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Structural Basis for Adenylate Kinase Activity in ABC ATPases

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ATP-binding cassette (ABC) enzymes are involved in diverse biological processes ranging from transmembrane transport to chromosome cohesion and DNA repair. They typically use ATP hydrolysis to conduct energy-dependent biological reactions. However, the cystic fibrosis transmembrane conductance regulator and the DNA repair protein Rad50 can also catalyze the adenylate kinase reaction (ATP + AMP ↔ 2ADP). To clarify and provide a mechanistic basis for the adenylate kinase activity of ABC enzymes, we report the crystal structure of the nucleotide-binding domain of the *Pyrococcus furiosus* structural maintenance of chromosome protein ($pfSMC^{nbd}$) in complex with the adenylate kinase inhibitor P^1,P^5-di(adenosine-5′)pentaphosphate. We show that $pfSMC^{nbd}$ possesses reverse adenylate kinase activity. Our results suggest that in adenylate kinase reactions, ATP binds to its canonical binding site while AMP binds to the Q-loop glutamine and a hydration water of the Mg^{2+} ion. Furthermore, mutational analysis indicates that adenylate kinase reaction occurs in the engaged $pfSMC^{nbd}$ dimer and requires the Signature motif for phosphate transfer. Our results explain how ATP hydrolysis and adenylate kinase reactions can be catalyzed by the same functional motifs within the structural framework of ABC enzymes. Thus, adenylate kinase activity is likely to be a latent activity in many ABC enzymes.

Keywords: ABC ATPase; structural maintenance of chromosomes; cystic fibrosis transmembrane conductance regulator; structural biology; adenylate kinase activity

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Introduction

ATP-binding cassette (ABC) enzymes are a large and versatile family of ATPases that conduct diverse biological processes.[1–4] Most prominently, the membrane spanning ABC transporters utilize ATP to transport a large variety of substrates across biological membranes. ABC transporters are composed of two ABC-type nucleotide-binding domains (NBDs) that bind to two transmembrane domains.[5,6] In the presence of ATP, the two NBDs engage into a tight head-to-tail dimer, promoted by binding of two ATP molecules to two composite ATP-binding sites in the NBD dimer interface.[7,8] In the closed ATP-bound conformation, Walker A motifs bind the α-, β-, and γ-phosphates from one side, while the Signature motifs of the opposing NBDs bind the γ-phosphates from the opposite side. This γ-phosphate-dependent conformational engagement and disengagement is thought to be part of the functional power stroke of ABC transporters.[9]

Besides the membrane-bound ABC transporters, there is a large variety of nuclear and cytoplasmic ABC enzymes. Among these, the structural maintenance of chromosome (SMC) protein family is involved in sister chromatid cohesion, chromosome condensation, and genome maintenance.[10,11] SMC proteins consist of an ABC-type NBD and a dimerization domain, both connected by a long coiled-coil linker. Two SMC proteins assemble into homodimers (in prokaryotes) or heterodimers (in eukaryotes) via their dimerization domains.[12] Two NBDs in the SMC protein dimer engage in the presence of ATP to form a tight NBD:2ATP:NBD sandwich, similar to that observed for the NBDs

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Abbreviations used: ABC, ATP-binding cassette; NBD, nucleotide-binding domain; SMC, structural maintenance of chromosome; CFTR, cystic fibrosis transmembrane regulator; Ap5A, P^1,P^5-di(adenosine-5′)pentaphosphate; PDB, Protein Data Bank.

from the ABC transporter.[13–16] Together with additional subunits, ATP-binding-induced engagement of the two NBDs in the SMC protein dimer is required for chromosome association *in vivo*, possibly by facilitating the formation of large proteinaceous rings.[17–19]

However, the sole dependence on ATP hydrolysis of all ABC enzymes is currently challenged by the analyses of the ABC transporter CFTR (cystic fibrosis transmembrane regulator) and the DNA repair enzyme Rad50. CFTR forms a transmembrane channel that regulates the passive flow of anions down an electrochemical gradient.[20] Channel opening is linked to ATP-dependent engagement of its two NBDs.[21] Although CFTR can be regulated by ATP hydrolysis *in vitro*, it can be also regulated by the reversible adenylate kinase reaction ($Mg^{2+}\cdot ATP + AMP \leftrightarrow Mg^{2+}\cdot ADP + ADP$).[22,23] The adenylate kinase and reverse adenylate kinase reactions release virtually no free energy under physiological nucleotide conditions, consistent with the passive ion gating function of CFTR. Which of the two ATP-dependent reactions is utilized by CFTR in its physiological context is currently debated.[24,25] Recently, the ABC ATPase Rad50 involved in DNA repair has been shown to catalyze a similar adenylate kinase reaction *in vitro*.[26]

The presence of the two distinct ATP hydrolysis and adenylate kinase activities within the ABC enzyme family is puzzling, and the molecular mechanism for adenylate kinase activity remains unclear. To help clarify this issue for this central and important enzyme family, we biochemically analyzed the adenylate kinase activity of the NBD of the SMC protein from *Pyrococcus furiosus* ($pfSMC^{nbd}$) and determined the crystal structure of $pfSMC^{nbd}$ in complex with the bi-substrate adenylate kinase inhibitor P^1,P^5-di(adenosine-5′)pentaphosphate (Ap5A). While the forward adenylate kinase reaction is masked by the much more robust ATPase activity of SMC proteins, we can show that $pfSMC^{nbd}$ can catalyze the reverse adenylate kinase reaction. Our analysis indicates that ATP in the adenylate kinase reaction occupies the Walker A motif/P-loop, while AMP binds to Q-loop glutamine and to the active-site magnesium moiety. Our data furthermore suggest a mechanism of adenylate kinase activity that reconciles structural and biochemical data in a coherent model and relates it to ATP hydrolysis.

