Metaphosphate in the Active Site of Fructose-1,6-bisphosphatase*

Received for publication, December 5, 2002, and in revised form, February 5, 2003
Published, JBC Papers in Press, February 20, 2003, DOI 10.1074/jbc.M212395200

Jun-Yong Choe, Cristina V. Iancu, Herbert J. Fromm, and Richard B. Honzatko‡
From the Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, Ames, Iowa 50011

The hydrolysis of a phosphate ester can proceed through an intermediate of metaphosphate (dissociative mechanism) or through a trigonal bipyramidal transition state (associative mechanism). Model systems in solution support the dissociative pathway, whereas most enzymologists favor an associative mechanism for enzyme-catalyzed reactions. Crystals of fructose-1,6-bisphosphatase grow from an equilibrium mixture of substrates and products near at atomic resolution (1.3 Å). At neutral pH, products of the reaction (orthophosphate and fructose-6-phosphate) bind to the active site in a manner consistent with an associative reaction pathway; however, in the presence of inhibitory concentrations of K⁺ (200 mM), or at pH 9.6, metaphosphate and water (or OH⁻) are in equilibrium with orthophosphate. Furthermore, one of the magnesium cations in the pH 9.6 complex resides in an alternative position, and suggests the possibility of metal cation migration as the 1-phosphoryl group of the substrate undergoes hydrolysis. To the best of our knowledge, the crystal structures reported here represent the first direct observation of metaphosphate in a condensed phase and may provide the structural basis for fundamental changes in the catalytic mechanism of fructose-1,6-bisphosphatase in response to pH and different metal cation activators.

Phosphatases, mutases, kinases, and nucleases all catalyze phosphoryl transfer reactions central to biochemical processes that sustain life. The transfer of the phosphoryl group of a phosphate ester to water in most cases requires metal ions as cofactors, and proceeds either by way of a trigonal-bipyramidal transition state (associative mechanism), or through the formation of an unstable intermediate of metaphosphate (dissociative mechanism) (1–6). The metaphosphate anion (PO₃³⁻) was first proposed as an intermediate in the hydrolysis of phosphate esters nearly 50 years ago (7, 8). Although it exists in a stable entity in the gas phase, where it is relatively non-reactive (9), metaphosphate is unstable in aqueous solutions, and its existence is inferred only by indirect evidence (9–15). The likelihood of trapping metaphosphate in the active site of an enzyme is remote, because of the proximity of acceptor molecules in the active site. Yet an enzyme offers an advantage in that it reduces the free energy of the transition state. Hence, the active site itself could serve as a thermodynamic trap if metaphosphate, once generated, is denied access to an acceptor.

Fructose-1,6-bisphosphatase (n-fructose-1,6-bisphosphatase 1- phosphohydrolase, EC 3.1.3.11, hereafter FBPase) is a key regulatory enzyme in gluconeogenesis. FBPase catalyzes the hydrolysis of fructose 1,6-bisphosphate (F₁₆P₂) to fructose 6-phosphate (F₆P) and orthophosphate (Pᵢ). The inhibition of FBPase in mammals results in reduced levels of serum glucose in the fasting state. Hence, FBPase is a target for the development of drugs in the treatment of non-insulin dependent diabetes, which afflicts over 15 million people in the United States (16, 17).

