The Dynamics of the MgATP-driven Closure of MalK, the Energy-transducing Subunit of the Maltose ABC Transporter

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The nucleotide binding domains (NBDs) are the energy supplying subunits of ATP-binding cassette (ABC) proteins. They power transport by binding and hydrolyzing ATP. Tracing the pathway between different conformational states of the NBDs during ATP binding, hydrolysis, and release has, however, proven difficult. We have used molecular dynamics simulations to study the ATP-driven association of the NBDs of the maltose ABC transporter, MalK, based on the crystal structures of its open and semiopen dimers. When MgATP was introduced into the binding pockets, the semiopen dimer transitioned to a closed conformation, whereas the open dimer evolved to a semiopen state. In the absence of docked MgATP, however, the twin NBDs of both the open and semiopen starting configurations drifted further apart. Both the presence of MgATP and direct cross-interface protein-protein hydrogen bonds, primarily involving the D-loop, quite likely play a key role in initiating closure. The simulations of the MgATP-docked semiopen form indicate that completion of closure is driven mainly by cross-interface contacts between the γ-phosphate of ATP and residues in the signature motif. Our simulations also give insight into possible interactions of MalK with the regulatory proteins MalT and enzyme IIAgc.

Selective uptake and efflux by ABC transporters is driven against concentration gradients using energy derived from the binding and hydrolysis of ATP. All ABC transporters consequently have an ATP-binding subunit whose sequence and structure is relatively well conserved, with sequence identities of these nucleotide binding domains (NBDs) in the range of 25–30% across the entire superfamily (3, 4). Together with the two NBDs, functional ABC transporters also typically have two transmembrane domains (TMDs). These have a more variable sequence, consistent with their role as the passageway for the many different kinds of substrates that ABC transporters transport.

The mechanism of energy transduction in ABC transporters is not exactly understood. Experimental evidence indicates that the catalytic cycle of ABC transporters in general involves ATP-driven association and dissociation of the NBDs (5, 6), which have two ATP-binding and hydrolysis sites. ATP binding in ABC transporters may proceed in two steps (7). The first step is the diffusion and binding of ATP to the core subdomain that hosts the Walker A motif. The second step is the closure of the NBDs leading to the formation of a dimer in which ATP is sandwiched between the Walker A and the signature motifs. This general sequence of events is supported by the fact that there are several crystal structures of monomeric NBDs (e.g. HisP) with ATP bound to the core subdomain (8) but none with ATP bound only to the signature motif. However, the details of this process and in particular the interaction between the two ATP-binding sites and the interaction between the NBDs and the TMDs remain unclear.

A number of crystal structures of dimeric NBDs (MJ0796, BtuCD, MalK, CysA, HlyB, and MsbA) have recently been reported (7, 9–12). In this study, we focus on the maltose transporter. The maltose transporter complex, MalFGK2, is composed of two TMDs (MalF and MalG) and two NBDs (MalK2). MalK is the only ABC transporter NBD that has been structurally characterized by x-ray crystallography as a physiological dimer with and without bound ATP (7), which makes it particularly interesting for understanding the interactions between NBDs. More recently, the structure of a MgADP-bound, presumably posthydrolysis dimer of MalK has also been reported (13).

Although crystal structures of open, semiopen, and closed forms of MalK exist, these are essentially static snapshots. In this study, we use molecular dynamics simulations, starting from the different crystal structures, to investigate the interactions between the MalK NBDs and MgATP in a realistic envi-
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environment by simulating the motions of the proteins, water, and MgATP. The utility of simulation studies in elucidating the mechanism of biological macromolecules has been discussed in a number of reviews (14–16). Molecular dynamics simulations give insight into dynamic properties of biomolecules, and can be used to investigate pathways between different states of a system, such as the different experimental structures of MalK. Because they are carried out in solution with all solvent and ions present, they are not constrained by crystal lattice contacts, and they can clearly follow the motions of water molecules and small cofactors, such as Mg^{2+}, which are often not visible in crystal structures. Finally, molecular dynamics simulations provide some hope that different states of a protein can be modeled and investigated based on a single crystal structure, which is of particular interest for ABC transporters where there is, for example, a single structure of the vitamin B_{12} importer BtuCD (9) in an unknown state in the overall transport process.

We investigate the effect of docked MgATP on the conformational state of open and semiopen MalK, focusing on two key questions. 1) Can the introduction of MgATP into the binding sites transform the open and semiopen dimeric configurations into the closed? 2) Can we identify features of the NBDs and MgATP that drive closure? We discuss the simulation results in the context of NBD-NBD interactions as well as their possible relevance for the mechanism of energy coupling to the TMDs.

