Minireview

The Structural Basis of the Myosin ATPase Activity*

Ivan Rayment‡

From the Institute for Enzyme Research and Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53705

Myosin is an ATPase that converts chemical energy into directed movement and can be viewed as a molecular motor. This protein comes in many shapes and sizes. Over 11 classes of myosin have been identified, and it is anticipated that more will be found as the search continues (2). Indeed it has been recognized in one form or another in every eukaryotic cell examined. The common feature of all of these molecules is a section close to the N terminus that can be identified as a motor domain.

Over the years considerable effort has been devoted to determining the chemical and physical basis of the energy conversion by myosin. All of the isoforms examined so far exhibit similar kinetic strategies and share common features of the cycle that converts chemical energy into directed movement. The key features of this process were identified 25 years ago by Lymn and Taylor (3). Contrary to initial expectations, ATP hydrolysis in myosin is not coincident with the force-generating step (3, 4). Instead, ATP binding initially reduces the affinity of myosin for actin, after which hydrolysis of ATP occurs rapidly and results in a metastable ternary complex between myosin, ADP, and inorganic phosphate (P_i). In this state, the equilibrium complex between ATP and ADP·P_i is between 1 and 10 for skeletal muscle (5–7). During this time there is rapid interconversion between substrate and products (5, 8). Release of the hydrolysis products from myosin is catalyzed by the rebinding of myosin to actin. The energy transduction step occurs during product release (4).

Thus myosin is an unusual enzyme in that the chemical step occurs at a different point in the contractile cycle from the energy transduction event. This most likely arose because myosin spends a very small time attached to actin (9). This feature presents several interesting biochemical problems. How is the hydrolysis of ATP coupled to energy transduction, how does myosin catalyze the hydrolysis of ATP, and what is the physical basis of the metastable state? Insight into the structural foundation of these questions has been provided recently by the structure determinations of the motor domain of *Dictyostelium discoideum* myosin II complexed with several nucleotides together with the earlier studies on chicken skeletal myosin subfragment-1 (10–14).

Structure of the Myosin Head

The structure of chicken skeletal myosin subfragment-1 revealed the overall organization of the myosin heavy chain and its two associated light chains. It is a highly asymmetric molecule (Fig. 1) where the thick part of the molecule is assembled from the heavy chain and the light chains wrap around a long α-helix to form an extended motif (10). The molecule is characterized by several deep clefts and pockets, one of which forms the nucleotide binding site. This was initially identified (in the absence of nucleotide) from the location of the phosphate binding loop and position of amino acid residues implicated by chemical modification (15, 16). A second prominent cleft splits the myosin head into two major domains and extends from the nucleotide binding site to the actin binding interface. It was proposed that domain movements associated with this cleft provide the communication route between the nucleotide site and the actin interface during the contractile cycle (11). The organization of the myosin head also suggested that the globular part of the myosin head formed a catalytic domain and that the light chain binding motif served to transduce, amplify, and moderate the conformational changes induced by ATP hydrolysis and interaction with actin. This role for the light chains has been confirmed by *in vitro* motility measurements on fragments that lack one or both of the light chains (17–19). Removal of the light chains generates a catalytic fragment that retains much of the enzymatic activity of myosin (18, 20). These observations confirm the autonomous, yet coupled, nature of these regions of the myosin head.

The catalytic domain of *D. discoideum* myosin II, S1Dc,[1] in contrast to most other myosins, crystallizes readily in forms suitable for high resolution structural analyses. So far the structures of S1Dc have been reported in the presence of Mg-PP_i, MgADP·BeF_x,[2] MgADP·AlF_4, and MgADP·VO_4 (12–14). These have defined the nucleotide binding site and provided a structural basis for catalysis. These complexes were investigated because extensive biochemical and physiological studies have demonstrated that these correspond to distinct points in the contractile cycle (21–26). Vanadate, beryllium fluoride, and aluminum fluoride have been studied extensively as analogs of the γ-phosphate of ATP (1, 21–23). The complexes fall into two conformational states represented by the beryllium and vanadate complexes discussed here.

