**Mycobacterium tuberculosis** DNA gyrase ATPase domain structures suggest a dissociative mechanism that explains how ATP hydrolysis is coupled to domain motion

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INTRODUCTION

Type II DNA topoisomerases are essential nucleic acid-dependent nanomachines present in all organisms. They solve topological problems of DNA by temporarily introducing a double-stranded break in one DNA duplex and transporting another DNA duplex [T-DNA (transported DNA)] through this break. The ATPase domains dimerize, in the presence of ATP, to trap the T-DNA segment. Hydrolysis of one of the two ATPs, and release of the resulting P\textsubscript{i}, is rate-limiting in DNA strand passage. A long unresolved puzzle is how the non-hydrolysable ATP analogue, AMP-PNP (adenosine 5′-[β,γ]-methylene)triphosphate), can catalyse one round of DNA strand passage without P\textsubscript{i} release. In the present paper we discuss two crystal structures of the *Mycobacterium tuberculosis* DNA gyrase ATPase domain: one complexed with AMP-PCP (adenosine 5′-[β,γ]-imido)triphosphate) and the other, an AMP-PNP complex, crystallized as a dimer. In the AMP-PNP structure, the unprotonated nitrogen (P-N=P imino) accepts hydrogen bonds from a well-ordered ‘ATP lid’, which is known to be required for dimerization. The equivalent CH\textsubscript{3} group, in AMP-PCP, cannot accept hydrogen bonds, leaving the ‘ATP lid’ region disordered. Further analysis suggested that AMP-PNP can be converted from the imino (P-N=P) form into the imido form (P-NH-P) during the catalytic cycle. A main-chain NH is proposed to move to either protonate AMP-P-N=P to AMP-P-NH-P, or to protonate ATP to initiate ATP hydrolysis. This suggests a novel dissociative mechanism for ATP hydrolysis that could be applicable not only to GHKL phosphotransferases, but also to unrelated ATPases and GTPases such as Ras. On the basis of the domain orientation in our AMP-PNP structure we propose a mechaenochemical scheme to explain how ATP hydrolysis is coupled to domain motion.

Key words: ATPase domain, ATP hydrolysis, dissociative mechanism, DNA gyrase.

DNA gyrase, a type II topoisomerase, regulates DNA topology by creating a double-stranded break in one DNA duplex and transporting another DNA duplex [T-DNA (transported DNA)] through this break. The ATPase domains dimerize, in the presence of ATP, to trap the T-DNA segment. Hydrolysis of only one of the two ATPs, and release of the resulting P\textsubscript{i}, is rate-limiting in DNA strand passage. A long unresolved puzzle is how the non-hydrolysable ATP analogue, AMP-PNP (adenosine 5′-[β,γ]-methylene)triphosphate) can catalyse one round of DNA strand passage without P\textsubscript{i} release. In the present paper we discuss two crystal structures of the *Mycobacterium tuberculosis* DNA gyrase ATPase domain: one complexed with AMP-PCP (adenosine 5′-[β,γ]-imido)triphosphate) and the other, an AMP-PNP complex, crystallized as a dimer. In the AMP-PNP structure, the unprotonated nitrogen (P-N=P imino) accepts hydrogen bonds from a well-ordered ‘ATP lid’, which is known to be required for dimerization. The equivalent CH\textsubscript{3} group, in AMP-PCP, cannot accept hydrogen bonds, leaving the ‘ATP lid’ region disordered. Further analysis suggested that AMP-PNP can be converted from the imino (P-N=P) form into the imido form (P-NH-P) during the catalytic cycle. A main-chain NH is proposed to move to either protonate AMP-P-N=P to AMP-P-NH-P, or to protonate ATP to initiate ATP hydrolysis. This suggests a novel dissociative mechanism for ATP hydrolysis that could be applicable not only to GHKL phosphotransferases, but also to unrelated ATPases and GTPases such as Ras. On the basis of the domain orientation in our AMP-PNP structure we propose a mechaenochemical scheme to explain how ATP hydrolysis is coupled to domain motion.

Key words: ATPase domain, ATP hydrolysis, dissociative mechanism, DNA gyrase.

**Abbreviations used:** AMP-PCP, adenosine 5′-[β,γ]-methylene)triphosphate; AMP-PNP, adenosine 5′-[β,γ]-imido)triphosphate; ASEC, analytical size-exclusion chromatography; AUC, analytical ultracentrifugation; CSD, Cambridge Structural Database; HSP90, heat-shock protein of 90 kDa; P-loop, phosphate-binding loop; T-DNA, transported DNA.

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†‡§ Co-ordinates and structure factor files for the *M. tuberculosis* GyrB ATPase domain have been deposited in the PDB under the accession codes 3ZKB, 3ZKD and 3ZM7.
and also have the transducer domain and a residue equivalent to Glu\textsuperscript{42} in E. coli GyrB [11]. Jackson and Maxwell [19] identified Glu\textsuperscript{42} as a key catalytic residue in the ATPase reaction of DNA gyrase and proposed that it acts as a general base polarizing a water molecule for nucleophilic attack on the ϕ-phosphate. In a comprehensive paper, Corbett and Berger [13] solved structures with several ADP and ATP analogues to structurally dissect ATP turnover in the prototypical GHL ATPase TopoVI. This led to the proposal of a detailed mechanism [13] for ATP hydrolysis (Supplementary Figure S1A at http://www.biochemj.org/bj/456/4560263add.htm), which is initiated when the conserved glutamate residue (equivalent to Glu\textsuperscript{42} in E. coli GyrB) abstracts a proton from a water (consistent with the original proposal of Jackson and Maxwell [19]). In contrast, in a dissociative mechanism [20], the breaking of the bond between the β- and ϕ-phosphates (Supplementary Figure S1B), gives a highly electrophilic metaphosphate [PO\textsubscript{3}\textsuperscript{−}]\textsuperscript{−} ion and a protonated ADP\textsuperscript{2−} ion (Supplementary Figure S2 at http://www.biochemj.org/bj/456/bj4560263add.htm).

