Active Site Comparisons Highlight Structural Similarities Between Myosin and Other P-Loop Proteins

Clyde A. Smith and Ivan Rayment
Institute for Enzyme Research and Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53705 USA

ABSTRACT The phosphate binding loop (P-loop) is a common feature of a large number of enzymes that bind nucleotide whose consensus sequence is often used as a fingerprint for identifying new members of this group. We review here the binding sites of nine purine nucleotide binding proteins, with a focus on their relationship to the active site of myosin. This demonstrates that there is considerable conservation in the distribution and nature of the ligands that coordinate the triphosphate moiety. This comparison further suggests that at least myosin and the G-proteins utilize a similar mechanism for nucleotide hydrolysis.

INTRODUCTION

A large number of enzymes capable of nucleotide binding and/or hydrolysis have been shown to contain a short, highly conserved piece of polypeptide, the phosphate binding loop (P-loop) or the Walker A sequence, as originally identified in the β-subunit of F_1-ATPase (Walker et al., 1982; Saraste et al., 1990). Indeed, the consensus sequence for this motif (Gly-X-X-X-Gly-Lys-Thr/Ser) is frequently used as a diagnostic tool for identifying new nucleotide-dependent proteins, as in Rubisco activase (Salvucci et al., 1993), aminoglycoside 3′-phosphotransferases (Martin et al., 1988; Kocabivik and Perlin, 1994), and thymidine kinase (Fry et al., 1986; Saraste et al., 1990). A significant number of structures of these enzymes have now been reported, including adenylate kinase (ADK) (Diederichs and Schulz, 1990; Muller and Schulz, 1992), uridylate kinase (UK) (Muller-Dieckmann and Schulz, 1994), elongation factors Tu (EF-Tu) and G (Berchtold et al., 1993; Kjeldgaard et al., 1993; Ævarsson et al., 1994; Czworkowski et al., 1994), F_1-ATPase (Abrahams et al., 1994), $G_{i\alpha 1}$ (Coleman et al., 1994; Mixon et al., 1995), transducin ($G_{t\alpha}$) (Noel et al., 1993; Sondek et al., 1994), guanylate kinase (GK) (Stehle and Schultz, 1992), myosin (Rayment et al., 1993), *ras* P21 (Pai et al., 1989, 1990), and recA (Story and Steitz, 1992; Story et al., 1992). Recently the structures of the motor domain of *Dictyostelium discoideum* myosin II in the presence of MgADP·BeF_x, MgADP·AlF_4, and magnesium pyrophosphate were added to this list (Fisher et al., 1995b; Smith and Rayment, 1995). (The exact composition of the beryllium fluoride species is unknown (Henry et al., 1993), whereas the aluminum fluoride complex has been shown to contain four equivalent fluorine atoms (Maruta et al., 1993). Consequently, these complexes will be designated as BeF_x and AlF_4^-.) From all of these structures it is clear that although these purine nucleotide-binding proteins exhibit widely varying functions (muscle contraction in myosin, signal transduction in the G proteins, and phosphoryl transfer in adenylate kinase and guanylate kinase), the three-dimensional structure of the P-loop is remarkably similar despite differences in sequence. This similarity is of interest because the topology of these proteins varies considerably.

The structural similarity between the P-loops in many of these proteins has been noted before; however, because a wider variety of purine nucleotide-binding proteins are now available it seemed timely to investigate the possibility that those features observed in the G-proteins are also associated with the binding sites in the other enzymes. We review here a comparison of nine purine nucleotide-binding proteins with a focus on their relationship to the active site of myosin. This reveals that there is considerable conservation in the distribution and nature of the ligands that coordinate the triphosphate moiety. This review focuses on the relationship between the motor domain of myosin II from *D. discoideum* and other nucleotide-dependent enzymes rather than that of chicken skeletal myosin, because the former structures were determined at much higher resolution and contain ATP analogs.

TOPOLOGICAL COMPARISON

The structures chosen for this comparison are listed in Table 1. To identify the structural features that these diverse enzymes have in common, the atomic coordinates of nine purine nucleotide-binding proteins were obtained from the Brookhaven protein data bank (Bernstein et al., 1977), and their active sites and surrounding structures were compared with the motor domain of myosin II from *Dictyostelium discoideum*. The overall molecular structure of the motor domain has a topology identical to that of skeletal myosin S1 structure (Fisher et al., 1995a,b; Rayment et al., 1993)

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Address reprint requests to Dr. Ivan Rayment, Institute for Enzyme Research, University of Wisconsin, 1710 University Avenue, Madison, WI 53785. Tel.: 608-262-0529; Fax: 608-265-2904; E-mail: ivan@enzyme.wisc.edu.

Dr. Smith's present address is Department of Chemistry and Biochemistry, Massey University, Palmerston North, New Zealand.

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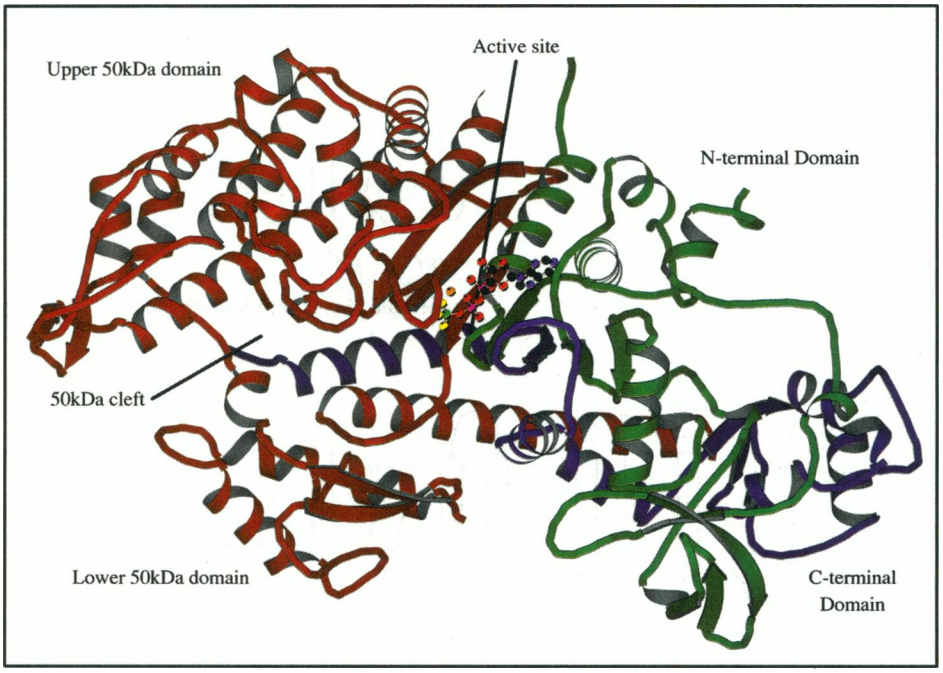

FIGURE 1 Ribbon diagram of the *Dictyostelium* truncated myosin head. The sections equivalent to the three major tryptic fragments of skeletal muscle (Balint et al., 1975; Mornet et al., 1979): the 25-kDa, NH_2-terminal section (Asp 2 to Ala 204); the 50-kDa, central section (Gly 209 to Ala 621); and a truncated fragment of the 20-kDa, C-terminal section (Ala 627 to Ala 759) are depicted in green, red, and blue, respectively. A nucleotide (ADP) has been trapped in the active site by beryllium fluoride, which binds to the bridging oxygen of the β-phosphate and mimics the γ-phosphate (Fisher et al., 1995a,b). Figs. 1, 2, and 4–6 were prepared with Molscript (Kraulis, 1991).