In addition to these general aspects of ABC transporter mechanism, MalK is of interest because of its regulatory roles. It contains a regulatory domain that interacts with enzyme IIAglc, which is often not visible in crystal structures. Finally, molecular dynamics simulations reveal that different states of a protein can be modeled and investigated based on a single crystal structure, which is of particular interest for ABC transporters where there is, for example, a single structure of the vitamin B_{12} importer BtuCD (9) in an unknown state in the overall transport process.

MATERIALS AND METHODS

The starting structures for the simulations were the structures of MalK from *Escherichia coli* in three different dimeric configurations: closed (1Q12), semiopen (1Q1B), and open (1Q1E) (7). The closed form was crystallized with ATP bound at each of its two active sites, whereas the open and semiopen forms were nucleotide-free. In addition, simulations in which MgATP was docked into the active sites of the open and semiopen conformations were performed. Docking of MgATP was performed as previously described (22). To enhance sampling and increase the statistical accuracy of the simulations, five copies of each set-up were simulated with different random initial velocities. We also investigated the effects of removing the regulatory domains in order to determine what influence they may have on the association and dissociation of the NBDs. Residues 236–370, which constitute the regulatory domain (RD), were deleted from each of the three crystal forms. All starting structures were solvated with Simple Point Charge water molecules (23). Random water molecules were replaced with Na^{+} and Cl^{-} ions to neutralize charge in the system and to emulate physiological conditions by raising the salt concentration to 150 mM NaCl. The production runs were 15 ns long. They were preceded by a short equilibration run of 240 ps with

RESULTS

Mobile Regions—To investigate the mobility of individual MalK residues in the ATP-bound closed conformation relative to the nucleotide-free open conformation, we compared open/closed B-factor ratios calculated from the simulations with experimental B-factor ratios obtained from the crystal structures. For C-α atoms, the minimum, maximum, mean, and S.D. values of crystallographic B-factors were 12.2, 87.0, 40.3, and 16.8 Å^{2}, respectively, for the open conformation, whereas those for the closed conformation were 33.7, 148.9, 70.1, and 23.9 Å^{2}, respectively. Corresponding minimum, maximum, mean, and S.D. values of simulation B-factors were 17.7, 850.5, 145.4, and 129.3 Å^{2}, respectively, for the open conformation, whereas respective values for the closed dimer were 10.1, 384.8, 60.6, and 46.1 Å^{2}.

The open/closed B-factor ratios are mapped onto color-coded ribbon diagram representations of the protein in Fig. 1. Fig. 1, A and B, shows the B-factor ratios from x-ray crystallographic data, whereas Fig. 1, C and D, shows results obtained from simulation. The red regions highlight segments of the protein whose B-factors were greater in the open conformation than in the closed. Conversely, the blue regions had higher B-factors in the closed form than in the open conformation. Fig. 1 thus identifies red and blue segments as regions exhibiting the highest differences in mobility between the two configurations.

Fig. 1, A and C, indicate that regions with higher mobility in the open conformation than in the closed form (red) are pre-
dominantly located within the nucleotide binding domains. Most regions within the regulatory domain had about the same mobility in both states (green) or higher mobility in the closed state relative to the open (blue). Some local differences were also observed. A comparison of Fig. 1, A and B, with Fig. 1, C and D, shows that the simulation study reveals more flexibility in the NBDs than indicated by the experimental analysis. Calculated $B$-factor ratios from the simulation are particularly high for the two helices C-terminal to the Q-loop in the helical subdomain of one of the two NBDs. Residues in this segment are predicted to interact with the transmembrane subunits.

A summary of residues in the protein that register very high or very low $B$-factor ratios is provided in Table 1. As indicated in Table 1, both experimental and simulation $B$-factor analyses identified the loop between strand S7 and helix H6 as highly mobile in the open conformation compared with the closed. In addition, the calculated $B$-factors also identified the segments containing the Walker A motif and the signature sequence as highly mobile in the nucleotide-free open conformation. Both sequences are involved in ATP binding in the closed MalK dimer, restricting their mobility when ATP is bound.

Both experiment and simulation indicate that the interface between the NBDs and the RDs is more mobile in the closed conformation than in the open. These include helices H8 and H9 at the C-terminal end of the NBD. Crystallographic analysis suggests that the loop between H3 and H4 is more mobile in the closed conformation, but simulations suggest enhanced mobility in the open form. Examination of crystal lattice interactions indicated that this region is involved in crystal contacts in the open dimer. These contacts are not present in our simulation.