Nucleotide Binding Site

The nucleotide binding site is located in a pocket at the interface of the 25-kDa N-terminal and the central 50-kDa tryptic fragments (Figs. 1 and 2). The nucleotide lies parallel to the plane of a seven-stranded β-sheet, where the α- and β-phosphates interact with the P-loop and a loop from the 50-kDa segment. In all of the complexes examined so far (MgADP·BeF_x·S1Dc, MgADP·AlF_4·S1Dc, and MgADP·VO_4·S1Dc) MgADP lies in a very similar position such that the major differences are observed around the putative location of the γ-phosphate and the C-terminal section of the protein. In contrast to the G-proteins, there are very few interactions between

[1] The abbreviations used are: S1Dc, *D. discoideum* myosin motor domain; S1, subfragment-1; MgADP·BeF_x·S1Dc, the beryllium fluoride-ADP complex of *D. discoideum* myosin motor domain; MgADP·AlF_4·S1Dc, the aluminum fluoride-ADP complex of *D. discoideum* myosin motor domain; MgADP·VO_4·S1Dc, the vanadate-ADP complex of *D. discoideum* myosin head.

[2] The exact composition of the beryllium fluoride species is unknown. Based on nmr evidence it appears to be a mixture of hydroxyfluoroberyllates (1).
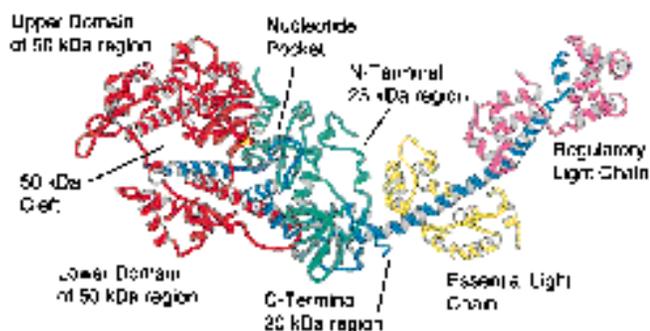
FIG. 1. **Ribbon representation of chicken skeletal myosin subfragment-1 showing the major domains and tryptic fragments.** Figs. 1–4 were prepared with the program Molscript (44).
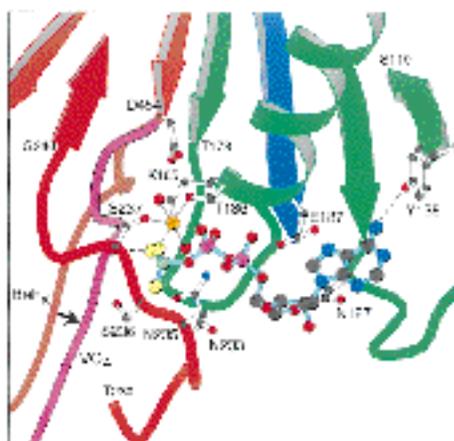

FIG. 2. **Expanded view of the nucleotide binding site of MgADP·BeF$_x$·S1Dc where the MgADP·beryllium fluoride complex mimics MgATP in a prehydrolysis state.** This shows the relationship of the phosphate binding loop to the nucleotide and the conserved ligands that coordinate the divalent cation. The N-terminal, central, and C-terminal sections of the polypeptide chain are colored in *green*, *red*, and *blue*, respectively. Carbon, nitrogen, oxygen, fluorine, phosphorus, and magnesium atoms are depicted in *black*, *blue*, *red*, *yellow*, *pink*, and *orange*, respectively. Shown in *magenta* is Asp[454]-Lys[462] of MgADP·VO$_4$·S1Dc, which illustrates the conformational change associated with binding a transition state analog in the γ-phosphate binding pocket.

the adenosine moiety of the nucleotide and the protein that discriminate between adenosine and other bases. There are, however, extensive interactions between the α- and β-phosphates and the analog of the γ-phosphate (BeF$_x$, AlF$_4^-$ and VO$_4^{3-}$) that are strikingly similar to those seen in other proteins that utilize a P-loop to coordinate a nucleotide (27). The magnesium ion is octahedrally coordinated to two protein ligands, two water molecules, one oxygen from the β-phosphate, and one atom from the analog of the γ-phosphate.

In the beryllium fluoride complex, the BeF$_x$ moiety forms a coordination complex with ADP that adopts the anticipated configuration of ATP prior to hydrolysis. The beryllium atom is tetrahedrally coordinated to three fluorine ligands and a terminal oxygen on the β-phosphate with a geometry close to that of ATP. The beryllium fluoride complex mimics a prehydrolysis state and has an overall protein conformation that is remarkably similar to that seen in chicken skeletal myosin subfragment-1 (10, 12, 13).