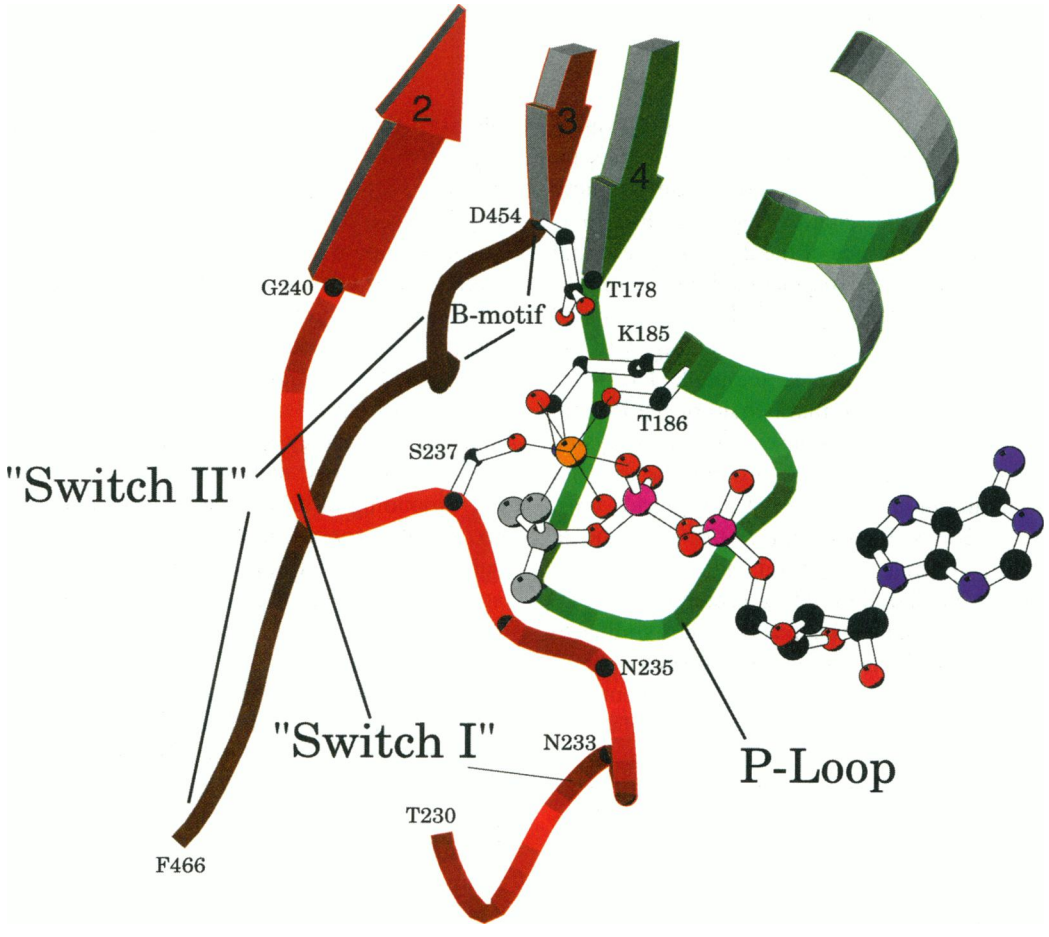

FIGURE 2 Expanded view of the nucleotide binding site of MgADP·BeF_x·S1Dc. This shows the relationship of the P-loop to the nucleotide and the conserved ligands that coordinate the divalent cation. In addition, the regions of the binding site analogous to the loops designated as "switch I" and "switch II" in the G-proteins are indicated. Switch I in myosin has not been shown to undergo a conformational change during the contractile cycle, whereas switch II exhibits a large change. The color scheme for the sections of the polypeptide is similar to that of Fig. 1.

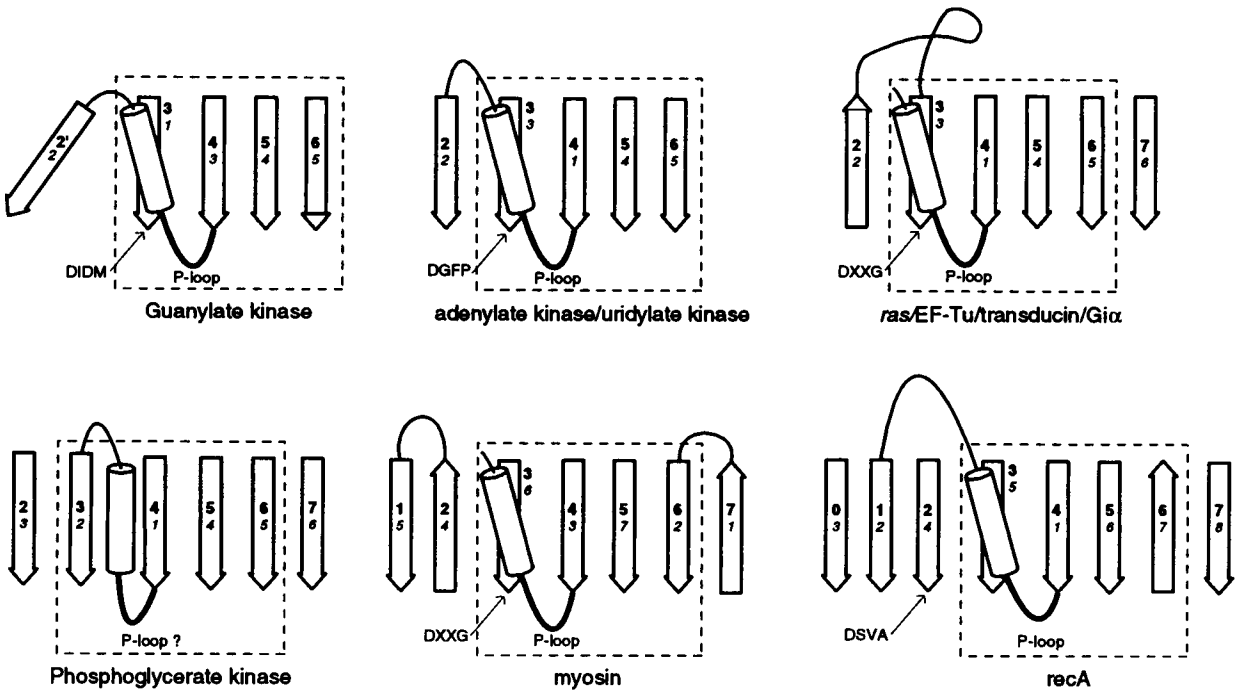

FIGURE 3 Schematic representations of the β-sheet structure of the GTP- and ATP-binding proteins. The strands are numbered according to their position in the sheet (*top number*) and in their topological position in the sequence (*bottom number*).

(Fig. 1). The active site was identified by the position of MgADP and beryllium fluoride. These form a complex on the enzyme that is an analog of ATP, where the BeF_x moiety occupies the location of the γ-phosphate. The P-loop 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, with the α- and β-phosphates interacting with the P-loop and a loop from the 50-kDa segment. The BeF_x moiety does not interact with the main chain of the P-loop, although the side chain of the conserved P-loop lysine residue (Lys 185 in myosin) wraps around the α- and β-phosphates to hydrogen bond to one of the fluoride ions. The P-loop motif joins a β-strand (residues 172–179) and an α-helix (186–200), a tertiary structural motif common to most of these purine nucleotide-binding proteins.