Results

Reverse adenylate kinase activity of $pfSMC^{nbd}$

To see whether adenylate kinase activity is not limited to CFTR and Rad50, but may even be present in other ABC enzymes, we tested the ability of the NBD of *P. furiosus* SMC protein for adenylate kinase activities. Surprisingly, we found that $pfSMC^{nbd}$ can catalyze the reverse adenylate kinase reaction *in vitro*. Reverse adenylate kinase activity produced about 0.16 ATP molecules per minute, which is significantly less than the ATP hydrolysis activity with 1 ATP hydrolyzed per minute.[15] However, careful co-elution analysis of protein and enzymatic activity (Fig. 1a) and reduction of the adenylate kinase activity by site-directed mutagenesis of $pfSMC^{nbd}$ (Fig. 1b) suggest that this activity is intrinsic to $pfSMC^{nbd}$ and not due to co-purified impurities. In particular, mutational analyses suggest that Walker A (K39A), Q-loop (Q145A), and Signature motif (S1070R) are required for adenylate kinase activity of $pfSMC^{nbd}$ (Fig. 1b).

To further examine the adenylate kinase activity of $pfSMC^{nbd}$, we used the adenylate kinase inhibitor Ap5A. Indeed, Ap5A inhibited the reverse adenylate kinase activity of $pfSMC^{nbd}$ but had no substantial effect on the ATPase reaction (Fig. 1c), similar to what was reported for Rad50.[26] These findings suggest that SMC proteins preferentially conduct ATP hydrolysis. However, our data show that the ability to catalyze the adenylate kinase reaction is not restricted to CFTR and Rad50 but may exist in other ABC enzymes. This observation raises two important questions. Why are two distinct biochemical activities observable within the same ABC enzymes and how are these two types of activities mechanistically related? Thus, the mechanistic characterization of adenylate kinase activity in ABC enzymes is important not only from an evolutionary point of view but also to better understand the mechanism of CFTR and Rad50.

Crystal structure of $pfSMC^{nbd}$ in complex with Ap5A

To reveal the structural mechanism of adenylate kinase activity of ABC enzymes, we crystallized $pfSMC^{nbd}$ in complex with Mg^{2+} and Ap5A (for nomenclature, see Fig. 1d). $Mg^{2+}\cdot$Ap5A is a bi-substrate inhibitor of adenylate kinases that blocks two sites: one adenosine (denoted adenosine 1 hereinafter) binds to the ATP site, while the other adenosine (now denoted adenosine 2) binds to the AMP site.

Crystals of the $Mg^{2+}\cdot$Ap5A$\cdot SMC^{nbd}$ complex diffracted to 1.6 Å resolution (Table 1). Molecular replacement and refinement yielded clear electron density for a whole $Mg^{2+}\cdot$Ap5A moiety in one of the two molecules in the asymmetric unit. A simulated annealing omit map countered at 2 σ for Ap5A, Mg^{2+}, and four important water molecules calculated from the final model is shown in Fig. 2a.

The ADP/ATP-binding site

Ap5A binds to $pfSMC^{nbd}$ in an "S"-like conformation (Fig. 2a–c). The two adenosine moieties are attached to two ~15 Å separated binding sites. Mg^{2+}, adenosine 1, plus α-, β-, and γ-phosphates bind to the canonical Mg^{2+}-ATP-binding site on Lobe I of $pfSMC^{nbd}$. Adenine 1 is recognized by three specific hydrogen bonds to the main chain of residues 65, 67, and 69 (Fig. 2b). The α- and β-phosphates are bound to the P-loop (Walker A motif) and among

