X-ray Structures of the Dictyostelium discoideum Myosin Motor Domain with Six Non-nucleotide Analogs*

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The three-dimensional structures of the truncated myosin head from Dictyostelium discoideum myosin II complexed with dinitrophenylaminoethyl, dinitrophenylaminopropyl, o-nitrophenylaminoethyl, m-nitrophenylaminoethyl, p-nitrophenylaminoethyl-, and o-nitrophenyl-N-methylaminoethyl-diphosphate-beryllium fluoride have been determined to better than 2.3-Å resolution. The structure of the protein and nucleotide binding pocket in these complexes is very similar to that of S1dC-MgADP-BeF₆ (-Fisher, A. J., Smith, C. A., Thoden, J., Smith, R., Sutoh, K., Holden, H. M., and Rayment, I. (1995) Biochemistry 34, 8960–8972). The position of the triphosphate-like moiety is essentially identical in all complexes. Furthermore, the alkyl-amino group plays the same role as the ribose by linking the triphosphate to the adenine binding pocket; however, none of the phenyl groups lie in the same position as adenine in S1dC-MgADP-BeF₆, even though several of these nucleotide analogs are functionally equivalent to ATP. Rather the former location of adenine is occupied by water in the nanolog complexes, and the phenyl groups are organized in a manner that attempts to optimize their hydrogen bonding interactions with this constellation of solvent molecules. A comparison of the kinetic and structural properties of the nanologs relative to ATP suggests that the ability of a substrate to sustain tension and to generate movement correlates with a well defined interaction with the active site water structure observed in S1dC-MgADP-BeF₆.

Myosin is a molecular motor that converts chemical energy into directed movement. It generates force in an unidirectional manner relative to filamentous actin through the hydrolysis of ATP. This is a cyclic process in which the binding of ATP first reduces the affinity of myosin for actin after which hydrolysis occurs (1). Interestingly, the hydrolysis event occurs when myosin has little affinity for actin. This leaves the molecule in a metastable state where the enzyme retains the hydrolysis products with an equilibrium constant of approximately unity between ATP and ADP-P, in the absence of actin (1–3). The energy transduction event occurs as myosin rebinds to actin and phosphate is released (4). Thus, the properties of the contractile cycle are dominated by protein-nucleotide interactions and their influence on the binding affinity of myosin for actin. Understanding the contractile cycle is a complex problem since even in the simplest models require 8 states to account for the kinetic properties of actomyosin (1), whereas in more elaborate analyses, up to 12 states have been proposed (5, 6). Determination of the structural conformation of myosin at each of these kinetic states has likewise proved difficult since most of the states are transitory in nature.

Many strategies have evolved to address and understand the transitory states of the contractile cycle of myosin. These include the application of agents such as vanadate, aluminum fluoride, and beryllium fluoride to trap nucleotides in the active site and in the development of a wide range of ATP analogs. The latter approach has proved to be of great value because myosin is able to utilize an enormously wide variety of nucleotides and nucleotide analogs in the contractile cycle, albeit with significantly different energetic and catalytic efficiencies (7, 8). Nucleotide analogs have served a role in defining both the structural and kinetic properties of the actomyosin interaction. For example non-hydrolyzable analogs of ATP, such as AMPNP and ATPγS, have proved to be particularly useful for dissecting the contractile cycle of myosin since they provide an opportunity to enhance the population of one kinetic state. These have been particularly useful in physiological studies of the contractile cycle since they have provided a strategy for defining the mechanical properties of the intermediate states. They have also allowed the supra-molecular structure of the actomyosin interaction to be studied by x-ray diffraction and electron microscopy. In contrast, the utilization of alternative nucleotides to ATP, such as GTP and CTP, in the contractile

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1 The abbreviations used are: AMPPNP, 5′-adenylyl-γ,3-imidodiphosphate; ATPγS, adenosine 5′-O-(thiotriphosphate); S1dC, D. discoideum myosin myosin II motor domain, residues 1–762; MgADP-VQ₅-S1dC, magnesium pyrophosphate complex of truncated D. discoideum myosin motor domain; PEG, polyethylene glycol; MgADP-BeF₆-S1dC, the beryllium fluoride-ADP complex of D. discoideum myosin motor domain; MgADP-AlF₄-S1dC, the aluminum fluoride-ADP complex of D. discoideum myosin motor domain; MgADP-AIF₅-S1dC, the vanadate-ADP complex of D. discoideum myosin motor domain; nanologs, non-nucleotide analogs; o-p-DNPhAP, ortho,para-dinitrophenylaminopropyl triphosphate; o-p-DNPhAE, ortho,para-dinitrophenylaminoethyl triphosphate; N-methyl-DNPhAE, meta-dinitrophenylaminoethyl triphosphate; p-DNPhAE, para-dinitrophenylaminoethyl triphosphate; N-methyl-NPhAE, ortho-nitrophenyl-N-methyl-aminoethyl triphosphate.
cycle of myosin has also proved useful for illustrating the importance of the individual rate constants in the energy transduction process (7, 8).