All of the enzymes in Table 1 have active sites similar to that observed in myosin. (It should be noted that elongation factor G, whose structure has been determined recently in two forms (Czworkowski et al., 1994), has a similar six-stranded β-sheet, although only C_α coordinates are available. Likewise, a discussion of the structural relationship between these enzymes and F_1-ATPase is excluded because of the unavailability of coordinates at the present time.) Each contains a β-sheet formed from a varying number of strands, above which the nucleotide is bound. This has been referred to as the *ras*-like domain (Coleman et al., 1994; Noel et al., 1993). The residues forming the β-sheets in these enzymes are given in Table 2. For the purposes of discussion the strands have been numbered according to their position in the sheet in myosin, as viewed from left to right in Figs. 2 and 3, as well as by their location in the primary sequence. This highlights the differences in topology between these enzymes.

The common core of all the β-sheet structures, with the exception of recA and PGK, are four parallel strands corresponding to myosin strands 3, 4, 5, and 6, with the P-loop motif connecting strand 4 with an α-helix that is generally of varying length and running essentially antiparallel to the four strands (Fig. 3). The same four β-strands in recA have a different topology from all the others (three parallel, one antiparallel; Story and Steitz, 1992), although the strand corresponding to myosin strand 4 still connects to an α-helix via the consensus P-loop sequence. The P-loop and subsequent α-helix in phosphoglycerate kinase adopt a substantially different orientation relative to the other enzymes discussed here, even though the P-loop is associated with the same strand.

Of the nine structures in Table 1, only guanylate kinase does not have any additional strands in its central β-sheet structure. It does have a fifth strand, directly following the P-loop helix, which could be comparable to myosin strand 2. However, this strand does not interact with the neighboring strand 3 to form the hydrogen bonding pattern typically seen in β-sheets, but rather runs away from the sheet at almost 90°. Adenylate kinase also has a fifth strand, which in this case does form part of the central β-sheet. The structural comparison of GK with two adenylate kinase structures, apo cytosolic ADK and the AMP complex of mitochondrial ADK (Stehle and Schultz, 1992), shows

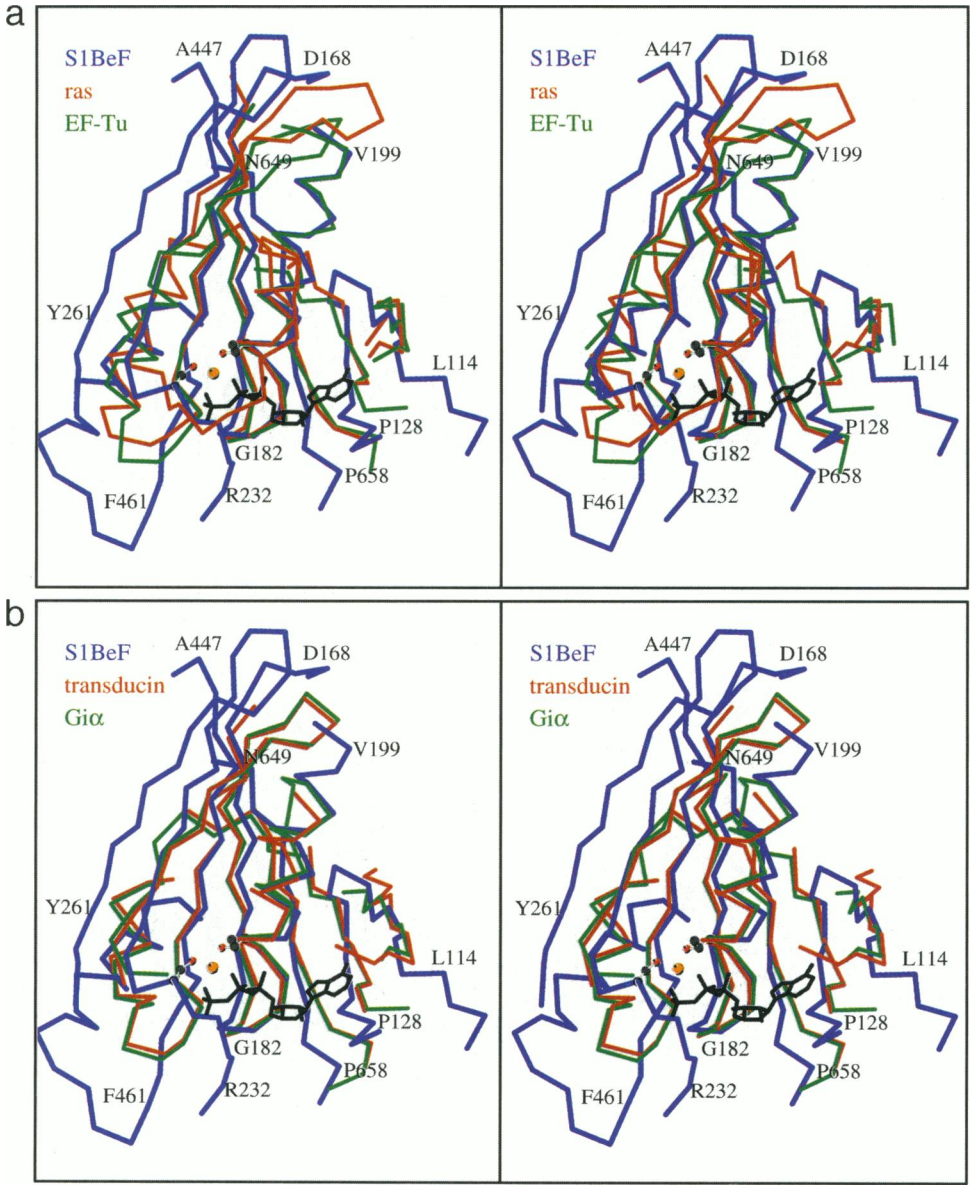

FIGURE 4 Stereoviews of the superpositions of the eight purine nucleotide binding proteins onto *Dictyostelium* myosin S1, based on the four common core strands of the β-sheet (as indicated in Fig. 2), the eight P-loop residues, and the helix following the P-loop. Myosin is shown in blue along with (*A*) *ras* (*red*) and Ef-Tu (*green*); (*B*) transducin $G_{t\alpha}$ (*red*) and $G_{i\alpha 1}$ (*green*); (*C*) ADK (*red*) and GK (*green*); and (*D*) recA (*red*) and PGK (*green*). The nucleotide shown corresponds to MgADP·BeF_x from *Dictyostelium* myosin.

clearly that this strand is in a different orientation in the two molecules. Elongation factor Tu, *ras*, $G_{t\alpha}$, $G_{i\alpha 1}$, and PGK all have six-stranded sheets, with one sheet running antiparallel (the equivalent of myosin strand 2) and the other (strand 7) running parallel, whereas myosin has a seven-stranded sheet, with strands 2 and 7 both running antiparallel and strand 1 running parallel. RecA has the largest β-sheet of all nine structures, with eight strands in all. As noted above, the core strands have a different topology, but the strand 4–P-loop–helix motif is intact. In addition, there are three extra parallel strands at one end (equivalent to myosin strands 1 and 2, plus an extra strand designated 0) and one parallel strand (7), similar to that in *ras*, EF-Tu, and the G-proteins.

