Mechanism for Coupling Free Energy in ATPase to the Myosin Active Site†

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ABSTRACT: Acrylamide quenching of tryptophan 510 (Trp510) fluorescence in rabbit skeletal myosin subfragment 1 (S1) indicates the conformation of the probe binding cleft, containing the highly reactive thiol (SH1) and Trp510, in the presence of nucleotides or nucleotide analogs trapped in the active site of S1 [Park et al. (1996) *Biochim. Biophys. Acta 1296*, 1−4]. The Trp510 quenching efficiency shows that the probe binding cleft closes slightly in the presence of beryllium fluoride trapped MgADP (MgADPBeF_x-S1) and most tightly in the presence of vanadate trapped MgADP (MgADPVi-S1) with aluminum fluoride and scandium fluoride trapped MgADP (MgADPAlF_4-S1 and MgADPScF_x-S1) falling in between in the order MgADPBeF_x > MgADPAlF_4 > MgADPScF_x > MgADPVi. These nucleotide analogs are identified with sequential structural changes in MgATP during hydrolysis in the same order with beryllium fluoride occurring earliest in the ATPase cycle. Correlation of the separation distance of the γ-phosphate analog metal from the oxygen connecting it to the β-phosphate in ADP, to the extent of cleft closure, suggests that this distance in the nucleotide transition state determines the conformation of the probe binding cleft. Trp510 quenching efficiency was also measured as a function of the base moiety of the vanadate trapped Mg-nucleotide diphosphate (MgNDPVi-S1). The extent of cleft closure is largest in the presence of the natural substrate NDP and follows the order MgADPVi > MgCDPVi > MgUDPVi > MgIDPVi > MgGDPVi with very little difference between MgADPVi and MgCDPVi. These data follow the order of the effectiveness of the corresponding nucleotide triphosphates to support force production in muscle fibers [Pate et al. (1993) *J. Biol. Chem. 268*, 10046−10053]. In both the fiber and S1, it appears that the 6-position amino group of the bases of ADP and CDP is required to properly anchor the nucleotide in the active site, possibly at tyrosine 135 as suggested by X-ray crystallographic studies [Fisher et al. (1995) *Biochemistry 34*, 8960−8972]. Finally, the Trp510 quenching efficiency was measured as a function of the size of the divalent cation trapped in the active site of S1 with ADPVi. These data failed to show a correlation suggesting that the divalent cation is not involved with the propagation of influence from the active site to the probe binding cleft. The forgoing experiments suggest that the changing conformation of ATP during hydrolysis, parameterized by the increasing distance between the β- and the γ-phosphate, stresses the active site of S1 through protein−nucleotide contacts at the γ-phosphate and nucleotide base. The stress-induced strain in the cross-bridge may be the mechanism by which energy in ATP is transferred to the myosin structure.

The mechanism by which myosin transduces chemical energy, liberated by the hydrolysis of ATP, into mechanical work likely involves the movement of energy from the nucleotide to the active site structure and then through the structure of the protein to other sites in myosin (Morales & Botts, 1979). Like others, we search for the consequence of this movement of potential energy in conformation changes in myosin that correlate with ATPase (Botts et al., 1989). One approach in this search is the measurement of static structural parameters of myosin as a function of nucleotide analogs occupying its active site (Fisher et al., 1995; Smith & Rayment, 1996). This approach is meaningful because the nucleotide analogs were shown to mimic transition state complexes of ATP (Werber et al., 1992).

Previously, we observed the conformation of the probe binding cleft in myosin, containing the highly reactive thiol (SH1) and a nucleotide-sensitive tryptophan (Trp510), as a function of bound nucleotide analogs. We showed that this cleft closes incrementally in a one-to-one relationship with the transition state complexes of ATP (Park et al., 1996c). A model for the mechanism of muscle contraction has a portion of the actin-bound myosin cross-bridge rotating during ATP hydrolysis to impel actin and generate force (Rayment et al., 1993). This model, a variation of the traditional rotating cross-bridge model of contraction (Huxley, 1969; Huxley & Simmons, 1971), is supported by data indicating that substrate binding to the active site of myosin causes a change in the shape of the myosin cross-bridge or subfragment 1 (S1) (Wakabayashi et al., 1992; Highsmith & Eden, 1993). We believe that the closing of the probe binding cleft during ATP hydrolysis facilitates this shape change and that the return of the cleft to its shape in the absence of nucleotide is the force-generating step (Park et al., 1996c).