Previous studies on the utilization of GTP, CTP, and 1^N^6-etheno-2-aza-ATP by myosin revealed that the choice of substrate has the potential to modulate more than one of the rate constants in the contractile cycle. Changes were observed not only in the catalytic rate constant for hydrolysis but also in the rate of dissociation of myosin from actin (7). For example the maximum steady state rate for actin-activated hydrolysis of GTP is \( \frac{3}{3} \% \) that of ATP where the change appears to be in the bond splitting step such that GTP is a very poor substrate in active fibers (8). Conversely CTP is a reasonable substrate both in solution and in fibers even though its second order rate constant for dissociation of acto myosin is 20 times lower than that of ATP (7, 8). Examination of the chemical structures of the GTP and CTP does not yield a satisfactory explanation for these striking differences.

Perhaps the most divergent approach to the use of nucleotide analogs to the study of myosin has been the development of a series compounds based on substituted phenylaminoethyl and

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**Table I**

| Acronym | W | X | Y | Z | n |
|---------|---|---|---|---|---|
| o-NPhAE | H | H | NO\(_2\) | H | 2 |
| p-NPhAE | NO\(_2\) | H | H | H | 2 |
| m-NPhAE | H | NO\(_2\) | H | 2 |
| o-p-NPhAE | NO\(_2\) | NO\(_2\) | H | 2 |
| o,p-NPhAE | NO\(_2\) | NO\(_2\) | H | 2 |
| N-Methyl-NPhAE | H | NO\(_2\) | CH\(_3\) | 2 |

**Table II**

| Data collection statistics | S1 \( \cdot \) o-NPhAE | S1 \( \cdot \) m-NPhAE | S1 \( \cdot \) p-NPhAE | S1 \( \cdot \) o,p-NPhAE | S1 \( \cdot \) o,p-NPhAP | S1 \( \cdot \) N-methyl-NPhAE |
|---------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| X-Ray source              | CHESS* F1      | CHESS F1      | SSRL* 7–1     | SSRL 7–1       | SSRL 7–1       | SSRL 7–1       |
| No. of crystals            | 1              | 1              | 1              | 1              | 1              | 1              |
| Maximum resolution         | 2.0 Å          | 2.0 Å          | 2.0 Å          | 2.0 Å          | 2.0 Å          | 2.0 Å          |
| Total reflections           | 146,074        | 190,396        | 218,034        | 254,629        | 193,041        | 133,036        |
| Individual reflections     | 59,396         | 66,106         | 66,932         | 66,972         | 69,602         | 42,098         |
| Complete                   | 86.6%          | 95.2%          | 95.7%          | 97.1%          | 97.0%          | 90.9%          |
| Highest shell              | 78.2%          | 96.0%          | 97.5%          | 95.5%          | 94.0%          | 93.0%          |
| R\(_{merge}\)              | 7.0%           | 3.9%           | 8.6%           | 6.8%           | 4.0%           | 7.5%           |
| Highest shell              | 14.8%          | 10.5%          | 37.7%          | 26.5%          | 14.4%          | 24.3%          |
| Average I/\(\sigma^2\)     | 20.7           | 31.4           | 4.3            | 16.8           | 20.7           | 4.9            |
| Unit cell                  |                |                |                |                |                |                |
| a                         | 103.8          | 104.0          | 104.0          | 103.7          | 103.9          | 103.9          |
| b                         | 180.3          | 180.6          | 180.9          | 180.0          | 180.5          | 180.9          |
| c                         | 54.0           | 54.0           | 54.1           | 54.0           | 54.1           | 54.1           |

* CHESS is Cornell High Energy Synchrotron Source.

**Table III**

| Refinement statistics | o-NPhAE | m-NPhAE | p-NPhAE | o,p-NPhAE | o,p-NPhAP | N-Methyl-NPhAE |
|-----------------------|---------|---------|---------|-----------|-----------|----------------|
| R-factor              | 17.8    | 18.7    | 19.3    | 22.6      | 20.4      | 18.1           |
| Number of atoms       | 6625    | 6658    | 6352    | 6236      | 6374      | 6521           |
| Number of protein atoms | 5897    | 5902    | 5872    | 5773      | 5802      | 5906           |
| Solvent molecules     | 702     | 730     | 454     | 434       | 541       | 588            |
| r.m.s.d. bond lengths (Å) | 0.014  | 0.017   | 0.017   | 0.011     | 0.019     | 0.012          |
| r.m.s.d. planarity (Å) | 0.013   | 0.016   | 0.016   | 0.011     | 0.017     | 0.013          |
| Average B-factor, protein (Å\(^2\)) | 42.0 | 38.0 | 30.9 | 51.9 | 42.6 | 36.8 |
| Average B-factor, main chain (Å\(^2\)) | 38.9 | 34.4 | 27.2 | 50.4 | 40.3 | 34.1 |
| Ramachandran parameters |         |         |         |           |           |                |
| Most favored (%)      | 89.3    | 90.5    | 91.3    | 86.4      | 88.5      | 89.2           |
| Allowed (%)           | 10.1    | 8.8     | 8.6     | 11.7      | 11.0      | 9.5            |
| Generously allowed (%) | 0.4    | 0.7     | 0.2     | 1.6       | 0.3       | 0.9            |
| Disallowed (%)        | 0.1     | 0.1     | 0.0     | 0.1       | 0.2       | 0.3            |

* r.m.s.d., root mean square deviation.