FBPase can be in either of two quaternary conformations, the R-state (catalytically active) or the T-state (inactive) (18). AMP and fructose 2,6-bisphosphate both inhibit catalysis by FBPase, the former through an allosteric mechanism (19, 20) and the latter by direct ligation of the active site (21, 22). Divalent metals (Mg²⁺, Mn²⁺, or Zn²⁺) are essential for FBPase activity. Monovalent metals (K⁺, Rb⁺, Tl⁺, or NH₄⁺) further enhance reaction rates at relatively low concentrations, but can be inhibitory at high concentrations (23–25). The enzyme-mediated reaction is pH-dependent; plots of initial velocity versus Mg²⁺ are sigmoidal (Hill coefficient of 2) at neutral pH, but hyperbolic at pH 9.6 (26). The kinetic mechanism at pH 7 with Mg²⁺ as the cation activator is steady-state random (25), whereas at pH 9.6 the kinetic mechanism is rapid-equilibrium random (26). The catalytic mechanism is also sensitive to the type of cation activator: The Mn²⁺-activated enzyme uses exclusively the α-anomer of F₁₆P₂ (27), but Mg²⁺-activated FBPase uses both α- and β-anomers of F₁₆P₂ (23). Mn²⁺-activated FBPase, but not the Mg²⁺-activated enzyme, hydrolyzes the substrate analogue (Sp)-[1,1⁴]O-fructose 1-phosphothioate 6-phosphate (28). The zinc cation is an activator of FBPase, and yet traces of Zn²⁺ reduce catalytic rates of the Mg²⁺-activated enzyme (24). The above suggests alternative catalytic pathways, the dominant mechanism being determined by pH, the kind of cation-activator, and the conformation of the substrate.

FBPase crystallizes readily from an equilibrium mixture of products and substrates, and in fact the enzyme itself is active under conditions of crystallization. In past crystal structures of FBPase, only orthophosphate and F₆P have appeared in the active site (29, 30). Presumably, the observed complexes represent a minimum free energy under the conditions of the crystallization experiment. Reported here are crystal structures of FBPase at near atomic resolution, in which a partial reaction (essentially the second step of a dissociative pathway) has lead to the formation of metaphosphate in the active site. The crystallographic structures do not constitute irrefutable evidence of a dissociative reaction pathway, but they do dem-

* This work was supported in part by National Institutes of Health Research Grant NS 10546 and National Science Foundation Grant MCB-9985565. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be regarded in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed. Tel.: 515-294-7103; Fax: 515-294-0453; E-mail: honzatko@iastate.edu.

† The abbreviations used are: FBPase, fructose-1,6-bisphosphatase; F₁₆P₂, fructose 1,6-bisphosphate; F₆P, fructose 6-phosphate.
onstrate that FBPase can generate and stabilize metaphosphate at its active site. Hence, variations observed in the mechanism of catalysis by FBPase may arise from changes in the rate-limiting step of a dissociative pathway or even a change in pathway, for instance, from associative to dissociative.

### EXPERIMENTAL PROCEDURES

**Protein Isolation and Crystallization**—FBPase was isolated as described previously (29–31). Crystals in 10 or 200 mM K\(^+\) grew (by the method of hanging drops) from equal parts of a protein solution (10 mg/ml FBPase, 50 mM Hepes, 5 mM MgCl\(_2\), 5 mM F6P, 10 mM or 200 mM KCl) and a precipitant solution (100 mM Hepes pH 7, 5% 200 mM KCl) (w/v) polyethylene glycol 3350). Crystals at pH 9.6 grew (by the method of hanging drops) from equal parts of a protein solution (10 mg/ml FBPase, 50 mM Hepes, 5 mM MgCl\(_2\), 5 mM F16P2, 10 mM or 200 mM KCl) and a precipitant solution. Crystals of uniform dimension (0.2 mm) grew in three to 5 days at room temperature. All crystals belong to space group \(I\overline{2}22\), with one subunit of FBPase in the asymmetric unit.

**Data Collection**—Data from the low K\(^+\) control complex (25.7 Å) for protein 25.7 Å

| Description                                              | Control | High pH | High K\(^+\) |
|----------------------------------------------------------|---------|---------|-------------|
| Resolution limit (Å)                                     | 1.3     | 1.6     | 1.3         |
| Number of measurements                                   | 347535  | 187681  | 345045      |
| Number of unique reflections                             | 83972   | 39885   | 76674       |
| Completeness of data (%)                                 | 92.6    | 83.5    | 85.6        |
| Last shell/resolution-range (Å)                          | 65.8(1.30–1.35) | 35.6(1.60–1.66) | 40.9(1.30–1.35) |
| \(R_{	ext{sym}}\)                                        | 0.024   | 0.017   | 0.054       |
| Number of reflections in refinement                      | 77354   | 39783   | 76821       |
| Number of atoms                                          | 2567    | 226    | 249         |
| Number of solvent sites                                   | 208     | 152    | 153         |
| \(R_{	ext{free}}\)                                       | 0.164   | 0.208  | 0.206       |
| Resolution of refinement (Å)                              | 1.3–10.0 | 1.8–10.0 | 1.3–10.0 |
| Mean B (Å\(^2\)) for protein 1                          | 25.7    | 23.8    | 24.9        |
| Mean B (Å\(^2\)) for ligands                             | 19.0    | 20.6    | 23.1        |