Inside the RD, both simulation and experiment generally reveal heightened mobility in the closed conformation relative to the open. Residues predicted to interact with MalT have been
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TABLE 1
Summary of regions with the highest and lowest B-factor ratios
These were defined as segments with absolute ratios equal to or greater than 2 on a normalized scale of 1–5. They are depicted as intensely red or blue segments in Fig. 1.

| Residue numbers | Description of segment | Conformation showing significantly enhanced flexibility | Average C-α B-factors, open/closed |
|-----------------|------------------------|---------------------------------------------------------|----------------------------------|
|                 |                        | Crystal | Simulation | Crystal | Simulation |
| NBD             |                        |  |            |  |            |
| 8–11            | C-terminal end of S1. Near a conserved aromatic residue (Trp<sup>13</sup>) that interacts with the adenine base of ATP | Open | 40.1/72.6 | 115.6/53.2 |
| 22–34           | Loop between S2 and S3 | Closed | 30.7/85.4 | 74.6/37.4 |
| 38–40           | Inside the Walker A motif | Open | 26.8/53.3 | 272.6/19.4 |
| 45–58           | H1 and the H1–S4 loop | Closed | 28.5/92.9 | 213.9/50.2 |
| 78–84           | C-terminal end of Ser<sup>70</sup> and Gln<sup>62</sup> of the conserved Q-loop | Closed | 39.5/144.4 | 123.5/35.8 |
| 100–103         | Loop between H3 and H4 | Closed | 70.6/142.7 | 67.5/63.8 |
| 134–140         | LSGGQ signature motif | Closed | 58.2/61.1 | 26.7/17.7 |
| 153–158         | Walker B motif | Closed | 31.8/71.9 | 84.2/48.8 |
| 160–167         | Loop between S7 and H6 | Closed | 66.8/48.6 | 349.2/26.9 |
| 172–184         | H6, the longest helix in the NBD | Closed | 57.9/67.3 | 41.4/59.5 |
| 188–192         | Switch region including the conserved His<sup>192</sup> of the H-loop | Closed | 26.9/56.0 | 56.8/20.1 |
| 196–202         | Helix H7 and the loop between H7 and S9 | Closed | 23.6/53.8 | 22.4/32.2 |
| 216–225         | Helix H8 at the NBD-RD interface | Closed | 18.4/50.8 | 27.9/44.2 |
| 232–236         | Helix H9 at the NBD-RD interface | Closed | 16.3/40.0 | 42.5/46.7 |
| RD              |                        |  |            |  |            |
| 250–254         | Loop between S11 and S12 | Closed | 28.0/70.1 | 151.4/124.2 |
| 265–269         | S13                    | Closed | 24.0/65.5 | 89.9/97.2 |
| 304–312         | S15                    | Closed | 23.8/50.5 | 37.4/57.5 |
| 329–340         | S17 and loop between S17 and S18 | Closed | 41.8/60.8 | 49.1/76.1 |

a Secondary structure elements are numbered sequentially in the order presented in Refs. 7 and 53.

b "Closed" or "Open" in boldface type indicates that the segment shows the same B-factor behavior in both monomeric units, whereas normal type indicates that the result applies for one of the two monomers.

Average open/closed B-factor ratios for all C-α atoms in the concerned segment. Values are shown for the monomer that best illustrates the observed difference in flexibility between the open and closed forms.

identified in some of the regions highlighted in Table 1. These are Ala<sup>250</sup>, Ile<sup>251</sup>, Trp<sup>267</sup>, and Leu<sup>268</sup> (37).

Transition toward a Closed Dimer—To investigate the transition from the open and semiopen crystal structure conformations to the closed conformation, we docked a MgATP molecule into each active site of the protein. Fig. 2 shows representative snapshots of the starting and final (15 ns) conformations obtained from the simulations. Also shown are the separation distances between the Walker A motif and the signature motif across the dimer interface.