**Table IV**

| RMS deviations between nucleotide analog complexes and S1dC \( \cdot \) Mg \( \cdot \) ADP \( \cdot \) BeF\(_2\) across different protein domains |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|
|                                 | Entire protein | 25-kDa domain | 50-kDa domain | 20-kDa domain | \( R_{factor} \) |
| o-NPhAE                         | 0.433          | 0.386          | 0.377          | 0.353          | 17.8           |
| m-NPhAE                         | 0.390          | 0.385          | 0.309          | 0.330          | 18.7           |
| p-NPhAE                         | 0.421          | 0.438          | 0.336          | 0.301          | 19.3           |
| o-p-DNPPhAE                     | 0.613          | 0.551          | 0.550          | 0.494          | 22.6           |
| o,p-DNPPhAP                     | 0.532          | 0.395          | 0.472          | 0.499          | 20.4           |
| N-Methyl-NPhAE                 | 0.457          | 0.366          | 0.409          | 0.361          | 18.1           |
phenylaminopropyl triphosphates where the adenine and ribose moiety of ATP are replaced by a substituted aromatic ring and an aminoethyl or aminopropyl group, respectively. These analogs bear remarkably little chemical similarity to ATP (9). Initially these compounds (nanologs) were designed as photoreactive reagents and served to identify amino acid residues associated with the active site. These studies proved to be of great value during the interpretation of the initial x-ray structure of myosin subfragment 1 (10, 11). Surprisingly some of these compounds match or exceed the chemical functionality of ATP in both the ATPase activity and the ability to support muscle contraction (9). However, there is a large variation in the steady state rate of hydrolysis and degree of actin activation for myosin in solution and in the maximum rate of shortening and tension development in muscle fibers depending on the substitution pattern of the aromatic ring and on the length of the alkyl linker to the triphosphate moiety. These compounds raise the question of what are the characteristics of the nucleotide-binding site that allow it to accommodate such a diverse range of triphosphates and, conversely, what are the characteristics of a substrate that allow it to function efficiently in both the hydrolytic step and in the generation of movement.

The nucleotide and actin binding activities of myosin are all associated with a globular section (head) of the molecule that is...
common to all members of the myosin superfamily of proteins (12, 13). Structural studies of chicken skeletal myosin subfragment 1 suggested that the nucleotide-binding site is located in a shallow groove that lies above the large mostly parallel β-sheet that forms the major structural motif of the myosin motor domain (10, 14). The motor domain is connected via a converter domain to a long α-helix that is stabilized by the myosin light chains. The converter domain and the α-helix have now been observed crystallographically to adopt multiple conformations in chicken smooth muscle myosin (15) and scallop S1 (16) providing additional support for the lever arm hypothesis of muscle contraction.

The nucleotide binding groove is formed by secondary structural elements from the N-terminal, central, and C-terminal sections of the polypeptide chain and terminates as a narrow tunnel below the β-sheet at the apex of a deep cleft that separates the upper and lower domains of the central section of the polypeptide chain. Structural studies on the motor domain from Dictyostelium discoideum myosin II showed that the triphosphate moiety binds in the narrow tunnel, whereas the adenine binding pocket is formed by amino acid residues contributed by the N-terminal section of the myosin heavy chain (17). Interestingly, very few specific interactions were observed between the base and the protein such that it was not obvious how the protein discriminates between nucleotide substrates.

In an effort to understand the structural determinants in myosin that are important in coordinating the adenosine component of ATP, we have determined the structure of truncated myosin head from D. discoideum complexed with six non-ATP nucleotide analogs (nanologs). These complexes include analogs that are functionally equivalent to ATP as well as those that exhibit excellent ATPase activity and yet are unable to
support force generation in myofibrils. These structures serve to identify the components of the adenosine-binding site that contribute to the specificity and efficiency of ATP as the primary substrate for motor activity. The systematic study of the structure of the complexes of a series of nanologs provides an opportunity to identify the interactions that contribute to a particular aspect of the contractile cycle and exemplify the relationship between substrate binding and hydrolytic activity.

**EXPERIMENTAL PROCEDURES**

**Nanologs**—The nanologs were prepared as described elsewhere (9,2). They were generally freeze-dried before use and dissolved in the minimal amount of distilled water to obtain a stock solution with a concentration of at least 30 mM. A list of the nanologs and their structures is given in Table I.

**Protein Purification and Crystallization**—Truncated myosin subfragment 1, residues Asp2 to Asn762, from *D. discoideum* (S1dC) was purified as described previously (17,19–21). The protein was frozen in small aliquots in liquid nitrogen and thawed daily for crystallizations experiments. The nanologs were co-crystallized with S1dC in the presence of beryllium fluoride. Prior to crystallization the nanogop was trapped in the active site of S1dC as the BeF₃⁺ adduct by the addition of stock solutions to a final concentration of 2 mM nanolog, 15 mM NaF, 3 mM BeCl₂, and 3 mM MgCl₂. Crystals were grown by microbatch in plexiglass depression slides from 6.3% PEG 8000, 50 mM Heps, pH 7.0, 125 mM NaCl, and 3 mM dithiothreitol where the protein concentration in the mother liquor was about 5 mg/ml. The crystals were micro-seeded from previous batch experiments by streak-seeding with a whisker from a fat domestic short-haired cat and left at 4 °C. Small crystals generally appeared within a few days and typically took approximately 2 weeks to reach maximum dimensions of 0.6 × 0.4 × 0.3 mm. Interestingly the thickness of the crystals varied considerably depending on the analog used.