**Table I**

| Bond lengths (Å)                                         | Control | High pH | High K\(^+\) |
|----------------------------------------------------------|---------|---------|-------------|
| Angle distances (Å)                                      | 0.013   | 0.009   | 0.013       |

Results

Three complexes (Table I) are presented here: (i) orthophosphate, F6P, Mg\(^{2+}\), and K\(^+\) (10 mM) at pH 7 (hereafter the control complex), (ii) metaphosphate, F6P, Mg\(^{2+}\), and K\(^+\) (200 mM) at pH 7 (hereafter the high K\(^+\) complex), and (iii) metaphosphate, F6P, and Mg\(^{2+}\) at pH 9.6 (hereafter the high pH complex). All complexes have the dynamic loop (residues 52–72) in its engaged conformation, as determined by the location of Tyr\(^{27}\) (29, 30). Conditions that result in the formation of metaphosphate differ only in pH (7 versus 9.6) or the concentration of K\(^+\) (10 versus 200 mM). The kinetic mechanism of FBPase changes from steady-state random at pH 7 to rapid-equilibrium random at pH 9.6 (26), and K\(^+\) is inhibitory at concentrations of 200 mM (K\(_{i}\) of 68 mM), but activating at concentrations of 10 mM (K\(_{i}\) of 17 mM) (25).

**Control Complex (PDB: 1NUX)**—Aside from its substantially higher resolution than the control complex, (ii) metaphosphate, F6P, Mg\(^{2+}\), and K\(^+\) (200 mM) at pH 7 (hereafter the high K\(^+\) complex), and (iii) metaphosphate, F6P, and Mg\(^{2+}\) at pH 9.6 (hereafter the high pH complex). All complexes have the dynamic loop (residues 52–72) in its engaged conformation, as determined by the location of Tyr\(^{27}\) (29, 30). Conditions that result in the formation of metaphosphate differ only in pH (7 versus 9.6) or the concentration of K\(^+\) (10 versus 200 mM). The kinetic mechanism of FBPase changes from steady-state random at pH 7 to rapid-equilibrium random at pH 9.6 (26), and K\(^+\) is inhibitory at concentrations of 200 mM (K\(_{i}\) of 68 mM), but activating at concentrations of 10 mM (K\(_{i}\) of 17 mM) (25).

**High K\(^+\) Complex (PDB: 1NUX)**—An increase in the concentration of K\(^+\) from 10 to 200 mM results in an anomalous signal at metal site 1 (Fig. 1B), indicating the displacement of the Mg\(^{2+}\) from that site by a different atom type. The metal at site 1 has four inner-sphere ligands: One oxygen atom each from Asp\(^{118}\), Asp\(^{121}\), Glu\(^{78}\), and the phosphoryl species is –2–2.5 Å from metal site 1 (Fig. 2B). The 1-OH group of F6P no longer coordinates the Mg\(^{2+}\) at site 1, optimally positioned for an associative reaction (Fig. 2A). Three of four oxygen atoms from P1 are approximately equidistant from the 1-OH group at site 1 of F6P, and the distance between the phosphorus atom and the oxygen atom of the 1-OH group is 2.72 Å. In its second position (displaced conformation; occupancy factor of 0.7), the 1-OH group no longer coordinates the Mg\(^{2+}\) at site 1, optimally positioned for an associative reaction (Fig. 2A). Three of four oxygen atoms from P1 are approximately equidistant from the 1-OH group at site 1 of F6P, and the distance between the phosphorus atom and the oxygen atom of the 1-OH group is 2.72 Å. In its second position (displaced conformation; occupancy factor of 0.7), the 1-OH group no longer coordinates the Mg\(^{2+}\) at site 1, optimally positioned for an associative reaction (Fig. 2A).
solution eliminates the anomalous signal at metal site 1. Furthermore, the tetrahedral coordination of the site-1 cation and the displaced conformation of the 1-OH group of F6P are characteristics of previously determined Zn\(^{2+}\)-product complexes of FBPase (29, 30). A mixture of Zn\(^{2+}\) and Mg\(^{2+}\) at occupancies of 0.25 and 0.75, respectively, account for the magnitude of the anomalous signal, and provide thermal parameters of 24 Å\(^2\), equivalent to that of the site-1 Mg\(^{2+}\) in the control structure.

