibitors of Mg\(^{2+}\), MgATP, and ribose 5-phosphate that all appeared noncompetitive when varying Pi at either saturating or unsaturating ribose 5-phosphate concentrations. Furthermore, none of the inhibitors induced inhibition at increasing Pi concentrations. Results from ADP inhibition of Pi activation suggest that these effectors compete for binding to a common regulatory site.

The enzyme 5-phospho-\(\alpha\)-1-diphosphate (PRPP) synthase (EC 2.7.6.1) catalyzes the reaction MgATP + Rib-5-P → AMP + PRPP. PRPP is a precursor of purine, pyrimidine and pyridine nucleotides and the amino acids histidine and tryptophan (1–3). In addition, PRPP is a precursor of methanopterin in *Methanococcoides* (4) and polypropylphosphate-pentoses in *Mycobacteria* (5). The PRPP synthase reaction proceeds by attack of the 1-hydroxyl of Rib-5-P on the \(\beta\)-phosphoryl of ATP resulting in the transfer of the \(\beta\),\(\gamma\)-diphosphoryl moiety of ATP to Rib-5-P (6, 7). Mg\(^{2+}\) ions are required to form the actual substrate MgATP and as an activator of the enzyme (8–13). PRPP synthases from *Salmonella typhimurium* (8, 14, 15), *Escherichia coli* (11, 16), *Bacillus subtilis* (10), human (17), and rat (18) possess an absolute requirement for Pi as an activator and are subject to inhibition by ADP and for *B. subtilis* and mammalian enzymes also by GDP, which binds to a specific allosteric site. In addition, ADP competes with MgATP for binding to the active site. A second class of PRPP synthases, so far only found in plants, is independent of Pi for activity (19, 20).

The enzymes from *S. typhimurium* and *E. coli* share identical primary sequences except for two conservative replacements (16, 21, 22), which is also reflected in their similar, if not identical, enzymological properties. We have previously shown that Mg\(^{2+}\), MgATP, and Rib-5-P bind in that order to *E. coli* PRPP synthase by a steady state ordered mechanism and allosteric inhibition by ADP appeared competitive against activation by free Mg\(^{2+}\) at subsaturating Rib-5-P concentrations (11). Inhibition by ADP and GDP was also shown to increase the half-saturation constant for Mg\(^{2+}\) activation of rat PRPP synthases I and II (18). From previous analysis of the enzymes from *S. typhimurium* (14, 15) and *E. coli* (16), it was found that PRPP appears to bind to the allosteric site only in the presence of Rib-5-P. As a consequence, the interaction of PRPP synthase with the allosteric inhibitor ADP appears to involve ADP binding to the allosteric site of the enzyme prior to Mg\(^{2+}\) binding as well as to the enzyme in complex with Mg\(^{2+}\) and substrates.

From analysis of the hydrodynamic properties of *S. typhimurium* PRPP synthase, it was found that Pi maintains the oligomeric structure of the enzyme (23) and that removal of Pi results in irreversible loss of activity (8, 16). The role of Pi in the steady state kinetics of PRPP synthase has not previously been analyzed in detail.

Indirect evidence that allosteric Pi activation and ADP inhibition occur by competition for binding to the same site has been presented. The analysis of mutant forms of human PRPP synthase I that have a reduced sensitivity to allosteric inhibition by ADP and GDP revealed a concomitant increase in affinity for Pi (17). The inhibitor 4-amino-8-(\(\beta\)-ribofuranosylamino)pyrimido[5,4-d]pyrimidine appears to bind at the allosteric site of both human PRPP synthases I and II, and the concentration of the inhibitor needed for half-maximal inhibition increased with increasing Pi concentration (24). The recent structure of the *B. subtilis* PRPP synthase in complex with ADP or sulfate ions revealed a hexameric arrangement. The structures indicated that the allosteric site defined by three subunits is the target for binding of both ADP and Pi, the latter being represented by a sulfate ion (25). Together these observations suggest a more subtle mechanism behind Pi activation apart from maintaining the structure. To gain a more detailed understanding of the regulation of the PRPP synthase, we have analyzed the steady state kinetics of Pi activation of the *E. coli* enzyme and suggest a complete model for the interaction of PRPP synthase with all of its known ligands.