**Data Collection and Refinement**—Crystals were almost isomorphous with the earlier crystals of S1dC and the BeF₃⁺ adduct by the addition of stock solutions to a final concentration of 2 mM nanolog, 15 mM NaF, 3 mM BeCl₂, and 3 mM MgCl₂. Crystals were grown by microbatch in plexiglass depression slides from 6.3% PEG 8000, 50 mM Heps, pH 7.0, 125 mM NaCl, and 3 mM dithiothreitol where the protein concentration in the mother liquor was about 5 mg/ml. The crystals were micro-seeded from previous batch experiments by streak-seeding with a whisker from a fat domestic short-haired cat and left at 4 °C. Small crystals generally appeared within a few days and typically took approximately 2 weeks to reach maximum dimensions of 0.6 × 0.4 × 0.3 mm. Interestingly the thickness of the crystals varied considerably depending on the analog used.

Data were collected with synchrotron radiation from either Cornell High Energy Synchrotron Source or Stanford Synchrotron Radiation Laboratory, reduced, merged, and scaled with the programs DENZO and SCALEPACK (27, 28). A typical data collection involved two scans, a high resolution scan consisting of 120 frames of 0.8° followed by a low resolution scan of 60 frames of 1.5°. The data collection statistics for all six structures are shown in Table II. Initial models were derived by a high resolution scan of 60 frames of 1.5°. The data collection statistics for all 6 nanolog complexes as seen from the root mean square differences between the models and the ADP-BeF₃⁺ complex as shown in Table IV. This table also reveals that there is very little difference in the fit of the 25-, 50-, and 20-kDa regions relative to the overall root mean square differences as seen previously in the comparison of the BeF₃⁺ and ADP complexes of S1dC (17, 20). As might be expected the root mean square difference between the S1dC·ADP·BeF₃⁺ and each nanolog correlates with the refinement R-factor which is an indication of the quality of each structure.

**RESULTS AND DISCUSSION**

The three-dimensional structures of the S1dC myosin head from *Dictyostelium* complexed with 6 nanologs were determined at high resolution; five were determined to 2.0 Å resolution, whereas one (N-methyl-NPhAE) was determined to 2.3 Å resolution. The overall protein conformation is very similar for all 6 nanolog complexes as seen from the root mean square differences between the models and the ADP-BeF₃⁺ complex as shown in Table III, whereas the electron density associated with the nanologs is shown in Fig. 1. Root mean square differences between the structures and S1dC·Mg·ADP·BeF₃⁺ were calculated with LSQKAB of the CCP4 package (33, 41). The x-ray coordinates have been deposited in the Protein Data Bank with code names as follows: o,p-dinitrophenylaminoethyl diphosphate-BeF₃⁺·S1dC, 1D1A; o-nitrophenylaminopropyl diphosphate-BeF₃⁺·S1dC, 1DOY; m-nitrophenylaminopropyl diphosphate-BeF₃⁺·S1dC, 1D0X; p-nitrophenylaminopropyl diphosphate-BeF₃⁺·S1dC, 1DOZ; o,p-dinitrophenylaminopropyl diphosphate-BeF₃⁺·S1dC, 1DB; and o,nitrophenyl-N-methyl-aminophosphate-BeF₃⁺·S1dC, 1DIC.

**Structure of the Nanolog Complexes of Myosin S1dC**

The electron density for each nucleotide analog is described below. The specific details of the refinement statistics are given in Table III, whereas the electron density associated with the nanologs is shown in Fig. 1. Root mean square differences between the structures and S1dC·Mg·ADP·BeF₃⁺ were calculated with LSQKAB of the CCP4 package (33, 41). The x-ray coordinates have been deposited in the Protein Data Bank with code names as follows: o,p-dinitrophenylaminoethyl diphosphate-BeF₃⁺·S1dC, 1D1A; o-nitrophenylaminopropyl diphosphate-BeF₃⁺·S1dC, 1DOY; m-nitrophenylaminopropyl diphosphate-BeF₃⁺·S1dC, 1D0X; p-nitrophenylaminopropyl diphosphate-BeF₃⁺·S1dC, 1DOZ; o,p-dinitrophenylaminopropyl diphosphate-BeF₃⁺·S1dC, 1DB; and o,nitrophenyl-N-methyl-aminophosphate-BeF₃⁺·S1dC, 1DIC.