To quantify the movement of the probe binding cleft in myosin, we isolate fluorescence emission from Trp510 and measure its availability to dynamic quenching by acrylamide. We isolate Trp510 emission in the presence of four other tryptophan residues in S1 using the xanthene dye 5′-(iodoacetamido)fluorescein (5′IAF), to specifically modify SH1 in S1 (5′F-S1). Trp510 forms a complex with 5′IAF

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in 5′F-S1 such that the probe totally quenches its emission. The probe is too far from the rest of the tryptophans to affect their emission (Burghardt & Ajtai, 1996; Ajtai & Burghardt, 1995). Since modification of SH1 does not otherwise alter tryptophan emission (Park et al., 1996b), the difference in the tryptophan emission between S1 and 5′F-S1 originates exclusively from Trp510. This method for isolating the Trp510 signal also works in the presence of nucleotide or nucleotide analogs (Park et al., 1996b).

In the present work, we correlate structural features of different natural nucleotides with the probe binding cleft conformation. These features, represented in Figure 1 by A, B, and C, define the regions of interest. Feature A shows *d*, the separation of the γ-phosphate (P_γ, or γ-phosphate analog metal) from the oxygen connecting it to the β-phosphate (P_β) in ATP. We find that cleft movement correlates with *d* such that increasing *d* closes the cleft. Feature B identifies the position of divalent cations linking nonbridging oxygens of P_γ and P_β in ATP, and we find that the ionic radii of the divalent cations do not correlate with cleft movement. Feature C identifies the base of the nucleotide. We find that quenching of Trp510 is strongly dependent on the base of the nucleotide and that the nucleotides supporting force production in muscle fibers (Pate et al., 1993; White et al., 1993) are also the most effective in modulating the probe binding cleft. The latter result clearly demonstrates the association of cleft movement with force production. The findings presented here suggest a mechanism for exchanging energy between ATP and myosin.

MATERIALS AND METHODS

Chemicals. The fluorescent label 5′-(iodoacetamido)fluorescein (5′IAF) was from Molecular Probes (Eugene, OR). Ultrapure acrylamide was from ICN Biomedicals (Cleveland, OH). Gallium chloride ($GaCl_3$) and scandium chloride ($ScCl_3$) were from Aldrich (Milwaukee, WI). The nucleotide diphosphates (NDPs) adenosine 5′-diphosphate (ADP), cytosine 5′-diphosphate (CDP), inosine 5′-diphosphate (IDP), guanosine 5′-diphosphate (GDP), and uridine 5′-diphosphate (UDP), and phenylmethanesulfonyl fluoride (PMSF), ethylenediaminetetraacetic acid (EDTA), ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), dithiothreitol (DTT), sodium orthovanadate (Na_3VO_4), beryllium chloride ($BeCl_2$) aluminum chloride ($AlCl_3$), and sodium fluoride (NaF) were from Sigma (St. Louis, MO). The chloride salts of divalent cations Mg^{2+}, Co^{2+}, Ni^{2+}, and Mn^{2+} were also from Sigma. All chemicals were analytical grade.

Solutions. A stock solution of $BeCl_2$ (Be, atomic absorption standard solution, in 1% HCl) was adjusted to pH 5.0 by addition of NaOH. A stock solution of sodium vanadate was made by the method of Goodno (1979). A stock solution of NaF was prepared freshly each day in a plastic bottle. Our standard buffer was 25 mM TES buffer at pH 7.0, labeling buffer was standard buffer plus 0.2 mM PMSF, and protein buffer was labeling buffer plus 1 mM DTT.