SEQUENCE SIMILARITY OF THE P-LOOP

The P-loop motif observed in most of these enzymes has an 8-amino acid consensus sequence Gly-X-X-X-X-Gly-Lys-Thr/Ser, where X denotes amino acids that vary among the different proteins. The eighth amino acid of the P-loop is usually a hydroxyl-containing residue. This residue is important in the majority of these enzymes because it serves as one of two protein ligands for the divalent cation normally associated with the bound nucleotide. Of the eight enzymes given in Table 1 whose structures were abstracted from the Protein Data Bank (PDB) (Bernstein et al., 1977) (other than the myosin structures), seven had P-loop sequences

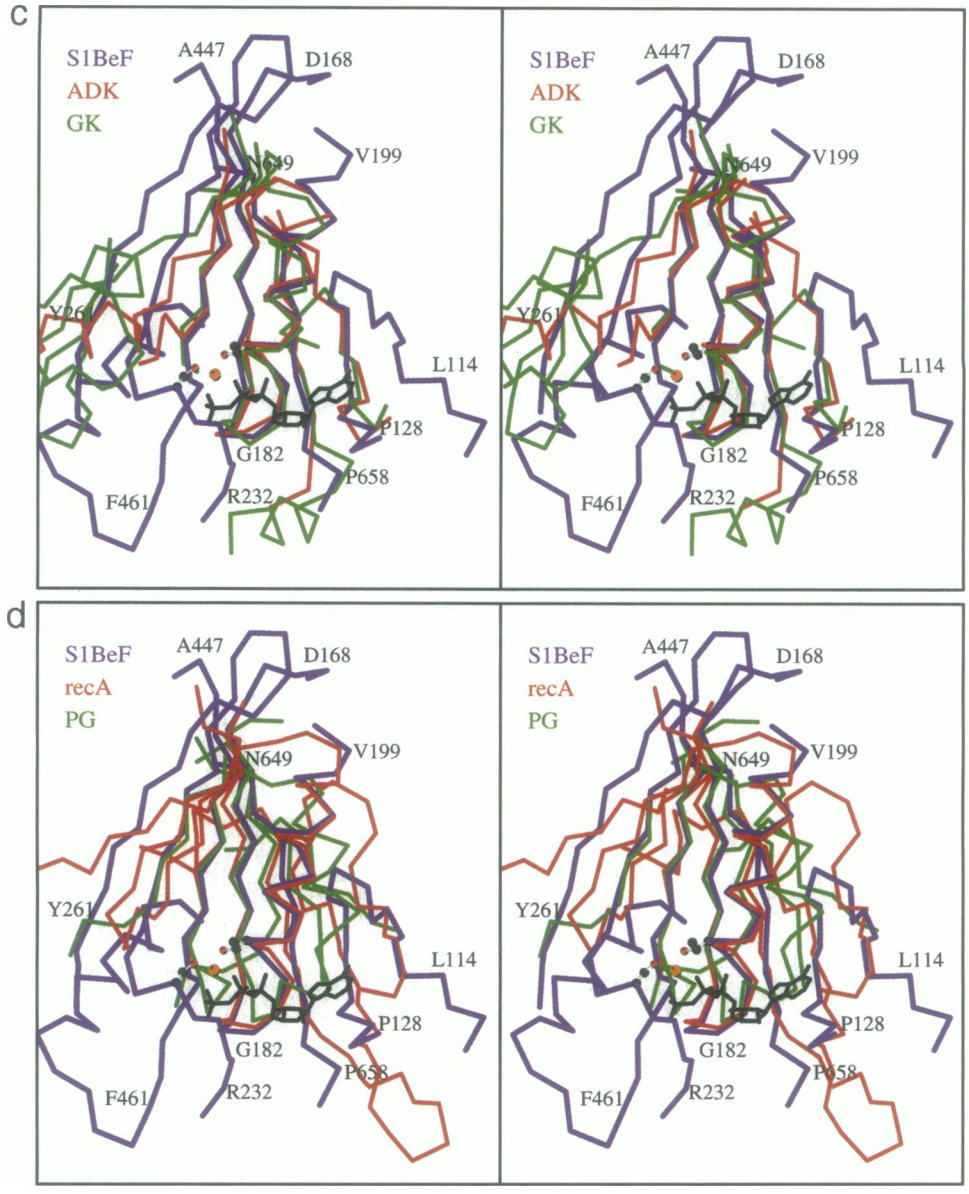

FIGURE 4. Continued.

that strictly adhered to the consensus sequence. Adenylate kinase has an insertion of a Gly between the Lys and the Thr. Phosphoglycerate kinase (PGK) has a putative P-loop that shows substantially more variation from the consensus sequence (Watson et al., 1982; Saraste et al., 1990). In the *Bacillus stearothermophilus* PGK, this region of the polypeptide chain has the sequence Ala-Ile-Ile-Gly-Gly-Ala-Lys-Val (Davies et al., 1994).

The first Gly and the Gly-Lys sequence are invariant in all of these enzymes (with the exception of phosphoglycerate kinase), whereas there is only a limited requirement for a particular amino acid in the intervening four residues (Shen et al., 1994). A recent study on the effect of mutations of the residues involved in the P-loop of F_1-ATPase (Shen et al., 1994) indicated that whereas the second residue could be replaced by virtually any other amino acid and still produce a functional enzyme, the other positions were more restrictive. For most of the enzymes discussed here, with the exception of EF-Tu, which has His, position 5 is a small hydrophobic (Gly, Ala, or Val) or a small polar (Ser or Thr) residue. In all other positions there is a considerable variation in the size and character of the amino acid residue. The Gly residues in the loop clearly play an important conformational role in maintaining the structure of the loop and allowing back-bone hydrogen bonds between adjacent amino acids and the β- and γ-phosphate groups. The function of the other residues is less obvious from sequence comparisons, which is not surprising, because the side chains face away from the phosphate-binding pocket and reflect the environment of the surrounding protein, as was suggested for F_1-ATPase (Shen et al., 1994). This is supported by the observation that for a particular enzyme from

a variety of different species, the sequence of the entire P-loop is highly conserved (Kocabivik and Perlin, 1994; Saraste et al., 1990; Bourne et al., 1991). Indeed, attempts to transfer a P-loop into the chemotactic protein CheY from *E. coli*, which has a classical mononucleotide binding fold, did not confer nucleotide binding or hydrolytic activity, which confirms the role for external interactions in stabilizing its conformation (Cronet et al., 1995). It has been suggested, based upon these differences in sequence among P-loops from unrelated enzymes, that the phosphate-binding motif may have evolved separately more than once (Saraste et al., 1990).

STRUCTURAL SIMILARITY OF THE P-LOOPS

The *ras*-like domains of seven of the structures (*ras*, EF-Tu, $G_{t\alpha}$, $G_{i\alpha 1}$, recA, GK, and ADK) were superimposed on the beryllium fluoride-trapped ADP complex of the truncated *Dictyostelium* myosin S1 ($MgADP{\cdot}BeF_x{\cdot}S1Dc$) based upon the core strands of the β-sheet, the P-loop residues, and the α-helix directly following the P-loop (Table 2). The program LSQKAB from the CCP4 program suite (CCP4, 1994) was used; the results are summarized in Table 3. Phosphoglycerate kinase has differences in the location of the strand-loop-helix motif, which is identical in all of the other cases. Ambiguity arises in the superposition of PGK onto myosin; if the strand that runs into the putative P-loop (189–195 in PGK numbering) is taken to be equivalent to myosin strand 4 and a superposition calculated based only upon the four core strands, the helix following the P-loop does not match with the corresponding helix in myosin (or the other seven structures). It is possible, however, to superimpose the helices by matching the PGK 312–318 strand to myosin strand 3, which then alters the connectivity of the P-loop relative to all the other structures. Fig. 4, *a–d*, shows the superpositions of the eight structures on $MgADP{\cdot}BeF_x{\cdot}S1Dc$, and Fig. 5, *a–c*, shows the results of the superpositions of all the structures on $MgADP{\cdot}BeF_x{\cdot}S1Dc$, based only on the eight P-loop residues.