Without MgATP, the semiopen starting conformation shown in Fig. 2A transitioned to the slightly more open conformation shown in Fig. 2B, with an increase in separation distance of 3 Å at both binding sites after 15 ns (Fig. 2C). In the presence of docked MgATP, however, all five copies of the semiopen starting conformation (Fig. 2D) evolved to a closed conformation (Fig. 2E). Considering backbone atoms for all of the 734 residues in the NBDs plus the RDs, the calculated r.m.s. deviation between the structure shown in Fig. 2E and the closed crystal conformation was 2.9 Å. Trends observed for the open crystal structure conformation were similar to those of the semiopen form. In the absence of MgATP, the open form (Fig. 2G) transitioned to an even more open state as shown in Fig. 2H. On the other hand, the presence of docked MgATP induced the open form (Fig. 2F) to adopt a semiopen conformation (Fig. 2K) after 15 ns in three of five simulations. The backbone r.m.s. deviation of the structure in Fig. 2K is 4.8 Å relative to the semiopen crystal conformation and 6.5 Å relative to the closed crystal conformation. It is evident, from these r.m.s. deviation values and from the final configuration presented in Fig. 2K, that despite the presence of MgATP, 15 ns was not sufficient time for the open conformation to evolve to the fully closed conformation as characterized by x-ray crystallography. At both active sites, however, the ATP molecule advanced closer to the signature motif of the opposing monomer, as shown in Fig. 2L. Movie clips of the four transitions described in Fig. 2 are included as supplemental material.

Although all five copies of the semiopen simulation with docked MgATP molecules evolved to a closed conformation, as judged by the decrease in distance between centers of mass of the NBDs, analyses of the separation distance between the Walker A and the signature motif at the two active sites revealed some differences. In two simulations, this gap converged to the same value at both sites. The final separation distance at both sites was ~8.5 Å in the first simulation and ~10 Å in the second. The former, in particular, was in very good agreement with the experimental closed structure in which the equivalent distance was also determined to be 8.5 Å at both binding sites. The rest of the simulations of the semiopen form with docked MgATP all had one-site convergence to a steady distance of 9 or 10 Å within the first 5 ns of simulation. In contrast, the separation distance at the second binding site was larger and constantly fluctuated during the 15-ns simulation time. The final distance at this site ranged between 12 and 15 Å.

In simulations with the regulatory domains removed, the orientation of the two NBDs relative to each other was severely disrupted in the open and semiopen nucleotide-free dimers in the absence of MgATP (details not shown). The closed dimer with bound MgATP was, however, stable on the time scale of the simulation.

Specific Interactions between NBDs—We analyzed the trajectories of the MgATP-induced transition of dimeric MalK from...
the open to the semiopen and the semiopen to the closed state to identify residues that are involved in closure. Simulations of the open form with docked MgATP revealed cross-interface protein-protein contacts not observed in the crystal structure. After modeling hydrogen atom coordinates by geometry into the Protein Data Bank files, two direct protein-protein hydrogen bond contacts between His192 and Asp165 were identified in the open crystal structure. Both of these were between His192 and Asp165. In addition to these, all simulations of the open dimer revealed cross-interface hydrogen bond interactions between the D-loops in each NBD, including Asn163–Asn163 (most common), Asn163–Asp165, and Leu164–Asp165. The MgATP molecules docked onto the Walker A motif of the open form did not advance close enough to be involved in hydrogen

FIGURE 2. A, the nucleotide-free crystal structure conformation of semiopen MalK; B, the conformation after 15 ns of molecular dynamics simulation; C, the change in distance between the centers of mass of residues of the Walker A sequence and the signature motif across the dimer interface at binding site 1 (black) and binding site 2 (red) during the simulation. D, the structure of semiopen MalK with MgATP docked to its binding sites; E, the conformation after 15 ns of simulation; F, the change in distance between the Walker A sequence and the signature motif across the dimer interface at the two binding sites as a function of simulation time. G, the nucleotide-free crystal structure conformation of open MalK; H, the conformation after 15 ns of molecular dynamics simulation; I, the change in distance between the centers of mass of the Walker A sequence and the signature motif across the dimer interface at binding sites 1 and 2 during the simulation. J, the structure of open MalK with MgATP docked to its binding sites; K, the conformation after 15 ns of simulation; L, the change in distance between the Walker A sequence and the signature motif across the dimer interface at the two binding sites. The two nucleotide-binding domains comprising residues 1–235, referred to as NBD1 and NBD2, are presented as red and orange ribbons, respectively. The green and cyan ribbons are the two regulatory domains, RD1 and RD2, respectively. They are composed of residues 236–370. Shown in blue are MgATP molecules docked at the interface of the NBDs. Movie clips of all four simulations are included as supplemental material. Images were rendered with depth cueing using VMD.
bond contacts across the dimer interface. However, in the absence of docked MgATP, the open dimer became even more open, as shown in Fig. 2, G and H. It is apparent, therefore, that attractive forces due to MgATP are at play, even without direct interaction.