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**Electron Density and Conformation of the Nanologs**—The electron density for each nucleotide analog is described below and shown in Fig. 1. The coordination of the nanologs relative to the adenine ring observed in the structure of S1dC·Mg·ADP·BeF₃⁺ is shown in Fig. 2. These clearly indicate the orientation of the nanologs in the active site and their effect on the water structure and conformation of the adenine binding pocket. Although the substituted phenyl rings of the nanologs exhibit a range of positions, the triphosphate-like moiety (PP₃·BeF₃⁺) in each of the nanologs binds in a very similar manner in all complexes; indeed, the locations of the β-phosphate and BeF₃⁺ are essentially identical in all complexes (Fig. 3). The differences between the complexes initiate at the α-phosphate; however, the overall position of the α-phosphate and the bridging oxygen to the ethyl or propyl ligand is still very similar. This occurs because the hydrogen bonding pattern for the triphosphate-like moiety is essentially identical for all nanologs and nucleosides observed thus far coordinated to myosin. The conserved position for the triphosphate moiety has profound implications on the orientation of the nanologs since it restricts the position of the alkyl linker as discussed below. A Mg²⁺ ion is also present in the active site of all six complexes, as was observed for the previous S1dC·nucleotide structures (17, 20, 21, 25, 26). The geometry of the metal coordination site is essentially identical in all cases. The physiologically important differences arise from the manner in which the remainder

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2 E. Pate, R. Cooke, and R. G. Yount, manuscript in preparation.
3 The exact composition of the beryllium fluoride species is unknown (53) but is believed to be a mixture of hydroxyfluorides; consequently, this complex will be designated as BeF₃⁺.
of the nanolog binds in the adenosine binding pocket as discussed below.

In S1dC-MgADP-BeF₄, the adenine lies in a cavity bordered by residues Phe¹²⁹-Tyr¹³⁵ and Glu¹⁸⁷-Lys¹⁹¹ (17). Few direct interactions are formed between the base and the protein with the exception of a hydrogen bond formed between the side
chain of Tyr$_{135}$ and between the C-6 amino group of the adenine ring. However, there are indirect hydrogen bonds made through intervening water molecules between N-1 of the adenine ring and both the carbonyl and amide nitrogen of Ile$_{152}$ and between N-7 of the adenine ring and the side chain of Asn$_{188}$. An analogous situation is observed for the nanologs, where there appear to be few specific interactions with the substituted phenyl ring. Similarly, in S1dC-MgADP-BeF$_x$, there are few interactions between the ribose moiety of the nucleotide and the protein. It would appear that the primary function of the ribose is to bridge the triphosphate-binding site and the adenine binding pocket. The notable exception is a hydrogen bond between the side chain of Asn$_{127}$ and the ribose ring oxygen. At first sight it might appear that there is a similar interaction between the bridging amino group of the nanologs and Asn$_{127}$; however, as described under “Experimental Procedures,” on the basis of model compounds, it is expected that the lone pair of electrons on the bridging nitrogen is substantially delocalized for the secondary amines such that they will be unable to participate in a direct hydrogen bond. Consistent with this suggestion, the bond distances between the N-8 of Asn$_{127}$ and the bridging nitrogen of the nanologs range from 3.0 to 4.0 Å and are indicative of a weak interaction. Remarkably there are no significant differences in the positions of any of the side chains that make up the adenine binding pocket in any of the nanologs, even though the substituted phenyl groups occupy a variety of positions in the pocket.

S1dC-o-NPhAE—The electron density for the entire nanolog is very well defined in the ortho-nitrophenylaminoethyl diphosphate-BeF$_x$ complex (Fig. 1a). As also observed in the o,p-DNPAE complex, the nitro group is directed into the adenine binding pocket, whereas the other side of the ring, which is hydrophobic, faces out to the aqueous environment. There is a hydrophobic interaction between the methylene components of Arg$_{130}$ as well with main chain carbon atoms of the same residue. The oxygen and nitrogen atoms attached to the ethyl linker adopt an eclipsed conformation that allows the nitro group to face into the adenine binding pocket. The substituted phenyl moiety does not adopt the same position as the adenine in S1dC-MgADP-BeF$_x$ but is located somewhat further out of the pocket (Fig. 2a). Interestingly, in this complex two water molecules are located at the same position as the hydrogen bonding components of the adenine ring and interact with a water network in the remainder of the pocket that is very similar to that seen in S1dC-MgADP-BeF$_x$. The ortho-nitro group is hydrogen-bonded to one of the additional water molecules in the adenine binding pocket. This suggests that the adenine binding pocket and its constellation of water molecules exist in the same orientation even in the absence of nucleotide, and this feature contributes to the high binding affinity of myosin for ATP. There are remarkably few changes in the positions of the side chains that make up the adenine binding pocket, which is a feature shared by all of the nanolog complexes.

S1dC-m-NPhAE—In the complex with meta-nitrophenylaminoethyl diphosphate-BeF$_x$, the electron density is well defined from the diphosphate through C-1 of the phenyl ring (Fig. 1b). The electron density for the remainder of the ring is less well defined although the location of the meta-nitro group is readily identifiable. This suggests that the ring is either mobile or adopts multiple conformations and implies that this nanolog does not bind as tightly as the nucleotide. The location of the nitro group and phenyl ring is very similar to that adopted by the nitro group in the ortho complex and faces into the adenine binding pocket (Fig. 2b). The hydrophobic component of the ligand is exposed to the aqueous environment. This arrangement allows for a very similar water structure to that observed in the o,p-DNPAE and o-DNPAG complexes and suggests that conservation of the water structure is an important determinant in the way that ligands bind in the active site. The aminoethyl linker adopts a staggered conformation relative to the bridging oxygen of the α-phosphate and...
is opposite to that observed in the ortho compound. As a consequence, the bridging nitrogen atom is exposed to solvent rather than being buried in the nucleotide binding pocket. This suggests that the conformation of the aminoethyl linker is arranged to allow the ortho- or meta-nitro groups to optimize their interactions with the water structure of the adenine binding pocket.