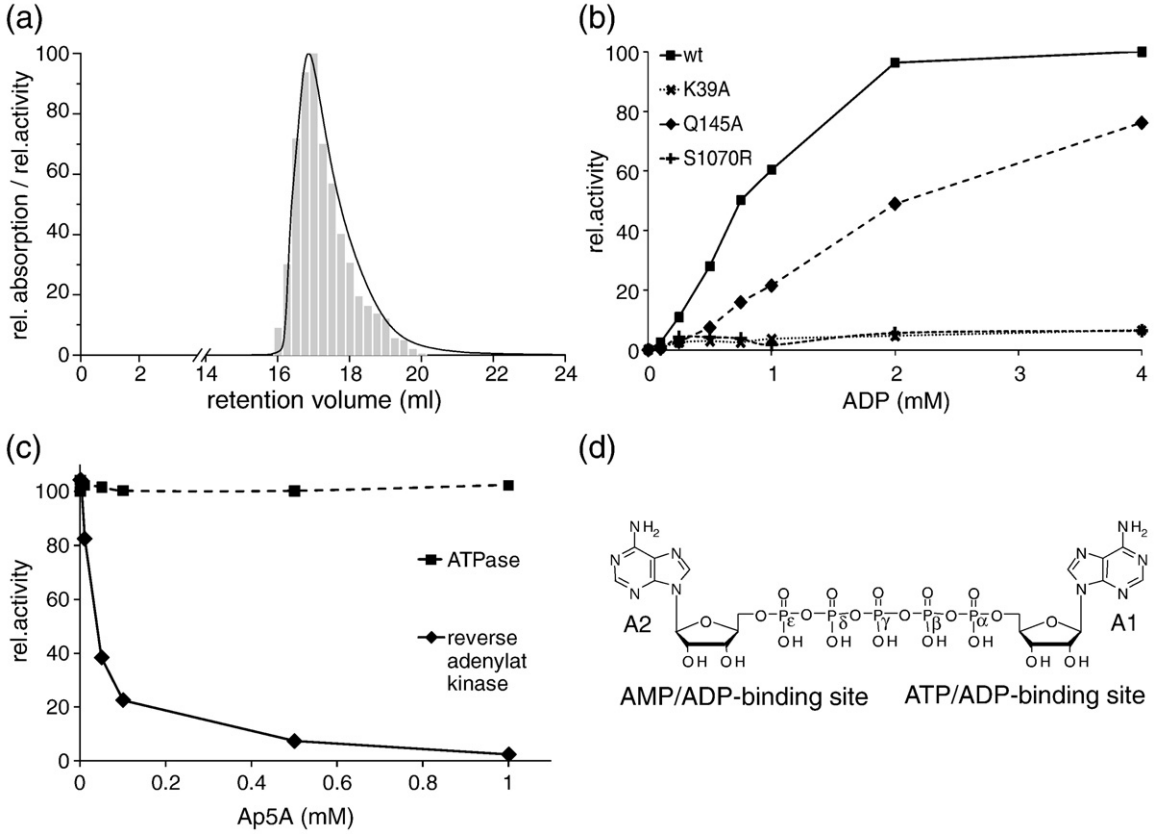

Fig. 1. Reverse adenylate kinase activity of pfSMC^{nbd}. (a) Superposition of a size-exclusion column elution profile of pfSMC^{nbd} monitored by the absorption at 280 nm (continuous line) with reverse adenylate kinase activity of the collected fractions (bars). The perfect co-elution of activity with the purified protein indicates that the measured adenylate kinase activity is intrinsic to pfSMC^{ndb} and not due to co-purified background activity. (b) Relative reverse adenylate kinase activity of pfSMC^{nbd} in percent wild-type activity as a function of ADP. Mutations in P-loop/Walker A (K39A) and Signature motif (S1070R) almost completely abolish activity. The Q-loop mutation Q145A also decreases reverse adenylate kinase activity. Increased relative activity of the Q145A mutant at high ADP concentrations suggests a role of the Q-loop glutamine in AMP/ADP binding. (c) Reverse adenylate kinase activity and ATPase activity of the wild-type protein was determined in the presence of the adenylate kinase inhibitor Ap5A. While increasing Ap5A concentrations clearly reduce reverse adenylate kinase activity, ATPase activity is not affected. (d) Chemical structure of the bi-substrate adenylate kinase inhibitor Ap5A with the nomenclature used in this study. A1 denotes adenosine 1 and A2 denotes adenosine 2.

SMC proteins conserved "arginine finger" (R59)[15] (Fig. 2b). The β- and γ-phosphates form two coordination ligands of the active-site Mg^{2+} ion, with the coordination sphere of Mg^{2+} completed by the side-chain oxygen of Q145 (Q-loop), the side chain of S40 (Walker A motif), and two water molecules (Fig. 2b). The arrangement of Mg^{2+}, adenosine 1, and α-, β-, and γ-phosphates of Ap5A is virtually identical with that of ATP in the Mg^{2+}·ATP·pfSMC^{nbd} complex[15] (Fig. 3a). The structural data are in agreement with the biochemical evidence that Ap5A blocks the ATP-binding site in CFTR[22,25] and that the K39A mutation in the Walker A motif disrupts the reverse adenylate kinase reaction of pfSMC^{nbd} (Fig. 1b).