In the present paper, we report crystal structures of the M. tuberculosis GyrB ATPase domain (MtbGyrB\textsubscript{47}) with two different non-hydrolysable ATP analogues; purification, crystallization and data collection are reported elsewhere [21]. A structure with AMP-PCP (adenosine 5′-[β,γ-methylene] triphosphate) is the first non-dimeric structure of a type II topoisomerase ATPase domain with an ATP analogue, whereas two AMP-PNP structures are dimeric and similar to previously determined ‘ATP-restrained’ structures. This gives new insights into the catalytic cycle of M. tuberculosis DNA gyrase, and provides a structural explanation of why ‘non-hydrolysable’ AMP-PNP can drive one round of strand passage in type II topoisomerases [22,23], whereas, with ATP, the release of P\textsubscript{i} from the hydrolysed ATP is the rate-limiting step [24,25]. We note that whereas the bridging nitrogen of AMP-PNP (Supplementary Figure S2) is normally protonated (P-NH-P imido), in the presence of divalent metal ions, it is often unprotonated (P-N = P, imino) [26].

**MATERIALS AND METHODS**

**Protein expression, purification, crystallization and data collection**

Two constructs, MtbGyrB\textsubscript{47C1} and MtbGyrB\textsubscript{47C2}, for the ATPase domain of M. tuberculosis H37Rv GyrB (residues 1–427) were designed independently in two different laboratories (GlaxoSmithK-line, U.K. and Pasteur Institute, France respectively). Both constructs coded for residues 1–427 of M. tuberculosis GyrB, but they had slightly different N-terminal His\textsubscript{6} tags. For MtbGyrB\textsubscript{47C1}, the His\textsubscript{6} tag was systematically cleaved. For MtbGyrB\textsubscript{47C2}, the protein was puriﬁed with the His\textsubscript{6} tag intact. The N-terminal tags were not seen in crystal structures and made, within experimental error, no significant difference in ATPase activity assays. Details of the expression, puriﬁcation, crystallization and data collection on crystals of MtbGyrB\textsubscript{47C1} with AMP-PNP and of MtbGyrB\textsubscript{47C2} with AMP-PCP are reported elsewhere [21].

**ATP hydrolysis assays**

ATPase activity of the M. tuberculosis DNA gyrase was assessed at the Pasteur Institute by measurement of free P, using the pyruvate kinase/lactate dehydrogenase assay described previously [27]. The reaction mixture (100 μl) contained 50 mM Tris/HCl (pH 7.5), 50 mM KCl, 5 mM MgCl\textsubscript{2}, 0.25 mM NAD\textsubscript{H}, 1 mM phosphoenolpyruvate, 2 units of pyruvate kinase and 2 units of lactate dehydrogenase, various amounts of ATP and M. tuberculosis DNA gyrase [an equimolar mixture of GyrA and GyrB subunits in 50 mM Tris/HCl (pH 8.0) and 50 mM NaCl]. Reactions were performed in the absence or presence of DNA (relaxed pBR322, 5 μg/ml) at 37 °C and the decrease in NAD\textsubscript{H} concentration was monitored continuously as a function of time for 90 min by measuring the absorbance at 340 nm in a UV–visible spectrophotometer. The K\textsubscript{m} and V\textsubscript{max} values were determined from the double-reciprocal plots.

ATPase activities of the ATPase domain and GyrB subunit of the M. tuberculosis DNA gyrase were assessed at GlaxoSmithKline by measurement of free P, using the fluorescence method described in [28]. The proteins were first buffer-exchanged into 20 mM Tris (pH 7.5), 100 mM NaCl and 1 mM EDTA to remove DTT, as this can produce erroneous results. Assays were carried out in 10 μl aliquots in triplicate, and reactions were followed for 30 min at room temperature (22 °C). Fluorescence was measured using a Gemini microplate spectrophotometer and Softmax Pro (Molecular Devices). Initial rates were correlated with a phosphate calibration curve and kinetics were analysed with Grafit (Erithacus Software).

**Structure determination and reﬁnement**

The structure of the P1(8) crystal form of MtbGyrB\textsubscript{47C1} with AMP-PNP was determined by molecular replacement...
with PHASER [29] using the 43 kDa *E. coli* ATPase domain (40% amino acid identity) as the search model (PDB code 1EI1) [8] and refined. The P1(16) crystal form of *Mtb* GyrB47 (with AMP-PNP) was solved by molecular replacement from the P1(8) crystal form and refined (statistics in Table 1). In the final models of both of the AMP-PNP crystal forms the bridging nitrogen has been modelled in the imino form (P-N=P). Refinement of the AMP-PNP structures was carried out with Refmac [30], phenix.refine [31] and Buster [32]. Restraint dictionaries for the imino (P-N=P) form of AMP-PNP were generated with eLBOW [33] and fit [34] into difference maps calculated with phenix.refine [31]. In the final 2.9 Å (1 Å = 0.1 nm) AMP-PNP structure the main-chain NH groups of Leu 120 and Gly 122 of both of the AMP-PNP crystal forms the bridging nitrogen was protonated (P-NH-P, imido; see Supplementary Figure S2) and fit into difference maps calculated with phenix.refine [31] and Buster [32]. Restraint dictionaries for the imino (P-N=P) form of AMP-PNP were generated with eLBOW [33] and fit [34] into difference maps calculated with phenix.refine [31].

