A Point Mutation Converts *Escherichia coli* FtsZ Septation GTPase to an ATPase*

(Received for publication, June 8, 1994, and in revised form, July 27, 1994)

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The cell division protein FtsZ, essential to initiate septum formation in *Escherichia coli*, is a GTPase. The thermosensitive ftsZ84 mutation, which impairs the ability of FtsZ to bind and hydrolyze GTP in *vitro*, maps to a short glycine-rich FtsZ segment. This region is conserved in eubacterial FtsZ homologs and is strikingly similar to the proposed GTP binding motif in the eukaryotic cytoskeletal protein tubulin. Here we show that in contrast to FtsZ, FtsZ84 protein has a Mg2+-dependent ATPase activity in *vitro*. This activity, unlike the wild-type GTPase, is specifically inhibited by sodium azide, a known antagonist of F-type ATPases and the bacterial SecA protein translocation ATPase (Oliver, D., Cabelli, R. J., Dolan, K. M., and Jarosik, G. P. (1990) *Proc. Natl. Acad. Sci. U. S. A.* 87, 8227–8231). Conversely, aluminum fluoride abolishes FtsZ GTPase activity but only partially affects FtsZ84 ATPase. Affinity-purified anti-FtsZ antibody blocks FtsZ84 ATPase activity, indicating that this enzymatic function is intrinsic to the mutant protein. This is, to our knowledge, the first example of a missense mutation that converts a GTPase to an ATPase.

Cytokinesis in most eubacteria is characterized by the formation of a septum at the cell division site leading to the formation of daughter cells. An early event in the establishment of the division plane in *Escherichia coli* is the localization of the essential FtsZ protein as a cytokinetic ring to the equator of the cell (1). FtsZ has recently been shown to be a GTP/GDP-binding protein with GTPase activity (2–4). A short glycine-rich segment in FtsZ was implicated in GTP binding on the basis of mutations that impaired both the biological and the biochemical functions of the protein (2–4). This segment is conserved in eubacterial FtsZ proteins (5–9) and is strikingly similar to the proposed GTP binding motif in α-, β-, and γ-tubulins of eukaryotic cells (2–4, 10, 11).

The temperature-sensitive ftsZ84 mutation causes *E. coli* cells to grow as long, nonseptate filaments at the restrictive temperature, with no visible constriction at the septal site (12). This mutation converts a glycine at residue 105 to serine (13) within the tubulin signature sequence of FtsZ (2–4), and the mutant FtsZ84 protein is impaired in GTP binding and GTPase activity in *vitro* (2, 3). Here we describe our serendipitous finding that, unlike wild-type FtsZ, FtsZ84 protein has a significant Mg2+-dependent ATPase activity in *vitro*. Such altered nucleotide specificity in a GTPase is without precedent. We also find that FtsZ proteins are related to the ATP-binding proteins such as adenylate kinase, myosins, and F-ATPases, as well as to members of the tubulin superfamily, in containing conserved sequence motifs for binding Mg2+ and phosphate.

**EXPERIMENTAL PROCEDURES**

**ATP and GTP Assays—**FtsZ and FtsZ84 proteins were overproduced and purified as described previously (2). To measure ATPase or GTPase activity, 5 μM proteins (assuming M, ~43,000 for FtsZ) were preincubated with or without nucleotide competitors or inhibitors for 5 min at 30 °C in 10 μl of buffer A (50 mM Tris-HCl, pH 7.5, 50 mM KCl, 5% glycerol) containing 2.5 mM MgCl2. The reaction was initiated by the addition of 1 μM [α-32P]ATP or GTP (0.2 μCi/μmol, ICN Radiomicroch. At the times indicated, 2-μl aliquots were analyzed as described (2) by TLC on polyethyleneimine cellulose (Merek) in 0.45 M KH2PO4 (pH 3.4) for ATPase or 0.75 M KH2PO4 (pH 3.4) for GTPase activities. The amount of ATP/GTP converted to ADP/GDP was determined by counting radioactivity in the nucleotide spots. For nucleotide competition assays (see Fig. 1, c and d), the reactions contained 0.5 μM [α-32P]ATP (0.2 μCi/μmol) and a 6-fold molar excess of unlabelled ribonucleoside triphosphate competitors. Aluminum fluoride (AlF3) exerts its effect by forming a complex with nucleoside diphosphate at the catalytic sites of GTPases and ATPases (14, 15). In our procedure, FtsZ copurifies with noncovalently bound GDP, whereas the FtsZ84 preparation is devoid of any associated nucleotide (2). Accordingly, AlF3 inhibition of FtsZ GTPase is independent of exogenous GDP addition, whereas 100 μM ADP was included with AlF3 in FtsZ84-containing reactions (see Fig. 2).

