Nucleotide-dependent Allosteric within the ABC Transporter ATP-binding Cassette

A COMPUTATIONAL STUDY OF THE MJ0796 DIMER

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ATP-binding cassette transporters perform energy-dependent transmembrane solute trafficking in all organisms. These proteins often mediate cellular resistance to therapeutic drugs and are involved in a range of human genetic diseases. Enzymological studies have implicated a helical subdomain within the ATP-binding cassette nucleotide-binding domain in coupling ATP hydrolysis to solute transport in the transmembrane domains. Consistent with this, structural and computational analyses have indicated that the helical subdomain undergoes nucleotide-dependent movement relative to the core of the nucleotide-binding domain fold. Here we use theoretical methods to examine the allosteric nucleotide dependence of helical subdomain transitions to further elucidate its role in interactions between the transmembrane and nucleotide-binding domains. Unrestrained 30-ns molecular dynamics simulations of the ATP-bound, ADP-bound, and apo states of the MJ0796 monomer support the idea that interaction of a conserved glutamine residue with the catalytic metal mediates the rotation of the helical subdomain in response to nucleotide binding and hydrolysis. Simulations of the nucleotide-binding domain dimer revealed that ATP hydrolysis induces a large transition of one helical subdomain, resulting in an asymmetric conformation of the dimer not observed previously. A coarse-grained elastic network analysis supports this finding, revealing the existence of corresponding dynamic modes intrinsic to the contact topology of the protein. The implications of these findings for the coupling of ATP hydrolysis to conformational changes in the transmembrane domains required for solute transport are discussed in light of recent whole transporter structures.

ATP-binding cassette (ABC) transporters couple hydrolysis of ATP to vectorial translocation of substrates across cellular membranes, typically against a concentration gradient. These integral membrane proteins are involved in the transport of a wide range of substrates, including ions, sugars, amino acids, peptides, proteins, lipids, and xenotoxins, and form one of the largest protein families across all species (1). Many ABC transporters are central to important biomedical problems, including resistance of cancers, microbes, and parasites to cytotoxic drugs, as well as human genetic diseases such as cystic fibrosis and Stargardt disease (2). Understanding the molecular mechanism of these proteins is necessary for the rational design of agents to control their transport function. The architecture of ABC transporters comprises a conserved core structure of two transmembrane domains (TMDs) and two cytosolic ABCs, also known as nucleotide-binding domains (NBDs). Most prokaryotic ABC transporters are importers with the core TMDs and NBDs expressed as separate subunits, whereas most eukaryotic ABC transporters are exporters that commonly have a single polypeptide for the core structure, with each NBD C-terminal to each TMD. The primary sequences of the NBDs are generally highly conserved and contain the Walker A and B consensus motifs for ATP binding (3) and the “LSGG(Q)” motif or “signature sequence,” which also interacts directly with ATP (4) (see Fig. 1A). The TMDs, which contain cytosolic as well as membrane-spanning regions, are less well conserved, reflecting their role in substrate binding and translocation.

Notwithstanding recent advances in structural studies, crucial questions remain concerning how ATP binding and hydrolysis in the NBDs are coupled to protein conformational changes in the TMDs, which drive vectorial transmembrane substrate movement. Many protein monomers are composed of relatively independent subdomains, which are able to change conformation cooperatively and move as rigid bodies, thus providing the structural flexibility that enables the functioning of the enzyme (5). In other P-loop NBDs such as Ras, myosin, RecA, and F$_{1}$-ATPase, NBD intradomain motions have been found, or are generally thought, to be central to the mechanism of the oligomeric complex, mediating the transduction of the free energy of ATP hydrolysis to functional changes in the protein conformation (6, 7). Crystallographic studies of the ABC ATPase have revealed a bilobal design (see Fig. 1A), in which a region comprising three α-helices, known as the helical domain (HD), is flexibly attached to the rest of the NBD and rotates as a rigid body (8–10). The HD contains the LSGG signature sequence, which interacts with the nucleotide bound to the Walker A and B residues of the opposite monomer, thus forming two active sites in the NBD dimer (Fig. 1A). Enzymological studies have suggested that the HD mediates interdomain allosteric communication between the ATPase active site and the...
substrate-binding site(s) in the TMDs (reviewed in Ref. 10). Nevertheless, the molecular details of this critical allosteric process remain obscure and the exact role of the rotation of the HD in current models of the catalytic cycle is unclear.

It has been suggested that a transition between “closed” and “open” conformations of the HD is dependent on the active site occupancy (ATP, ADP, or empty), being mediated by a conserved glutamine, Gln-90 in MJ0796 (8). The side chain of the conserved glutamine coordinates the active site catalytic magnesium ion, in a manner similar to other P-loop ATPases (6), and is proposed to act as a sensor of the γ-phosphate of the bound ATP (11). Gln-90 is located at the C terminus of β-strand 5 of the central β-sheet, at the junction with a linker sequence (Q-loop; residues 91–108 in MJ0796) that connects the ABC core to the HD (see Fig. 1B). Karpowich et al. (9) suggested that γ-phosphate release from the active site results in withdrawal of the glutamine and the coupled outward rotation of the HD to the open state. The structural data, however, are inconclusive regarding the role of the conserved glutamine in controlling the orientation of the HD. Of six ABC transporter NBDs for which crystal structures of different ligand-bound states, including an ATP- or ATP analogue-bound state, are available (8, 11, 12, 13–17), only MJ0796 (dimer and isolated monomer, respectively) show a clear rotation of the HD between different nucleotide-bound states of the same protein.