The G-proteins ($G_{t\alpha}$ and $G_{i\alpha 1}$), *ras*, the elongation factors, recA, and ADK have P-loops similar in structure to myosin. The *rms* deviations in C_α and main-chain atom positions between myosin and these enzymes range from 0.30 to 0.40 Å and 0.31 to 0.38 Å, respectively. These differences are of a magnitude anticipated from coordinate errors. The close similarity of the loops, between these seven enzymes and $MgADP{\cdot}BeF_x{\cdot}S1Dc$, is remarkable, considering the variety of their functions and the diversity of their sources. Guanylate kinase differs somewhat from these preceding seven enzymes, with greater deviations in C_α and main-chain atom positions. Even so, the conformation of the P-loop in GK is very similar to that in $MgADP{\cdot}BeF_x{\cdot}S1Dc$ and the other structures (Fig. 5 *c*). In contrast, phosphoglycerate kinase from *Bacillus stearothermophilus* has a much shorter loop, with a conformation different from that seen in the other structures (Fig. 4 *d*), as expected from its differing sequence.

NUCLEOTIDE BINDING

The nucleotides associated with each of the enzymes (see Table 1), with the exception of three kinases, are all in a position similar to that of ADP in $MgADP{\cdot}BeF_x{\cdot}S1Dc$. The difference in position of the α and β phosphorus atoms between all the other nucleotide-bound structures and myosin averages 0.40 Å and 0.28 Å, respectively. The ribose groups, with the exception of recA, exhibit a C3′-endo ring pucker (Fisher et al., 1995b). Furthermore, the location of the purine ring, which in all cases is in an anti-conformation relative to the ribose, differs only to a small extent in these structures; the difference in position of the guanine ring in the four GTP-binding enzymes is only about 0.6 Å. The adenine ring in $MgADP{\cdot}BeF_x{\cdot}S1Dc$ has been rotated by approximately 20° about the bond joining the ribose relative to the guanine in the four GTP enzymes. In recA, both the ribose and the adenine adopt a very different conformation, relative to the preceding group, resulting from torsions about the α-phosphorus-ribose O5 bond (~195°) and the ribose O5-C5 bond (~55°). This places the adenine ring almost 6 Å from the equivalent position in myosin.

In the GTP-binding proteins, the guanine is bound in a hydrophilic pocket formed in part by a loop at the C-terminal end of strand 6. This loop has a partially conserved sequence Asn-Lys-X-Asp in these four enzymes and in a number of other GTP-binding proteins (Bourne et al., 1991). Several hydrogen bonding interactions between this loop and groups on the guanine help to maintain the rigidity of this part of the nucleotide and may explain why the purine ring is in a similar orientation in these four structures. These interactions also provide specificity for guanine nucleotides. Myosin, on the other hand, has a somewhat different polypeptide conformation at the end of the equivalent strand 6 and does not form the same loop as in the GTP enzymes. There is one hydrogen bonding interaction between the side chain of Tyr 135 and the amine nitrogen on the adenine, but very few other contacts apart from hydrogen bonds with some solvent molecules. The lack of specificity in myosin is reflected in the wide range of nucleotides that can be utilized by this enzyme to generate force (Pate et al., 1993), whereas recognition of a specific base is considerably more important for G-proteins.

Associated with the majority of the P-loops in these enzymes is a divalent cation binding pocket, with one ligand supplied by the conserved hydroxyl residue at position 8. Myosin, recA, EF-Tu, *ras*, and the G-proteins all contain an Mg^{2+} ion coordinated to the side chain of P-loop residue 8 (Thr in the case of myosin, recA, and elongation factor TU, and Ser in *ras* and the G-proteins). Adenylate kinase does not possess a residue in the same position capable of coordinating to a metal ion, because the Thr has been replaced by a Gly. Interestingly, the three-dimensional structures do not contain a cation (Muller and Schulz, 1992; Diederichs and Schulz, 1990; Dreusicke et al., 1988; Berry et al., 1994), in spite of the requirement for a divalent cation for catalysis (Vasavada et al., 1984). Guanylate kinase, which has a