**ATP Binding Assay—**ATP binding reactions (30 °C, 75 min) were carried out in duplicate with 1 μM protein in 20 μl of buffer A containing 0.5 mM MgCl2 and various concentrations of [α-32P]ATP (0.62 μCi/μmol). The samples were filtered, washed, and counted as described (2).

**Affinity Purification of Anti-FtsZ Antibody—**Polyclonal antibodies were raised in a rabbit by injecting SDS-polyacrylamide gel-purified (16) FtsZ protein with Freund’s adjuvant, and the IgG fraction from the rabbit serum was obtained by a 30–50% ammonium sulfate precipitation. The IgG fraction was dialyzed against 20 mM Tris-HCl, pH 8.0, 20 mM NaCl and then passed through an FtsZ-Aff-Gel 10 (Bio-Rad) affinity column (4 mg of protein coupled to 1 ml of Affi-Gel, according to the manufacturer’s instructions). The column was washed with 25 mM Tris-HCl, pH 7.5, 300 mM NaCl, and the antibodies were eluted with 0.1 M glycine HCl, pH 2.8, directly into 1 volume of 1 M Tris-HCl, pH 8.0, to neutralize the pH. Purified antibodies were dialyzed and stored in phosphate-buffered saline.

**Partial Trypsin Digestion—**FtsZ (20 μg of protein) and FtsZ84 (30 μg) were digested at 25 °C with trypsin (0.05 μg) in 20 μl of buffer containing 50 mM Tris-HCl, pH 7.5, 5 mM MgCl2, 50 mM KCl, 0.1 M EDTA, 0.5 mM dithiothreitol, and 5% glycerol. The proteins were incubated with nucleotides or inhibitors for 2 min at 25 °C before the addition of trypsin. Aliquots were withdrawn after 4 and 8 min of trypsin treatment into an equal volume of 2 x sample buffer (17), boiled immediately for 3 min, and analyzed on 15% SDS-polyacrylamide gels.

**RESULTS AND DISCUSSION**

We found to our surprise that, unlike FtsZ GTPase, the mutant FtsZ84 protein exhibited ATPase activity *in vitro* (Fig. 1a). Interestingly, FtsZ84 showed a temperature-dependent increase in ATPase activity (~2-fold higher initial specific activity at 43 °C compared with that at 30 °C; Fig. 2b, controls), whereas its GTPase activity is impaired at all temperatures (2, 3). As was observed with FtsZ GTPase (2), FtsZ84-catalyzed ATP hydrolysis occurred with no detectable lag (Figs. 1 and 2) and required the presence of Mg2+ ions (Fig. 1a). Affinity-puri-
The affinity of FtsZ84 for ATP binding was slow compared to that for GTP binding to FtsZ (Fig. 1c). This is consistent with the idea that the mutant protein contains a substoichiometric amount of GTP inherent in the mutant protein. The affinity of FtsZ84 for ATP in the protein preparation was measured by a filter retention assay. The amount of FtsZ84 bound radioactivity on the filters was drastically diminished (Fig. 1c). The TLC profile of competitive inhibition of FtsZ84 ATPase activity with ribonucleoside triphosphates after 10 min of assay (Fig. 1c) shows that the nucleotide associated with FtsZ84 on filters was predominantly ADP due to ATP hydrolysis during incubation.