In contrast the vanadate complex exhibits a different conformation around the γ-phosphate binding site that is propagated to the C-terminal region of the molecule. This results in a major rearrangement of the polypeptide chain beyond Lys-690. The difference is seen as partial closure of the cleft that splits the central section of the myosin heavy chain (Fig. 3). This is
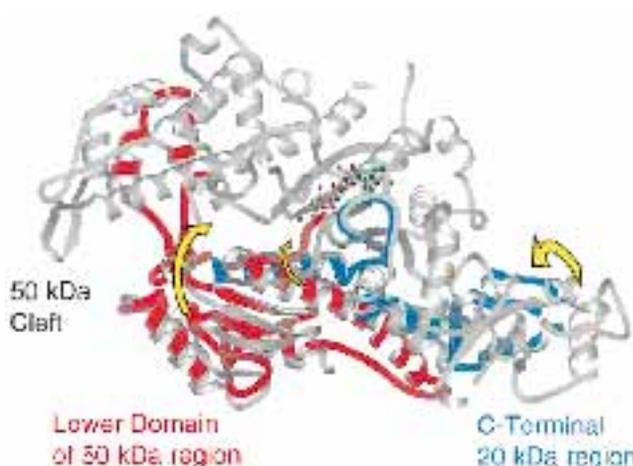

FIG. 3. **Ribbon representation of the movement of the lower 50-kDa domain in the vanadate complex relative to the position in the beryllium fluoride complex.** The beryllium fluoride complex is shown in *gray* whereas the lower domains of the 50-kDa and C-terminal sections of the vanadate complex are shown in *red* and *blue*, respectively. The *arrows* indicate the nature of the conformational differences between these two complexes.

accomplished by changing two backbone conformational angles in the connector region between the upper and lower domains of the 50-kDa region and results in closure of the cleft by 10°. However, the tertiary structure of the individual domains of the MgADP·VO$_4$·S1Dc and MgADP·BeF$_x$·S1Dc complexes remains the same. The conformational change is induced or stabilized by interactions with the trigonal bipyramidal vanadate moiety seen in the γ-phosphate pocket.

The vanadate ion adopts a trigonal bipyramidal coordination in the γ-phosphate binding pocket where three oxygen atoms lie in a plane and the axial positions are occupied by a bridging oxygen from the β-phosphate and an additional water molecule (Fig. 4). The bond distances for the two apical bond V–O distances are 2.3 and 2.1 Å, respectively. These differ significantly from the equatorial V–O bond distances of 1.7, 1.6, and 1.7 Å. The typical single and double bond distances for vanadium are 1.79 and 1.57 Å, respectively (28). Thus the apical bonds to the vanadium ion in MgADP·VO$_4$·S1Dc have a valency that is considerably less than unity, whereas the equatorial bonds as a whole are greater than unity (28). The coordination complex between the vanadate and MgADP is a realistic analog of the metaphosphate-like transition state for phosphoryl transfer. The structure of the vanadate moiety is similar to that seen in the complex of ribonuclease with uridine vanadate (29).

Mechanistic Considerations

As mentioned earlier myosin is an unusual enzyme because it forms a metastable state (ADP·P$_i$) after hydrolysis. In the absence of actin, product release is slow. The structure of the vanadate complex provides an explanation for this state since there is reduced access of solvent to the γ-phosphate pocket caused by partial closure of the cleft. If this conformational change is essential for hydrolysis and attainment of the ADP·P$_i$ state it suggests that the phosphate group will be trapped in the γ-phosphate pocket. Kinetic studies indicate that the phosphate ion is released prior to ADP during the contractile cycle (4, 8). The arrangement of MgADP and the vanadate demands that the phosphate ion cannot leave via the same route that the substrate enters. It is unlikely that the phosphate ion could squeeze past MgADP. Indeed the x-ray structures indicate that phosphate may depart from the bottom of the active site pocket as has been also suggested from modeling ATP in the active site of myosin (30). These structures imply that release of phos-