Preparation and Labeling of Myosin Subfragment 1. Rabbit myosin was prepared by a standard method (Tonomura et al., 1966) and S1 by digesting myosin filaments with α-chymotrypsin (Weeds & Taylor, 1975). Probe modification of S1 was carried out with 10−25 μM S1 in labeling buffer with a 1.2-fold molar excess of 5′IAF for 12 h at 4 °C. This procedure specifically modified 60−70% of the S1 at SH1 (Ajtai & Burghardt, 1992). Unreacted dye was not removed from the sample, but the reaction was stopped by the dilution of S1 with the DTT-containing protein buffer. Unlabeled S1 was prepared and handled identically to the labeled protein so that unlabeled and labeled samples had identical protein concentration and their tryptophan fluorescence emission intensities could be directly subtracted. The fluorescence intensities were corrected for the dilution due to the addition of ultrapure acrylamide. The addition of 0.2 M acrylamide to the sample produced a total absorbance of <0.065 at 298 nm and negligible inner filtering of the excitation light.

Preparation of S1−Nucleotide Analog Complexes. Beryllium, aluminum, and vanadate NDP trapped S1 were prepared as described previously (Werber et al., 1992; Park et al., 1996c). Scandium trapped S1 was prepared after Gopal and Burke (1995). For gallium trapped S1, 3−5 μM S1 in 25 mM TES, pH 7.0, was mixed with 1 mM $MgCl_2$, 0.2 mM ADP, 50 mM NaF, and 0.1 mM $GaCl_3$ for 20 min at 25 °C (Park et al., 1996a). Vanadate trapped S1 with other NDPs was prepared by substituting CDP, IDP, GDP, or UDP for ADP. Vanadate trapped S1 in the presence of various divalent cations was prepared by substituting $CoCl_2$, $NiCl_2$, or $MnCl_2$ for $MgCl_2$. With the exception of MgGDPVi and MgIDPVi, analogs trapped S1 with 85% efficiency as measured by S1 K^+EDTA ATPase inhibition. MgGDPVi and MgIDPVi trapped S1 with 60−75% efficiency.

Fluorescence Measurements. We measured fluorescence on an SLM 8000 spectrofluorometer (SLM Instruments, Urbana, IL) with monochromator slits of 2−4 nm. Tryptophans in S1 or labeled S1 were excited with 298 nm light, and we quantitated tryptophan emission using the emitted light intensity at 336 nm or using the area from the emission band integrated from 320 to 354 nm. All fluorescence measurements were made at 6 °C.

We form the difference emission intensity between S1 and 5′F-S1 such that, $\Delta F \equiv F_{S1} - F_{5'F-S1}$, where *F* is the tryptophan fluorescence intensity. In the presence of quencher, Q, ΔF quantifies the accessibility of Trp510 to collisional quenching using the Stern−Volmer equation such that

$$\Delta F_0/\Delta F = 1 + K_{SV}[Q] \quad (1)$$

where ΔF_0 is the fluorescence intensity in the absence of quencher, K_{SV} is the quenching constant, and [Q] is quencher concentration. $\Delta F_0/\Delta F$ from S1 in the presence and absence of nucleotides or nucleotide analogs obeyed eq 1, indicating that quenching occurs on a single accessible chromophore and that either collisional or static quenching occurs (Lakowicz, 1983). We showed earlier that acrylamide is a collisional quencher of Trp510 (Park et al., 1996c).

Quenching data were fitted using a linear least-squares protocol and eq 1. Fitted data had correlation values of more than 0.999, and residuals were randomly distributed about 0.

RESULTS

Effect of Changing d on the Probe Binding Cleft. Identified in Figure 1 is the distance, *d*, from P_γ of ATP to the bridging oxygen. We surmised reasonable values for *d* in transient states of ATP during hydrolysis using estimates for the equivalent length in five nucleotide analogs. Our estimates of *d* and the coordination number and geometry of the nucleotide analogs are listed in Table 1. We obtained

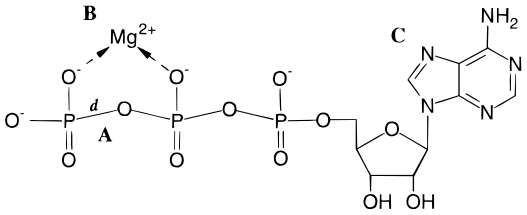

FIGURE 1: ATP molecule showing feature A at the γ-phosphate, feature B the divalent cation, and feature C the base of the nucleotide. Distance, *d*, defines the separation between the γ-phosphate and the bridging oxygen.