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### AUC (analytical ultracentrifugation) and analytical ASEC (analytical size-exclusion chromatography)

Sedimentation velocity experiments were performed at 15 μM protein (*Mtb*GyrB47$^{C2}$ or His$_6$–*Mtb*GyrB47$^{C2}$) either in Tris buffer [50 mM Tris (pH 8) and 50 mM NaCl, ± 5 mM AMP-PCP, or 5 mM AMP-PNP] or in PBS (pH 7.4, ± 2 mM AMP-PNP and ± 2 mM Mg or 2 mM EDTA). The PBS samples were gently resuspended at the end of the AUC run and the run repeated with the same sample cell after 3 or 6 days of incubation at room temperature.

Experiments were performed in a Beckman XL-I analytical ultracentrifuge using a double sector charcoal-Epon cell at 20°C and 42000 rev/min. Interference scans were taken every 6 min. The program Sednterp 1.09 (available at http://www.jphilo/mailway.com/download.htm) was used to calculate solvent density, solvent viscosity and partial specific volume using the amino-acid composition. The sedimentation data were analysed.
with the program Sedfit [39] using the continuous c(s) and c(M) distributions. The theoretical sedimentation coefficient value calculated from the crystallographic monomer structure without the ATP lid and insertion region (see below) was 3.8 S. AUC experiments showed that the MtbGyrB47 was dimeric as a monomer in the absence or presence of AMP-PCP, irrespective of the presence or absence of the N-terminal His6 tag.

The oligomerization state of MtbGyrB47 and MtbGyrB47 in solution was also assessed by ASEC. MtbGyrB47 mixed with 5 mM MgCl2 plus 1 mM AMP-PCP (Sigma) and applied to a TOSOH SW30000 column in 20 mM Hepes (pH 7.5) and 100 mM Na2SO4 at 0.2 ml/min. Cytochrome c (12.4 kDa), carbonic anhydrase (29 kDa), BSA (66 kDa), alcohol dehydrogenase (150 kDa), &-amylase (200 kDa), apoferritin (443 kDa) and thyroglobulin (669 kDa) from Sigma were used to calibrate the column.

ASEC showed slow time-dependent dimerization on a time scale of days in the presence of AMP-PCP (results not shown), in agreement with the results from AUC. Dimerization was not observed in the presence of novobiocin, consistent with results for the E. coli GyrB ATPase domain [27].

RESULTS

ATPase activity of M. tuberculosis DNA gyrase

ATPase activities of full-length M. tuberculosis GyrB and the ATPase domain (MtbGyrB47, residues 1–427) measured independently in two laboratories with different assays (see the Materials and methods section) gave similar results.

To confirm that purified MtbGyrB47 was active, we analysed its ATPase activity using a sensitive fluorescence assay which measures the production of Pi [28]. As the ATPase activity of the isolated ATPase domain is quite low, 15 μM protein was used. Figure 1 shows an ATP titration for the ATPase domain. An ATP-dependent increase in activity was observed, and activity was Mg2+-dependent and inhibited by novobiocin (results not shown). The kcat was 0.002 s–1. Full-length GyrB is also active, with a kcat of 0.025 s–1 at 15-fold less protein (results not shown), suggesting that the C-terminal end of the protein (missing from the isolated ATPase domain) enhances ATPase activity, possibly by making M. tuberculosis GyrB dimeric. This higher activity of the full-length protein compared with the ATPase domain has also been observed in Saccharomyces cerevisiae and Plasmodium falciparum Topo II [40,41].

The ATPase activity of M. tuberculosis DNA gyrase (GyrA and GyrB subunits at an equimolar concentration) was investigated using a PK/LDH (pyruvate kinase/lactate dehydrogenase)-linked enzyme assay as a function of enzyme concentration at a fixed substrate concentration. The ATPase activity of the M. tuberculosis DNA gyrase shows a linear dependence of the rate of hydrolysis depending on the enzyme concentration (Supplementary Figure S4 at http://www.biochemj.org/bj/456/bj4560263add.htm). Typically, 5 μM of the M. tuberculosis gyrase was found to have an activity of 30 nM/s, and activity was enhanced at least 1.5-fold (from 1.5 to 2.5) in the presence of DNA (Supplementary Figure S4). The ATPase activity of the M. tuberculosis gyrase demonstrated a hyperbolic dependence on substrate concentration with a Km (app) of 0.77 mM and a kcat (app), or enzyme turnover number, of 0.02 s–1 (Supplementary Figure S4). In comparison with the values observed for the E. coli DNA gyrase (Km = 0.83 mM and kcat = 1.2 s–1) [10], the Km (app) is similar, but the kcat (app) was decreased by 60-fold, resulting in a decreased catalytic efficacy for the M. tuberculosis gyrase. The much lower ATPase activity of M. tuberculosis gyrase compared with E. coli gyrase has also recently been reported elsewhere [5].

The structures of the M. tuberculosis ATPase domain in complex with AMP-PCP and AMP-PNP

Structures of the M. tuberculosis ATPase domain with AMP-PCP and AMP-PNP were determined by molecular replacement and refined as described in the Materials and methods section (see Table 1 for details). The structure of MtbGyrB47 with AMP-PCP was, surprisingly, not dimeric (Figures 2A–2C). There was clear density for the AMP-PCP in all six subunits in the asymmetric unit (Figure 2C and Supplementary Figure S5 at http://www.biochemj.org/bj/456/bj4560263add.htm), but the ATP lid region (residues 104–125) was largely disordered, and the GHKL and transducer domains were differently oriented than in the AMP-PNP structure (see the next section for more detail).