The AMP/ADP-binding site

Remarkably, in one of the two molecules in the asymmetric unit, adenosine 2 stacks onto the side chain of Gln145 of the Q-loop at the interface of Lobe I and Lobe II (Fig. 2b). In the second pfSMC^{nbd} in the asymmetric unit, crystal packing prevents the Ap5A from adopting the same bound conformation. The stacking interaction with the Q145 carboxamide moiety is similar to the interaction of adenine 1 with the K13–S14 peptide bond. Furthermore, N7 nitrogen of adenosine 2 forms a hydrogen bond to a Mg^{2+} water ligand (W1). Finally, the adenosine N6 amine is specifically recognized via hydrogen bonds to the same water of the MG^{2+} coordination sphere (W1) and a second water (W2) interacting with a water (W3) bound by the main-chain carbonyl of Q145 and N1 nitrogen (Fig. 2b). Together, these hydrogen-bonding interactions could explain the observation that GMP cannot substitute for AMP in the CFTR adenylate kinase reactions, as previously tested, and argue for a fairly specific adenine recognition.[22,25] To biochemically verify the importance of this interaction, we mutated Q145 to alanine and tested the reverse adenylate kinase activity of pfSMC^{nbd}. As expected, Q145A substantially affected the

Table 1. Crystallographic data collection and model refinement statistics

Data collection	
Beamline	14-2 (European Synchrotron Radiation Facility)
Wavelength	0.933
Space group	$P2_1$
Unit cell parameters	
a, *b*, *c* (Å)	65.0, 86.1, 70.1
α, β, γ (°)	90, 116.6, 90
Resolution (Å)	50.0–1.6
R_{sym}	4.7 (25.5)
I/σ	16.3 (3.8)
Completeness (%)	95.7 (83.0)
Redundancy	3.7 (3.2)
Refinement	
Resolution (Å)	35.4–1.6
No. of reflections (test)	84,044 (4271)
R_{work}/R_{free}	17.6/20.6
No. of atoms	
Protein	5117
Inhibitor	2×Ap5A
Ion	$2\times Mg^{2+}$
Water	871
Mean *B*-factor (Å^2)	
Protein	22.4
Ap5A	25.7
Water	33.5
rmsd from ideal	
Bond lengths (Å)	0.007
Bond angles (°)	1.138
Ramachandran plot (%)	
Most favored	94.7
Additionally allowed	4.7
Generously allowed	0.6
Disallowed	0
PDB accession code	3KTA

activity (Fig. 1b). However, activity was partially restored at higher concentrations of ADP, suggesting that Q145 is involved in substrate binding, as suggested by the structural analysis, and not direct catalysis.

The δ- and ε-phosphates of Ap5A are not recognized by pfSMC^{nbd}, and ribose 2 is bound by the C-terminal tail of pfSMC^{nbd} (Figs. 2c and 3a). Since this tail is not conserved among ABC enzymes, its involvement could be due to the crystal packing, and its position is typically occupied by the D-loop in the engaged state of the NBD dimer. To test whether adenylate kinase indeed occurs in the engaged state of NBDs, we analyzed the effect of the Signature motif mutation S1070R (Fig. 1b). This mutation, which prevents ATP-dependent dimer formation and abolishes ATP hydrolysis of pfSMC^{nbd},[15] also abolishes reverse adenylate kinase activity. The effect of this mutation can only be explained if the reverse adenylate kinase reaction of pfSMC^{nbd} occurs in the engaged dimeric form of NBDs. Consistent with our finding, biochemical and *in vivo* analysis suggests that the adenylate kinase reaction of CFTR and Rad50 also requires dimerization of NBD1 and NBD2.[25–27]

In fact, two pfSMC^{nbd} in the crystal lattice face each other, positioned to engage, but a close engagement, as seen for ATP as ligand, is not observed in the case of Ap5A. The fact that we do not observe a pfSMC^{nbd} dimer in the crystal is presumably a result of steric clashes because of the additional phosphate present in Ap5A compared to the situation occurring during the adenylate kinase reaction with only four phosphates involved (2 ADP or ATP + AMP). When the bound Ap5A is aligned with the ATP in the ATP-bound dimer, the linked pentaphosphate chain clashes with the signature motif of the opposing pfSMC^{nbd} and therefore could prevent the highly transient interaction of the two NBDs. The inability to sterically form an engaged pfSMC^{nbd} dimer might also explain why Ap5A cannot compete with ATP (in the ATPase activity) but can compete with ADP (in the reverse adenylate kinase activity).

Comparison of Ap5A-bound and ATP-bound pfSMC^{nbd} and implications for CFTR

To learn more about the relationship between adenylate kinase and ATP hydrolysis activities, we superimposed Ap5A- and ATP-bound pfSMC^{nbd} via Lobe I. ATP and adenosine 1 plus α-, β-, and γ-phosphates of Ap5A overlap very well (Fig. 3a). There are also no significant differences in the conformation of the ATP-binding site of Ap5A- and ATP-bound conformations of pfSMC^{nbd}. This structural similarity indicates that the same type of ATP recognition is used in ATP-hydrolysis- and adenylate-kinase-type reactions within the structural framework of ABC enzymes.