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†The abbreviations used are: PRPP, 5-phospho-\(\alpha\)-1-diphosphate; Rib-5-P, ribose 5-phosphate; eRib-5-P, (+)-1-\(\alpha\)-2,\(\alpha\)-3-\(\alpha\)-trihydroxy-4-\(\beta\)-cyclopentanemethanol 5-phosphate; mATP, \(\alpha\),\(\beta\)-methylene ATP; MgmATP, \(\alpha\),\(\beta\)-methylene MgATP.
**EXPERIMENTAL PROCEDURES**

**Materials and Enzyme Purification**—ATP was obtained from Roche Molecular Biochemicals, mATP and Rib-5-P were obtained from Sigma, and cRib-5-P was a gift from R. J. Parry (Rice University, Houston, TX) (26). [8-14C]ADP was from Amersham Pharmacia Biotech. *E. coli* PRPP synthase was purified as described previously (11, 27) and had a specific activity of approximately 150 μmol min⁻¹ mg⁻¹ when assayed in the presence of 2 mM ATP, 5 mM Rib-5-P, 5 mM MgCl₂ as described below. The protein concentration was determined by the bicinchoninic acid procedure (28) with reagents provided by Pierce and with bovine serum albumin as a standard.

**Assay of PRPP Synthase Activity**—The 32P transfer assay was performed at 37 °C in 55 mM triethanolamine, pH 8.0, as described previously (11, 27), except that enzyme was diluted in 2 mM ATP, 10 mM MgCl₂, 50 mM triethanolamine, pH 8.0, bovine serum albumin (1 mg ml⁻¹). The concentrations of divalent metal ion and nucleotide complexes and free divalent metal ions were calculated as described previously (9, 11, 15). Unless otherwise noted, the free Mg²⁺ concentration varied within a saturating level of 3–5 mM, because of the varying P_i concentrations. The MgATP concentration was maintained at 2 mM. The nucleotides ADP and mATP was calculated to be more than 90% complexed with Mg²⁺. The binding of Ca²⁺ by ATP was neglected when calculating the free Ca²⁺ concentration, because Mg²⁺ was present in at least 15-fold excess. The stability constant used for the calculation of the Ca²⁺ complex with P_i was 0.03 mM⁻¹ (29). The concentration of P_i, Rib-5-P and inhibitors varied as indicated.

**Kinetic Enzyme Data**—Results of initial velocity experiments were analyzed by fitting data by nonlinear regression to the appropriate Equations 1–6 using the computer program UltraFit (BioSoft, version 3.01). The standard errors for kinetic parameters presented are those calculated by the program. Equation 1 is the Michaelis-Menten equation for hyperbolic substrate saturation kinetics, Equation 2 is the Hill equation for cooperative substrate saturation kinetics, Equation 3 is a general equation for nonhyperbolic saturation kinetics (30, 31), Equation 4 applies to noncompetitive inhibition, and Equations 5 and 6 apply to nonlinear competitive and nonlinear noncompetitive inhibition, respectively, and where the effect of the inhibitor on S₅₀ is caused by successive binding of two molecules of inhibitor at different sites on the enzyme. Equations 4–6 apply to cooperative substrate saturation kinetics, where n is not affected by the presence of inhibitor (32).