The structure of MtbGyrB47 with AMP-PNP was dimeric (Figures 2D and 2E), had an ordered ATP lid region and the dimer was stabilized by the N-terminal arm crossing from one subunit to the other. The overall dimeric structure of MtbGyrB47 with AMP-PNP is similar to previously reported AMP-PNP complexes of ATPase domains (Supplementary Figure S6 at http://www.biochemj.org/bj/456/bj4560263add.htm) from E. coli GyrB [7,8], E. coli Topo IV [15], human Topo II [12] and other type II topoisomerases. Two main-chain NHs were within hydrogen-bonding distance of the bridging nitrogen in the AMP-PNP structure (Figure 2F) showing it to be in the unprotonated (P–N = P) imino form [26]. In the MtbGyrB47–AMP-PNP structures the ordered ATP lid region (residues 104–124) wraps around the γ-phosphate and also makes extensive interactions with the N-terminal arm from the other subunit of the dimer (Figures 2D–2F). Whereas most of the contacts to the AMP-PNP come from the GHKL domain (residues 21–255), Lys972 from the transducer domain (Lys375 in E. coli) contacts the γ-phosphate of ATP, as observed in related structures. In the AMP-PCP structure this switch lysine residue does not contact the γ-phosphate.

Compared with previously determined crystal structures of bacterial type II topoisomerase ATPase domains, the M. tuberculosis GyrB ATPase domain possesses an insert of 32 amino acids between the last two β-strands of the GHKL domain (Supplementary Figure S7 at http://www.biochemj.org/bj/456/bj4560263add.htm). In our MtbGyrB47 structures this insert (residues 214–245) is largely disordered (Figures 2A and 2D). PSI-Blast searches showed that this insert (residues 214–245) is found only in bacterial GyrB ATPase domains of the Gram-positive Corynebacterineae, which include Mycobacteria, Nocardia, Rhodococcus, Gordonia and Corynebacteria [42]. In the MtbGyrB47–AMP-PCP structure there were six subunits in the asymmetric unit, all structurally similar, arranged in two very similar trimers. The insert region (residues 214–245) was close to the three-fold axis of the trimers (Supplementary Figure S8 at http://www.biochemj.org/bj/456/bj4560263add.htm); however, there was not clear electron density for the insert in the 3.3 Å maps, so the insert was not modelled. AUC experiments of MtbGyrB47 with AMP-PNP showed monomers in solution (Supplementary Figure S9 at http://www.biochemj.org/bj/456/bj4560263add.htm), so the trimers seen in the crystal structure are probably not biologically relevant. The two crystal forms solved with AMP-PNP had either four dimers or eight dimers in the asymmetric unit and, in one subunit of each dimer, a short β-strand at the end of the insert region (residues 243–246) mediated a common crystal contact (Supplementary Figure SBC). In one of the 24 AMP-PNP subunits a longer ordered region was stabilized by
ATP hydrolysis by the M. tuberculosis DNA gyrase ATPase domain

Figure 2  Structures of the M. tuberculosis ATPase domain with AMP-PCP and AMP-PNP

(A and B) Two orthogonal views of the monomeric structure of MtbGyrB47 with AMP-PCP. AMP-PCP, red sticks; Mg^{2+}, blue sphere. The ATP lid (residues 104–125, green) is largely disordered. (C) \( F_o - F_c (3 \sigma) \) omit map (mesh) for the AMP-PCP (carbon, magenta; nitrogen, blue; oxygen, red; and phosphate, orange) and Mg^{2+} (small blue sphere). The protein is shown with yellow carbons, except for residues in the ATP lid which have green carbons (residues 104 and 122–125 are included in the model, residues 105–121 are disordered). (D and E) Two orthogonal views of the dimeric structure of MtbGyrB47 with AMP-PNP. AMP-PNP, red sticks; Mg^{2+}, blue sphere. The ATP lid (residues 104–125, green), which is shown as a solid main-chain trace and semi-transparent spheres (green) for all atoms, buries the AMP-PNP and interacts with the N-terminal arm (black) from the other subunit of the dimer. (F) \( F_o - F_c (3 \sigma) \) omit map (mesh) for AMP-PNP and Mg^{2+} (coloured as in (C), except that protein atoms are with brown carbon atoms when not in the ATP lid). The black broken lines indicate that the main-chain NH groups of Leu^{120} and Gly^{122} are 3.09 and 3.12 Å from the bridging nitrogen. (G) Superimposition of the three conformations: RelaxT, observed in the AMP-PCP structure (yellow); ATS, ATP-restrained (AMP-PNP) conformation (orange); RelaxED, relaxed conformation (brown). For clarity, the AMP-PCP-bound structure was also used for the RelaxED structure, superimposing its GHKL and transducer domains on corresponding domains of the T. thermophilus gyrase (RelaxED) structure. The red square and zoom-in view highlight different orientations of the C-terminal helix. (H) Comparison of the binding modes for AMP-PCP and AMP-PNP. Note that the CH2 in AMP-PCP cannot accept hydrogen bonds from the NH groups of Leu^{120} and Gly^{122}.

Comparison of the M. tuberculosis ATPase domain with AMP-PCP and AMP-PNP

The MtbGyrB47 structure with AMP-PCP is the first non-dimeric structure of a DNA gyrase ATPase domain in complex with an ATP analogue. Comparing the AMP-PCP subunit structure to that with AMP-PNP (Figure 2G), the individual GHKL and transducer subdomains are similar (Ca RMSD of 0.3 Å over 152 atoms for GHKL, and Ca RMSD of 0.3 Å over 136 atoms for transducer), but the relative orientation of a unique crystal contact. A sequence search of structures in the PDB revealed that the N-terminal domain of HSP90 from P. falciparum also possesses a similar, but longer, insert (50% sequence similarity over the 32 residues in common), also located inbetween the last two strands of a GHKL domain (PDB code 3PEH) (Supplementary Figure S8B). However, no function was suggested for this insert in P. falciparum HSP90 [43], and although our structural studies have not identified a clear functional role for the M. tuberculosis GyrB insert, crystal packing contacts suggest it may play a role in protein–protein interactions.
6 days (and AT) a new conformational state, which we term RelaxT (Figure 2G). The relaxed conformation of type II topoisomerase ATPase domains is highly conserved and all of the important residues for binding and catalysis are observed in M. tuberculosis gyrase. In the AMP-PNP structures the ATP lid is ordered. Residues at the C-terminus of the ATP lid, GLHGVG (residues 119–124), form a glycine-rich P-loop (phosphate-binding loop), that has main-chain NH groups from His121, Val123 and Gly124, making hydrogen bonds to oxygens on the γ-phosphate of AMP-PNP. Gly122 has its main-chain NH pointing directly at the bridging nitrogen between the β- and γ-phosphates, whereas the main-chain nitrogen of Leu120 is within 3.0 Å of both the bridging nitrogen and one of the oxygens on the γ-phosphate (Figure 4A). The binding mode observed for AMP-PNP in MtbGyrB47 is essentially the same as seen in the 1.8 Å yeast Topo II complex (Figure 4B) and the 1.87 Å human complex (Supplementary Figure S3); we conclude that these structures also bind the imino form of AMP-PNP. A more distantly related Topo VIB structure with AMP-PNP [16] is also in the imino (P=N=P) form [26].