Fig. 3A shows the crystal structure conformation of semio-
pen MalK (rendered in color) fit onto the closed crystal structure (white ribbon). The distance between the centers of mass of the NBDs of the semiopen crystal form was determined to be 33.3 Å. After 15 ns of simulation in the presence of docked MgATP, this distance decreased to 28.1 ± 0.2, 30.3 ± 0.4, 29.2 ± 0.2, 29.7 ± 0.1, and 31.2 ± 0.3 Å, respectively, in each of the five copies of the semiopen form simulated (averaged over the last 2 ns). These values are close to the equivalent NBD to NBD distance of 28.1 Å in the closed crystal structure. A fit of the closed conformation determined by x-ray crystallography and one derived from the semiopen form by molecular dynamics simulation is shown in Fig. 3B (r.m.s. deviation of 2.9 Å).

Our simulations suggest that the MgATP-driven closure of semiopen MalK is initiated by favorable contacts across the dimer interface that mainly involve the γ-phosphate and the signature motif. However, direct protein-protein interactions between the D-loop and the Walker A motif also appear to play a key role. The sequence of structures presented in Fig. 3, C–F, highlights changes at the interface between the NBDs during one simulation of semiopen MalK with docked MgATP. At the beginning of the simulation, the two NBDs are separated by a large volume of solvent water molecules (Fig. 3C). These were added to the simulation box at the outset in order to mimic the aqueous cellular environment of MalK. For clarity, only water molecules within a 5-Å radius of MgATP are shown in subsequent panels. No direct protein–protein hydrogen bonds were observed between the NBDs of the semiopen crystal structure. At time 0.5 ns during the simulation, a direct protein–protein contact across the dimer interface was observed in the form of a hydrogen bond between Ser38 of the Walker A motif and Asp165 of the D-loop (Fig. 3D). The γ-phosphate of ATP also formed a direct hydrogen bond with Glu337 and a water-mediated hydrogen bond with Gln138. Both residues are located in the signature motif. The snapshot at 2.5 ns (Fig. 3E) confirms the involvement of the same set of conserved residues in hydrogen bond contacts across the dimer interface during the initial stages of closure. Within the first 5 ns, most of the water molecules initially surrounding the MgATP have been displaced (Fig. 3F). Sites vacated by these water molecules are occupied by protein–protein and protein–ATP contacts.

Further analyses confirmed the dominance of cross-interface protein–protein contacts between Ser38 and Asp165 and between His192 and Asp165 during the initial stages of closure. The analyses were performed based on snapshot structures taken at 0.5-ns intervals during the first 5 ns of each simulation of semiopen MalK with docked MgATP. Taking all five copies of the simulation into account, this represented a total of 50 frames (10 per simulation). The geometric center of each side chain was determined and a contact defined as a distance of less than 6 Å between these centers of mass. For every snapshot, distances between the side chain of each residue in NBD1 and all residues in NBD2 were calculated. Contact between Ser38 of the Walker A motif and Asp165 of the D-loop was the most common, occurring in 34 frames. Contact between the conserved His192 of the switch region and Asp165 of the D-loop occurred in 29 frames. In both cases, the type of contacts involved were side chain hydrogen bonds. ATP–protein contacts across the dimer interface during the first 5 ns almost exclusively involve the signature motif and the γ-phosphate. We calculated the shortest distance between ATP and protein residues across the dimer interface during the first 5 ns. Using a 3.5 Å maximum cut-off criterion for interactions, all residues are screened out except Lys132-Ala133-Leu134-Ser135-Gly136-Gly137-Gln138-Arg139, which is the signature sequence plus a few flanking residues. The shortest distance between ATP and a residue in the opposing NBD in the first 5 ns of simulation was 1.9 Å obtained for Ser135.