\[
\begin{align*}
\text{v} & = \text{V}_{\text{app}}S/K_{S} + \text{S} \\
\text{v} & = \text{V}_{\text{app}}S^{a}/(S_{50}^{a} + S^{a}) \\
\text{v} & = (aS + bS^{2})/(1 + cS + dS^{2}) \\
\text{v} & = \text{V}_{\text{app}}S^{a}/S_{50}^{a}[1 + I/K_{i}] + \text{S}^{a}[1 + I/K_{i}] \\
\text{v} & = \text{V}_{\text{app}}S^{a}/S_{50}^{a}[1 + I/K_{i}] + P/K_{i} + S^{a}[1 + (I/K_{i})] \\
\text{v} & = \text{V}_{\text{app}}S^{a}/S_{50}^{a}[1 + I/K_{i}] + P/K_{i} + S^{a}[1 + (I/K_{i})]
\end{align*}
\]

where v is the initial velocity; \(V_{\text{app}}\) is the apparent maximal velocity; S is the concentration of the varied substrate or activator; \(K_{S}\) is the apparent Michaelis-Menten constant for S; \(S_{50}\) is the half-saturation constant for S; n is the apparent Hill coefficient for S; a, b, c, and d are complex functions of rate constants and the concentration of nonvaried substrates and as such have no physical meaning; \(K_{i}\), \(K_{a}\), and \(K_{n}\) are inhibition constants for the effect on \(S_{50}\), where a suffix, 1 or 2, on \(K_{i}\) refers to the two different binding constants for nonlinear inhibition; and \(K_{n}\) is the inhibitor constant for the effect on \(V_{\text{app}}\). All velocities are in μmol min⁻¹ mg⁻¹.

**Ligand Binding Studies**—Ligand binding was performed as described previously (33, 34). PRPP synthase (4.5 nmol) was incubated at pH 8.2 in 150 μl of 50 mM P_i, 25 mM Tris-HCl, 5 mM MgCl₂ and varying concentrations of ADP (0.2 nCi of [8-14C]ADP per incubation). When present the Rib-5-P concentration was 2 mM. Each incubation was transferred to a Millipore Ultratfree-MC centrifugal filter unit and equilibrated to 25 °C and centrifuged for 5–10 min at 5000 × g in a thermoshake microturbine (OLE DICH Instruments, Copenhagen, Denmark). Samples (30 μl) from the incubation prior to centrifugation (total ligand) and from the eluent after centrifugation (free ligand) was withdrawn, and radioactivity was quantitated with a Packard 2000 liquid scintillation analyzer. The ADP binding data were analyzed by fitting to Equation 7 or 8 for data obtained in the absence or presence of Rib-5-P, respectively. Equation 7 applies to simple hyperbolic binding, and Equation 8 is a two-site binding model with one site, the allosteric site, showing cooperative binding.

\[
N = A_{\text{max}}L/(K_{a} + L)
\]

\[
N = A_{\text{max}}L/(K_{a} + L) + B_{\text{max}}L^{n}(K_{b}^{\text{nb}} + L^{m})
\]

where N is mol ADP bound per mol monomer; \(A_{\text{max}}\) and \(B_{\text{max}}\) are the numbers of active sites and allosteric sites per monomer of enzyme (34,000 kDa), respectively; L is the unbound ADP concentration; \(K_{a}\) and \(K_{b}\) are the half-saturation constants for the active site and the allosteric site, respectively; and m is the Hill coefficient for binding to the allosteric site.

**RESULTS**

**Stability of PRPP Synthase in the Absence of P_i**—PRPP synthase from *E. coli* is normally stored and diluted in the presence of 50 mM P_i to maintain stability (16). Therefore, to study P_i activation it was necessary to find a condition where the enzyme was stable in the absence of P_i. With 2 mM MgATP or more the enzyme was found to be fully stable upon dilution and subsequent incubation at 37 °C. Because concentrations of free Mg²⁺ that were kinetically subsaturating, in combination with 2 mM MgATP, fully stabilized the enzyme, it was possible to study the influence of Mg²⁺ and Rib-5-P, but not MgATP, on the activation of PRPP synthase by P_i.