Table 1: Coordination Number, Geometry, and Size of Various ATP Analogs

ATP analog[a]	coordination no. and geometry[b] of metal	metal to bridging oxygen distance (*d*) (Å)
MgADPBeF$_x$	4 (td)	1.57[c]
MgADPGaF$_x$	4 (td)[d]	1.82−1.97[e]
MgADPAlF$_4$	6 (Oh)	2.00[c]
MgADPScF$_x$	6 (Oh)	2.06−2.18[f]
MgADPVi	5,6 (tbp,Oh)	2.09,[g] 2.24[h]

[a] The exact stoichiometry of F^- in the aluminum fluoride is known (Maruta et al., 1993), but not so for beryllium fluoride, gallium fluoride, and scandium fluoride. The composition of beryllium fluoride was proposed to be a mixture of hydroxyfluoroberyllates rather than fluoroberyllates (Henry et al., 1993). [b] td = tetrahedral, Oh = octahedral, and tbp = trigonal bipyramidal. [c] Fisher et al. (1995). [d] GaF$_x$ also shows octahedral geometry, but we found no estimate of *d* for this substance. [e] Parise (1985); Burford et al. (1990). [f] Anderson et al. (1973); Atwood & Smith (1974). [g] Smith & Rayment (1996). [h] Scheidt et al. (1971).

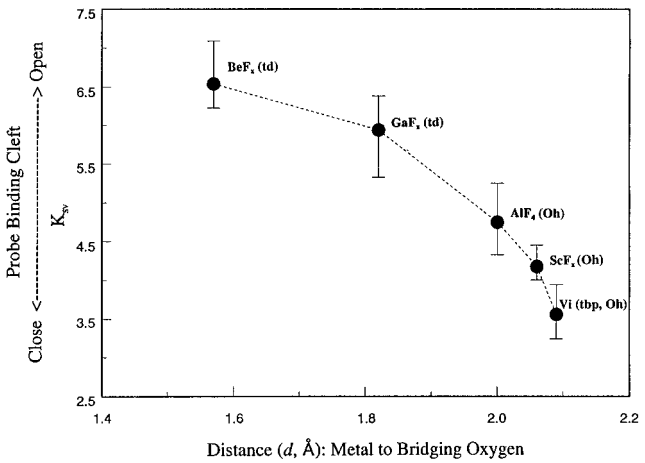

FIGURE 2: Stern−Volmer constant for acrylamide quenching of Trp510 as a function of *d*, the separation between the γ-phosphate analog metal and the bridging oxygen. Equation 1 defines the quenching constant, K_{SV}. The coordination geometry for each nucleotide analog metal is indicated in parentheses where td is tetrahedral, Oh is octahedral, and tbp is trigonal bipyramidal.

this information from crystallographic studies of myosin or inorganic compounds as indicated by the references in the table. We also observed K_{SV} for the quenching of Trp510 using eq 1 as a function of these five nucleotide analogs trapped in the active site of S1. Figure 2 shows K_{SV} for Trp510 in nucleotide analog trapped S1 as a function of *d*. Several of these quenching constants were reported earlier (Park et al., 1996c). These data indicate a correlation between *d* and the conformation of the probe binding cleft. Probe binding cleft closure (identified by a diminished quenching constant K_{SV}) is a structural change accompanying energy transduction, suggesting that *d* may be a changing structural feature of ATP during hydrolysis that is communicated to the ATP binding site of S1 and subsequently to the probe binding cleft.