However, the superposition indicates that in the presence of Ap5A, Lobes I and II of both molecules in the asymmetric unit are rotated ~15° compared to their conformation in the presence of ATP (Fig. 3b). ATP binding does not induce such a reorientation of the two lobes compared to the apo pfSMC^{nbd}.[15] The rotation could be induced by

Fig. 2. Structure of pfSMC^{ndb} in complex with Ap5A. (a) Stereo plot of the Ap5A binding site of pfSMC^{nbd} (ribbon representation) with a portion of a ($F_o - F_c$) simulated annealing omit density (green mesh) surrounding the Ap5A (color-coded sticks), Mg^{2+} (gray sphere), and important waters (red spheres) and countered at 2 σ. (b) Stereo plot of the Ap5A binding site of pfSMC^{nbd} (same view as in a). Selected amino acids and water molecules are annotated. Notable hydrogen bonds are represented as broken lines. Adenosine 1 (A1) and α-, β-, and γ-phosphates occupy the canonical ATP-binding site of pfSMC^{nbd}, including interactions with the P-loop, the Mg^{2+} ion (gray sphere), and the R-loop (R59). Adenosine 2 stacks with the carboxamide group of Q145 and hydrogen bonds to the Mg^{2+}-coordination sphere water (W1) and two additional waters (W2 and W3). δ- and ε-phosphates have no specific interactions with pfSMC^{nbd}. Water W4 is located at the expected site of the nucleophil. (c) Stereo plot of pfSMC^{nbd} (ribbon representation) with Ap5A (color-coded sticks), Mg^{2+} (gray sphere), and important waters (red spheres) (for nomenclature, see a and b). Functional relevant motives are annotated and colored green.

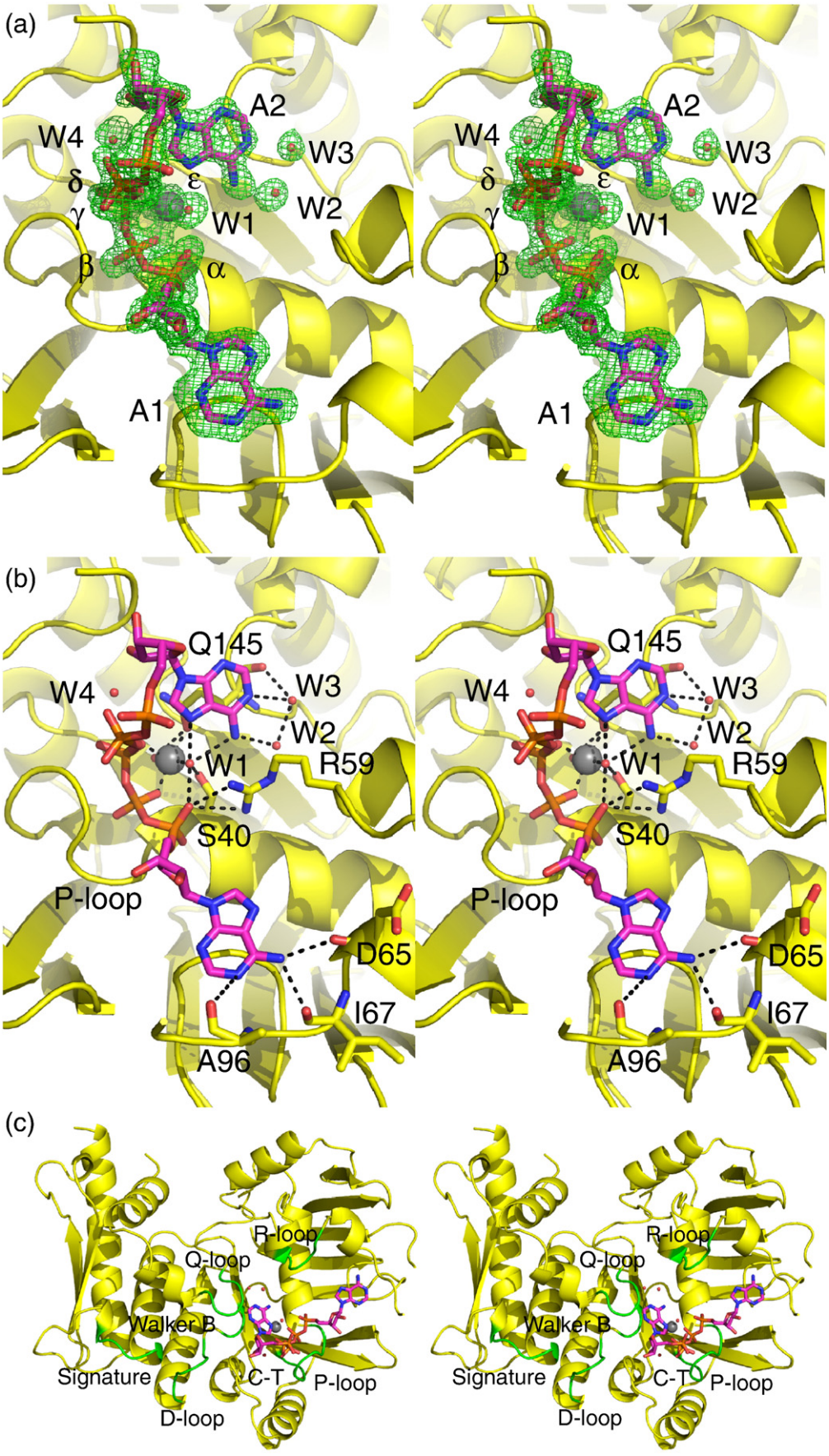

Fig. 2 (*legend on previous page*)