**Influence of Mg²⁺ or Rib-5-P on P_i Activation**—Saturation with free Mg²⁺ resulted in nearly hyperbolic activation of PRPP synthase by P_i, whereas cooperative activation by P_i was observed at unsaturating free Mg²⁺ concentrations (Fig. 1A). Apparently, also the S₅₀ for P_i increased when the free Mg²⁺ concentration was lowered to 14 μM (Fig. 1A). Mg²⁺ activation appeared hyperbolic at P_i concentrations of 5 mM and above but was clearly cooperative at 1.5 mM P_i (Fig. 1B). However, the concentration for half-saturation with free Mg²⁺ changed by less than a factor of two over the entire range of P_i concentrations (Fig. 1B). The apparent cooperativity of P_i activation increased when the Rib-5-P concentration was lowered to 0.5 mM and showed a 2-fold decrease in S₅₀ for P_i compared with the results obtained at 5 mM Rib-5-P (Fig. 2A). At 0.5 mM Rib-5-P, beginning inhibition by P_i concentrations exceeding 25 mM was observed, probably because of competitive binding to the Rib-5-P binding site. Apparently, the saturation of PRPP synthase with Rib-5-P was sensitive to the P_i concentration because increasing Rib-5-P concentrations induced partial substrate inhibition that was relieved by 50 mM P_i (Fig. 2B).

**Inhibition of P_i Activation by Ca²⁺, MgmATP, or cRib-5-P**—Divalent calcium, MgmATP, and cRib-5-P have been shown to competitively inhibit the binding of Mg²⁺, MgATP, and Rib-5-P, respectively (11). Results from experiments where P_i was varied in the presence of different fixed concentrations of inhibitor at saturating or nonsaturating Rib-5-P concentrations are presented in Table I. All three inhibitors exhibited linear noncompetitive inhibition of P_i binding regardless of the Rib-5-P concentration. At the tested concentrations of inhibitor and Rib-5-P neither Ca²⁺, MgmATP, nor cRib-5-P induced inhibition by increasing P_i concentrations.

**Inhibition of P_i Activation by ADP**—When P_i was varied at either 0.5 mM or 5 mM Rib-5-P, the presence of increasing fixed concentrations of ADP resulted in a pronounced nonlinear effect on S₅₀ for P_i (Fig. 3 and Table I). The effect of ADP on both S₅₀ and Vₐₚₚ for saturation of the enzyme by P_i in the presence of 0.5 mM Rib-5-P could readily be determined when the data were fitted to Equation 5 (Fig. 3B and Table I). Data from ADP inhibition of P_i saturation at 5 mM Rib-5-P were fitted as nonlinear competitive inhibition because no effect of ADP on Vₐₚₚ could be estimated for the data within the range of P_i concentrations available to us (Fig. 3A and Table I). Increasing the concentration of P_i beyond 50 mM in the assay incubation results in the rapid formation of a MgP_i precipitate.
Effector and Substrate Binding to PRPP Synthase

Fig. 1. Activation of PRPP synthase by P_i or Mg^{2+}. Assays were performed as described under "Experimental Procedures." A, P_i varied as indicated in the presence of the indicated concentrations of Mg^{2+}. △, data were fitted to Equation 2; \( V_{app} = 130 \pm 3, S_{0.5} = 3.1 \pm 0.2 \, \text{mM}, n = 1.3 \pm 0.1 \). ○, data were fitted to Equation 2; \( V_{app} = 73 \pm 1, S_{0.5} = 2.6 \pm 0.1 \, \text{mM}, n = 2.0 \pm 0.2 \). ▽, data were fitted to Equation 2; \( V_{app} = 39.1 \pm 0.8, S_{0.5} = 8.5 \pm 0.3 \, \text{mM}, n = 2.0 \pm 0.1 \). B, Mg^{2+} varied as indicated in the presence of the indicated concentrations of P_i. △, data were fitted to Equation 1; \( V_{app} = 129 \pm 4, K_i = 41 \pm 4 \, \mu\text{M} \). ○, data were fitted to Equation 1; \( V_{app} = 103 \pm 6, K_i = 59 \pm 10 \, \mu\text{M} \). ▽, data were fitted to Equation 2; \( V_{app} = 55 \pm 2, S_{0.5} = 69 \pm 5 \, \mu\text{M}, n = 2.1 \pm 0.2 \).