We found that [γ-32P]ATP binding to FtsZ84 was inhibited ~60% in 10 min by 1 mM azide at 30 °C, and the inhibition was ~80% within the first 10 min at 43 °C. 1 mM vanadate caused ~30% inhibition throughout a 30-min period (Fig. 2, a and b), and 5 mM vanadate did not significantly enhance the inhibition of FtsZ84 ATPase (not shown). In contrast, FtsZ GTPase activity was largely unaffected by azide or vanadate at 1 mM (Fig. 2, c and d) or at 3 mM or 5 mM, respectively (not shown). KNO₃ had no detectable effect either on FtsZ84 ATPase (Fig. 2, a and b) or on FtsZ GTPase (not shown). These results demonstrate that sodium azide is a specific inhibitor of FtsZ84 ATPase but does not antagonize FtsZ GTPase to any significant extent. This is an unique example of an inhibitor with singular selectivity toward the mutant form of an enzyme.

It has been proposed that the azide sensitivity of F-ATPases is due to inhibition of the catalytic cooperativity of the enzyme by ATP binding (18). We found that 1 mM azide inhibits [α-32P]ATP binding to FtsZ84 (60 min, 30 °C) ~70% at 50 μM ATP, whereas the inhibition of binding is ~40% at 500 μM ATP (data not shown). The diminished ATP binding in the presence of azide can partially explain the azide sensitivity of FtsZ84 ATPase. It is also possible, as suggested for FtsZ GTPase (3), that FtsZ84 self-associates into multimeric forms that hydrolyze ATP cooperatively, and such multisite activation of FtsZ84 ATPase is inhibited by azide.

The abbreviations used are: AMP-PNP, adenosine 5′-[(β,γ-imido)triphosphate]; EF-Tu, elongation factor Tu.

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FIG. 2. Effects of ATPase inhibitors and AlF₄⁻ on FtsZ84 ATPase and FtsZ GTPase activities. a, TLC profile of inhibition of FtsZ84 ATPase activity at 30 °C after 10 min of assay. FtsZ84 was preincubated with or without inhibitors for 5 min at 30 °C, except in lanes 3 and 7 where the protein was preincubated for 30 min. FtsZ84 controls showed similar extents of ATP hydrolysis after 5 or 30 min of preincubation at 30 °C. Where indicated, concentrations were 1 mM sodium azide, 1 mM sodium orthovanadate, 1.25 mM KNO₃. Lane 7 contained AlF₄⁻ (100 μM AlCl₃ and 10 mM NaF) and 100 μM ADP. b, time course for FtsZ4-catalyzed ATP hydrolysis as described in a, in the absence (open circle) or in the presence of inhibitors: azide (closed circle), vanadate (open square), KNO₃ (closed circle), AlF₄⁻, and ADP (open triangle). Solid lines represent assays carried out at 30 °C, and the broken lines indicate assays performed at 43 °C. c, TLC profile of inhibition of FtsZ GTPase at 30 °C after 10 min of assay. FtsZ84 was preincubated at 30 °C for 5 min with or without inhibitors that were present at concentrations indicated in a, except that ATP was not included with AlF₄⁻. d, time course for FtsZ GTPase assayed as in c without inhibitor (open circle) or with inhibitors: azide (closed circle), vanadate (open square), AlF₄⁻ (closed square). The values shown in b and d were corrected for spontaneous nucleotide hydrolysis and are averages of three separate determinations.