Here we use molecular dynamics (MD) simulations and elastic network analyses (Gaussian network model (GNM)) (18) of the MJ0796 NBD dimer and monomer in tandem to characterize the motions of the HD and the effect of the ligand on its conformational changes. GNM analysis is a computational method for identifying the principal dynamic modes, which play a predominant role in effectuating the functional motions of the protein (19). The GNM approach is based on a coarse-grained model in which the protein is represented as a set of points, corresponding to the Cα atom positions, connected to their nearest neighbors by simple harmonic springs (20). Elastic network analysis has been used previously to identify functional dynamics in other membrane transporters (21, 22). GNM analysis of the MJ0796 dimer provides an independent methodology that supports the view that the rotation of the HD is fundamental to functionally important conformational transitions of the NBD monomer and dimer. This analysis also suggests the possibility that the function of the NBD dimer involves asymmetric dynamics of the monomers.

In contrast to the GNM analytical approach, MD simulations use a full atomic model, including explicit water molecules, with empirical force fields to model the dynamics of the protein. This methodology is a valuable complement to crystallographic structural analysis, which is often unable to analyze the wild-type protein bound to its natural reactant ligand(s). To more fully characterize the relationship between active site nucleotide occupancy, interactions of the conserved glutamine and transitions of the HD, we performed 30-ns MD simulations of the ATP-bound, ADP-bound, and apo states of the isolated monomer, using the ATP-bound monomer from the MJ0796 dimer as the starting structure. Our data support the idea that the conserved glutamine senses the presence of ATP in the active site and maintains the equilibrium conformation of the HD in a more closed state than the apo or ADP-bound monomer conformations.

Here we also report that in three independent 22-ns simulations of the ATP/ADP-bound dimer, no significant alteration in the conformation of the HD occurred. In contrast, replacement of ATP with ADP in one active site of the dimer results in a significantly altered conformation of the HD in the monomer with ADP bound to its Walker A and B residues. The observed HD rotation opens a large gap between helix 2 and the region of the α3–4 loop (Fig. 1A) of the ADP-bound monomer. This conformational change, which is reproduced in four independent runs, is different from that which can be inferred from any published crystal structures, with the exception of the Thermococcus litoralis MalK NBD (Protein Data Bank (PDB) ID code 1G29 23). A possible role for this transition in mediating TMD-NBD interactions in response to ATP hydrolysis is discussed in the light of recent whole transporter structures.

**EXPERIMENTAL PROCEDURES**

The starting coordinates for the MD simulations were taken from the x-ray structure of dimeric MJ0796 E171Q (monomer A was used for the monomer simulations) published in the PDB (ID code 1L2T, 1.9-Å resolution) (11). This structure contains ATP in the active site and, therefore, was preferred for the current MD studies. The wild-type active site glutamate residue 171 was restored, and a magnesium ion replaced the sodium ion in the active site in the crystal structure. MD simulations were performed using the parallel MD program NAMD 2.5 (24), the CHARMM27 force field for ligands and protein (25), and the TIP3P model for water (26). The SHAKE algorithm was used to constrain the bonds containing hydrogens to their equilibrium length (27). A cutoff of 11 Å (switching function starting at 9.5 Å) for van der Waals interactions and real space electrostatic interactions was used. Periodic boundary conditions were used, and minimum distances between periodic images of the protein were 20 Å in all cases. Sodium ions were added to the system to maintain electrical neutrality where required. The particle-mesh Ewald method (28) was used to compute long range electrostatic forces with a grid density of ~1/Å². An integration time step of 1.5 fs was used, permitting a multiple time-stepping algorithm to be employed in which interactions involving covalent bonds, and short range nonbonded interactions were computed every time step, whereas long range electrostatic forces were computed every two time steps. Langevin dynamics was utilized to keep a constant temperature of 358 K in all simulations, to mimic the native environment of Methanococcus jannaschii (29). Constant pressure simulations at 50 atm were conducted using the hybrid Nosé-Hoover Langevin piston method with a decay period of 100 fs and a damping timescale of 50 fs.

The solvated starting structure was minimized using conjugate gradient minimization to a 0.5-kcal/mol-Å² root mean square (r.m.s.) gradient with all protein heavy atoms fixed. Water molecules and protein hydrogens were then further minimized during a 50-ps MD run at 358 K, in which all protein heavy atoms were again fixed. This starting model was then minimized with harmonic positional constraints, derived from the crystallographic temperature factors (B), on the NCoC backbone. A 2000/B kcal/mol-Å² force constant was used to
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minimize the system to a 0.5-kcal/mol-Å r.m.s. gradient. The constraints were gradually removed, by subsequent minimizations to a 0.1-kcal/mol-Å r.m.s. gradient, scaling the initial force constants by factors of 0.5, 0.15, 0.05, and 0. The minimized structure was then heated from 50 to 350 K in steps of 25 K using velocity reassignment during a 16.2-ps MD run. The equilibrated system was then used for the production runs. For analysis, the coordinates were saved every 0.75 ps.