The thermal parameter associated with the Mg\(^{2+}\) at site 3 is higher than that of its counterpart in the control complex (30 versus 26 Å\(^2\)). The Mg\(^{2+}\) cation may not fully occupy site 3. (As defined more clearly in the high pH complex below, Mg\(^{2+}\) could occupy a site near Glu\(^{286}\) at low occupancy, and not be resolved from the electron density associated with the water molecule that hydrogen bonds with Glu\(^{286}\) and coordinates the Mg\(^{2+}\) at site 3. See Fig. 2, B and C.) Further indications of weakened interactions involving Mg\(^{2+}\) at site 3 are an increase in the coordination distance to the oxygen atom of the phosphoryl species and the concomitant decrease in the donor-acceptor distance to Arg\(^{276}\) of that same oxygen atom (Fig. 2B).

The electron density associated with the ligand at the 1-phosphoryl pocket is a distorted tetrahedron. An elongated teardrop of electron density extends from a plane of electron density. Metaphosphate fits the planar density well, and a water molecule (perhaps representing a molecule of hydroxide) fits equally well to the teardrop of electron density. Thermal parameters of the metaphosphate molecule and its associated water/hydroxide molecule, both refined at full occupancy, are comparable to those of the ligating Mg\(^{2+}\) and nearby side chains. The water molecule is 2.35 Å away from the phosphorus
atom of metaphosphate, being coordinated to the magnesium cations at sites 2 and 3, and is on the verge of hydrogen bonding to the side chain of Asp74 (Fig. 2B). The electron density probably represents an equilibrium mixture of orthophosphate and \( \text{PO}_4^{3-}/\text{H}_2\text{O}^+ \). High pH Complex (PDB: 1NUW)—Glu\(^{97} \), which in the control and high K\(^+ \) complexes coordinates magnesium cations at sites 2 and 3, now bridges the magnesium cations at sites 1 and 2. The Mg\(^{2+} \) at site 1 is at least 5-coordinated and the Mg\(^{2+} \) at site 2 remains six-coordinated (Figs. 1C and 2C). As in the high K\(^+ \) complex, the 1-OH group of F6P migrates from site 4 to site 3. D, product complex. The crystal structure of the control complex represents the end-state of the reaction.

![Graph](https://example.com/image.png)

**Fig. 2.** Distance relations between selected atoms in the active site. A, control complex. B, high K\(^+ \) complex. C, high pH complex. The dotted outline in panel C represents a channel of electron density that extends between metal sites 3 and 4, which in the refined model is represented by a discrete set of water molecules and magnesium cations.