We next investigated if AlF₄⁻ ions affect FtsZ84 ATPase and FtsZ GTPase activities differently. AlF₄⁻ has been postulated to act as a high affinity analog of the γ-phosphate of GTP by forming a fluorocomplex with GDP at the active site of G proteins (14). It also binds to the GDP-tubulin subunit of microtubules (20) but does not interact with p21 Ras (21), ADP-ribosylation factor (22), or the elongation factor Tu (EF-Tu) (23). Moreover, F-ATPases are inhibited by AlF₄⁻, which forms a ribosylation factor (22), or the elongation factor Tu (EF-Tu) complex with ADP at the catalytic sites (15). As shown in Fig. 2, FtsZ GTPase was remarkably sensitive to AlF₄⁻ (100% inhibition within 10 min; panels c and d), whereas FtsZ84 ATPase activity was inhibited ~25% after 20 min (panels a and b).

Taken together, these results indicate that the ftsZ84 mutation profoundly alters FtsZ conformation to allow differential selectivity for nucleotides and differential sensitivity to azide and AlF₄⁻. We performed partial tryptic proteolysis of FtsZ and FtsZ84 to assess the difference in their conformations. As shown in Fig. 3, FtsZ4 (Mₙ ~43,000) in the GDP-bound state (2) yielded two major tryptic fragments (Mₙ ~40,500 and ~38,000), while in the presence of GTP the proteolysis was markedly slower. ATP protected FtsZ over a shorter period of time. Azide or AlF₄⁻ did not affect the rate or pattern of FtsZ cleavage. In contrast, trypsin digested FtsZ84 (nucleotide-free) (2) at multiple sites in the initial stages of proteolysis, and the presence of GTP, ATP, azide, or AlF₄⁻ had no detectable effect (Fig. 3). We interpret the significantly higher trypsin sensitivity of FtsZ84 as indicative of an open conformation compared with FtsZ. The indifference of FtsZ84 to the presence of GTP/ATP during proteolysis is consistent with its low affinity interaction with nucleotides (2) (Fig. 1b). These results with FtsZ84 are reminiscent of the trypsin digestion properties of the DnaK756 (17) and Gₛₙ-₁ί₅ (24) mutant proteins.

The ftsZ84 mutation (Gly-105 to Ser substitution) (13) resides in a 7-amino-acid glycine-rich segment (residues 105–111 in E. coli FtsZ; GGGTGTG; single-letter code) (5) that is nearly identical to the proposed phosphate-binding loop (P-loop) sequence (G/A)GGTGSG conserved in α-, β-, and γ-tubulins of eukaryotic cells (2–4, 10, 11) (Fig. 4a). This tubulin signature sequence differs from the P-loop motif GXXXGK(T/S) (X being any amino acid residue) present in a large number of nucleotide-binding proteins (25) (Fig. 4a). Numerous mutations in the P-loops of diverse proteins have been reported, but none has been shown to alter the nucleotide specificity (25). The ftsZ84 mutation in the presumed phosphate binding sequence of FtsZ is unique in this respect. The substitution of Asp-138 by Asn in the guanine ring binding NKKD motif of EF-Tu is the only known mutation in a GTPase that changes the base specificity of EF-Tu from GTP/GDP to xanthosine (tri/di)phosphates (26). It is noteworthy that the Gly-107 to Ser mutation in bacterial adenylate cyclase results in a significant level of guanylate cyclase activity (27), and a change of Ala-334 to Thr or Ser in mammalian cAMP-dependent protein kinase creates a high affinity cGMP-binding site (28).