Three 30-ns simulations of the ATP-bound, ADP-bound, and apo forms of the MJ0796 monomer were performed. Four simulations were performed of the ADP/ATP-bound MJ0796 dimer (Table 1). Simulations 1 and 2 used the crystallographic dimer, whereas simulations 3 and 4 used a constructed dimer of two monomer Bs. Durations were: simulation 1 (ADP in monomer A), 28 ns; simulation 2 (ADP in monomer B), 19 ns; simulation 3, 20 ns; and simulation 4, 25 ns. Three 22-ns simulations were performed of the ATP/ATP-bound dimer (Table 1). Simulation 5 used the crystallographic dimer, whereas simulations 6 and 7 used a constructed dimer of two monomer Bs. In all simulations, the starting x-ray structure was re-equilibrated for the production run. All simulations remained stable throughout, and simulation run durations were chosen to optimize computational resources.

Principal component analysis of the simulation α-carbon atom trajectories was performed using the GROMACS package (30). Buried solvent-accessible surface area calculations were performed using XPLOR4 with a 1.4-Å probe radius. The program Hingefinder was used for analysis of domain rotations using the slow partitioning algorithm (32).

GNM analysis was performed on the MJ0796 dimer (PDB 1L2T) using the iGNM online facility (33) using a 10-Å nearest neighbor cutoff criterion. This analysis showed that principal modes 1–5, which represent the most collective motions of the protein, account for over 30% of all residue fluctuations within the dimer.

**RESULTS**

The GNM analysis was performed on the MJ0796 dimer structure using the iGNM webserver using a 10-Å nearest neighbor cutoff criterion. This analysis showed that principal modes 1–5, which represent the most collective motions of the protein, account for over 30% of all residue fluctuations within the dimer.

**GNM Mode 1 Shows Anticorrelated Motions of the NBDs in the Dimer—**As found previously for other homodimers, the hinge region in mode 1 lies at the interface between the monomers (Fig. 2A), and this mode is exclusively induced by the dimeric form (19). Thus, dimerization induces a new cooperative mode, not found in the isolated monomer. This result is consistent with previous biochemical and structural data, which indicate that the dimeric form of the ABC NBD is required for catalytic activity (35–40) and illustrates the relationship between the dominant dynamic modes and the functioning of the protein. Interestingly, visualization of the GNM modes indicates that the motion described by mode 1 (Fig. 2A) would most likely involve one NBD moving toward the TMD.

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**FIGURE 1. Three-dimensional structure of the ABC transporter NBD.**

A, ABC transporter NBD dimer, with a ribbon diagram of the MJ0796 E179Q dimer (PDB 1L2T). β-Strands are depicted as arrows, and α-helices are shown as coiled ribbons. Loops connecting secondary structural elements are depicted as thick lines. The ATP-binding core subdomain is shown in dark blue (β-strands) and light blue (α-helices), the antiparallel β-subdomain (ABCβ) is in green, and the α-subdomain (helical domain) is in red. Two ATP molecules are shown in stick form at the dimer interface, sandwiched between the P-loop of one monomer and the LSGG region of the other. The α3–4 loops are indicated by large asterisks, the A-loops are indicated by vertical arrows, and α-helix 2 (colored tan) is indicated by diagonal arrows. The view is from the plane of the membrane. B, interaction of the conserved glutamine with the ABC transporter NBD active site. Monomer A of the MJ0796 E179Q dimer with structural features, cofactors, and residue side chains as indicated. The crystal structure contains a sodium ion in the position that the catalytic magnesium ion (Mg2+) is expected to take (green sphere). The oxygen atoms of the two coordination water molecules are depicted as red spheres. ATP is shown in stick form with: carbon as yellow, nitrogen as blue, oxygen as red, and phosphorous as gold. The PDB coordinates of the side-chain amido oxygen and nitrogen atoms have been swapped to maintain the expected electrostatic complementarity in the Q90-Mg2+ interaction.
while the other moves away, thus suggesting the possibility of asymmetric engagement of the NBDs and the TMDs.

**Principal Modes 2 and 3 Reveal Both In-and Out-of-phase Patterns of Mobility within the NBD Dimer**—Fig. 2, B and C, illustrate the second and third principal modes, respectively, as calculated by the GNM analysis of the MJ0796 dimer. As found previously for other homodimers (19), mode 2 of the dimer is indistinguishable from that of mode 1 of the isolated monomer. In both principal modes 2 and 3 of the dimer, the HD moves in an opposite sense to the rest of the NBD, clearly showing that the HD is structurally and dynamically a distinct entity within the NBD. As in mode 1, the monomers are 180° out of phase with respect to mode 2, but symmetrical with respect to mode 3. These data thus suggest that both in-phase and out-of-phase motions of the two HDs may be involved in the functional dynamics of the dimer.