**Fig. 3.** Possible dissociative reaction pathway for FBPase. The coordination spheres of metal cations (M1 through M4) are not shown in full. The Mg\(^{2+} \) at site 2 is six-coordinated, and the coordinating ligands do not change over the proposed reaction pathway. A, initial substrate complex. Mg\(^{2+} \) at site 3 is six-coordinated. As \( \text{PO}_4^{3-} \) forms, negative charge builds on the O-1 atom of F16P, and Glu\(^{97} \) leaves the coordination sphere of M1. B, \( \text{PO}_4^{3-}/\text{H}_2\text{O}^+ \) complex. Metaphosphate is present, and Asp\(^{74} \) abstracts a proton from the water molecule bound to the Mg\(^{2+} \) at site 4. The events of panel B may precede those of panel A, or occur in synchrony with those of panel A. C, \( \text{PO}_4^{3-}/\text{OH}^-/\text{H}_2\text{O}^+ \) complex. Mg\(^{2+} \) migrates from site 4 to site 3. D, product complex. The crystal structure of the control complex represents the end-state of the reaction.
3 and 4. A similar conformational change in Glu97 occurs in Mg2+/Tl complexes, in which Tl1 at site 4 displaces Mg2+ at site 3 (40). Glu98 coordinates to Mg2+ at site 4, whereas Asp68 coordinates to Mg2+ at site 3, but the binding of metal cations to sites 3 and 4 are probably mutually exclusive, as they are only 2.5 Å apart. Thermal parameters for Mg2+ at sites 3 and 4 are 23 and 21 Å2, respectively, with fractional occupancies of 0.6 and 0.3. The electron density at the 1-phosphoryl pocket appears as an elongated teardrop extending from a plane (Fig. 1C). A molecule of metaphosphate, distorted from planarity, and a water molecule (or hydroxide anion) provide the best fit to the electron density. The oxygen atom of the water molecule is 3.09 Å from the phosphorus atom of the metaphosphate, and is within the coordination spheres of the magnesium cations at sites 2 and 4 (or 3), and within hydrogen bonding distance of Asp74 (Fig. 2C). As in the high K+ structure, the electron density at the 1-phosphoryl pocket is consistent with an equilibrium mixture of orthophosphate and PO3/OH−.

**DISCUSSION**

The control complex may represent a step on the reaction pathway, but it almost certainly does not represent the central kinetic complex (the interconversion of F16P2 and F6P/Pi at the active site). Liu and Fromm (22) determined a value of 2 for the equilibrium constant of the central kinetic complex. The electron density is consistent, however, with the presence of only F6P and orthophosphate. Conditions of crystallization and packing interactions of the crystal could perturb the equilibrium constant of the central complex in favor of products, or the packing interactions of the crystal could perturb the equilibrium mixture of orthophosphate and PO3/OH−.

The arrangement of catalytic side chains and ligands in the active site of FBPase, however, are also consistent with a dissociative pathway, and indeed FBPase can generate metaphosphate and the hydroxide anion from orthophosphate. To the best of our knowledge, the high pH and high K+ complexes reported here are the first direct observations of metaphosphate in a condensed phase. FBPase in its crystalline complex catalyzes the second step of a dissociative mechanism; however, the formation of F16P2 from PO3 and F6P (the first step of a dissociative mechanism) may be inaccessible because of the mutual rotation of the plane of metaphosphate and the 1-OH group of F6P away from in-line geometry (Fig. 1, B and C). Evidently, the crystalline complex is dead-end with respect to the overall reaction, but clearly the active site of FBPase can at least equalize the free energies of bound meta- and orthophosphate. Variations in the kinetic and/or catalytic mechanisms due to changes in pH, chemical composition and/or conformation of the substrate, and to the type of metal activator (23–28), may stem from differences in the rate-limiting step of a dissociative pathway and/or a change in the type of pathway (dissociative versus associative).

The presence of Mg2+ cations at mutually exclusive loci (sites 3 and 4) and the alternative ligation of cation sites by Glu97 suggest the possibility of significant change in the active site during the course of the reaction. The Mg2+ at site 4, a cation-binding site identified in Mg2+/Tl complexes of FBPase (40), may in fact play a direct role in catalysis (Fig. 3). In F16P2 complexes of FBPase, Mg2+ may appear initially at sites 1, 2, and 4. Stereoinversion of the 1-phosphoryl group may favor the migration of Mg2+ from site 4 to 3. Hence, in all crystalline complexes of P1 and F6P, Mg2+ (or Zn2+) is at site 3, whereas in F16P2 complexes the preferred binding site for Mg2+ may be site 4. Asp74 then could activate a water molecule coordinated to magnesium cations at sites 2 and 4 for a nucleophile attack on the metaphosphate anion (Fig. 3).