Recently, mutations in a second conserved but reversed P-loop motif (100KGHYTEG₁₀⁵) in β-tubulin have been shown to affect polymerization-dependent GTP hydrolysis (29). Coincidentally, FtsZ proteins from E. coli (5), Bacillus subtilis (6), and Wolbachia (9) also contain a conserved P-loop-like sequence (KR/GLGAGA) with similar orientation and spacing as the reversed motif in tubulin, ~40 residues N-terminal to the (G/A)GGTG/S/TG signature sequence. The terminal residues in
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The ATP-binding proteins (31, 32) (Fig. 4b). In particular, E. coli represented by to find that FtsZ proteins contain a conserved sequence that is  human P-tubulin and Lys residues at two or more sites are  protein kinase (34), have a similar Me-binding sequence in  8), F,-ATPase and GTP binding, requires the presence of Me ions (2-41, the  FtsZ sequences are from E. coli (Euc.) (5, 13), R. meliloti (Rm.) (7, 8), B. subtilis (Bs.) (6), and Wobachia (9), a. conserved Gly, Ser, Thr, and Lys residues at two or more sites are boxed. Identical sequences to that shown for E. coli FtsZ P-loop exist in all known FtsZ homologs, b. conserved residues that conform to the Walker B motif (31, 32) are boxed. The pentapeptide segment of (hydrophobic, aliphatic)-X-aromatic-X-acidic residues is indicated as the Mg²⁺-binding loop (32, 33). Gaps, represented by dashes, were introduced into the sequences to optimize the alignment. Numbers within parentheses indicate the residue positions from the polypeptide amino terminus.

In the context of FtsZ4 ATPase activity, it was remarkable to find that FtsZ proteins contain a conserved sequence that is homologous to the Walker B motif present in a large number of ATP-binding proteins (31, 32) (Fig. 4b). In particular, E. coli FtsZ residues 208-212 (VLDfad) conform to the motif ("hydrophobic, aliphatic").X-aromatic-X-acidic" (part of Walker B sequence), which has been implicated as the Mg²⁺-binding site consensus sequence in adenylate kinase, ATPases, and myosins (31-33). Protein kinases, as exemplified by cAMP-dependent protein kinase (34), have a similar Mg²⁺-binding sequence in subdomain VII, with the third position being occupied by an aliphatic, hydrophobic residue (35). Significantly, the ftsZ mutation in E. coli converts Asp-212 to a Gly residue (13), and the FtsZ2 protein is proficient in GTP binding but fails to hydrolyze GTP (30). Because FtsZ-mediated GTP hydrolysis, in contrast to GTP binding, requires the presence of Mg²⁺ ions (2-4), the properties of the FtsZ2 protein would be consistent with our proposal that the β-amino-acid segment (VLDfad) in FtsZ proteins is likely to form the Mg²⁺-binding site (Fig. 4b), with the terminal aspartic residue (Asp-212 in E. coli) being involved in Mg²⁺ coordination. Strikingly, the Asp-224 to Asn mutation in β-tubulin impairs Mg²⁺-dependent GTP binding, and this residue is part of a conserved sequence in β-tubulin that also resembles the Mg²⁺-binding consensus site in ATP-binding proteins (33) (Fig. 4b). Because both tubulins and bacterial FtsZ proteins contain sequences for binding Mg²⁺ and phosphate identified in diverse ATP-binding proteins (Fig. 4), they form a distinct class of GTP-binding proteins that are closely related to the ATP-binding proteins, notably adenylate kinase, myosin, and F-ATPases.

Understanding the basis of altered nucleotide specificity must await the solution of the three-dimensional structures of FtsZ and FtsZ4. Because the ftsZ4 mutation occurs in the signature sequence (G/AAGGTG/C/T) shared by tubulins and FtsZ proteins (2-4, 10), it would be interesting to study the mitotic and biochemical consequences of a similar mutation in tubulins. Such a mutation in tubulins has not been reported (33, 36, 37). Finally, because AlF₃-GDP complex at the catalytic centers of GTPases mimics the GTP-bound state (14), the AlF₃ sensitivity of FtsZ GTPase provides an excellent biochemical tool for in vitro studies with FtsZ in its active GTP-bound conformation.

Acknowledgments—We thank P. O. Ljungdahl for suggesting the use of ATPase inhibitors, B. R. Oakley, L. J. Rasmussen, and A. Wittinghofer for communicating references, H. R. Bourne and L. J. Rasmussen for discussions, and J. M. Coffin, L. Feig, M. Schaechter, and A. Wright for comments on the manuscript.

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