Binding of ADP—An observed cooperativity in binding of MgmATP to PRPP synthase (14) at 0 °C was shown to be an effect of the temperature at which the experiment was performed, because it is absent at 25 °C (11). To investigate whether the high degree of cooperativity associated with ADP binding to the allosteric site previously determined (14) would also be influenced by temperature, we performed ADP binding experiments at 25 °C. However, the extent of cooperativity in ADP binding to the allosteric site at 25 °C (Fig. 4) was not significantly different from that determined previously at 0 °C (14).

DISCUSSION

Steady state kinetics and ligand binding studies of the S. typhimurium and E. coli PRPP synthases have identified the mechanism for Mg^{2+}, MgATP, and Rib-5-P binding to the enzyme as occurring in that order (8, 9, 11). Allosteric binding of ADP has been shown to occur either at conditions where the enzyme is fully saturated with Mg^{2+} and substrates (14–16) or prior to binding of Mg^{2+} and substrates as revealed by cooperative competitive ADP inhibition of Mg^{2+} activation (11).

By comparing MgmATP and ADP inhibition of P_i activation at 0.5 mM Rib-5-P (Table I) the \( K_i \) for ADP is likely to represent binding to the active site in competition with MgATP. The nonlinear effect of ADP on \( S_{0.5} \) for P_i described by \( K_{i1} \) and \( K_{i2} \) is observed both at 0.5 and 5 mM Rib-5-P. This nonlinear effect of ADP on \( S_{0.5} \) for P_i is sufficient to explain the inhibition by ADP at 5 mM Rib-5-P. Although a \( K_i \) for ADP at 5 mM Rib-5-P would have been expected by comparing with MgmATP inhibition under similar conditions, this could not be extracted from the data. The nonlinear effect of ADP on \( S_{0.5} \) for P_i (Table I) suggests that ADP competes with P_i for binding to the regulatory site. However, because neither \( K_{i1} \) nor \( K_{i2} \) is comparable with \( K_i \) for MgmATP under similar conditions, we hesitate to further assign an exact physical meaning for \( K_{i1} \) and \( K_{i2} \). It appears that ADP and P_i compete for binding to the same enzyme form in a manner largely independent of the Rib-5-P concentration. On the basis of the previous data from ADP inhibition and ligand binding studies mentioned above, we suggest that ADP and P_i compete for binding to the allosteric site of the enzyme either prior to or after binding of Mg^{2+} and substrates.

The results of Fig. 2 show the characteristics of a steady state random mechanism (30, 31, 35) with a preferred pathway in which P_i binds to the enzyme prior to Rib-5-P. The kinetic pattern shown in Fig. 2 is likely to result from a difference in the magnitude of the rate constants of otherwise similar kinetic equilibria for a random binding of P_i and Rib-5-P. Because substrate inhibition by Rib-5-P is only partial, it is unlikely to result from formation of a dead end complex.
The apparent noncompetitive inhibition of Pi activation observed by Ca\(^{2+}\), MgmATP, or cRib-5-P (Table I) suggests two mechanisms that both would agree with the results of Fig. 2. Either substrates can bind fully random to the enzyme with respect to Pi (i.e. before or after binding of Pi) by a steady state random mechanism, or an ordered binding of Mg\(^{2+}\) and MgATP is proceeded by a random binding of Rib-5-P and Pi. In favor of a fully random mechanism is the absence of inhibition at increasing Pi concentrations induced by the presence of inhibitor. This would be observed to the extent that obligatory binding of the inhibitor to the enzyme occurs prior to Pi and if downstream binding of substrates and Pi also occurs to the enzyme-inhibitor complex.