the GHKL and transducer subdomains are different, with a swivel of approximately 17°, moving the distal end of the C-terminal-most helix approximately 15 Å outward (Figure 2G). This movement is different from the one observed for the ATP-binding pocket of type II topoisomerase ATPase domains (Figure 2G). The MtbGyrB47–AMP-PCP structure seems to be in a new conformational state, which we term RelaxT (Figure 2G), intermediate between the previously observed RelaxED (or open) and ATP-restrained (ATS or closed) conformations [13,18]. In the AMP-PCP structure the ATP lid region (residues 104–125) is disordered, as it is in many structures with inhibitors [44] and in some structures with ADP [13]. This suggested that because the CH2 between the β- and γ-phosphates of AMP-PCP cannot accept hydrogen bonds from main-chain NHs of Leu120 and Gly122 (Figures 2C, 2F and 2H), the ATP lid cannot become ordered and close, and therefore the dimer cannot form.

AUC showed that incubation of the M. tuberculosis GyrB ATPase domain with AMP-PNP initially gave monomers (as observed with AMP-PCP and apo protein), but that dimers formed slowly (compared with the 6–10 h run time of an AUC experiment) over a period of several days (Figure 3). This suggested that AMP-PNP was initially largely in the imino (P-N=P) form, and that the presence of the hydrogen on the nitrogen blocked the ATP lid from becoming ordered and closing (as with AMP-PCP). However, the AMP-PNP could convert into the imino (P=N=P) form, and when in the imino (P=N=P) form the ATP lid could close and the dimer form; the percentage of dimer increased with time (Figure 3). Crystals of MtbGyrB47 with AMP-PNP were grown at pH 8.5, in the presence of between 5 and 200 mM MgCl2 [21], conditions expected to stabilize the imino (P=N=P) form. This slow interaction of AMP-PNP has also been observed with E. coli DNA gyrase [45].

Several Topo II crystal structures contain the imino (P=N=P) form of AMP-PNP

The position of the nitrogen between the β- and γ-phosphates in a 2.3 Å E. coli gyrase and a 2.1 Å E. coli Topo IV structure with AMP-PNP are quite similar to each other (Figures 4C and 4D), but this nitrogen is in a different position from other structures (Figure 4). Moreover, in these two structures there is not a Mg2+ adjacent to the β- or γ-phosphates (although the 2.1 Å E. coli Topo IV structure has a nearby Mg2+; Figure 4D). The different position of the bridging nitrogen (and the absence of the adjacent Mg2+ ion) in the E. coli gyrase and Topo IV structures (Figures 4C–4E) suggests that the nitrogen is in the imido form (Supplementary Figure S2). In the 2.1 Å E. coli Topo IV structure (Figure 4D), the closest main-chain NH from the ATP lid is some 3.9 Å away; the bridging nitrogen is too far away to accept hydrogen bonds from the ATP lid. However, the ATP lid is ordered and in the same conformation as observed in the other AMP-PNP structures, moreover these two E. coli structures are dimeric (Supplementary Figure S6). This observation was initially puzzling because the MtbGyrB47 AMP-PCP structure suggested that to initially become ordered and close, the ATP lid needed to make contact with the atom between the β- and γ-phosphates. Only once the ATP lid has closed can the dimer form.

One possibility is that dimers of the two E. coli proteins formed while the AMP-PNP was in the imino form (P=N=P), but at some point during crystal formation the NH group of the adjacent glycine residue protonated the P=N=P group to be P-NH-P. In the scheme shown in Figure 5 (Figures 5A–5C), movement of the P-loop and the AMP-PNP would then allow a hydrogen from the γ-phosphate to reprotonate the glycine residue. The extensive interactions with the N-terminal arm of the other subunit (Figures 2D and 2E) would keep the ATP lid ordered after the AMP-PNP had adopted the imido form.

**DISCUSSION**

A fully dissociative mechanism for ATP hydrolysis

The proposed scheme for the catalytic conversion of the imino (P=N=P) into the imino (P-NH-P) form of AMP-PNP (Figures 5A–5C) suggests a simple dissociative mechanism for ATP hydrolysis by M. tuberculosis GyrB and related ATPases (Figures 5G–5I). In this proposed mechanism an initial movement of Gly122 causes its main-chain NH to protonate the bridging oxygen of the ATP. Stabilization of the resulting negative charge on the protein is proposed to be, in M. tuberculosis gyrase, by transfer to Tyr233.
via His$^{104}$ (Figure 5D). In human Topo II a similar charge-relay network (Figures 5E and 5F) involves His$^{42}$ (equivalent to Ala$^{25}$ in M. tuberculosis), although direct transfer of the charge to Tyr$^{151}$ might also be possible. The protonation of ATP gives rise to an ADP$^3^−$ and a free metaphosphate ion (Figure 5H). The activated water immediately attacks the highly reactive metaphosphate ion (Figure 5H) to give a phosphate ion. The ADP$^3^−$ (Supplementary Figure S2) then reprototases the main-chain nitrogen of Gly$^{125}$ (Figure S1), becoming an ADP$^3^−$ ion in the process. If the activated water is not correctly positioned to attack the metaphosphate ion, once the ADP$^3^−$ ion is formed it would be correctly positioned to re-attack the metaphosphate ion to reform ATP [20].