A change in metal cation coordination by Glu97 could facilitate the putative migration of Mg2+ from site 4 to 3 during catalysis. In a dissociative pathway, charge builds on the O-1 atom of F16P2, and as a consequence the Mg2+ at site 1 may release Glu97 from its crowded coordination sphere. A modest conformational change puts Glu97 into the nascent coordination sphere of metal site 3, which in turn promotes the migration of the cation (with its attached hydroxide anion) from site 4 to 3 (Fig. 3). The migration of Mg2+ between sites 3 and 4 would account for the significance of both Asp68 (which coordinates Mg2+ at site 3) and Glu98 (which coordinates Mg2+ at site 4) in catalysis. Mutations of Glu98 (36) and Asp68 each reduce catalytic rates of FBPase by orders of magnitude under comparable conditions of assay. The dissociative pathway, as in Fig. 3, replaces the double proton transfer of the associative pathway, with the migration of a metal-bound hydroxide anion.

The loss of Mg2+ cooperativity at pH 9.6 in FBPase kinetics may simply reflect a change in the rate-limiting step of a dissociative pathway. Mutations of Asp68 eliminate Mg2+ cooperativity at pH 7 and thereby implicate the cation in site 3 in cooperative phenomenon. The generation of the hydroxide anion at pH 7 may require the participation of Asp74 and metal cations at sites 2 and 4 (or 3), whereas at pH 9.6 the generation of the hydroxide anion may occur with less involvement from the active site. Hence, the formation of metaphosphate may be limiting at pH 9.6, whereas the formation of hydroxide anion may be limiting at pH 7.

The hydrolisis of most phosphate esters in organic model systems occurs by a dissociative mechanism (3–6), and the same chemistry may occur in the active site of FBPase. In fact, Herschlag and Jencks (37) present a scheme (Chart II or III) that is strikingly similar to the complexes reported here in the relative placement of Mg2+, a metal-coordinated hydroxide ion and a planar intermediate. Furthermore, there are striking parallels between the active sites of FBPase and alkaline phosphatase (38), suggesting that the chemistry of model systems is broadly applicable to phosphatases. Ancestral phosphatases long ago may have commandeered the dominant reaction pathway in solution, but through evolution the free-energy landscape of the reaction coordinate may now place the associative and dissociative pathways on a near equal footing. Hence, small perturbations in the relative free energies of transition states, due to changes in metal cofactors, pH, chemical composition of the substrate, and/or conditions of crystallization may leverage profound effects on the catalytic mechanism.