**Modes 4 and 5 Indicate Asymmetric Patterns of Mobility between the Monomers in the NBD Dimer**—Fig. 2, D and E, illustrate the fourth and fifth principal modes. In contrast to modes 1–3, modes 4 and 5 reveal asymmetric dynamics between the two monomers. Although the most significant fluctuations in these two modes involve the dynamics of the A-loops (Fig. 1A 41), other possibly significant dynamic asymmetries also appear. In mode 4, monomer B undergoes relative motions of its HD and the core β-sheet, whereas monomer A is relatively quiescent. In mode 5, in monomer A, the region of helix 2 and the α3–4 loop (see Fig. 1A) within the HD undergo opposite sense motions, while the equivalent regions in monomer B undergo concerted motions in the same direction. These dynamic asymmetries between the NBD monomers are notable in view of earlier experimental work that also indicated asymmetric functioning of the monomers (42, 43) and thus may be of significance in the understanding of the catalytic cycle. Moreover, as discussed below, modes 4 and 5 are broadly in accord with the results of our MD analysis of the MJ0796 NBD dimer.

**MD Simulations of the Apo, ADP-bound, and ATP-bound MJ0796 Monomer**—A previous MD study (44) found nucleotide-dependent mobility of the HD in simulations of the HisP monomer using a crystal structure of the isolated monomer containing ATP but not Mg²⁺. However, this study did not address in detail the interaction of the conserved glutamine with the catalytic Mg²⁺, the ADP-bound state of the monomer, nor the transitions from the ATP-bound dimer conformation of the NBD to that of the isolated monomer. We performed unrestrained MD simulations of the apo, ADP-bound, and ATP-bound forms of the MJ0796 monomer, to further characterize the effect of the ligand on its intradomain motions. Monomer A from the MJ0796 E171Q dimer (PDB 1L2T) was used as the starting structure for these simulations after substituting the crystal structure Gln-171 with the wild-type Glu-171 residue, and placement of a Mg²⁺ ion in the canonical position in the catalytic site in the ADP- and ATP-bound states.

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**FIGURE 2.** GNM analysis of MJ0796 dimer (PDB 1L2T) using a 10-Å nearest neighbor cutoff criterion. Stereo pairs of the MJ0796 dimer with each residue colored according to its normalized displacement along the nominated principal mode, with blue being positive and red negative. The coloring is for qualitative purposes and is not strictly to scale. The view of the dimer is from the plane of the membrane, with the interface between the monomers lying horizontal to the page and bisecting the dimer, with monomer A below and monomer B above. This is similar to the view in Fig. 1A. Top to bottom: Principal modes 1–5 (A–E).
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FIGURE 3. Comparison of simulation structures with the starting crystal structure (monomer A from the MJ0796 E171Q dimer; PDB 1L2T) for the apo, ADP-bound, and ATP-bound monomer simulations. A, per residue r.m.s. deviations relative to the initial structure for the average simulation structures over the 27–30 ns period: ATP is blue, ADP is red, apo is green, and black shows the ADP-bound crystal structure (PDB 1F3O). The average structures were r.m.s. fitted to the initial structure using the Ca coordinates of the core domains (for the purposes of our analysis we define this core region in MJ0796 as comprising residues 2–10 (β-strand 1), 23–37 (β-2 and -3), 44–64 (α1 to β-4), 165–171 (β-6), 198–202 (β-7), and 214–229 (β-8 and -9)). The inset shows, for residues 85–165, the per residue r.m.s. deviations for the average structures after r.m.s. fitting to the initial structure using the Ca coordinates of conserved residues of the helical domain (residues 99–107 and 146–161 in MJ0796). B, rotation of the helical domain. Upper panel: stereo pair showing axes of rotation of the helical domain. The MJ0796 monomer A (PDB 1L2T) is shown in α-carbon trace with the HD (rotating domain) colored red and the rest of the NBD blue. The long green line shows the rotation axis between the maximum and minimum projection structures of principal mode 1 from the ADP-bound monomer simulation (0–30 ns). The two short green lines connect the centers of mass of the rotating domain to the pivot point on the rotation axis (rotation 14.2°, error 2%). The yellow lines similarly illustrate the HD rotation when monomer A from the ATP-bound dimer is compared with the ADP-bound MJ0796 crystal structure (PDB 1F3O, rotation 17.5°, and error 11.2%). All structures were aligned to the MJ0796 monomer A (PDB 1L2T) crystal structure (shown), using the Ca coordinates of the core domains, and are not shown for clarity (see lower panel). Lower panel: left, ribbon diagram showing overlay of the ATP-bound (blue) and ADP-bound (red) crystal structures (as in the upper panel). The structures were aligned using the coordinates of the core domain. Only the helical domain of the ADP-bound monomer is shown. The short arrow indicates the Pro-loop, which is the approximate hinge region for the helical domain rotation. The upper long arrow indicates the LSGG of the ADP-bound monomer, and the lower long arrow indicates the LSGG of the ATP-bound monomer. The view is along the axis of the LSGG helix 5 of the ATP-bound monomer. Right, ribbon diagram showing overlay of the trajectory frames from the ADP-bound monomer simulation corresponding to the maximum (red) and minimum (blue) projection structures of principal mode 1. The structures were aligned using the coordinates of the core domain. Only the helical domain of the maximum structure is shown. The short arrow indicates the Pro-loop, which is the approximate hinge region for the helical domain rotation. The upper long arrow indicates the LSGG of the maximum, and the lower long arrow indicates the LSGG of the minimum structure. C, retraction of the conserved glutamine from the active site in the ADP-bound monomer. Ribbon diagram showing overlay of the trajectory frame from the ADP-bound monomer simulation corresponding to the maximum projection structure of principal mode 1 (ochre) and the starting structure (purple). The structures were aligned using the coordinates of the core domain. Mg-ADP and the Glu-90 side chain are shown in stick form, color-coded as indicated in the Fig. 1B legend. Structural elements are as indicated. The P-loop and upstream β-strand and downstream α-helix are also depicted.