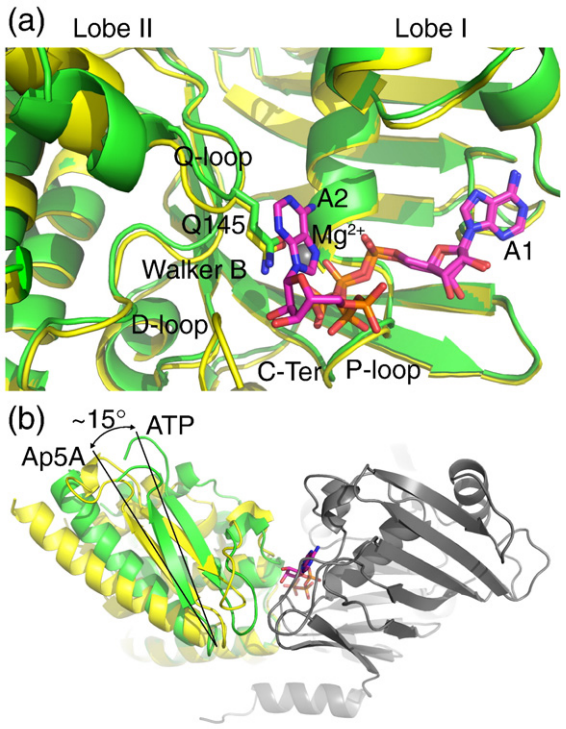

Fig. 3. Comparison of ATP- and Ap5A-bound structures of pfSMC^{nbd}. (a) Active-site view of superimposed models of Ap5A-bound pfSMC^{nbd} (yellow ribbon model) and ATP-bound pfSMC^{nbd} (green ribbon model). Ap5A and ATP are shown as color-coded stick models. ATP and the corresponding portion of Ap5A bind in identical conformations to the ATP-binding site of Lobe I, suggesting that the same type of ATP binding mode functions in ATP-hydrolysis- and adenylate-kinase-type reactions of ABC enzymes. (b) Domain view of the superposition with the color code of (a). The opposing NBD in the ATP-bound dimer of pfSMC^{nbd} is shown in gray. In response to superposition of Lobes I, Lobes II differ by an ~15° rotation between Ap5A- and ATP-bound states of pfSMC^{nbd}.

binding of AMP at the Q-loop glutamine. In fact, the conformation of the helix protruding from the Q-loop is markedly different between Ap5A- and ATP-bound conformations of pfSMC^{nbd} (Fig. 3a). Hereby, subtle conformational changes of the main-chain geometry around the Q-loop between ATP- and Ap5A-bound forms could lead to the observed geometrical differences. If this is the case, AMP binding to CFTR could also help to orient Lobes I and II, a process that may follow or facilitate engagement of the NBDs in response to ATP binding. Since the transmembrane domains bind to the NBDs of ABC transporters near the Lobe I/Lobe II interface,[6] both NBD engagement and interlobe rotations could contribute to channel gating in CFTR.

The location of the AMP-binding site in pfSMC^{nbd} might also explain the effect of the cystic fibrosis mutation N1303K^{CFTR}. This mutation abolishes adenylate kinase activity of CFTR *in vitro*.[22] The effect of this mutation was puzzling, because N1303 is not located near the canonical ATP-binding site or the proposed NBD1:NBD2 interface of CFTR. There is no structure available for NBD2 of CFTR, to which N1303 belongs. However, the structure of CFTR NBD1 has been determined[28] and the equivalent N499 in NBD1 binds to the main chain and stabilizes the β-strand that follows the Q-loop. Sequence conservation of NBD1 and NBD2 of CFTR in this region suggests that N1303 and N499 residues fulfill similar structural roles. Thus, N1303K would be expected to affect main-chain stability at the Q-loop, thereby perturbing the proposed AMP-binding site.

Discussion

We report here the first structural analysis of adenylate kinase activity in ABC enzymes. The presence of both adenylate-kinase- and ATP-hydrolysis-type reactions in ABC enzymes has been puzzling and raised the question as to how these two types of biochemical reactions have evolved and how they are related within the same structural framework. The identification of adenylate kinase reactions in now three different ABC proteins (CFTR, Rad50, and SMC) indicates that this type of biochemical reaction is perhaps a widespread and inherent feature of many ABC-type enzymes.

How does the adenylate kinase reaction occur? The crystallographically observed conformation of Ap5A could resemble the substrate conformation of the reverse adenylate kinase reaction. Adenosine 1 and α- and β-phosphates are in a position to mimic the acceptor ADP, while adenosine 2 and δ- and ε-phosphates are in a position to approximately mimic the donor ADP, although it is possible that this conformation slightly changes in an engaged NBD dimer. In particular, the β-phosphate oxygen is in a good position for collinear nucleophilic attack on the δ-phosphate. The observed conformation of Ap5A is unlikely to resemble the substrate conformation of the forward adenylate kinase, because Pε (AMP) in pfSMC^{nbd} complex is not positioned for nucleophilic attack on Pγ (ATP).[29] However, a simple rotation of the sugar-ε-phosphate moiety around the N-glycosidic bond of adenosine 2 would position the ε-phosphate oxygen at the expected place of the nucleophile (water W4 in Fig. 2a and b), in bonding distance to the Walker B glutamate. Such a position would be consistent with the biochemical results showing that the Walker B glutamate is required for forward adenylate kinase activity of CFTR.[22] Perhaps, the additional δ-phosphate sterically prevents Ap5A to adopt a position in pfSMC^{nbd} where the ε-phosphate is poised for attack on the γ-phosphate.