*S1dCp-NPhAE*—The electron density for the complex between S1dC and para-nitrophenyl diphosphate-BeF₄⁻ is very well defined for the entire ligand (Fig. 1c). The para-nitro group is exposed to the solvent and makes no contacts with the protein. There are no ordered water molecules in close proximity to the phenyl ring. The closest ordered water molecule lies 4.8 Å from the ring. Overall there are fewer water molecules in the adenine binding pocket than observed in nanologs that carry a nitro group in either the ortho or meta position. Again there are no changes in the position of the protein side chains that make up the adenine binding pocket. The hydrophobic component of the phenyl ring adopts a similar position to that observed in o-NPhAE except that the ring is displaced 0.7 Å away from the triphosphate moiety relative to this latter complex that allows the nitro group to interact with solvent. This shift is accompanied by a small movement in the main chain atoms between Pro¹²⁸ and Ile¹³² and is similar to that observed in the o,p-dinitro compound discussed below.

*S1dCo,p-DNPPhAE*—The electron density for o,p-dinitrophenylaminomethyl diphosphate-BeF₄⁻ is reasonably well defined (Fig. 1d). As might be anticipated from the o-NPhAE and p-NPhAE complexes, the nitro group in the ortho position faces into the adenine binding pocket, whereas the para group points out into the solvent. Again the dinitrophenyl group does not occupy the same location as adenine in S1dC-MgADP-BeF₄⁻. As in the ortho-substituted nanolog, the space that would be occupied by the adenine is taken up by well ordered water molecules that are coordinated in part by the ortho-nitro group. However, in the case of the dinitro compound the ortho-nitro group is displaced somewhat from its position in o,NPhAE in order to accommodate the para-nitro group. Overall the substituted phenyl group is located ~0.7 Å further from the ortho-phosphate relative to the position observed in o,NPhAE. This allows the para-nitro group to interact with solvent and causes a small displacement (~0.5 Å) of the polypeptide chain between Pro¹²⁸ and Arg¹³¹ away from the adenine binding pocket. As a consequence of these small changes the ortho-nitro group does not interact as well with the water structure in the active site. Other than the small shifts associated with Arg¹³¹, there are no major changes in the conformation of the protein ligands that coordinate this nanolog.

*S1dCo,p-DNPPhAP*—The electron density for the triphosphate moiety of o,p-dinitrophenylaminopropyl triphosphate is well defined; however, the electron density for the remainder of the analog deteriorates progressively as the distance increases from the α-phosphate (Fig. 1e). From this it is apparent that the dinitrophenyl moiety for this analog adopts multiple conformations in the adenine binding pocket. The atoms in the dinitrophenyl group were refined with an occupancy of 50% and clearly show partial occupancy of the nitro groups. Interestingly, the dinitrophenyl moiety does not bind in the same location as the adenine in S1dC-MgADP-BeF₄⁻ but rather is exposed to solvent where the adenine binding pocket is now occupied by four well ordered water molecules. There are remarkably few changes in the positions of the side chains that make up the adenine binding pocket.

*S1dCN-methyl-NPhAE*—The electron density for the ortho-nitrophenyl-N-methyl-aminomethyl diphosphate-BeF₄⁻ complex is well defined (Fig. 1f). In this nanolog the bridging nitrogen is not coplanar with its substituents as seen in all of the other analogs. Consequently the bridging nitrogen atom is, in principle, a chiral center that has implications for the coordination of the complex. Close examination suggests that the R configuration fits the electron density better than the S configuration. In the R configuration the nitrogen lone pair forms a hydrogen bond to Asn¹²⁷, whereas in the S configuration the nitrogen lone pair would be directed toward the solvent and would not interact with any other protein ligand or ordered solvent molecule. The bond distance between the nitrogen of N-methyl-NPhAE and N-δ of Asn¹²⁷ is 3.0 Å, which is not indicative of a strong hydrogen bond. A hydrogen bond to Asn¹²⁷ is observed to the ribose oxygen in S1dC-MgADP-BeF₄⁻ but is most likely not observed in the other complexes on account of the delocalization of the lone pair on their bridging nitrogen. The nitro group is exposed to solvent, whereas the hydrophobic component of the nitrophenyl moiety as well as the methyl group on the bridging nitrogen point into the adenine binding pocket and are not in close contact with any of the water molecules in the adenine binding pocket. Interestingly the water structure in the adenine binding pocket is similar to that observed in S1dC-MgADP-BeF₄⁻. There are no water molecules closer than 3.5 Å to the ortho-nitrophenyl-N-methyl-amino moiety. Again there are no significant changes in the positions of any protein side chains.