ATP-bound forms. Uniquely, this computational analysis allows us to characterize the effect of unhydrolyzed ATP on the dynamics and structure of the monomer. Evaluation of the change in the overall structure for the simulations is provided by analysis of the Ca r.m.s. deviations relative to the initial structure as a function of time. These data indicate that all simulations were stable, with the average r.m.s. difference and standard deviation from the starting structure over the last 20 ns of each simulation being: ATP, 2.21 ± 0.30 Å; ADP, 2.47 ± 0.33 Å; and apo, 3.09 ± 0.39 Å.

Simulations Show Good Agreement with Crystallographic Data—Fig. 3A shows the per residue r.m.s. deviation of Ca atoms between the average structures over the last 3 ns of each simulation and the starting structure. This shows that the HD (residues 96–162) has moved significantly relative to the core domain in all simulations but to a markedly greater extent in the apo and ADP simulations, where the changes approximate those found between the ATP- and ADP-bound crystal structures. The inset in Fig. 3A shows the per residue r.m.s. deviation from the starting structure when the average structures are r.m.s. aligned using conserved residues of the HD. This plot indicates that, with the notable exception of the loop joining helices 3 and 4 in the HD (α3–4 loop, residues 112–116), the HD maintains its conformation,
moving as a rigid body about flexible hinges at its N and C termini.

Principal component analysis (45) of the ADP simulation trajectory revealed that the dominant dynamic mode described a rotation of the HD about a pivot point lying proximal to the C terminus of the HD (Fig. 3B). This latter region, known as the Pro loop, has been found in other ABC NBDs to act as a hinge for the HD rotation (43, 46). Although the pivot point and rotation axis for the HD between the ADP- and ATP-bound crystal structures are not identical to those obtained from the principal component analysis of the ADP-bound monomer simulation, they are similar in that both axes are approximately parallel to the plane described by the central β-sheet of the NBD, and the pivot points are proximal to the C terminus of the HD (Fig. 3B). In addition, in the ADP-bound crystal structure, the Gln-90 Cα atom is withdrawn from the active site, and its side chain does not coordinate the active site catalytic Mg$^{2+}$ ion as it does in the ATP-bound dimer. This is accompanied by alterations in the backbone torsion angles of residue 89 and rotation of β-strand 5, immediately N-terminal to Q90, away from the active site. Similarly, in the ADP simulation, the Gln-90 side chain has withdrawn from the Mg$^{2+}$ (Fig. 3C), and in both the ADP and apo simulations the Gln-90 Cα atom is withdrawn from the active site, accompanied by similar changes in β-strand 5 as observed between the ATP- and ADP-bound crystal structures. These changes do not occur in the ATP simulation, where Gln-90 remains attached to the active site Mg$^{2+}$ throughout.

These data demonstrated the ability of the simulations to reproduce the rotation of the HD observed between the ATP-bound (from the dimer) and ADP-bound (isolated monomer) x-ray structures and that the shift between these conformational sub-states is quite rapid, occurring on the 10- to 20-ns timescale. The data indicated that, for the isolated monomer, the nucleotide influences the equilibrium conformation of the HD, with the LSGG region remaining closer to the starting structure in the ATP-bound monomer than in the ADP-bound monomer. The results also showed that the interaction of the conserved glutamine with the active site Mg$^{2+}$ requires the presence of the ATP γ-phosphate to remain intact and supports the idea that this interaction may mediate, at least partly, allosteric coupling of the active site and the HD.

**Simulations of the MJ0796 Dimer**—To investigate the effect of ATP hydrolysis on the dimer, we performed multiple multi-nanosecond simulations of the MJ0796 dimer in two different ligand-bound states, one in which two ATP molecules are bound, as in the crystal structure, and the other in which ATP is bound at one active site and ADP at the other. As for the monomer simulations, the crystal structure Gln-171 was substituted with the wild-type Glu-171 residue, and an Mg$^{2+}$ ion placed in the canonical position in the catalytic site. The ADP/ATP-bound state was simulated in four independent runs referred to as simulations 1–4 in Table 1. In simulations 1 and 2, the x-ray dimer structure was used. Although the monomer structures within this dimer are almost identical (r.m.s. deviation for all heavy atoms, excluding residues 112–116, is 0.82 Å), there is a significant difference in the conformation of the α3–4 loop (for residues 112–116 the r.m.s. deviation for all heavy atoms is 4.16 Å). These loops form contacts between the dimers in the crystal lattice. Although the α3–4 loop in monomer B is similar to that observed in the ADP-bound monomer x-ray structure (PDB 1F3O), the monomer A α3–4 loop has a different conformation and has higher temperature factors. This suggests that the monomer A conformation in this region is less stable and may be distorted by the crystal contacts.