Table 2: Stern-Volmer Constant for Me^{2+}ADPVi Trapped S1 in the Presence of Varying Divalent Cations

divalent cation	ionic radius (Å)[a]	K_{SV} (M^{-1})[b]
Ni^{2+}	0.83	3.11 ± 0.14 (n = 3)
Mg^{2+}	0.86	3.56 ± 0.33 (n = 5)
Co^{2+}	0.89	2.98 ± 0.08 (n = 3)
Mn^{2+}	0.97	3.31 ± 0.17 (n = 4)

[a] Cotton & Wilkinson (1988). [b] Errors are standard deviation of the mean for n experiments.

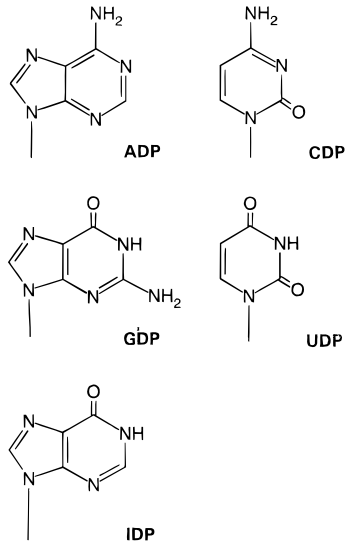

FIGURE 3: Structures of the base parts of the nucleotides ADP, CDP, GDP, UDP, and IDP.

Effect of Divalent Cations on the Probe Binding Cleft. Feature B identifies the divalent cation Mg^{2+} linking oxygens from P_γ and P_β of ATP. The ionic radius of the divalent cation in vanadate trapped S1 does not correlate with the K_{SV} of Trp510. These data, shown in Table 2, indicate that the structural change accompanying energy transduction at Trp510 is not sensitive to the size of the divalent cation. However, previous work indicated that the ionic radius of the divalent cation affected the myosin ATPase and the actin-activated myosin ATPase (Peyser et al., 1996). Our data suggest that the effect of divalent cation ionic radius on myosin ATPase does not work by influencing the movement of the probe binding cleft.

Effect of the Nucleotide Base on the Probe Binding Cleft. Feature C, identified in Figure 1, is the base of a nucleotide. This feature alone is unique to each nucleotide diphosphate (NDP). The structures of the bases from the NDPs used are indicated in Figure 3. We observed K_{SV} for the quenching of Trp510 using eq 1 as a function of the vanadate trapped nucleotide diphosphates (MgNDPVi-S1) ADP, CDP, UDP, IDP, and GDP. Trp510 quenching is strongly dependent on the vanadate trapped NDP in the active site of S1. These data, summarized in Table 3, indicate that the probe binding cleft is most effectively closed by the natural substrate NDP (ADP) with the effectiveness of other NDPs ordered such that ADP > CDP > UDP > IDP > GDP. The effects of ADP and CDP are nearly identical. These data correlate with the effectiveness of the corresponding triphosphate nucleotides to support force production in muscle fibers (Pate et al., 1993; White et al., 1993). We also observed K_{SV} for the quenching of Trp510 as a function of

Table 3: Stern−Volmer Constants for MgNDPVi Trapped S1

NDP	K_{SV} (M^{-1})
GDP	7.35 ± 0.22 ($n = 3$)[a]
IDP	5.87 ± 0.30 ($n = 3$)
UDP	4.45 ± 0.55 ($n = 4$)
CDP	3.87 ± 0.15 ($n = 3$)
ADP	3.56 ± 0.33 ($n = 5$)

[a] Errors are standard deviation of the mean for n experiments.

the NDPs without vanadate (MgNDP-S1, data not shown) and found that K_{SV} is identical for ADP, CDP, UDP, IDP, and GDP in MgNDP-S1.