The location of the δ- and γ-phosphates could mimic the position of the transferred phosphate before and after the reverse adenylate kinase reaction. The opposing Signature motif, as deduced from its position in the ATP-bound engaged form of NBD dimers, would be ideally suited to facilitate adenylate kinase activity. In particular, the

Signature motif could bind to transferred phosphate and help to stabilize the transition state with its positive helix dipole charge. Such a role would explain not only why the mutation in the Signature motif disrupts ATP hydrolysis[15] but also why it disrupts reverse adenylate kinase activity (Fig. 1b). Moreover, this model would mechanistically unify the function of the Signature motif in ATP hydrolysis and adenylate kinase activities of ABC enzymes. The conserved nature of the involved motifs in most if not all ABC enzymes now provides a molecular explanation for the puzzling phenomenon that two distinct enzymatic activities have been identified in several ABC enzyme family members. A plausible model that combines the structural and biochemical data for both ATP hydrolysis and adenylate kinase activity is shown in Fig. 4.

For all three ABC proteins able to catalyze the adenylate kinase reaction *in vitro*, the biological relevance of this activity is still under discussion. A clear dissection of the two functions, namely, ATPase and adenylate kinase activity, *in vivo* is hardly possible because both reactions use the same functional motifs. However, the adenylate kinase activity of CFTR and presumably Rad50 is suggested to be physiologically important by some experimental data, raising the question as to how a more robust adenylate kinase activity has evolved from ATP hydrolysis. Since Walker A and Signature motifs are shared between ATP-hydrolysis- and adenylate-kinase-type reactions, optimizing or specifically blocking the sites for AMP (adenylate kinase) or nucleophilic water (ATP hydrolysis) may determine whether adenylate kinase or ATP hydrolysis is preferred by the ABC enzyme. In particular, our model hints at how CFTR evolved out of an ATP-hydrolyzing ABC transporter. Additional loops as well as the precise primary sequence and geometry of Q- and D-loops could additionally strengthen AMP binding of CFTR, making it a much more efficient adenylate kinase than pfSMC^{nbd}. For instance, CFTR possesses a loop around Lys411, which is suitably located to form a lid for AMP binding.[28]