Both Ca\(^{2+}\) (15) and MgmATP (11) induce substrate inhibition by Rib-5-P in agreement with the ordered binding mechanism where Rib-5-P binds to the enzyme last. A random mechanism where Mg\(^{2+}\) and substrates bind to PRPP synthase either before or after Pi binding therefore appears most consistent with the results presented here and those previously obtained as mentioned above.

In Scheme 1 an outline of the interaction of PRPP synthase with substrates, activators, and ADP is suggested. The allosteric effectors Pi and ADP compete for binding to the allosteric site of either the free enzyme or enzyme in complex with Mg\(^{2+}\) and substrates. To explain the data of Fig. 2, we have made the assumption that a fast pathway and a slow pathway exist from the free enzyme to the catalytic complex. Furthermore, it is assumed that the binding order of Mg\(^{2+}\), MgATP, and Rib-5-P is conserved whether Pi is bound to the enzyme or not. The noncompetitive inhibition of Pi activation by Ca\(^{2+}\), MgmATP, and Rib-5-P is also consistent with the mechanism in Scheme 1.

According to Scheme 1 the Mg\(^{2+}\) activation of PRPP synthase should resemble the saturation of the enzyme with Rib-5-P under similar conditions. Accordingly, the results of Fig. 1A and 1B are consistent with the binding data presented in Table I.

### Table I

**Mode of inhibition of Pi activation by inhibitors of PRPP synthase**

| Inhibitor     | Mode of inhibition | [Rib-5-P]\(a\) | \(K_i\) | \(K_{cat}\) | \(K_{cat}\) |
|---------------|-------------------|----------------|--------|-----------|-----------|
| Ca\(^{2+}\)   | noncompetitive    | 5.0            | 32 \(\pm\) 6 | 47 \(\pm\) 4 |           |
| Ca\(^{2+}\)   | noncompetitive    | 0.5            | 53 \(\pm\) 10 | 110 \(\pm\) 6 |           |
| MgmATP\(d\)  | noncompetitive    | 5.0            | 229 \(\pm\) 65 | 230 \(\pm\) 19 |           |
| MgmATP\(d\)  | noncompetitive    | 0.5            | 790 \(\pm\) 279 | 560 \(\pm\) 34 |           |
| cRib-5-P\(e\) | noncompetitive    | 1.0            | 1180 \(\pm\) 325 | 716 \(\pm\) 33 |           |
| ADP\(f\)     | nonlinear         | 5.0            |          |           |           |
| ADP\(f\)     | competitive       |               |          |           |           |
| ADP\(f\)     | noncompetitive    | 0.5            | 334 \(\pm\) 28 | 15 \(\pm\) 3 | 40 \(\pm\) 14 |

\(\text{a}\) The Pi concentration varied from 0 to 50 mM, except with 0.5 mM Rib-5-P, where the Pi concentration varied from 0 to 25 mM.

\(\text{b}\) The Ca\(^{2+}\) concentration varied from 0 to 0.2 mM.

\(\text{c}\) The free Mg\(^{2+}\) concentration was maintained at 1.2 mM.

\(\text{d}\) The MgmATP concentration varied from 0 to 1.0 mM.

\(\text{e}\) The cRib-5-P concentration varied from 0 to 1.0 mM.

\(\text{f}\) Conditions were as described in the legend to Fig. 3A.

\(\text{g}\) Conditions were as described in the legend to Fig. 3B.

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**Fig. 3. Inhibition of Pi activation of PRPP synthase by ADP.** Assays were performed as described under “Experimental Procedures.” Pi varied as indicated in the presence of the indicated concentrations of ADP and 5 mM Rib-5-P (A; data were fitted to Equation 5; the calculated constants are presented in Table I) or 0.5 mM Rib-5-P (B; data were fitted to Equation 6; the calculated constants are presented in Table I).