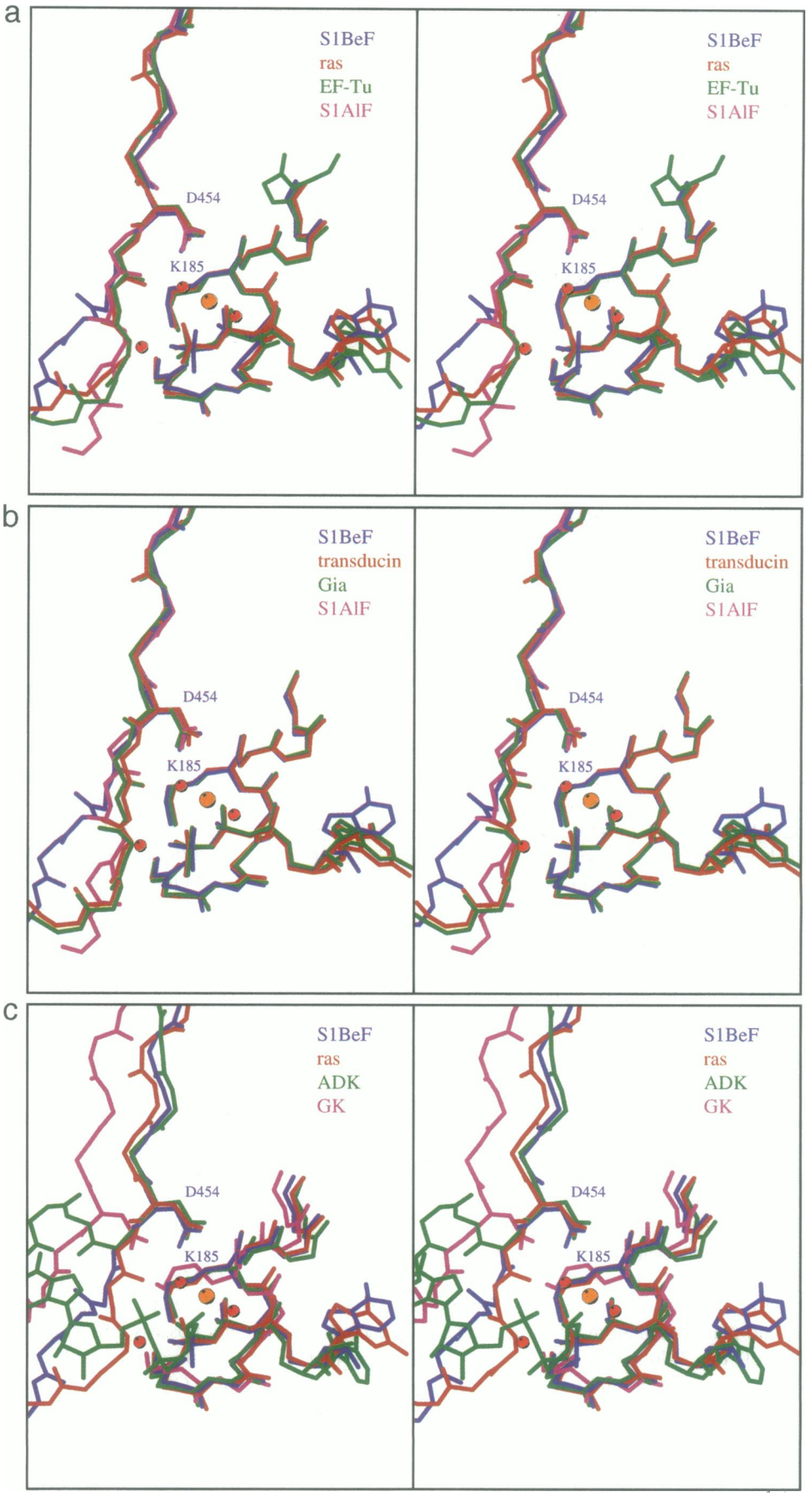

FIGURE 5 Stereoviews of the superpositions of six of the purine nucleotide binding proteins onto *Dictyostelium* myosin S1, based on the eight residues comprising the P-loop. MgADP·BeF_x·S1Dc is shown in blue along with (*A*) *ras* (*red*), EF-Tu (*green*), and S1Dc·AlF_4·ADP (*purple*); (*B*) transducin $G_{t\alpha}$ (*red*), $G_{i\alpha 1}$ (*green*), and S1Dc·AlF_4·ADP (*purple*); and (*C*) ras (*red*), ADK (*green*) and GK (*purple*). In this figure the backbone, carbonyl, and Cα atoms are shown for the polypeptide chain from 180 to 188 and 450 to 459 for MgADP·BeF_x·S1Dc and the structurally equivalent residues in the other proteins. Side chains are included for Lys 185 and Asp 454 in myosin, together with the equivalent residues in the other proteins. The disposition of the other metal ligands is shown in Fig. 6. The nucleotide (if present) is also indicated, as is the equivalent of myosin strand 3, which has the consensus sequence D-X-X-G at its C-terminus in the majority of the enzymes. The start of the D-X-X-G motif is indicated by the location of Asp 454 in myosin.

serine at position 8 with the side chain oriented similarly to the other proteins, is potentially capable of coordinating a metal ion in a similar site.

The coordination sphere of the Mg^{2+} in myosin, EF-Tu, *ras*, and the G-proteins is completed by two oxygen atoms of the bound trinucleotide, one from each of the β and γ phosphates, a second hydroxyl side chain (either Thr or Ser), and two water molecules (Figs. 2 and 6). The second protein ligand generally comes from a region of the polypeptide chain quite removed from the P-loop, and although analysis of the sequences of a wide variety of GTPases (Bourne et al., 1991) shows that among a particular family of proteins (i.e., the *ras* proteins or the G-proteins) there is some level of consensus in this part of the molecule, there is no apparent sequence identity between different GTPases, or between other purine nucleotide-binding proteins. The superpositions of these structures onto MgADP·BeF_x·S1Dc show that the polypeptide chain on the C-terminal side of the hydroxyl residue is structurally conserved, leading into the equivalent of myosin strand 2 of the six-stranded β-sheet. In most cases, however, the structure on the N-terminal side does not show any similarity. In *ras*, the helix immediately following the P-loop runs back approximately antiparallel to the sheet and carries Thr 35, the second Mg^{2+} ligand, whereas in the G-proteins, the polypeptide chain forms the helical domain (Coleman et al., 1994; Noel et al., 1993) before returning to form the remainder of the *ras*-like domain just before Thr 177 (transducin numbering), the second metal ligand. In myosin, the polypeptide chain runs from the P-loop helix into another roughly antiparallel helix, then into an extended loop region before forming strand 2.

The three kinases do not possess the second protein ligand to the metal ion that is common to the *ras*-like enzymes. Consequently, the structure of these enzymes in this region is markedly different from that of the other proteins. The three kinases possess a binding site for substrate in place of water to which the γ-phosphate is transferred. That lies approximately 10 Å away from the P-loop. The substrate site is located in about the same place as the loop-strand motif in myosin, *ras*, and the others, and hence overlaps the location of the second ligand to the metal ion observed in these enzymes.

There is one further region near the active site, which has a limited sequence similarity in the GTPases (Bourne et al., 1991). This is the B motif, Asp-X-X-Gly (Walker et al., 1982), which appears to be conserved among the majority of the purine nucleotide-binding proteins, including the *ras* proteins, EF-Tu, the G-proteins, and myosin (Asp454-Ile-Ser-Gly in *Dictyostelium* myosin). This motif has been shown to be involved in the binding of the γ-phosphate (Pai et al., 1990; Noel et al., 1993; Kjeldgaard et al., 1993) and has been linked to a conformational change after hydrolysis. In these proteins, this piece of structure participates in or precedes a flexible loop at the C-terminal end of strand 3 and is followed by an α-helix running almost perpendicular to the β-sheet. In the superpositions of these five structures, the side chain of the conserved Asp adopts an almost identical conformation and is hydrogen bonded to one of the two water molecules contributing to the metal coordination sphere when a nucleotide triphosphate is bound in the active site. In myosin the other oxygen of the carboxyl group of Asp 454 forms a hydrogen bond to the proton on the hydroxyl group of Thr 186 (first protein ligand to the Mg ion) and a salt bridge to Nζ of Lys 241. The *rms* difference in the positions of the side-chain atoms of the equivalent Asp between the first four structures and myosin is between 0.6 and 0.8 Å. In *ras*, EF-Tu, and the G-proteins the conformation of the Asp-X-X-Gly motif is very similar (Fig. 5, *a* and *b*), looping around the γ-phosphate binding pocket such that the amide nitrogen of the conserved glycine hydrogen bonds with one of the γ-phosphate oxygen atoms. In both ADK and GK there is an Asp in the same location, but the sequence immediately following it (Gly-Phe-Pro and Ile-Asp-Met, respectively) does not match the consensus sequence for the B motif. The conformation of this loop in