A scheme for the reverse reaction, the synthesis of ATP by type II topoisomerases [25], is shown in Supplementary Figure S10 (at http://www.biochemj.org/bj/456/bj4560263add.htm). In GHKL domain histidine kinases, the activated water is not present, and the phosphate group is transferred from ATP to a histidine side chain [46]. In two-component signal transduction the phosphate group is then transferred from the histidine side chain to an aspartic acid side chain (Supplementary Figure S10).

The proposed dissociative mechanism seems chemically reasonable for GHKL domain histidine kinases. The previously proposed mechanisms for ATP hydrolysis [13,19] by type II topoisomerases show the reaction being initiated when the activated water (or OH$^−$ ion) makes a nucleophilic attack on the highly negatively charged γ-phosphate (Supplementary Figure S1). This type of mechanism, initiated by a nucleophilic attack on a negatively charged tetrahedral γ-phosphate, has been proposed for many other ATPases and GTPases.

Could other ATPase/GTPase families also have a fully dissociated mechanism?

The glycine-rich P-loop in M. tuberculosis GyrB and related GHKL phosphotransferases does not correspond to the Walker A motif (GxxxxGK[T/S]) found in many ATP- and GTP-binding proteins [47]. However, because of structural similarities in the way the atom corresponding to the bridging oxygen between the β- and γ-phosphates is co-ordinated in transition state complexes of Ras and F$_{1}$-ATPase and M. tuberculosis GyrB–AMP-PNP (Supplementary Figure S11 at http://www.biochemj.org/bj/456/bj4560263add.htm), it is tempting to speculate that Walker A motif-containing proteins may have, by convergent evolution, arrived at a similar mechanism for ATP/GTP hydrolysis. Mainchain NHs from glycine residues point at the bridging oxygen between the β- and γ-phosphates (Supplementary Figure S11) in transition state analogue complexes of the small G-protein Ras [48] (bold Gly$^{13}$ in GagvGKT) and in F$_{1}$-ATPase [49] (bold Gly$^{159}$ in GagvGKT). We propose that movement of this glycine residue past the bridging oxygen between the β- and γ-phosphates forces it to leave its main-chain NH proton behind on the bridging oxygen promoting GTP or ATP hydrolysis. The bridging oxygen between the β- and γ-phosphates of GTP is negatively charged [50]. A fully dissociative mechanism for GTP hydrolysis by Ras (Supplementary Figure S12 at http://www.biochemj.org/bj/456/bj4560263add.htm) can explain why point mutations at Gly$^{12}$, Gly$^{13}$ or Gln$^{61}$ impair intrinsic rates of GTP hydrolysis by Ras, while increasing intrinsic rates of hydrolysis of some GTP analogues [51].

How is ATP hydrolysis coupled to domain motion in M. tuberculosis DNA gyrase?

Analysis of previously available type II topoisomerase ATPase structures (GHKL + transducer domains) supported the existence of ‘ATP-restrained’ and ‘relaxed’ conformational states [13,18]. In the M. tuberculosis GyrB complex with AMP-PCP, a second relaxed conformation (RelaxT) was observed, with the transducer and GHKL domains in different relative positions; we term this conformation ‘RelaxT’ and suggest it may correspond to the monomeric ATP-bound form. In ‘ATP-restrained’ conformations the size of the ‘hole’ between the two transducer domains is often too small to accommodate the T-segment DNA [12,14].

In the present study we compare schemas showing how the catalytic cycle of DNA gyrase could be governed by (i) ATP hydrolysis [24,25] or (ii) protonation of imino AMP-P-N=P to imido AMP-P-NH-P. The catalytic cycle in the presence of ATP is described first (Figure 6A). A closed ATP lid is indicated by a green square, an open ATP lid by a green line. When the ATP lid is open, nucleotide exchange can take place. (i) At the start of the catalytic cycle both GHKL domains are shown occupied by ATP (represented by the letter T), but the two GHKL domains are too far apart to dimerize. (ii) When the T-DNA comes between the transducer domains, they are attracted inwards towards it [6,52]. Once the two GHKL domains are close enough together a closed ATP lid in one subunit will bind the terminal N-arm from the other subunit, closing the ATP-gate dimer interface. (iii) In forming this dimer the first ATPase domain adopts the ATS
conformation (orange) and the ATP lid hydrolyses the first ATP. This first GHKL domain now contains ADP and Pi. (iv) When the ATP lid in the second GHKL domain (which still contains ATP) becomes ordered, the domain tries to adopt the ATP-restrained conformation, but it cannot adopt this ATS conformation until the T-DNA segment has been squeezed out from between the two transducer domains. During this conformational change the P, is released from the first ATPase domain, allowing the first ATPase domain, now containing ADP (D) to move to a relaxed conformation. (v) The second ATPase domain can adopt the ATP-restrained conformation (orange) once the T-segment has passed through the cleaved DNA, helping to close the central G-gate. (vi) After the T-DNA segment has passed through the exit gate, closure of the exit gate may signal for the second ATP to be hydrolysed via interactions between DNA-gate domains and the transducer domain [53]. In the presence of two ADPs (D), the ATP lid is no longer restrained so tightly and the ATP-gate can re-open, returning the enzyme to step (i) to rerun the catalytic cycle.

