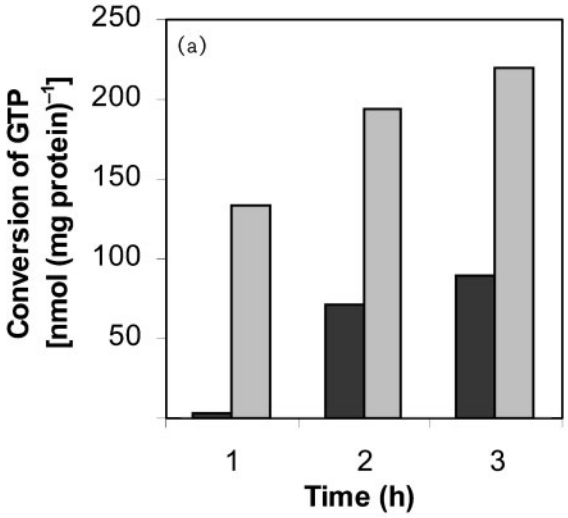
The C-terminally truncated MtFtsZ-ΔC169 mutant of *Mycobacterium tuberculosis* FtsZ shows GTPase and GTP-induced, GTP-specific polymerization activities *in vitro*

We recently reported that the deletion mutant MtFtsZ-ΔC169 of *Mycobacterium tuberculosis* FtsZ (MtFtsZ), which lacked 169 C-terminal residues and retained only the first 210 residues, formed long polymers in about 30 s after the addition of GTP, like the FtsZ of *Escherichia coli* (EcFtsZ) (Anand *et al.*, 2004). Both of these FtsZs were purified under denaturing conditions and refolded. By contrast, the full-length MtFtsZ, prepared under identical conditions of denaturation and refolding, formed equivalent-type long polymers in 10 min after the addition of GTP. These observations prompted us to suggest that the residues in the C-terminal region of the MtFtsZ protein might be responsible for imposing slow polymerization on MtFtsZ (Anand *et al.*, 2004).

Modelling the probable structure of the MtFtsZ-ΔC169 mutant protein (Borhani & White, 2004) based on the crystal structure data of the full-length MtFtsZ protein (Leung *et al.*, 2004), and showing exposure of a large hydrophobic patch, which normally remains buried in the native full-length protein, Borhani & White (2004) raise the following contentions against our observations. (i) Polymerization of the C-terminally truncated MtFtsZ-ΔC169 mutant is unlikely to be physiologically relevant since the molecule has lost the conserved C-terminal domain and the non-conserved C-terminal tail that is believed to be interacting with other cell-division proteins; (ii) the polymerization elicited by the mutant could be aggregation and not true protofilament formation owing to the exposure of the large hydrophobic patch; and (iii) GTPase activity of the mutant should have been determined as the critical T7 loop, which is required for the activity, would be of uncertain conformation in the mutant owing to the loss of helix H11 that links the stabilizing C-terminal domain.

Disproving these contentions, with qualitative and quantitative biochemical evidence, we demonstrate here that the MtFtsZ-ΔC169 mutant does hydrolyse GTP, and elicits Mg^{2+}-dependent, GTP-induced, GTP-specific polymerization *in vitro*. Examination of the MtFtsZ-ΔC169 mutant for GTP hydrolysis revealed that it hydrolysed GTP at the rate of 97·2 nmol GTP h^{-1} (mg protein)$^{-1}$ (Fig. 1). By contrast, the negative control sample – namely, the deletion mutant MtFtsZ-ZN, which lacked the C-terminal 211 residues and therefore the T7 loop critical for GTPase activity – did not show GTPase activity (Fig. 1). Recombinant full-length MtFtsZ and EcFtsZ, which were also purified under denaturing and refolding conditions that are identical to those used for the purification of the mutant, were used as the positive control samples. Full-length MtFtsZ elicited GTP hydrolysis at the rate of 35·8 nmol GTP h^{-1} (mg protein)$^{-1}$ (Fig. 1), while EcFtsZ



(b)

GTP converted [nmol (mg protein)$^{-1}$]

Protein	1 h	2 h	3 h
MtFtsZ-FL	3·45	71·66	89·05
MtFtsZ-ΔC169	133·33	194·48	219·27
MtFtsZ-ZN	NA	NA	NA

Fig. 1. GTPase activities of full-length MtFtsZ (black bars) and MtFtsZ-ΔC169 deletion mutant (grey bars). GTPase activity was assayed essentially as described by RayChaudhuri & Park (1992) with minor modifications. Ten micromolar full-length MtFtsZ and 3·9 μM MtFtsZ-ΔC169 were used for the assay.

showed 1 μmol GTP h^{-1} (mg protein)$^{-1}$ (data not shown).

Furthermore, we examined Mg^{2+}-dependent, GTP-induced, GTP-specific polymerization of the MtFtsZ-ΔC169 mutant protein using a 90° light scattering assay (Mukherjee & Lutkenhaus, 1999). The mutant protein elicited GTP-induced, GTP-specific polymerization (Fig. 2a). Polymerization was not observed either in the absence of GTP or in the presence of another nucleotide such as ATP. The MtFtsZ-ΔC169 mutant did not polymerize in the absence of Mg^{2+} ions either, a characteristic feature reported for the full-length MtFtsZ protein (White *et al.*, 2000). The positive control sample – namely, denatured–refolded full-length MtFtsZ – also showed