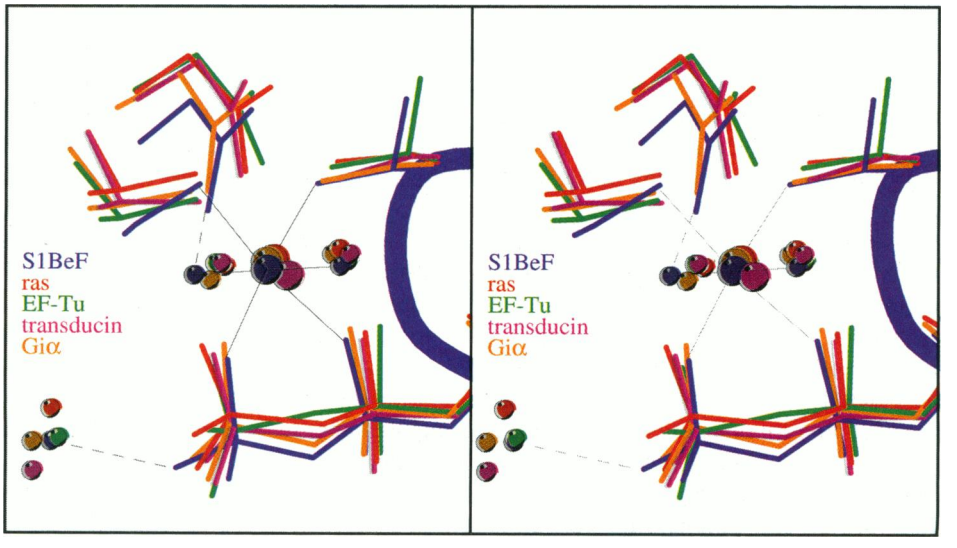

FIGURE 6 Stereoviews of the superpositions of the cation binding sites of the four GTPases onto *Dictyostelium* myosin S1. The superposition was based on the eight P-loop residues, which are shown for myosin only as a blue coil. The Mg^{2+}, the two protein ligands, and two water molecules associated with the Mg^{2+} are indicated for myosin (*blue*), *ras* (*red*), EF-Tu (*green*), transducin $G_{t\alpha}$ (*purple*), and $G_{i\alpha 1}$ (*orange*). Also shown is a water molecule associated with the γ-phosphate that has been implicated in the catalytic mechanism.

GK, although similar to that observed in myosin, is displaced away from the γ-phosphate-binding pocket, whereas in ADK, the differences in this loop are much greater, with the chain turning away from the active site. RecA does not contain the Asp-X-X-Gly observed in the other P-loop proteins. The loop in RecA that is equivalent to the B-Motif has an isoleucine side chain (192 in RecA numbering) in place of Asp 454 in myosin. RecA does have a conserved Asp (Asp 144) at the end of the adjacent strand, but this is at a greater distance from the putative metal binding site and is in a different orientation from that observed in the other enzymes. However, it has been suggested that in the presence of ADP Asp 144 is coordinated to the metal ion via a water molecule (Story and Steitz, 1992).

CONFORMATIONAL CHANGES

In many of these proteins the loop that contains the second ligand to the Mg^{2+} ion (Thr 35 in *ras*) and the Asp-X-X-Gly loop together with the subsequent polypeptide chain exhibit conformational changes as a consequence of nucleotide hydrolysis that are important for the function of the molecule. In *ras* and the G-proteins these two regions have been referred to as switches I and II (Milburn et al., 1990; Lambright et al., 1994; Mixon et al., 1995). In *ras* these motifs, together with the loop that contains the second ligand to the Mg^{2+} ion (Thr 35 in *ras*), exhibit significant changes upon hydrolysis of GTP to GDP. The presence of the γ-phosphate group in the GTP-bound form of *ras* causes the side chain of Thr 35 to flip in toward the nucleotide, bind to the Mg^{2+}, and alter the conformation of the surrounding loop. In addition, a new hydrogen bonding interaction between the amide nitrogen of Gly 60 of the Asp-X-X-Gly motif and the phosphate oxygen is formed, altering the conformation of the flexible loop and changing the orientation of the downstream helix. The Asp-X-X-Gly motif or the polypeptide chain shortly thereafter in all of the G-proteins experiences a conformational change as a consequence of nucleotide hydrolysis.

In myosin there is also a structural change associated with the B-motif or switch II during hydrolysis, as revealed from a comparison of the structures of MgADP·BeF_x·S1Dc and MgADP·AlF_4·S1Dc, where the beryllium fluoride complex mimics ATP in the active site and aluminum fluoride complex is an analog of the transition state for hydrolysis (Fisher et al., 1995a,b). Conversely, there does not appear to be a conformational change in myosin associated with the second metal ligand or switch I, based on the structure of myosin in the absence of nucleotide (Rayment et al., 1993). The change associated with the B-motif involves a rotation in the main-chain torsion angles at Ile 455 (the ϕ angle changes by ~90°), Gly 457 (ψ changes by ~60°) (Fisher et al., 1995a,b), and Ser 456 (ψ changes by ~35°). These alterations in the polypeptide conformation in this loop cause the partial closure of the large cleft in the central 50-kDa segment of the structure. The obstruction of the narrow cleft provides a structural explanation for the metastable ADP, P_i state of the enzyme, because release of the inorganic phosphate from the enzyme, which is known to accompany the force-generating step, cannot occur without a further conformational change in the myosin head. It has been suggested that this occurs when myosin binds to actin (Fisher et al., 1995a,b). Superposition of the aluminum fluoride-trapped S1 complex (S1Dc·AlF_4·ADP) onto MgADP·BeF_x·S1Dc based on the eight P-loop residues is also shown in Fig. 5, *a* and *b*. Interestingly, after the initial conformational change in the myosin head during ATP hydrolysis, this hinge portion of the S1 molecule adopts a conformation very similar to that observed in *ras*, elongation factor Tu, and the G-proteins. The amide nitrogen of the conserved Gly 457 hydrogen bonds to one of the fluoride ions of the aluminum fluoride moiety, much the same as the interaction observed in ras, EF-Tu, and the G-proteins.

Two other regions have been identified in the G-proteins that exhibit a conformational change between the GTP and GDP states of the enzyme. These have been named switches III and IV (Lambright et al., 1994; Mixon et al., 1995). Switch III is located in a loop in the *ras*-like domain between strands β-4 and α-3 (transducin strand numbering) that is influenced by the conformation of switch II (Lambright et al., 1994). Switch IV is located in the α-helical domain (Mixon et al., 1995). These changes appear to be important for recognition of the GTP and GDP states. Interestingly, the loop that is structurally equivalent to switch III in myosin precedes the reactive cysteine residues that are known to undergo a conformational change during the contractile cycle. The region equivalent to switch IV in the G-proteins does appear to be represented in myosin.

MECHANISTIC IMPLICATIONS

The structural similarity between the P-loops and the other ligands surrounding the γ-phosphate pocket in these widely different enzymes is quite remarkable. From the very different topologies of these proteins it seems likely that this motif has arisen several times during evolution. It is particularly striking that the B-motif or the Asp-X-X-Gly sequence observed in *ras*, the G-proteins, and myosin is structurally conserved in those enzymes that exhibit a conformational change that is propagated beyond the active site to signal the state of the nucleotide bound to the enzyme. In this case it appears that the inherent flexibility of the glycine residue may be an important factor in the post-hydrolysis conformational change, because this residue is conserved in all myosins, all the *ras* proteins, elongation factors, and G-proteins (Bourne et al., 1991), irrespective of source or function.