Moreover, we note that in the simulations of the isolated monomer, this loop changes to a more compact conformation similar to that observed in monomer B and in the ADP-bound x-ray structure (data not shown). Thus, to ensure that the results of the simulations are robust with respect to this initial asymmetry, a second dimer was constructed to be exactly symmetrical by overlaying the coordinates of monomer B on to monomer A and thus creating a dimer of two B monomers. This symmetrical dimer was used for simulations 3 and 4. In addition, three 22-ns simulations (Table 1, simulations 5–7) of the ATP/ADP dimer were also performed. Simulation 5 used the x-ray dimer, whereas simulations 6 and 7 used the constructed symmetrical dimer. All these data are summarized in Table 1.

The results of our multiple (>15 ns) simulations of the ATP/ATP- and ADP/ATP-bound dimers differ significantly from a previous MD study of the same states of the MJ0796 dimer (47). This may be ascribed to a number of factors including: (i)

### Table 1

| Simulation* | Run time | Active site nucleotide | Ser-117 transition > 12 Å | Average Ser-117 transition | Q90-Mg$^{2+}$ disengagement |
|-------------|----------|------------------------|---------------------------|----------------------------|----------------------------|
|             |          | A                     | B                         | A                          | B                          |
|             | m        | m                     | Å                         | m                         | Å                          |
| 1) X-ray    | 28       | ADP                   | ATP                       | 15.9                       | 8.0 ± 2.0                  | 13.8                       |
| 2) X-ray    | 19       | ATP                   | ADP                       | 7.3                        | 2.7 ± 1.1                  | 7.7                        |
| 3) BB       | 20       | ADP                   | ATP                       | 15.8                       | 12.8 ± 2.2                 | 0.7                        |
| 4) BB       | 25       | ADP                   | ATP                       | 13.7                       | 8.7 ± 2.5                  | 5.6                        |
| 5) X-ray    | 22       | ATP                   | ATP                       | 2.9 ± 1.3                  | 3.2 ± 1.4                  | 1.4 ± 1.1                  |
| 6) BB       | 22       | ATP                   | ATP                       | 2.5 ± 2.5                  | 4.2 ± 2.5                  | 4.9 ± 2.4                  |
| 7) BB       | 22       | ATP                   | ATP                       | 3.6 ± 1.3                  | 2.6 ± 1.1                  | 2.6 ± 1.1                  |

* Simulations that used the crystal structure (PDB 1LT) are indicated by "X-ray;" and those that used a constructed dimer of two monomer B subunits from the same crystal structure are labeled "BB."

† Time point at which the measured transition of serine 117 (see Fig. 5) first exceeded 12 Å.

‡ For the ADP-bound monomer, the average of the serine 117 transition following the point at which it first exceeded 12 Å to the end of the simulation is given. For the ATP-bound monomer the average for the entire simulation is given.

§ Time point at which the Gln-90 side-chain amido oxygen-Mg$^{2+}$ distance exceeded, and remained at, >3.0 Å.
greater conformational sampling in the present study (160 ns compared with 20 ns in the earlier work); (ii) temperature/stability relationships, as natural conditions can be important to accurate simulation of enzymes of thermophilic origin (48) (our simulations were performed at the optimum temperature and pressure conditions for the thermophilic host organism *Methanococcus jannaschii*)(29); and (iii) the lack of the Q90-Mg$^{2+}$ coordination interaction in the ATP-bound sites in the earlier study, possibly due to an uncorrected error in the MJ0796 crystal structure (see Fig. 1B). As discussed above, the Q90-Mg$^{2+}$ interaction may be of importance to the accurate reproduction of transitions of the HD. In all our simulations, the canonical six-ligand Mg$^{2+}$ coordination sphere (Fig. 1B) is maintained in the ATP-bound active site throughout all simulations.

*ATP Hydrolysis in One Active Site of the Dimer Induces an Asymmetrical Transition of the HD*—In all four ADP/ATP dimer simulations, after an initial period of between ~5 and 15 ns, there is a rotation of the HD of the ADP-bound monomer (HD-ADP), which does not occur in the ATP-bound monomer. This rotation is distinct from that observed between the ATP- and ADP-bound x-ray structures of MJ0796 and from that derived from the principal mode observed in the ADP monomer simulation (Fig. 3B). The rotation observed in these latter instances is similar to that which can be inferred from comparison of most isolated NBD monomer crystal structures with monomer structures derived from NBD dimers: the HD rotates about a pivot proximal to the Pro-loop, with the LSGG undergoing the most substantial movement. However, in the rotation of the HD-ADP observed in our ADP/ATP dimer simulations, the pivot is proximal to the LSGG (Fig. 4), such that the LSGG remains close to its starting position or moves slightly forward in the opposite direction to that observed in the isolated monomer. The movement of the ADP-HD in this instance may be likened to a pendulum, with the LSGG representing the fulcrum and the $\alpha$3–4 loop the bob.