Comparison of the S1dC Nanolog Structures with S1dC-MgADP-BeF₄⁻—At first glance, the chemical structures of the nanologs appear to bear little relationship to that of adenosine (Table I); however, a superficial comparison of the chemical structures of ATP and the nanologs suggests that the aminoethyl or propyl group might fulfill the role of the ribose, and the substituted phenyl moiety takes the place of the adenine. Indeed, it is observed that the C-1 atoms of the alkyl linkers are located in all complexes at approximately the same position as C-5 of the ribose. Significantly, the O—C-1 bonds of the nanologs are oriented in a similar manner to the O—C-5 bond of the ribose. Examination of the nucleotide binding pocket reveals that it would be sterically unfavorable to rotate C-1 of the linker into any other general position without introducing a steric clash with either the C-o of Gly¹⁸⁴ or the C-γ of Glu¹⁸⁷. Thereafter, the nitrogen atoms of the amino alkyl linkers are located in a variety of positions relative to the C-1 of the ribose, but always at a site that attempts to optimize the interaction of the substituted phenyl ring with the solvent structure of the adenine binding pocket. The positions of the substituted phenyl groups appear to be unrelated to the location of the purine until careful consideration of the solvent structure and restraints imposed by the O—C-1 bond.

The phenyl groups adopt a variety of locations depending on the substitution and the length and type of the linkage to the triphosphate moiety (Figs. 2 and 3). Several common themes arise from these structures. First, all of the phenyl rings lie approximately in the same plane, where this plane is inclined at an angle of ~20° to that of the purine ring in S1dC-MgADP-BeF₄⁻. Second, all of the ethyl-linked nanologs that carry an ortho-nitro group, with the exception of N-methyl-NPhAE, adopt the same orientation and location for the phenyl ring where the nitro group points into the adenine binding pocket. Interestingly the nitrophenyl moiety is located further out of the pocket than the adenine in S1dC-MgADP-BeF₄⁻ such that only the nitro group overlaps with the purine ring. In so doing the nitro group coordinates to two new water molecules that appear to take the place of the amino group at C-6 and nitrogen at N-7 of the adenine (Fig. 4). These additional water molecules then coordinate to the protein and water structure in a manner that is analogous to the
The structures of the nanologs provide an opportunity to the ortho-nitro group, and the bridging nitrogen is no longer coplanar with its substituents. This allows this compound, unlike all others, to form a definite hydrogen bond to Asn$^{127}$. This hydrogen bond, coupled with the restraints imposed by the orientation of the $\alpha$-phosphate, forces the ortho-nitro group to be oriented into the solvent and the phenyl ring to be directed into the comparatively hydrophilic adenine binding pocket. As a consequence, this substituted phenyl ring has fewer binding interactions than any of the other nanologs even though the N-methyl-substituted nanolog forms the analogous hydrogen bond to Asn$^{127}$ observed with ADP.

**Structural and Kinetic Correlations**—This series of structures raises the question of what attributes determine whether an organotriphosphate is a good substrate for the ATPase activity and is able generate movement. The basic kinetic and physiological characteristics of these compounds have been determined for rabbit skeletal muscle myosin and reveal that while they are all hydrolyzed by myosin to a significant extent, they differ greatly in their ability to generate movement and force (8, 9, 18, 42). Independent of the structural information, it is difficult to find any definite correlations between the various kinetic and physiological parameters themselves. Table V shows a compilation of the kinetic properties of the nanologs for skeletal muscle myosin together with a summary of how they interact with the nucleotide-binding site for S1dC. Even though the structural studies have been carried out on Dictyostelium S1dC (17, 43) and the overall similarity in their kinetics (44—46). Thus it is feasible to utilize the structural studies on S1dC to account for the kinetic properties of the nanologs in skeletal muscle and better understand the manner in which ATP interacts with myosin.

The structures of the nanologs provide an opportunity to way in which the adenine interacts with its binding pocket. Significantly the nitro group does not form a hydrogen bond with Tyr$^{135}$ as did the amino group of adenine, rather the interaction is mediated by a water molecule. This observed binding differs from a prediction that the nitro group would interact directly with Tyr$^{135}$ (42). The same orientation for the phenyl ring is also observed in the para-nitro complex, p-NPhAE, where the para-nitro group points into the solvent and the hydrophobic component of the phenyl ring points in the pocket.

The structure of the meta-nitro complex, m-NPhAE, further illustrates the importance of interactions with the water structure in the adenine binding pocket. In this case the benzene ring overlaps the position in the ortho compound and is rotated such that its meta-nitro group is in the same proximity as the ortho-nitro group of o-NPhAE. This changes the position of the bridging nitrogen and conformation of the alkyl linker such that the bridging nitrogen is exposed to solvent. In this manner the meta-nitro group is able to maintain and interact favorably with the network of water molecules observed in the adenine binding pocket.

In the case of $o,p$-dinitrophenylaminopropyl triphosphate, the phenyl adopts the same overall orientation as that seen in the corresponding ethyl-linked nanolog; however, the additional methylene group displaces the phenyl ring and its nitro group from the location favored by the ethyl linkage (Fig. 2c). It appears that this displacement reduces the stability of the ring in the adenine pocket such that multiple conformations are adopted by this substrate. Interestingly the water structure observed in the adenine pocket is as well ordered as that seen the other nanolog complexes, even though it does not interact with the nitro groups.