The proposed schema when the catalytic cycle is regulated by AMP-PNP (Figure 6B) is very similar to that for ATP. Note that the ATP lid can only initially close when the AMP-PNP is in the imino (P-N = P) form (shown by letter N in Figure 6B), but once the dimer is formed and the ATP lid is held in place by extra interactions with the N-arm of the other subunit, the ATP lid will remain closed with the imido (P-NH-P) form (shown by letters NH

Figure 5  Main-chain NH protonation of AMP-P. N = P to AMP-P-NH-P suggests a fully dissociative mechanism for ATP hydrolysis

(A–C) A scheme for enzymatic conversion of imino (P-N = P) into imido (P-NH-P) forms of AMP-PNP. (D) The MbtGyrB47–AMP-PNP structure showing residues that could stabilize the negative charge after Gly122 protonates ATP. (E) Human Topo II structure (PDB code 1ZXM) with AMP-PNP (equivalent view). (F) Superimposition of structures in (D) and (E). (G–I) A dissociative mechanism for ATP hydrolysis by M. tuberculosis DNA gyrase.

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ATP hydrolysis and domain movement in *M. tuberculosis* ATPase gyrase, these studies open up new possibilities for the rational design of covalent inhibitors against DNA gyrase, HSP90 and possibly other challenging, but important, drug targets.

**Conclusions**

The crystal structure of the *M. tuberculosis* DNA gyrase ATPase domain with AMP-PCP was in a novel monomeric form, whereas crystal structures with AMP-PNP were dimeric and similar to many related structures in the literature. By including hydrogens in the refinement and comparing our structures with others from the literature we were able to come up with an explanation for the ability of AMP-PNP to catalyse one round of the reaction cycle. In this explanation the enzyme protonates the imino (P-N = P) form of AMP-PNP to give the more common imido form (P-NH-P). This insight allowed a novel mechanism for ATP hydrolysis to be proposed. In this fully dissociative mechanism for ATP hydrolysis a main-chain NH moves to protonate ATP, causing it to dissociate into ADP and P_i. To the best of our knowledge this is the first time a main-chain amide has been proposed to play a direct role in catalysis. Moreover, this mechanochemical mechanism may have a broad applicability to proteins such as F_1-ATPase and G-proteins such as Ras, as well as proteins involved more directly in movement. As well as providing insights into the coupling of ATP hydrolysis and domain movement in *M. tuberculosis* ATPase gyrase, these studies open up new possibilities for the rational design of covalent inhibitors against DNA gyrase, HSP90 and possibly other challenging, but important, drug targets.

**AUTHOR CONTRIBUTION**

In GlaxoSmithKline, Alka Agrawal carried out cloning, protein expression, purification, crystallization experiments, refinement and activity assays. Claus Spitzfaden performed AUC and ASEC. Benjamin Bax solved structures, completed refinements and devised dissociative mechanism. In Paris, Claudine Mayer conceived and supervised the work. Mélanie Roué carried out cloning, protein expression, purification, activity assays, crystallization experiments, AUC experiments, crystallographic data collection, and refinement with the help of Claudine Mayer. Alka Agrawal, Mélanie Roué, Benjamin Bax and Claudine Mayer wrote the paper with assistance from Stéphanie Petrella, Alexandra Aubry and Michael Hann.

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REFERENCES


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SUPPLEMENTARY ONLINE DATA

*Mycobacterium tuberculosis* DNA gyrase ATPase domain structures suggest a dissociative mechanism that explains how ATP hydrolysis is coupled to domain motion

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Figure S1 Comparison of two possible mechanisms for ATP hydrolysis by *M. tuberculosis* DNA gyrase

(A) A mechanism in which the ATP hydrolysis is initiated when a water (or OH− ion) makes a nucleophilic attack on the phosphate (associative). This scheme is essentially that proposed in [1], an excellent paper dissecting the ATP turnover of a GHL ATPase. (B) A mechanism in which the hydrolysis is initiated when the oxygen between the β- and γ-phosphates is protonated. This causes ATP to dissociate into ADP and a metaphosphate ion (PO3−).

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Co-ordinates and structure factor files for the *M. tuberculosis* GyrB ATPase domain have been deposited in the PDB under the accession codes 3ZKB, 3ZKD and 3ZM7.

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A is adenosine. Adenosine is uncharged between pH 4.8 and 12. The common protonation states of P₃, ADP and ATP are shown [3,4]. In the presence of bound Mg²⁺ ions the more negatively charged forms can be stabilized [5]. Solution NMR studies suggest that AMP-PNP probably has the hydrogen on the bridging nitrogen [6]. Although E. coli alkaline phosphatase can remove the terminal phosphate of AMP-PNP [7], most enzymes do not efficiently hydrolyse AMP-PNP.

**Figure S2** Common protonation states for P₃, ADP and ATP with pK values, and protonation and tautomeric states of AMP-PCP and AMP-PNP
ATP hydrolysis by the *M. tuberculosis* DNA gyrase ATPase domain

Figure S3  The 1.87 Å human Topo IIA structure with AMP-PNP is consistent with a bridging imino (P-N=P), but not a bridging imido (P-NH-P)

(A) The 1.87 Å human Topo IIA structure with AMP-PNP (PDB code 1ZXM). Distances from main-chain nitrogens of Gly164 and Arg162 are indicated by broken lines (estimated error ~0.1 Å based on two molecules in asymmetric unit). (B) The original AMP-PNP was removed, hydrogens were added to the protein and a difference map was calculated (insert 5 σ Fo − Fc). The imino P-N=P form of AMP-PNP was modelled [8] into the Fo − Fc map using PHENIX [9] and displayed in Coot [10]. Distances from hydrogens on main-chain nitrogens of Gly164 and Arg162 are indicated by broken lines. (The hydrogen on the main-chain nitrogen of Asn163 is some 2.9 Å from the bridging nitrogen). (C) Superimposition of (A) and (B).