Most notably, the rotation of the ADP-HD in the ADP/ATP dimer produces a significant movement of the $\alpha$3–4 loop away from its initial position. To follow the time course of the relative movement of the $\alpha$3–4 loop, we measured the distance moved by the C$\alpha$ atom of serine 117 (immediately C-terminal to the $\alpha$3–4 loop) from its starting position (Fig. 5 and Table 1). As can be seen in the inset in Fig. 3A, residue 117 undergoes very low fluctuations from the frame of reference of the HD, and thus its position is a good indicator of the rigid body motion of the HD. The movement of the $\alpha$3–4 loop away from the region of helix 2 in the ADP-bound monomer, but not the ATP-bound monomer, results in a significant conformational asymmetry in the dimer (Fig. 6). With the possible exception of the monomer structures of the apo form of the *Thermo-
coccus litoralis* MalK NBD (PDB 1G29 23), a similar orientation of the HD is not observed in any crystal structure of ABC transporter NBDs. When the *T. litoralis* MalK monomers are compared with the *Escherichia coli* MalK monomer structures from the ATP-bound MalK dimer (PDB 1Q12 12), there is a similar difference in the relative locations of the $\alpha$3–4 loop and the region of helix 2, as observed in the ADP-bound monomer in our ADP/ATP dimer simulations. The rotation of the HD and the displacement of the $\alpha$3–4 loop observed in the ADP/ATP dimer does not occur in three 22 ns ATP/ATP dimer simulations (Fig. 5).

**DISCUSSION**

Understanding how the hydrolysis of ATP in the NBDs is coupled to conformational changes at the substrate binding sites within the TMDs is of crucial importance to the elucidation of the ABC transporter molecular mechanism. Earlier enzymological studies implicated the ABC HD in NBD:TMD coupling (reviewed in Ref. 49). Crystallographic data and previous MD simulations have indicated that the NBD HD is capable of independent movement, and it has been suggested that movements of the HD during the catalytic cycle may correlate with the cycle of ATP hydrolysis and mediate NBD:TMD coupling (see the introduction). Such ideas are consistent with the functional mechanism of related P-loop ATPases. The structural data for ABC NBDs, however, have been derived either from studies of the isolated ADP-bound monomer (NMR, crystallographic) that may not reflect the behavior of the oligomerized protein, and/or from mutant or catalytically deficient forms of the ATP-bound dimer. To further investigate the relationship between stages of the ATP hydrolysis cycle and conformational changes of the HD, we performed multiple MD simulations of both the MJ0796 monomer and dimer in different ligand-bound states. As a cross-reference to the all-atom simulations, we also performed a coarse-grained analysis of the MJ0796 dimer using an elastic network model.

A previous MD study found nucleotide-dependent mobility...
of the HD in simulations of the HisP monomer using a crystal structure of the isolated monomer containing ATP but not Mg\(^{2+}\) (44). To more fully characterize the relationship between active site nucleotide occupancy, interactions of the conserved glutamine and transitions of the HD, we performed 30 ns MD simulations of the apo, ADP-bound, and ATP-bound states of the isolated monomer, using the ATP-bound monomer from the MJ0796 dimer as the starting structure. The apo and ADP monomer simulations reproduced the full extent of outward rotation of the HD and the disengagement of the conserved glutamine from the active site observed between the ATP-bound monomer (from the MJ0796 dimer) and ADP-bound monomer crystal structures. These simulations indicate that the fully closed conformation of the HD, as observed in dimer...

FIGURE 5. Conformational changes during the ATP/ADP and ATP/ATP dimer simulations. Transit of the $\alpha_3$–4 loop. Time course of the distance between the C\(_\alpha\) atom of residue serine 117 (immediately C-terminal to the $\alpha_3$–4 loop) and its position in the starting structure, after r.m.s. fitting of the trajectory frames to the initial structure using C\(_\alpha\) coordinates of the core domain for dimer simulations 1 to 7. Monomer A is red; monomer B is black. Top to bottom: A, ADP/ATP simulation 1 (x-ray dimer, ADP in monomer A); B, ATP/ADP simulation 2 (x-ray dimer, ADP in monomer B); C, ADP/ATP simulation 3 (symmetrical dimer, ADP in monomer A); D, ADP/ATP simulation 4 (symmetrical dimer, ADP in monomer A); E, ATP/ATP simulation 5 (x-ray dimer); F, ATP/ATP simulation 6 (symmetrical dimer); and G, ATP/ATP simulation 7 (symmetrical dimer).
Nucleotide-dependent Allostery in ABC ATP-binding Cassettes

FIGURE 6. Asymmetry between the NBD monomers in the ADP/ATP dimer. Averaged structure of the ATP-bound (left) and ADP-bound (right) monomers from the ADP/ATP dimer simulation 3 over the period 19–20 ns. The Cα atom positions are traced in a “warm” representation and color-coded according to the r.m.s. deviation between the monomers with low values in blue and high values in red. The view in each panel is from the same position after r.m.s. alignment of the monomers using the Cα coordinates of the core domain. Structural features discussed in the text are indicated.