**Mg2+ Cofactor**—We further observed in our simulation of semiopen MalK that the Mg2+ ion cofactor was coordinated by five oxygen atoms. Three were contributed by the α-, β-, and γ-phosphates of ATP and two by solvent water molecules. Fig. 4A illustrates the nature of interactions of the Mg2+ ion cofactor and solvent water molecules with the binding site of the wild type protein using a snapshot taken at 10 ns. The two water molecules in the first coordination shell of the Mg2+ ion are labeled Wat1 and Wat2. Their oxygen atoms are 2.0 and 1.9 Å away from the Mg2+ ion, respectively. Oxygen atoms of both the β- and γ-phosphate are 2.0 Å away from the Mg2+ ion, whereas the α-phosphate oxygen is 2.1 Å away. A key difference between the crystal structure of MalK and our simulations is the absence of Mg2+ ion and water molecules in the binding site of the former (Fig. 4B). Nevertheless, these distances compare very well with Mg2+ coordination distances of 2.1 and 2.2 Å observed in the crystal structure of the GlcV-AMPNP-Mg2+ complex (38). An analysis of all five simulations showed that Ser43 of the Walker A motif also came within ~2 Å of the Mg2+ ion cofactor during the simulation and may be transiently involved in the coordination of the cation. No distances around 2 Å were obtained for Mg2+ -protein interactions across the dimer interface. The closest amino acid from the opposing NBD was at least 4.7 Å away. Fig. 4A also shows a water-mediated hydrogen bond between a side chain oxygen atom of the catalytic glutamate Glu159 and an oxygen atom from the γ-phosphate. In the crystal structure, amino acids involved in hydrogen bonding with ATP are Gly39, Thr44, Lys42, and His192 (Fig. 4B). Apart from Gly39, all these residues also form contacts with ATP in the simulation snapshot.
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FIGURE 4. A comparison of binding site interactions in a snapshot taken at 10 ns during the simulation of MgATP-induced closure of semiopen MalK (A) and in the crystal structure of ATP-bound closed MalK (B). The backbones of NBD1 and NBD2 are represented as red and orange ribbons, respectively. The simulation snapshot shows interactions of the magnesium ion co-factor (yellow sphere) and solvent water molecules (red and white spheres) with the binding site of the wild type protein. Two water molecules involved in the coordination of the Mg ion are labeled Wat1 and Wat2. Amino acids involved in hydrogen bonds are also labeled. The hydrogen bonds are shown as white broken lines. The angle cut-off used was 30°, whereas the distance cut-off was 3.2 Å. This figure was rendered using VMD (36).

Putative Interaction Sites with MalFG, MalT, and Enzyme IIA^{bc}.—To investigate possible implications of MalK domain motions for its interactions with MalFG, MalT, and enzyme IIA^{bc}, we calculated changes in solvent accessibility (33) and separation distances between clusters of residues predicted to play a role in these interactions. Fig. 3, A and B, highlight the locations of residues whose mutations are known to inhibit the regulatory functions of MalK (19, 37) or interact with MalFG.

Suppressor mutant studies have revealed that Ala^{85}, Lys^{108}, Val^{114}, and Val^{117} interact with MalFG. They are all located in the helical domain of the NBDs (39, 40). These residues are represented as green spheres in Fig. 3A. Their solvent-accessible surface area did not change substantially during the simulation of semiopen MalK with docked MgATP. The separation distance between the two clusters of these residues in NBD1 and NBD2 decreased from 48.4 Å to an average value of 4.4 ± 0.6 Å over the last 1 ns of the simulation. Information on the distance between the EAA loops (in MalFG), with which they have been predicted to interact, is unavailable. However, the observed decrease is consistent with the ATP-mediated change in dis-

Docking of MgATP to MalK did not significantly alter the separation distance between the two patches of residues located in RD1 and RD2 implicated in interaction with MalT.

Amino acids implicated in binding to enzyme IIA^{bc} are located in the NBD (Glu^{119} and Ala^{124}), at the interface between the NBD and the RD (Arg^{228} and Phe^{241}), and in the regulatory domains (Gly^{278}, Gly^{284}, Gly^{302}, and Ser^{322}) (19, 37). These residues are represented as purple spheres in Fig. 3A. During the simulation of semiopen MalK with docked MgATP, no significant change in solvent accessibility of these residues was observed. Nevertheless, MgATP docking caused a narrowing of the enzyme IIA^{bc} binding site predicted by Samanta et al. (42). These authors suggested that enzyme IIA^{bc} binds in between the NBDs and the RDs in dimeric MalK. The E. coli enzyme IIA^{bc} is a small protein of 168 amino acids. Its crystal structure revealed a parallelepiped architecture measuring about 30 Å on a side but significantly thinner (3 Å) from face to face (43). Glu^{119} in NBD1 and Ser^{322} in RD2 are positioned at opposite peripheries of the proposed binding site, as shown in Fig. 3A. The distance between their C-α atoms decreased from 24.5 Å in the crystal structure of the semiopen dimer to 20.4 Å at the end of the MgATP docked simulation. It is noteworthy that a comparative analysis of the distance between these residues in the three crystal structure conformations revealed no clear trend of increase or decrease. A crystal contact analysis was performed on the three crystal structures using the program PyMol (35). Contacts in the lattice were defined as residues from neighboring dimers that were within 4 Å of each other. The result revealed that in all three structures, contact regions included patches in both the NBDs and the RDs. These may have the effect of restricting the displacement of the NBDs relative to the RDs. Some of the contact patches either included or were close to Glu^{119} and Ser^{322}.