DISCUSSION

Tryptophan emission intensity from S1 is altered by ATP binding to its active site (Werber et al., 1972). Trp510 is one of the ATP-sensitive tryptophans (Johnson et al., 1991; Hiratsuka, 1992; Bivin et al., 1993; Papp & Highsmith, 1993; Park et al., 1996b,c). The nucleotide-sensitive tryptophan emission showed that the stable complexes of ADP with vanadate, beryllofluoride, or aluminofluoride bound to S1 mimic ATPase intermediates (Werber et al., 1992). Similarly, the stable complexes of ADP with scandium or gallium fluoride bound to S1 also mimic ATPase intermediates (Gopal & Burke, 1995; Park et al. 1996a). The trapped analog states are associated with identifiable steps in the ATPase cycle. If M is myosin, we can summarize myosin ATPase with the scheme:

$$\mathrm{M + ATP \leftrightarrow M^{*}{\cdot}ATP \leftrightarrow M^{**}{\cdot}ADP{\cdot}P_i \leftrightarrow M^{*}{\cdot}ADP + P_i \leftrightarrow M + ADP + P_i} \quad (2)$$

The M*·ATP transient is associated with S1 structure induced by $MgADPBeF_x$ or $MgATP\gamma S$ (Fisher et al., 1995; Phan et al., 1996; Mendelson et al., 1996). The M**·ADP·P_i transient, immediately preceding the rate-limiting phosphate release step (Bagshaw & Trentham, 1974), is associated with S1 structure induced by $MgADPAlF_4$ (Fisher et al., 1995; Ponomarev et al., 1995; Mendelson et al., 1996; Phan et al., 1996) or MgADPVi (Smith & Rayment, 1996). The identification of transients with static trapped analog states means that we can correlate signals originating from Trp510 and the probe binding cleft in nucleotide analog trapped S1 with a particular chemical intermediate in the ATPase cycle. The trapped nucleotide analogs are important tools for investigating the structural changes in S1 accompanying energy transduction since they provide a means to study important transient S1 structures with static structure-determining techniques (Fisher et al., 1995; Smith & Rayment, 1996).

Previously, we used the difference spectrum, formed from fluorescence-detected circular dichroism (FDCD), between S1 and 5′F-S1 to observe the optical activity of the near-ultraviolet transition bands of Trp510 of S1 in the presence and absence of nucleotide analogs (Park et al., 1996b). The optical activity of Trp510 indicated the tertiary structural change in the indole side chain of Trp510 during ATP hydrolysis. Previously, we also observed the quencher accessibility of Trp510, in the presence and absence of nucleotide or nucleotide analogs (Park et al., 1996c). These data implied, using the identification of nucleotide-trapped states with ATPase intermediates, that the probe binding cleft is fully open without nucleotide and closes incrementally during ATP hydrolysis. We proposed that the opening of the probe binding cleft upon nucleotide release is the force-generating step. In the present work, we establish aspects of the molecular mechanism of energy transduction by further study of the movement of the probe binding cleft. Here we correlate structural features of the nucleotide during hydrolysis and while bound to the active site of S1 with the opening and closing of the probe binding cleft.

Specification of the conformation of the γ-phosphate requires the distances separating metal from ligands; the bond angle between P_γ, the bridging oxygen, and P_β ($P_\gamma-O-P_\beta$); the torsion angle $O-P_\gamma-O-P_\beta$; and the geometry of P_γ (for example, tetrahedral or trigonal bipyramidal). Crystallography of S1 supplies some of this information including data on beryllium or vanadate trapped S1 showed differing distances from Be to F in BeF_x or V to O in the vanadate (Fisher et al., 1995; Smith & Rayment, 1996). Additionally, three of the fluoride ligands in BeF_x and three oxygens of the Vi lie close to the same plane in the crystals due to different geometries and similar $P_\gamma-O-P_\beta$ angles in the beryllium and vanadate complexes. However, very limited information is available on the structure of the other analogs. Consequently, we found that we could effectively compare all of the analogs only with d, the distance from P_γ to the bridging oxygen. The results in Figure 2 show the correlation of d with the conformation of the probe binding cleft.