**REFERENCES**

1. Admiraal, S. J., and Herschlag, D. (1995) Chem. Biol. 2, 729–739
2. Knowles, J. R. (1980) Annu. Rev. Biochem. 49, 877–910
3. C. V. Inacio and R. B. Honzatko, unpublished data.
3. Westheimer, F. H. (1981) *Chem. Rev.* **81**, 313–326
4. Westheimer, F. H. (1987) *Science* **235**, 1173–1178
5. Hengge, A. C. (1998) *Comprehensive Biological Catalysis. A Mechanistic Reference*, Vol. 1, pp. 517–542, Academic Press, San Diego, CA
6. Vincent, J. B., Crowder, M. W., and Averill, B. A. (1992) *Trends Biochem. Sci.* **17**, 105–110
7. Butcher, W. W., and Westheimer, P. H. (1955) *J. Am. Chem. Soc.* **77**, 2420–2424
8. Barnard, D. W. C., Bunton, C. A., Llewellyn, D. R., Oldham, K. G., Silver, B. L., and Vernon, C. A. (1955) *Chem. Ind.* (Lond.) **760–763
9. Henchman, M., Viggiano, A. A., Paulson, J. F., Freedman, A., and Wormhoudt, J. (1985) *J. Am. Chem. Soc.* **107**, 1453–1455
10. Calvo, K. C., and Berg, J. M. (1984) *J. Am. Chem. Soc.* **106**, 4202–4204
11. Hengge, A. C., Edens, W. A., and Elsing, H. (1994) *J. Am. Chem. Soc.* **116**, 5045–5049
12. Jankowski, S., Quin, L. D., Paneth, R., and O'Leary, M. H. (1994) *J. Am. Chem. Soc.* **116**, 5045–5049
13. Du, X., Heinz, F., and Kim, S-H. (2000) *J. Biol. Chem.* **275**, 8492–8500
14. Guthrie, J. P. (1977) *Ann. Intern. Med.* **86**, 771–777
15. Wu, Y, and Houk, K. N. (1993) *J. Am. Chem. Soc.* **115**, 3991–4000
16. Wright, S. W., Carlo, A. A., Carty, M. D., Danley, D. E., Hageman, D. L., Karam, G. A., Levy, C. B., Mansour, M. N., Mathiowetz, A. M., McClure, L. D., Nestor, N. B., and Fromm, H. J. (2002) *J. Med. Chem.* **45**, 3865–3877
17. DeFronzo, R. A. (1999) *Ann. Intern. Med.* **131**, 281–303
18. Zhang, Y., Liang, J-Y., Huang, S., and Lipscomb, W. N. (1983) *J. Biol. Chem.* **258**, 609–624
19. Nimmo, H. G., and Tipton, K. F. (1975) *Eur. J. Biochem.* **58**, 575–585
20. Stone, S. R., and Fromm, H. J. (1986) *Biochemistry* **25**, 620–625
21. McGrane, M. M., El-Maghrabi, M. R., and Pilkus, S. J. (1985) *J. Biol. Chem.* **258**, 10445–10454
22. Liu, F., and Fromm, H. J. (1988) *J. Biol. Chem.* **263**, 9122–9128
23. Benkovic, S. J., and de Maine, M. M. (1982) *Adv. Enzymol. Relat. Areas Mol. Biol.* **53**, 45–82
24. Tewari, G. A. (1983) *Adv. Enzymol. Relat. Areas Mol. Biol.* **54**, 121–124
25. Zhang, R., Villeret, V., Lipscomb, W. N., and Fromm, H. J. (1996) *Biochemistry* **35**, 3038–3043
26. Liu, F., and Fromm, H. J. (1990) *J. Biol. Chem.* **265**, 7401–7406
27. Frey, W. A., Fischbein, R., de Maine, M. M., and Benkovic, S. J. (1977) *Biochemistry* **16**, 2479–2484
28. Domanico, P. L., Rahil, J. F., and Benkovic, S. J. (1986) *Biochemistry* **25**, 620–625
29. Choe, J-Y., Honzatko, R. B., and Fromm, H. J. (2000) *Biochemistry* **39**, 8565–8574
30. Choe, J-Y., Poland, B. W., Fromm, H. J., and Honzatko, R. B. (1998) *Biochemistry* **37**, 11441–11450
31. Kurbanov, P. T., Choe, J-Y., Honzatko, R. B., and Fromm, H. J. (1998) *J. Biol. Chem.* **273**, 17511–17516
32. Owens, Z., and Minor, W. (1997) *Methods Enzymol.* **276**, 307–326
33. Sheldrick, G. M., and Schneider, T. R. (1997) *Methods Enzymol.* **277**, 319–343
34. McRee, D. E. (1992) *J. Mol. Graphics* **10**, 44–46
35. Moffatt, E. A., and Bacon, D. J. (1997) *Methods Enzymol.* **277**, 505–524
36. Woychik, R., and Schulte, G. K., Steele, C. W., and Treadway, J. L., Wang, I-K., and Bauer, P. H. (2002) *J. Biol. Chem.* **277**, 1303–1311
37. Kraulis, P. J. (1991) *J. Appl. Crystallogr.* **24**, 946–950
38. Choe, J-Y., Nelson, S. W., Fromm, H. J., and Honzatko, R. B. (2003) *J. Biol. Chem.* **278**, 16008–16014

**Metaphosphate**