FIG. 4. **A close-up stereo view of the γ-phosphate binding pocket of MgADP·VO₄·S1Dc onto which ADP·BeFₓ has been superimposed.** The rotation and translation matrices were derived by aligning the protein ligands that coordinate the nucleotide. The magnesium ion, its associated water molecules, and the protein atoms are shown for MgADP·VO₄·S1Dc. For clarity, only the atoms in the ribose diphosphate·BeFₓ are shown since the positions of the protein atoms are not significantly different from those of the vanadate complex. Reproduced with permission from Ref. 14.
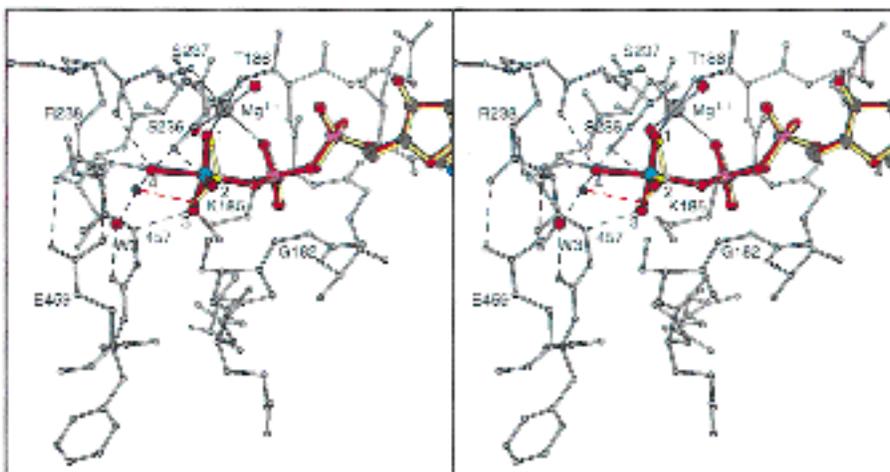
phate must be accompanied by a second conformational rearrangement, which has been proposed to occur when myosin rebinds to actin (31).

Comparison of ADP·BeFₓ with MgADP·VO₄·S1Dc (Fig. 4) provides insight into the structural basis of ATP hydrolysis and the role the conformational change plays in the generation of movement. It is well established that hydrolysis of ATP occurs by attack of a water molecule on the γ-phosphorus (32–34). Examination of the γ-phosphate pocket in both MgADP·BeFₓ·S1Dc and MgADP·VO₄·S1Dc does not reveal any amino acid side chains within 5.5 Å of the beryllium or vanadium that might function as a catalytic base. This suggests that the nucleotide must be the base and that water might transfer its proton directly to the γ-phosphate. A similar mechanism has been proposed for the G-proteins (35–37). Alternatively, the proton transfer might occur via hydrogen exchange with O-γ of Ser-236 that is coordinated to the terminal water molecule on the vanadate and one of the equatorial oxygen atoms. This mechanism would provide better stereochemistry for proton transfer from the incoming water to the γ-phosphate than direct transfer (12).

The overlap of ADP·BeFₓ with MgADP·VO₄·S1Dc (Fig. 4) also shows that the α- and β-phosphates and ribose of these complexes adopt almost identical locations in the phosphate binding pocket. This suggests that the leaving group, MgADP, does not change much as the transition state is approached. The overlap also shows that the three fluorine ligands of BeFₓ and three equatorial oxygen ligands on vanadate lie close to the same plane. This suggests that in proceeding from the ATP ground state to the transition state the oxygen atoms remain close to the observed position of the fluorines and vanadium oxygens while the phosphorus to oxygen distance increases. This fits into the expected picture for phosphate hydrolysis. However, this raises an interesting question of why the BeFₓ adduct does not adopt the conformation observed in the vanadate complex. It is not due to the formation of the additional hydrogen bond between the amide hydrogen on Gly-457 and an equatorial vanadate oxygen, since the same bond could be formed to a fluorine on BeFₓ. A likely reason for differences in conformation adopted by the BeFₓ and vanadate complexes lies in the water structure surrounding these moieties in the γ-phosphate pocket.

There is an approximately tetrahedral distribution of ligands and an extensive hydrogen bond network surrounding the apical water coordinated to the vanadate moiety. This suggests that the axial attack of a water molecule on the γ-phosphorus of ATP would be stabilized by these interactions also. This constellation of interactions that stabilizes the location of the water molecule in the vanadate complex is not available in the beryllium fluoride complex or presumably in the prehydrolysis ATP state due to the different protein conformation. It would appear that the conformation observed in MgADP·VO₄·S1Dc is optimized to orient a water molecule for nucleophilic attack on the γ-phosphorus atom. Conversely this suggests that premature hydrolysis of ATP and loss of hydrolysis products when the apex of the γ-phosphate pocket is open is prevented by the stereochemically restricted environment of the active site.