There has been considerable discussion of the mechanism of phosphoryl transfer in these nucleotide-dependent en-

TABLE 1 P-loop-containing proteins compared in this study

Protein	Source	P-loop residues	Nucleotide	Reference
Myosin	Chicken	G(181)ESGAGKT(188)	SO_4^{2-}	Rayment et al., 1993
1MMD	*Dictyostelium*	G(179)ESGAGKT(186)	BeF_x·ADP	Fisher et al., 1995b
1MND	*Dictyostelium*	G(179)ESGAGKT(186)	AlF_4·ADP	Fisher et al., 1995b
1MNE	*Dictyostelium*	G(179)ESGAGKT(186)	PPi	Smith and Rayment, 1995
Adenylate kinase				
1AKE	*E. coli*	G(7)APGAGKGT(15)	AP_5A	Muller and Schulz, 1992
1ANK	*E. coli*	G(7)APGSGKGT(15)	AMPPNP & AMP	Berry et al., 1994
Elongated factor Tu				
1EFT	*Thermus aquaticus*	G(18)HVDHGKT(25)	GMPPNP	Kjeldgaard et al., 1993
$G_{i\alpha 1}$				
1GIA	Rat*	G(40)AGESGKS(47)T	$GTP_\gamma S$	Coleman et al., 1994
Guanylate kinase				
1GKY	Yeast	G(8)PSGTGKS(15)T	GMP	Stehle and Schultz, 1992
H-*ras*-P21 phosphoglycerate kinase				
121P	Human	G(10)AGGVGKS(17)	GMPPCP	Pai et al., 1989
1PHP	*B. stearothermophilus*	A(191)IIGGAKV(198)	ADP	Davies et al., 1994
3PGK	Yeast	A(207)ILGGAKV(214)	AMPPNP PG[2]	Watson et al., 1982
recA				
2REB		G(66)PESSGKT(73)T	apo[3]	Story et al., 1992
Transducin, $G_{t\alpha}$				
1TND	Bovine retina	G(36)AGESGKS(43)T	$GTP_\gamma S$	Noel et al., 1993

**Rattus norvegicus.*

zymes. Thus it is worth examining the active sites of these enzymes from a structural viewpoint to see if there are any similarities that might indicate common mechanisms. The comparison above shows that most of the binding sites for the β- and γ-phosphates are functionally identical. The differences arise primarily opposite the γ-phosphate in the location anticipated to be occupied by groups that might function as the catalytic base. In the case of the G-proteins and myosin where the reaction is one of simple hydrolysis, it is well established that bond cleavage is associated with the attack of a water molecule on the γ-phosphate (Feuerstein et al., 1989; Sleep et al., 1980; Webb and Trentham, 1981; Dale and Hackney, 1987). At least for *ras* p21 and myosin the maximum rate of bond cleavage is similar (19 s^{-1} for *ras* p21 at 25°C (Gideon et al., 1992) and 5 s^{-1} at 3°C for myosin (Taylor, 1977)). Much of the discussion has centered on the identity and function (if any) of the catalytic base that serves to activate the water molecule as it ap-

TABLE 2 Residue numbers of the structurally conserved β-sheets in the purine nucleotide-binding proteins

Strand	Myosin	H-*ras*-P21	EF-Tu	$G_{i\alpha}$	$G_{t\alpha}$	ADK	PGK	GK	recA
1	253–262	—	—	—	—	—	259–262	—	87–96
2	240–246	37–43	65–71	184–190	180–186	27–31	214–219	—	139–145
3	448–455	51–58	75–82	194–201	190–197	78–85	187–194	92–99	186–193
4	173–179	4–10	12–18	34–40	30–36	1–7	312–318	2–8	60–66
5	649–657	75–83	99–107	218–226	214–222	103–111	345–353	114–122	219–227
6	122–126	111–115	131–135	264–268	260–264	193–197	367–370	163–167	240–246
7	114–119	139–143	168–174	318–323	314–319	—	—	—	256–260
P-loop	179–186	10–17	18–25	40–47	36–43	7–14	194–203	8–15	66–73
Helix	186–200	17–25	25–38	47–58	43–50	14–26	203–212	15–25	73–86

The strands are arbitrarily numbered accordingly to their position in the sheet, not according to their position in the sequence, because the numbering based on sequence position is different for the individual proteins. The sequence order and polarity, relative to the strand leading into the P-loop (i.e., strands pointing down in Figs. 2 and 3 are designated +), of the strands are as follows: myosin, +5 −4 +6 +3 +7 +2 −1; *ras*, EF-Tu, $G_{i\alpha}$, and $G_{t\alpha}$, −2 +3 +1 +4 +5 +6; ADK, +2 +3 +1 +4 +5; GK, +2 +1 +3 +4; PGK, +3 +2 +1 +4 +5 +6; recA, +3 +2 +4 +5 +1 +6 −7 +8.

TABLE 3 Superposition of nucleotide-binding proteins onto MgADP·BeF_x·S1Dc, based upon the phosphate-binding loop residues and the structurally conserved central β-sheet

Protein	pdb Filename	rms Deviations (Å)			
		P-loop residues		β-sheet and P-loop*	
		Cα atoms	Main chain	Cα atoms	Main chain
Myosin (chicken)		0.53	0.74	0.49(46)	0.54(184)
Adenylate kinase	1AKE	0.30	0.32	1.12(34)	1.07(136)
	1ANK	0.22	0.27	1.09(40)	1.13(160)
Elongated factor Tu	1EFT	0.39	0.35	1.24(41)	1.25(164)
$G_{i\alpha 1}$	1GIA	0.31	0.31	1.27(40)	1.32(160)
Guanylate kinase	1GKY	0.75	0.69	1.66(40)	1.69(160)
H-*ras*-P21	121P	0.40	0.38	1.35(40)	1.41(160)
Phosphoglycerate kinase	1PHP			0.65(18)	0.99(72)
recA	2REB	0.34	0.35	1.09(39)	1.07(156)
Transducin, $G_{t\alpha}$	1TND	0.35	0.35	1.22(38)	1.27(152)

*Residues used in the superpositions are listed in Table 2. Some residues at the ends of strands or the helix that showed poor agreement between the two structures after superposition were removed, and the superposition was then recalculated. The number of Cα and main chain atoms used to give the best fit of the two structures are given in parentheses.

proaches the γ-phosphate. Initially it was believed that Gln 61 in *ras* p21 was the catalytic base, even though this would not be expected to be a very effective activating group, based on its normal pK_a (Pai et al., 1990). Indeed, the structures of MgGDP·$AlF4^-$ complexes between transducin α (Sondek et al., 1994) and Giα (Coleman et al., 1994) both implicated the equivalent glutamine in proton transfer. However, replacement of this residue with an unnatural amino acid nitroglutamine generated a protein with normal GTPase activity, which is inconsistent with this being the active site base (Chung et al., 1993). More recently it has been suggested that the γ-phosphate functions as its own base and accepts the proton directly from the attacking water molecule, and as such does not have a catalytic base provided by the protein (Schweins et al., 1994, 1995). In this mechanism a major role of the glutamine residue is to orient the water molecule for nucleophilic attack on the γ-phosphate.

The active site of myosin likewise does not contain any group within 5.5 Å of the γ-phosphate that is an acceptable candidate as a catalytic base (Fisher et al., 1995b). Thus it would appear that ultimately the nucleotide also functions as it own base. In this case an absolutely conserved serine has been proposed to participate in the transfer of the proton. Again the role of the protein surrounding the γ-phosphate appears to be to orient the nucleophilic water molecule. The similarity in the environment surrounding the γ-phosphate suggests that at least the G-proteins and myosin utilize a similar strategy to facilitate hydrolysis of the nucleotide triphosphate. In contrast, RecA and the kinases, in which the nucleotide serves a different function, exhibit a more divergent coordination of the metal ion and γ-phosphate. Perhaps as a consequence, RecA and the kinases utilize the protein in a different way to achieve catalysis.

CONCLUSION

This brief comparison of myosin with other enzymes that utilize a P-loop to bind nucleotides illustrates that a strikingly similar coordination scheme has evolved to firmly hold the triphosphate in the active site in a very broad range of proteins. The major variation occurs at the end of the triphosphate-binding pocket in a location occupied by the incoming nucleophile. Even so, it appears that myosin and the G-proteins may utilize a similar mechanism for nucleotide hydrolysis.

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