The results of our MJ0796 dimer simulations provide further insight into the effect of ATP hydrolysis on functional transitions of the HD and on NBD-NBD interactions. Because transitions of the HD between different nucleotide-bound states can occur in <15 ns (see above), conformational transitions that occur in >15 ns simulations of the ADP/ATP dimer may well approximate that which occur upon ATP hydrolysis and phosphate release in one active site of the ATP/ATP dimer. In three >20 ns simulations of the ATP/ATP-bound dimer, using both the x-ray structure and a constructed symmetrical dimer, significant alterations from the starting structure in the orientation of the HD were not observed. In contrast, in the simulations of the ADP/ATP-bound dimer, the HD of the ADP-bound monomer undergoes a large rotation that results in a transition of ~13 Å of the α3–4 loop within the HD to a new equilibrated conformation, not observed in most other ABC ATPase x-ray structures, including those of the ATP- or ADP-bound MJ0796. This conformational change does not occur in the opposite ATP-bound monomer, resulting in significant asymmetry in the dimer.

The conformational changes observed in the dimer simulations are consistent with experimental evidence. The transition of the α3–4 loop observed in the ADP/ATP dimer simulations is consistent with NMR experiments on the MJ1296 NBD, which showed that the dynamics of the α3–4 loop were allosterically coupled to the active site (50, 51). Also of note, the asymmetric dynamics of the ATP/ADP dimer correlate with the asymmetric dynamic patterns of principal modes 4 and 5 of the GNM analysis (Fig. 2, D and E). Thus, in GNM mode 4, the HD of one monomer moves relative to its core domain while the opposite monomer remains relatively static with respect to these intradomain motions; this is essentially the motion the dimer undergoes in the transition from the ATP/ATP state to the ADP/ATP state in the MD simulations. Moreover, in GNM mode 5, the region of helix 2 and the α3–4 loop undergo opposite sense motions in one monomer, thus corresponding to the transition of the α3–4 loop away from helix 2 in our ADP/ATP dimer simulations, while the equivalent regions in the opposite monomer undergo concerted motions in the same direction. This is consistent with the dynamic asymmetry with respect to these regions observed in our simulations.

Insight into how the observed transition of the α3–4 loop may be involved in the mechanism of the transporter is provided by recent whole transporter crystal structures. To date there are four structures of whole ABC transporters, namely Sav1866 multidrug exporter from *Staphylococcus aureus* (52), BtuCD vitamin B12 importer from *E. coli* (53), ModBC putative molybdate importer from * Archaeoglobus fulgidus* (54), and HI1470/1 putative metal-chelate importer from * Haemophilus influenzae* (55). In all of these structures, a helix from the TMD intracytoplasmic loops (ICLs) contacts the NBDs, binding to a pocket formed by β-strand 5, the Q-loop, the C-terminal regions of helices 3 and 5 in the HD, the N terminus of the α3–4 loop and the C terminus of helix 2. As noted in the reports of the Sav1866 and BtuCD structures, the NBD dimer overlays closely with that of the MJ0796 dimer. Indeed, the NBD structures from the four whole transporters do not differ markedly from MJ0796, as expected from their conserved sequences, and thus it seems reasonable to speculate that MJ0796 may share a similar NBD:TMD interface. In support of this wider extrapolation, a number of mutations in the histidine and maltose permeases, which uncouple the NBDs and TMDs (46, 49, 31), map to the TMD:NBD interface observed in the whole transporter structures, including parts of the Q-loop, helix 2, the Pro-loop, and the α3–4 loop (8, 46).

Extrapolating the conformational change of the HD observed in the ADP-bound monomer during the ATP/ADP dimer simulations to the Sav1866 TMD:NBD interface, we found that the net effect of the α3–4 loop transition would be to significantly open the binding pocket for ICL2 in the ADP-bound NBD (Fig. 7). This would have the effect of loosening or breaking the interaction with the TMD in the ADP-bound NBD monomer, but not the ATP-bound NBD monomer. The idea that one NBD...
could disengage from the TMD while the other does not is consistent with the opposite sense motions of the monomers observed in the GNM mode 1 (Fig. 2A). This is also consistent with biochemical data that show that, in the histidine permease complex, the NBD monomer cycles between loose and tightly bound states (31). Thus, our data suggest that ATP hydrolysis in one NBD results in an alteration in the interaction between the ADP-bound NBD and its respective ICL, which may constitute, at least in part, the mechanism by which ATP hydrolysis is coupled to conformational changes in the TMDs.

In this work we provide further evidence that the conserved glutamine mediates the allosteric coupling of the active site and the HD and that this interaction occurs by way of the catalytic metal. We have also discovered and reproduced, in multiple extended MD simulations, a remarkable symmetric transition of the HD within the ABC ATPase dimer in response to ATP hydrolysis. We show how this subdomain motion dovetails facility with recent whole transporter structures to provide a possible mechanism for allosteric communication between the NBDs and TMDs. Our findings are consistent with a coarsely-grained elastic network analysis of the ABC dimer. They are consistent with previous x-ray structures, NMR, and experimental studies and are of additional significance due to the difficulty of investigating the ADP/ATP-bound state of the dimer using experimental methods. Examination of our simulation trajectories has revealed a wealth of data concerning the dynamics of other regions of the NBD, including the D-loop and the H-loop. Although beyond the scope of the present work, we look forward to analyzing data for these conserved motifs and the roles they might play in the protein's mechanism.

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