There has been considerable discussion over the nature of the transition state for phosphoryl transfer in enzymes and in solution (38, 39). In reactions with model compounds in aqueous solution there is compelling evidence for a metaphosphate-like transition state that is essentially dissociative without the formation of metaphosphate itself (40). However, the experiments on model compounds have not necessarily emulated the environment of the active site of an enzyme. In particular it is difficult to simulate the restricted stereochemistry and ionic configuration provided by an enzyme nucleotide binding site. Consequently the situation is less clear for enzymatically catalyzed reactions. The structure of MgADP·VO₄·S1Dc provides a glimpse of the environment that is likely to surround a metaphosphate-like intermediate at the transition state. For myosin it would appear that there is no difference in the environment or structure of the ligands that coordinate ADP in either MgADP·BeFₓ·S1Dc or MgADP·VO₄·S1Dc. This suggests that the leaving group (ADP) experiences a very similar environment in the ground and transition state. Rather it seems that hydrolysis requires a conformational change in the protein that orients the nucleophilic water for attack on the γ-phosphate. This structure appears to be more consistent with greater bond order to the attacking and leaving groups in the transition state for hydrolysis than might be predicted for a purely dissociative mechanism. This is consistent with the conclusions derived from a detailed study of phosphoglucomutase (41, 42).

Conclusion

The structural studies on S1Dc confirm the value of phosphate analogs as models for understanding phosphoryl transfer. They demonstrate that beryllium fluoride is an analog of phosphate whereas aluminum fluoride and vanadate are analogs of the transition state for phosphoryl transfer. The coordination complex between the vanadate moiety and ADP is a particularly realistic analog of the transition state for phosphoryl transfer. In combination with biochemical and physical studies these structures provide insight into those factors responsible for myosin's enzymatic activity. There remain, however, many questions concerning the structural connection be-

tween the ATPase activity and the generation of movement. Initially it was suggested that closure of the nucleotide binding pocket was the source of the conformational change that underlaid this process (11). The studies of *Dictyostelium* myosin show that this is not the case. Rather it appears that the conformational change that occurs in the γ-phosphate pocket is transmitted to the light chain binding motif through the C-terminal region of the molecule that contains the reactive sulfhydryl groups. The present hypothesis suggests that attainment of the metastable ADP·P$_i$ state is coupled to a conformational change that is reversed on rebinding to actin and is ultimately responsible for the energy transduction, in which case the interactions between the light chains and the motor domain become critical for the energy transduction process and may account in part for the participation of the light chains in the regulation of the myosin ATPase activity. This is a fascinating aspect of the myosin molecule as revealed by the structure of the light chain binding motif of scallop muscle (43). Unfortunately space limitations preclude an adequate treatment of the role of the myosin light chains in energy transduction and regulation.