The crystallography of *Dictyostelium discoideum* S1 indicates fluoride from an ATP analog representing a P_γ oxygen forms a hydrogen bond to the NH of the peptide backbone at Gly457 (Gly466 in chicken myosin) (Fisher et al., 1995). This residue is in the DXXG motif, equivalent to 1 of 4 regions that show a conformational change between GTP- and GDP-bound states in the G-protein α-subunit (switch II) (Rayment, 1996), and separated from Trp510 by a 40 residue helical stretch. The increase in d ($+\Delta d$) during ATP hydrolysis and the crystallographic data indicating a contact between P_γ of ATP and Gly466 in the active site suggest to us that Δd is a means of coupling free energy liberated during ATPase to the active site of myosin. We propose that the nucleotide does work, ΔG, on myosin through Δd acting against the force, F, from myosin resisting deformation, i.e., $\Delta G = \Delta dF$. We detected myosin's deformed or strained condition as a perturbation in the conformation of the probe binding cleft. If we model myosin's resistance to deformation as the straining of an ideal spring, then $\Delta G = \Delta dF = -\Delta V$, where $-\Delta V$ is the potential energy increase in the spring representing myosin (Taylor, 1993). Alternatively, we could characterize energy stored in the strained myosin from ΔG (acting through Δd) as conformational free energy (Franks-Skiba et al., 1994). This suggestion does not exclude the possibility that another structural determinant in ATP, such as the change in the terminal phosphate coordination geometry during hydrolysis also indicated in Figure 2, plays a role in coupling additional free energy liberated during ATPase to the active site of myosin.

The importance of the base on the effectiveness of nucleotides to close the probe binding cleft, summarized by the data in Table 3, could be related to the effectiveness of a nucleotide to couple free energy liberated during ATPase to the active site of myosin. Earlier work on the effectiveness of these nucleotides (the triphosphate versions, NTPs) to support contraction in muscle fibers indicated that the presence of an amino group in the base was crucial (Pate et al., 1993; White et al., 1993). There it was shown that the structure of the purine base in GTP and ITP resembles that

in ATP; however, superposition of the bases of CTP and ATP shows a similar spatial location for the 6-position amino group of ATP and the 4-position amino group of CTP (see Figure 3). Constrained energy minimization of these structures can bring the N−N distance between the amino groups of CTP and ATP to within 0.7 Å (Pate et al., 1993).

Crystallography of MgADPBeF_x trapped *Dictyostelium discoideum* S1 shows only one interaction between the base and the amino acid residues around the base moiety in the active site (Fisher et al., 1995), i.e., hydrogen bonding between the highly conserved Tyr135 residue and the nitrogen of the 6-position amino group of the base. The perturbation of the S1 absorption band upon nucleotide binding was interpreted as a tyrosine perturbation in the vicinity of the nucleotide binding pocket (Sekiya & Tonomura, 1967). The specific interaction of the base moiety with the protein to support contraction was studied by the use of synthetic nonnucleotide analogs (Wang et al., 1993). There it was shown that force production required an ortho nitro group at the aryl ring of the nitrophenylamino class of triphosphates, suggesting that the oxygens of the ortho nitro group in the analogs act as hydrogen bond acceptors to form a specific interaction with protein residues. We do not know if the amino group of a physiological base functions as a hydrogen bond donor or acceptor. Generally, the amino groups of ADP and CDP can function as both. The carbonyl of the GDP, UDP, and IDP, that replaces the amino group in CDP and ADP, functions only as a hydrogen bond acceptor (Pate et al., 1993).

The data from Table 3 and the data from earlier work on muscle fibers indicate that cleft closure and force production require the anchoring of the base to the active site of S1. Crystallographic data provide evidence for hydrogen bonding between Tyr135 and the amino group in the nucleotide base, and between Gly466 (and other residues) and P_γ oxygens. Consequently, the increasing *d* during ATP hydrolysis imposes a stress on the S1 structure. A change of shape in S1 resulting from this stress, the strain in S1, perturbs the shape of the probe binding cleft. Assuming myosin's response to this strain is equivalent to the straining of an ideal spring, then some of the free energy liberated during ATPase increases the potential energy of the myosin. Alternatively, we may characterize energy stored in the strained S1 structure as conformational free energy. The strained conformation of S1 produced by ATP hydrolysis is the mechanism by which free energy in ATP is transferred to myosin.

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