DISCUSSION

By introducing MgATP into the active sites of MalK, we were able to observe in computer simulations, a transition from the
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The transformation of semiopen MalK to the closed conformation due to the presence of docked MgATP suggests a mechanistic link between the two configurations and supports the suggestion by Chen et al. (7) that the semiopen and ATP-bound closed forms represent two different physiological states visited by MalK during the transport cycle. Intermediate snapshots between the two conformations obtained from the molecular dynamics simulations indicate that the closure is driven primarily by interactions between the γ-phosphate and the signature motif. These two moieties are the first points of contact between the two NBDs during closure of the semiopen conformation. In particular, Ser135 of the signature motif formed a strong hydrogen bond interaction (~1.9 Å in length) with an oxygen atom from the γ-phosphate of ATP. The critical role played by the γ-phosphate in driving closure may explain why ADP does not induce dimerization of the NBDs in experimental studies, whereas ATP does (11). Hydrolysis of ATP to ADP leads to dissociation of the dimer, as indicated by recent results by Davidson and co-workers (13). The dimer is also stabilized by direct protein-protein hydrogen bond contacts across the interface. These simulations highlight the role of two such contacts as being key to the stabilization of the dimer during initial stages of closure: the contact between conserved residues His192 of the H-loop and Asp165 of the D-loop as well as between conserved Ser38 of the Walker A motif and Asp165. The significance of the latter is perhaps underscored by the fact that it forms early during our simulation of the closure of semiopen MalK, and it is also observed in the ATP-bound closed crystal structures of both MalK and MJ0796 (7, 44). Similarly, the significance of the hydrogen bond contact between His192 and Asp165 during our simulations of the semiopen form is supported by crystallographic results that show it exists in both the open crystal structure of MalK and the ATP-bound dimer of MJ0796.

Results from previous experimental studies have indicated that the regulatory domains play a significant role in stabilizing the physiological MalK dimer in solution, in contrast to other NBDs without regulatory domains. The disruption of the dimer interface in the absence of regulatory domains as observed in our simulations provides supporting evidence for this.

Coordination of Mg2+ and the Role of Solvent Molecules in the Active Site—Snapshots from our simulations show that Mg2+ has five oxygen atoms as ligands: two from solvent molecules and one each from the three phosphates of ATP. In a study of F1-ATPase, Oster and co-workers (45) reported that the total coordination number of Mg2+ oscillates between 5 and 6, indicating an unstable octahedral coordination environment. Free energy calculations on the conformational states of MgATP in water further indicate that the Mg2+ ion is equally likely to coordinate oxygen atoms from the two end phosphates or all three (46). A significant contact observed in our simulations, but not in the closed crystal structure of MalK, is the water-mediated hydrogen bond between the catalytic glutamate and a γ-phosphate oxygen atom. The catalytic glutamate has been proposed to align the attacking water molecule during the hydrolysis reaction (8). Mutation of this glutamate to a glutamine renders the NBDs catalytically inactive, thus enhancing the stability of the ATP-bound dimer (44).

Mechanistic Implications—Chen et al. (7) have suggested that MgATP initially docks to the Walker A motif of the open conformation of MalK. From results of the simulations of open MalK with and without docked MgATP, we propose that subsequent to this, the two NBDs are initially drawn toward each other by a combination of cross-interface protein-protein hydrogen bonds and long range attractive forces due to MgATP. Protein-protein hydrogen bonds were observed between His192 and Asp165, Asn163 and Asp165, Asn163 and Asn163, and Leu164 and Asp165. Although the MgATP molecules may not make direct contacts across the dimer interface at this stage, their attractive role is indicated by the observation that excluding them from the simulation causes the NBDs of the open form to drift even further apart. The system may evolve from an open state with MgATP bound to a semiopen state in which MgATP molecules are close enough to the opposing NBD that contacts between the γ-phosphate and the signature motif assume dominance. These contacts are strong enough to drive the semiopen dimer to a closed state, as shown in our simulations of MgATP-docked semiopen MalK.