REFERENCES

1. Henry, G. D., Maruta, S., Ikebe, M., and Sykes, B. D. (1993) *Biochemistry* **32,** 10451–10456
2. Mooseker, M. S., and Cheney, R. E. (1995) *Annu. Rev. Cell Dev. Biol.* **11,** 633–675
3. Lymn, R. W., and Taylor, E. W. (1971) *Biochemistry* **10,** 4617–4624
4. Goldman, Y. E. (1987) *Annual Reviews of Physiology* **49,** 637–654
5. Bagshaw, C. R., and Trentham, D. R. (1973) *Biochem. J.* **133,** 323–328
6. Bagshaw, C. R., and Trentham, D. R. (1974) *Biochem. J.* **141,** 331–349
7. Taylor, E. W. (1977) *Biochemistry* **16,** 732–740
8. Trentham, D. R., Eccleston, J. F., and Bagshaw, C. R. (1976) *Q. Rev. Biophys.* **9,** 217–281
9. Spudich, J. A. (1994) *Nature* **372,** 515–518
10. Rayment, I., Rypniewski, W. R., Schmidt-Bäse, K., Smith, R., Tomchick, D. R., Benning, M. M., Winkelmann, D. A., Wesenberg, G., and Holden, H. M. (1993) *Science* **261,** 50–58
11. Rayment, I., Holden, H. M., Whittaker, M., Yohn, C. B., Lorenz, M., Holmes, K. C., and Milligan, R. A. (1993) *Science* **261,** 58–65
12. Fisher, A. J., Smith, C. A., Thoden, J., Smith, R., Sutoh, K., Holden, H. M., and Rayment, I. (1995) *Biochemistry* **34,** 8960–8972
13. Smith, C. A., and Rayment, I. (1995) *Biochemistry* **34,** 8973–8981
14. Smith, C. A., and Rayment, I. (1996) *Biochemistry* **35,** 5404–5417
15. Yount, R. G., Cremo, C. R., Grammer, J. C., and Kerwin, B. A. (1992) *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **336,** 55–61
16. Rayment, I., Smith, C. A., and Yount, R. G. (1996) *Annu. Rev. Physiol.* **58,** 671–702
17. Uyeda, T. Q. P., and Spudich, J. A. (1993) *Science* **262,** 1867–1870
18. Itakura, S., Yamakawa, H., Toyoshima, Y. Y., Ishijima, A., Kojima, T., Harada, Y., Yamnagida, T., Wakabayashi, T., and Sutoh, K. (1993) *Biochem. Biophys. Res. Commun.* **196,** 1504–1510
19. Lowey, S., and Trybus, K. M. (1995) *Biophys. J.* **68,** 120S–127S
20. Woodward, S. K. A., Geeves, M. A., and Manstein, D. J. (1995) *Biochemistry* **34,** 16056–16064
21. Goodno, C. C., and Taylor, E. W. (1982) *Proc. Natl. Acad. Sci. U. S. A.* **79,** 21–25
22. Phan, B., and Reisler, E. (1992) *Biochemistry* **31,** 4787–4793
23. Maruta, S., Henry, G. D., Sykes, B. D., and Ikebe, M. (1993) *J. Biol. Chem.* **268,** 7093–7100
24. Wilson, G. J., Shull, S. E., and Cooke, R. (1995) *Biophys. J.* **68,** 216–226
25. Dantzig, J. A., and Goldman, Y. E. (1985) *J. Gen. Physiol.* **86,** 305–327
26. Chase, P. B., Martyn, D. A., Kushmerick, M. J., and Gordon, A. M. (1993) *J. Physiol.* **460,** 231–246
27. Smith, C. A., and Rayment, I. (1996) *Biophys. J.* **70,** 1590–1602
28. Brown, I. D., and Wu, K. K. (1976) *Acta Crystallogr. B* **32,** 1957–1959
29. Wlodawer, A., Miller, M., and Sjolin, L. (1983) *Proc. Natl. Acad. Sci. U. S. A.* **80,** 3628–3631
30. Yount, R. G., Lawson, D., and Rayment, I. (1995) *Biophys. J.* **68,** 44s–49s
31. Fisher, A. J., Smith, C. A., Thoden, J., Smith, R., Sutoh, K., Holden, H. M., and Rayment, I. (1995) *Biophys. J.* **68,** 19s–28s
32. Sleep, J. A., Hackney, D. D., and Boyer, P. D. (1980) *J. Biol. Chem.* **255,** 4094–4099
33. Webb, M. R., and Trentham, D. R. (1981) *J. Biol. Chem.* **256,** 10910–10916
34. Dale, M. P., and Hackney, D. D. (1987) *Biochemistry* **26,** 8365–8372
35. Sondek, J., Lambright, D. G., Noel, J. P., Hamm, H. E., and Sigler, P. B. (1994) *Nature* **372,** 276–279
36. Schweins, T., Langen, R., and Warshel, A. (1994) *Nat. Struct. Biol.* **1,** 476–484
37. Schweins, T., Geyer, M., Scheffzek, K., Warshel, A., Kalbitzer, H. R., and Wittinghofer, A. (1995) *Struct. Biol.* **2,** 36–44
38. Westheimer, F. H. (1987) *Science* **235,** 1173–1178
39. Admiraal, S. J., and Herschlag, D. (1995) *Chem. Biol.* **2,** 729–739
40. Herschlag, D., and Jencks, W. P. (1989) *J. Am. Chem. Soc.* **111,** 7579–7586
41. Deng, H., Ray, W. J., Jr., Burgner, J. W., II, and Callender, R. (1993) *Biochemistry* **32,** 12984–12992
42. Ray, W. J., Crans, D. C., Zheng, J., Burgner, J. W., Deng, H., and Mahroof-Tahir, M. (1995) *J. Am. Chem. Soc.* **117,** 6015–6026
43. Houdusse, A., and Cohen, C. (1996) *Structure* **4,** 21–32
44. Kraulis, P. J. (1991) *J. Appl. Crystallogr.* **24,** 946–950