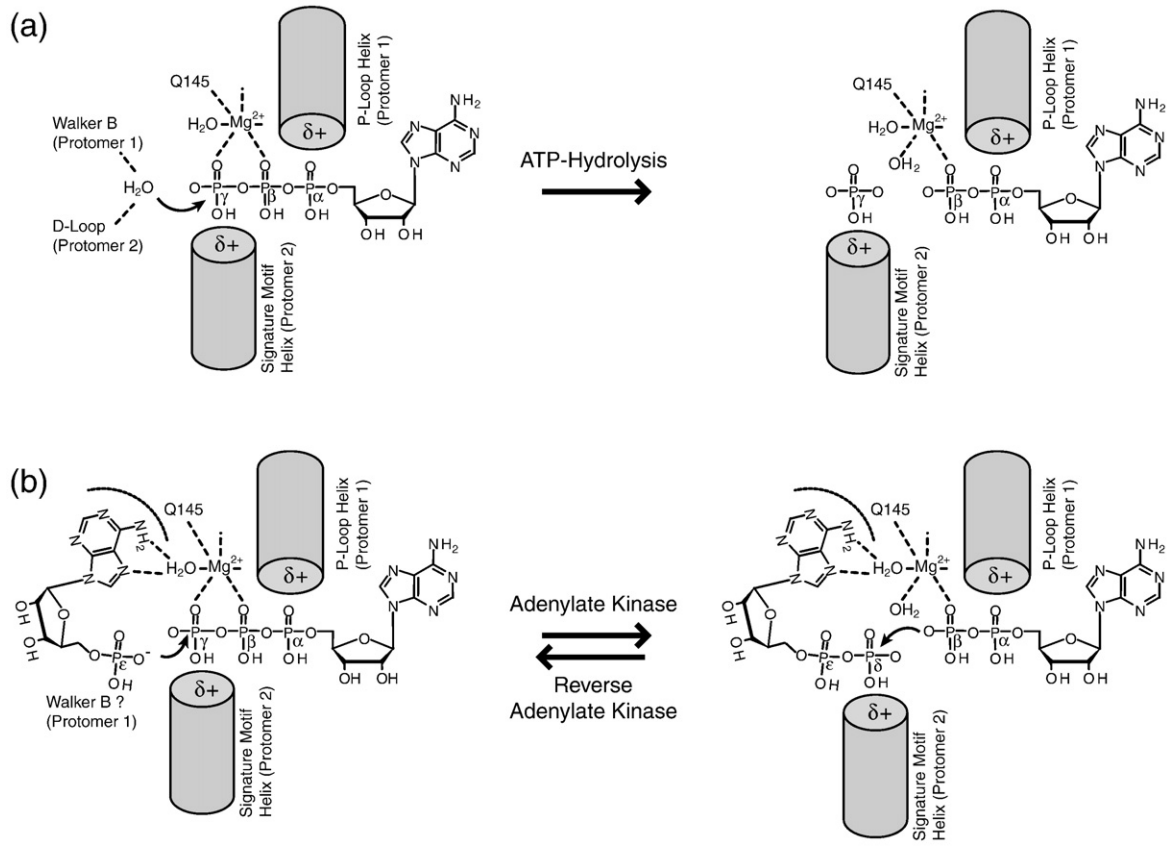

Fig. 4. Proposed reaction mechanism for adenylate kinase activity in ABC enzymes and its comparison to ATP hydrolysis. Part of the active site in the engaged NBD dimer with nucleotides, selected protein motifs, and the active-site magnesium (plus part of its coordination sphere) is schematically indicated. The proposed nucleophilic attacks of water or phosphate oxygens are indicated by arrows. In ATP hydrolysis (a), a water molecule is positioned and activated for nucleophilic attack by the Walker B of protomer 1 of the NBD dimer and D-loop of protomer 2. The Signature motif of protomer 2 binds the ATP γ-phosphate and likely stabilizes the transition state as well as promoting phosphate release. In the proposed adenylate kinase reaction, the adenine of AMP (or ADP in the reverse reaction) binds to the Q-loop Q145 and the magnesium coordination sphere (b). The Signature motif likely binds the transferred phosphate, a model that would unify its role in ATP-hydrolysis- and adenylate-kinase-type reactions in ABC enzymes. Parts of the model are speculative and need to be addressed in future studies.

Other ABC enzymes could also specifically block the AMP-binding site. For instance, in the translation-initiation- and ribosome-biogenesis-associated ABC enzyme ABCE1, a conserved tyrosine residue specifically blocks the proposed adenine binding surface of the Q-loop glutamines.[30]

In summary, we have identified the previously elusive AMP-binding site for adenylate kinase activity in ABC enzymes. While this binding site is perhaps suboptimal or even blocked for most ABC enzymes that utilize ATP hydrolysis, CFTR and other ABC enzymes with physiologically used adenylate kinase activity may have optimized AMP binding. Thus, it will be important to further analyze whether other ABC proteins also display or use adenylate kinase activity rather than ATP hydrolysis *in vitro* and *in vivo*.

Methods

Crystallization, data collection, phasing, and structure refinement

pfSMC^{nbd} was expressed and purified as previously described.[15] pfSMC^{nbd}-Ap5A [9 mg/ml in 5 mM Tris–HCl (pH 8.0), 100 mM NaCl, 0.1 mM ethylenediaminetetraacetic acid, 2.5 mM Ap5A, and 25 mM $MgCl_2$] was crystallized by sitting-drop vapor diffusion at 25 °C after mixing 2 μl protein solution with 2 μl precipitant solution {2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)-propane-1,3-diol (pH 6.6) and 16% (w/v) polyethylene glycol 3350}. Crystals were flash frozen in liquid nitrogen after the addition of 30% (v/v) polyethylene glycol 400. Diffraction data were recorded at 100 K at ID14-2 (European Synchrotron Radiation Facility) and processed with XDS[31] (Table 1). Phases where obtained by molecular replacement with PHASER,[32] using pfSMC^{nbd}-ATP [Protein Data Bank (PDB) ID: 1XEX] as a search model. An initial model was automatically built with ARP/wARP[33] and completed by cycles of manual model building with Coot[34] and positional, *B*-factor, and occupancy refinement with CNS[35] and PHENIX.[36] The coordinate, topology, and parameter files for the ligand Ap5A were obtained from the HIC-Up database.[37] Refinement and model statistics are shown in Table 1. Structure figures were generated with PyMOL†.

Adenylate kinase activity assay

Protein (1 μM) was incubated with increasing concentrations of ADP in 50 mM Tris (pH 7.5), 100 mM NaCl, and 10 mM $MgCl_2$ at 55 °C for 2 h. To determine the amount of produced ATP, we mixed 40 μl of the reaction mixture with 40 μl of the "ATP determination kit" reagent from Biaffin (Kassel, Germany). ATP generated in the reverse adenylate kinase reaction is used by firefly luciferase to oxidize D-luciferin. The resulting chemiluminescence was recorded with a Tecan infinite M1000 (Tecan Group Ltd., Männedorf, Switzerland) immediately after mixing.

For inhibitor studies, reverse adenylate kinase activity was assayed at a concentration of 1 μM wild-type protein in the presence of 2 mM ADP and increasing Ap5A concentrations (Sigma-Aldrich, Taufkirchen, Germany).

ATPase assay

Protein (1 μM) was incubated with 2 μM ATP for 2 h at 55 °C in the presence of increasing Ap5A concentration. Released phosphate was determined by mixing 40 μl of reaction with 110 μl BIOMOL Green Reagent (Enzo Life Science, Lörrach, Germany). OD_{260} (optical density at 260 nm) was measured after 30 min incubation at room temperature using a Tecan infinite M1000.

Accession number

Coordinates and structure factors for the crystal structure of pfSMC^{nbd} in complex with Ap5A have been deposited in the PDB with accession code 3KTA.

† www.pymol.org

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