Figure S4  ATP activity assays on *M. tuberculosis* DNA gyrase

(A) ATPase activity of the *M. tuberculosis* DNA gyrase as a function of protein concentration. Rates are initial velocities. The substrate (ATP) concentration was 1 mM, and the subunits GyrA and GyrB were mixed in equimolar concentrations. (B) ATPase activity of the *M. tuberculosis* DNA gyrase in the presence and absence of DNA (relaxed pBR322 at 5 μg/ml). Rates are initial velocities. The substrate (ATP) concentration was 1 mM, and the subunits GyrA and GyrB were mixed in equimolar concentrations. Increased ATPase activity in the presence of DNA has previously been shown for *E. coli* DNA gyrase, yeast, human and protozoan topoisomerases II, and the *P. falciparum* DNA gyrase [11–17]. (C) ATPase activity of the *M. tuberculosis* DNA gyrase as a function of substrate (ATP) concentration at a constant enzyme concentration. Rates are initial velocities. The subunits GyrA and GyrB both at 5 μM. The Kₘ was calculated by the Lineweaver–Burk plot. Initial rates were correlated with a phosphate calibration curve.
Figure S5 $F_o - F_c (\pm 2.5 \sigma)$ maps showing difference density for the six AMP-PCPs in the asymmetric unit

The final structure is shown with a map phased on the original molecular replacement solution from which the ATP lid, N-arm and, in molB, residues from the transducer domain have been deleted. The map shows the density for all AMP-PCPs, but no density for the ATP lid. For molA the AMP-PNP structure is shown superimposed.
Figure S6  Comparison of structures of type IIA topoisomerases with AMP-PNP

Three orthogonal views of (A) M. tuberculosis GyrB (the present study), (B) 2.3 Å E. coli GyrB (PDB code 1EI1) and (C) 2.1 Å structure E. coli Topo IV (PDB code 1S16). (D) Superimposition of (A–C) (the structures are superimposed using one GHKL domain, indicated by an arrow in the centre of A). (E) The 1.87 Å human Topo IIA (PDB code 1ZX0). Because the superimpositions in this Figure were done on a single GHKL domain (indicated by an arrow in A), this Figure emphasizes differences between the structures.
Figure S7 Structure-corrected sequence alignment of the ATPase domain from type IIA topoisomerases

The sequence names are as follows: M. tuberculosis Gyrb, M. tuberculosis DNA gyrase; M. leprae Gyrb, M. leprae DNA gyrase; 1EI1_E.coli_Gyr, E. coli DNA gyrase (PDB code 1EI1); 1S16_E.coli_TopoIV, E. coli Topo IV (PDB code 1S16); 1PVG_S.cerev_TopoII, S. cerevisiae Topo II (PDB code 1PVG); and 1ZXM_Human_TopoII, human Topo II (PDB code 1ZXM). α-Helices and β-strands are shown above the sequences as red and blue cylinders respectively, and are defined from the E. coli DNA gyrase ATPase domain (PDB code 1EI1) from PDBSum. The ATP lid is indicated by a green arrow. The vertical green bar delimits the end of the GHKL and the beginning of the transducer sub-domains. The insert regions (32 or 34 residues) of M. tuberculosis and M. leprae are indicated in brown. The two constructs used in the present paper both had the M. tuberculosis Gyrb sequence shown in alignment, but they had different N-terminal tags: MtbGyrB47C1 had a 19 residue tag MGHHHHHLVFFAQGSPG and MtbGyrB47C2 had a 15 residue N-terminal tag with MAHHHHHHVDDDDKV with the cleavage site just prior to Val8 (where / represents a cleavage site).
Figure S8  The insert region of the *M. tuberculosis* ATPase domain is involved in crystal contacts

(A) View of the Mtb GyrB47–AMP-PNP dimer showing positions of the largely disordered insert regions (framed in red). (B) Superimposition of the GHKL domain of *P. falciparum* HSP90 (PDB code 3PEH) showing an insert at the same position. (C) Dimer–dimer interactions observed in all MtbGyrB47–AMP–PNP dimers [shown between the AB and EF chains in the P1(8) form]. Residues from the insert are in brown. (D) The insert region is also close to crystal contacts in the MtbGyrB47–AMP–PCP structure. The first residue of β10 and last residue of β11 are represented in brown CPK (Corey–Pauling–Koltun).

Figure S9  AUC of the *M. tuberculosis* GyrB ATPase domain

AUC traces are shown of untagged MtbGyrB47C2 at 17 μM (bottom) and MtbGyrBpC2 in the presence of 2 mM MgCl₂ and 2 mM AMP–PCP (top).
Figure S10  Dissociative reaction schemes for the (A) synthesis of ATP by the GyrB ATPase domain and (B) for the transfer of a phosphate from a histidine to an aspartic acid residue in two-component signal transduction.

Figure S11  Comparison of transition state complexes of Ras and F₁-ATPase with the AMP-PNP structure of *M. tuberculosis* GyrB47

(A) Ras (cyan carbons)/RasGAP (dark green carbons) complexed with ADP and AlF₃ (PDB code 1WQ1). The Mg²⁺ is shown as a small green sphere. Arg⁷₈⁹ from RasGAP accelerates ATP hydrolysis. (B) F₁-ATPase with ADP and BeF₃. The Mg²⁺ is shown as a small green sphere. Two F₁-ATPase subunits are indicated by carbons in different shades of green. (C) MtbGyrB47–AMP-PNP. (D) Superimposition of (A–C).
ATP hydrolysis by the M. tuberculosis DNA gyrase ATPase domain

Figure S12  A simplified dissociative mechanism for the hydrolysis of GTP by Ras

(A) In the presence of wild-type Ras, Gln61 helps to position a water, so that it can attack the metaphosphate ion [PO3]−, once it is formed. Movement of the NH of Gly13 past the bridging oxygen is responsible for the initial protonation, and by the time the glycine has moved again (to a position where it can take back the proton from GDP) the phosphate ion has formed. (B) In the presence of an Ala61 mutant the water is not optimally positioned, so the metaphosphate ion is likely to be re-attacked by GDP before the water can attack.

REFERENCES


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