**Fig. 4. Binding of ADP to PRPP synthase.** Binding experiments were performed as described under “Experimental Procedures.” The binding of ADP was determined in the absence or presence of Rib-5-P as indicated. ○, data were fitted to Equation 7; \(A_{max} = 1.14 \pm 0.05, K_A = 59 \pm 11 \mu M\). △, data were fitted to Equation 8; \(A_{max} = 1.0 \pm 0.1, K_A = 42 \pm 2 \mu M, B_{max} = 1.0 \pm 0.2, K_B = 244 \pm 52 \mu M, n_b = 24 \pm 1.4\).
where unsaturation of PRPP synthase with Mg$^{2+}$ yields cooperative P$_i$ activation can be explained by a preferred pathway in a random mechanism. However, unlike the saturation with Rib-5-P at low P$_i$ concentrations (Fig. 2B), the Mg$^{2+}$ activation at 1.5 mM P$_i$ is cooperative (Fig. 1B), and no inhibition by increasing Mg$^{2+}$ concentrations is observed. Because apparently no cooperativity is associated with Ca$^{2+}$ inhibition of P$_i$ activation, it may suggest that the apparent cooperativity of Mg$^{2+}$ activation at low P$_i$ is not due to homotropic site-site interactions. The apparent cooperativity of Mg$^{2+}$ activation in the presence of 1.5 mM P$_i$ (Fig. 1B) may be interpreted in terms of the apparent increase in $S_{0.5}$ for P$_i$ at low Mg$^{2+}$ (Fig. 1A). If the affinity of the enzyme for P$_i$ binding via the slow pathway is relatively higher, it may favor this pathway over the fast pathway at low Mg$^{2+}$ concentrations.

We realize that Scheme 1 must somehow be a simplification of the actual overall mechanism for PRPP synthase. One consistent observation that is not explained by Scheme 1 is the high degree of cooperativity for binding of ADP to the allosteric site (Fig. 4) with Hill coefficients between 3 and 4.4 when determined in the presence of 50 mM P$_i$ (11, 14). Our results from ADP inhibition of P$_i$ activation at 0.5 mM and 5 mM Rib-5-P can be explained without including any cooperativity associated with the ADP inhibition. It seems controversial that ADP and P$_i$ can only bind to the allosteric site of either free enzyme or enzyme complexed with Mg$^{2+}$ and substrates. However, at present there seems to be no experimental evidence supporting the possibility that ADP can bind to the intermediates of Scheme 1 other than those indicated, and because ADP and P$_i$ apparently compete for binding to the same form(s) of the enzyme, this should be true for P$_i$ as well. Apart from explaining the observed competition between binding of Mg$^{2+}$ and ADP (11, 18) as a simple consequence of a random mechanism, Scheme 1 also allows for an equilibrium between active and inactive forms of the enzyme that can be shifted by allosteric effectors and apparently by specific amino acid changes, as suggested from analysis of human mutant enzymes (17).

The recent solving of the crystal structure of *B. subtilis* PRPP synthase (25) seems very promising for our attempts to understand the mechanism behind the allosteric regulation of the enzyme. The kinetic analysis presented here suggests what complexes can be expected to be formed between the enzyme and its ligands. When the structural details of more complexes are known other than those likely to represent free enzyme complexed with ADP and P$_i$, we may address specific questions about the regulatory mechanism in Scheme 1.

The mechanism in Scheme 1 also addresses the question of actual substrate and activator concentrations under physiological conditions. Because the response of the PRPP synthase to substrates, activators, and ADP seems very dependent on the concentration of Rib-5-P and P$_i$, it may suggest that the mechanism proposed in Scheme 1 can play a regulatory role in determining the rate of PRPP synthesis in response to changes in metabolite concentrations. This is the topic of work currently in progress in our laboratories.

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