Based on the present study, we were unable to conclude whether or not MgATP binds asymmetrically to the two active sites. In two of our simulations of MgATP-docked semiopen MalK, the separation distance between the Walker A and signature motifs at the two binding sites equilibrated to the same value. In the remaining three simulations, they did not. In our previous study of BtuCD, we found that the presence of docked MgATP caused the Walker A and signature motif to draw closer to each other in one binding site and further apart at the second binding site (22). Campbell and Sansom (47), in a molecular dynamics simulation study of MJ0796 in the absence of TMDs, reported symmetric MgATP binding. MgADP is also similarly bound at both active sites in the more recent crystal structure of MalK in the posthydrolysis state (13). Reyes and Chang, on the other hand, have recently shown that ADP-vanadate binds asymmetrically to MsbA in the presence of transmembrane domains (48). Taken together, both experimental and theoretical results seem to suggest that the presence of TMDs may be required for asymmetric nucleotide binding to take place, unless the degree of asymmetry is very small and, for example, limited to side chain conformations in the active site.

Our study supports an assertion previously made by several researchers that MgATP-induced dimerization of the NBDs may be a mechanism for transfer of conformational energy...
from the NBDs to the TMDs. As a consequence of tighter dimerization of the NBDs due to docking of MgATP to the semiopen form, we observed a ~4-Å decrease in separation distance between patches of amino acids implicated in the interaction of MalK with MalF and MalG. It is evident that even larger distance shifts can be realized in transitioning from the open to the closed form. ATP hydrolysis subsequently resets the closed MgATP-bound dimer to an open resting state conformation (13). Since transmembrane domains were not present, our simulations did not capture the influence of the TMDs on the dynamics of MalK.

**Regulation of MalK by Enzyme IIA^{abc}**—In the model proposed by Samanta et al. (42), enzyme IIA^{abc} might inhibit maltose transport in the presence of glucose by impeding the opening and closing of MalK during the catalytic cycle. No consensus sequence or structural motif has been identified in the targets of enzyme IIA^{abc}, numbering at least 10 (49, 50). Our study indicated that the size of the putative enzyme IIA^{abc} binding site between the NBDs and the RDs decreased upon nucleotide binding. This was measured as the change in separation distance between Glu^{199} and Ser^{322} (51). We note, however, that a similar comparative analysis of the three crystal structure conformations revealed a clear trend of increase or decrease in this dimension. Evidence from crystal contact analysis suggests that this may be due to the involvement of regions close to or encompassing Glu^{199} and Ser^{322} in crystal contacts. The observed ~4-Å decrease observed in the simulation is a potentially significant change, considering that enzyme IIA^{abc} is only ~13 Å thick from face to face (43). The hydrophobic interface believed to interact with MalK and other permeases surrounds the active site and is predominantly located on one face (51). It is conceivable that the observed decrease in the size of the space between the NBD and the RD may be prevented by bound unphosphorylated enzyme IIA^{abc}. This could suppress MgATP binding by inhibiting movement of the NBDs toward each other. The crystal structure of enzyme IIA^{abc} in complex with one of its targets, glycerol kinase, showed that the His^{90} phosphorylation site of enzyme IIA^{abc} is located in a hydrophobic environment at the interface of the two proteins (52). Should this be true for the MalK-enzyme IIA^{abc} interface as well, then phosphorylation of His^{90} may cause a steric mismatch that leads to the dissociation of the complex. NBD movements accompanying MgATP-binding during the catalytic cycle could then resume unhindered. Since the decrease in size of the binding site is not corroborated by crystallographic data, we suggest further investigation to test this model of the structural basis of regulation of MalK by enzyme IIA^{abc}. It may be interesting, for example, to determine whether MgATP-bound MalK can be immobilized by cross-linking the positions occupied by Glu^{199} and Ser^{322}.

**Interaction between MalK and MalT**—Additionally, this study indicates that MgATP binding to MalK may lead to the increased solvent exposure of amino acids involved in its interaction with MalT, thus presenting a suitable binding interface for MalT. Experimental evidence suggests that it is the dimeric form of MalK that interacts with MalT and that the interaction between the two occurs when MgATP is bound to MalK but not hydrolyzed (20). Simulations of the MgATP-induced transition from the semiopen to the closed state are thus relevant for making inferences about changes leading up to the MalK-MalT interaction. We also observed that the RDs are generally more flexible in the closed state following ATP-induced dimerization of the NBDs than in the open state. This points to the possibility that the regulatory domains are more adaptable to interaction with MalT in the nucleotide-bound closed state than in the open state. Our results show no change in the separation distance between patches in RD1 and RD2 that potentially interact with MalT, suggesting that reorientation of RD1 relative to RD2 may not be crucial for the interaction.

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