The addition of a methyl group to the bridging nitrogen of the nanolog has a profound effect on the orientation of the nanolog in the active site. In this case the presence of a methyl group prevents the formation of an intramolecular hydrogen bond to the $o$-nitro group, and the bridging nitrogen is no longer coplanar with its substituents. This allows this compound, unlike all others, to form a definite hydrogen bond to Asn$^{127}$. This hydrogen bond, coupled with the restraints imposed by the orientation of the $\alpha$-phosphate, forces the ortho-nitro group to be oriented into the solvent and the phenyl ring to be directed into the comparatively hydrophilic adenine binding pocket. As a consequence, this substituted phenyl ring has fewer binding interactions than any of the other nanologs even though the N-methyl-substituted nanolog forms the analogous hydrogen bond to Asn$^{127}$ observed with ADP.

**Structural and Kinetic Correlations**—This series of structures raises the question of what attributes determine whether an organotriphosphate is a good substrate for the ATPase activity and is able generate movement. The basic kinetic and physiological characteristics of these compounds have been determined for rabbit skeletal muscle myosin and reveal that while they are all hydrolyzed by myosin to a significant extent, they differ greatly in their ability to generate movement and force (8, 9, 18, 42). Independent of the structural information, it is difficult to find any definite correlations between the various kinetic and physiological parameters themselves. Table V shows a compilation of the kinetic properties of the nanologs for skeletal muscle myosin together with a summary of how they interact with the nucleotide-binding site for S1dC. Even though the structural studies have been carried out on Dictyostelium S1dC (17, 43) and the overall similarity in their kinetics (44—46). Thus it is feasible to utilize the structural studies on S1dC to account for the kinetic properties of the nanologs in skeletal muscle and better understand the manner in which ATP interacts with myosin.

The structures of the nanologs provide an opportunity to
dissect the contributions of the triphosphate, ribose, and adenine moieties to the binding affinity of a nucleotide or analog to the active site. Clearly a substantial component of the binding affinity is provided by the triphosphate moiety alone. Evidence for this can be deduced from the relative binding affinity of myosin for ADP and ATP which are $1 \times 10^6$ and $3 \times 10^{11} \text{ M}^{-1}$, respectively (47, 48). This suggests that more of the binding energy is contributed by the triphosphate. Certainly most of the interactions between MgADP-Be$_F^-$ and S1dC occur via the triphosphate moiety (17). What then is the contribution of the remainder of the nucleotide to the kinetic cycle? Clearly it takes more than just a triphosphate to support movement. One possibility is that the primary purpose of the ribose and base is to control the on and off rates of the nucleotide and modulate the rate constants during the contractile cycle.

A comparison of the kinetic and structural properties of the nanologs relative to ATP suggests that the ability of a substrate to sustain tension and to generate movement correlates at a minimal level with a well defined interaction with the water structure observed in S1dC. Minimal level with a well defined interaction with the water to sustain tension and to generate movement correlates at a nanologs relative to ATP suggests that the ability of a substrate to control the on and off rates of the nucleotide and modulate the rate constants during the contractile cycle. It is also evident that a small correlation exists between the chemical steps in the contractile cycle. It seems likely that a key aspect is how they interact with the protein itself.

Finally, the study of a series of closely related substrates reveals that the adenine binding pocket is essentially identical, regardless of the substrate analog bound in the active site. This suggests that very little of the binding energy for ATP is devoted to reorganization of the active site. In addition it appears that the water structure in adenine binding pocket is similar for all compounds that are able to support active contraction. This suggests that the water structure in the adenine binding pocket plays a special role in the function of substrate recognition.

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**TABLE V**

Kinetic and structural properties of myosin-nanolong complexes

|                | Mg$^{2+}$-NTPase | Actin-activated NTPase | Active tension | Shortening velocity | Number hydrogen bonds$^a$ |
|----------------|------------------|------------------------|----------------|--------------------|--------------------------|
|                | $\mu$mol P$_i$ mg S1$^{-1}$ min$^{-1}$ | $N$ mm$^{-2}$ | $mm$ s$^{-1}$ |                    |                          |
| ATP$^b$        | 0.03             | 14.7                   | 0.24           | 1.07               | 3                        |
| o,NPhAE$^b$    | 0.12             | 3.2                    | 0.12           | 1.05               | 3                        |
| p,NPhAE$^b$    | 0.35             | 1.3                    | 7.8 $\times$ 10$^{-3}$ | 5 $\times$ 10$^{-4}$ | 1                        |
| o,p-DNPhAE$^c$ | 0.94             | 1.2                    | 0.01           | 1.1 $\times$ 10$^{-4}$ | 0                        |
| o,p-DNPhAP$^d$ | 0.12             | 9.7                    | 0.062          | 0.35               | 2                        |
| N-Methyl-NPhAE$^d$ | 0.11         | 1.2                    | 0.0025         | 0.0                | 2$^2$                    |

$^a$ Number of hydrogen bonds to the water network in the adenine binding pocket.
$^b$ Kinetic data from Pate et al. (18).
$^c$ Kinetic data from Wang et al. (9).
$^d$ In the case of o,p-DNPhAP there is an additional water molecule in the adenine binding pocket compared with all other complexes that allow the displaced o-nitro group to form two hydrogen bonds.
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