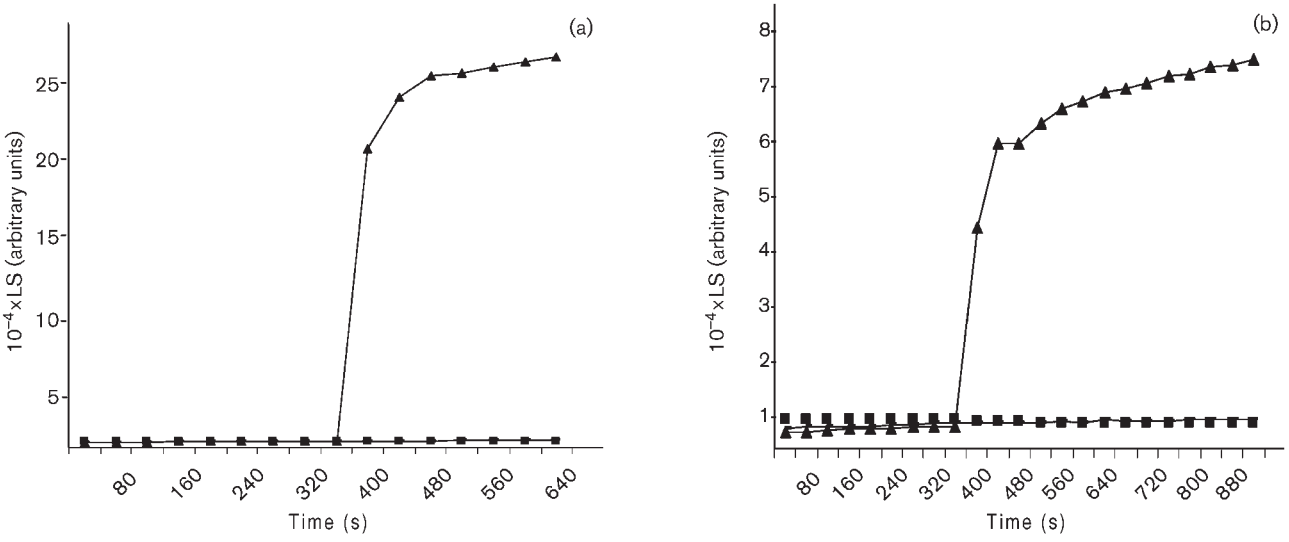


Fig. 2. Light-scattering (90°) assay to monitor polymerization of denatured–refolded preparations of MtFtsZ-ΔC169 (a) and full-length MtFtsZ (b) (both 6 μM) under identical conditions. The proteins were incubated in 1 × CGH buffer (1 mM citrate, 1 mM glycine, 1 mM HEPES, pH 6·5), 5 mM $MgCl_2$ and 50 mM KCl in a 150 μl fluorimeter cuvette at 30°C. The 90° light-scattering (LS) values were initially monitored for 320 s in the absence of GTP to obtain a baseline. Subsequently, 1 mM GTP was added and monitoring continued. The time-course of the reaction in the presence of GTP is indicated by ▲–▲ in both plots. Control reactions were carried out by adding an equal volume of buffer in place of GTP (■) or by adding ATP to a final concentration of 1 mM (---). Light-scattering values were measured every 40 s as described by White *et al.* (2000).

Mg^{2+}-dependent, GTP-induced polymerization (Fig. 2b). The biochemical activities of the MtFtsZ-ΔC169 mutant and of full-length MtFtsZ and EcFtsZ proteins indicate that the proteins prepared under denatured–refolded conditions are active. Recently, a denatured–refolded preparation of EcFtsZ protein was also found to possess biochemical activity (Santra & Panda, 2003).

Thus, the GTPase activity and the Mg^{2+}-dependent, GTP-induced, GTP-specific polymerization of the MtFtsZ-ΔC169 mutant clearly demonstrate that the polymerization of the mutant reported by us (Anand *et al.*, 2004) was not a protein aggregation-induced artefact. These results also point out that the truncation in the MtFtsZ-ΔC169 mutant did not cause loss of either polymerization and GTPase properties per se or any of the features of polymerization property in particular, such as Mg^{2+}-dependence and GTP-specificity, of the full-length MtFtsZ protein. Since loss of functional properties of FtsZ has been found to precede any secondary or tertiary structural changes in the molecule (Santra & Panda, 2003), the full complement of biochemical activities elicited by the MtFtsZ-ΔC169 mutant protein clearly implies that the mutant molecule in general, and the critical T7 loop of the mutant in particular, had indeed maintained a conformation that was conducive for polymerization and GTPase activities.

It may also be mentioned here that the MtFtsZ-ΔC169 mutant was originally obtained as a spontaneous deletion mutant when the full-length Mt*ftsZ* clone in vector pET-$20b^{+}$ was accidentally maintained in the *recA*-positive *Escherichia coli* BL21(DE3) C41 strain (Miroux & Walker, 1996). As a case of serendipity and to our pleasant surprise, this mutant gave clues on fast polymerization and GTPase activities *in vitro* (Anand, 2001). In order to authenticate the spontaneous mutant, we recreated the clone using recombinant DNA methods in order to study biochemical properties of the recombinant mutant protein *in vitro*; part of this study was reported in our previous article (Anand *et al.*, 2004).

Since our sole interest was to study fast polymerization of the mutant *in vitro*, we were not concerned about the physiological relevance of the mutant. For the same reason, we did not create an equivalent deletion mutant of EcFtsZ, a control sample suggested by Borhani & White (2004), as full-length EcFtsZ in any case polymerizes rapidly and there was no point in creating a faster-polymerizing mutant EcFtsZ protein! Nevertheless, from our studies, it is interesting to note that the first 210 residues of MtFtsZ, forming a 'Mini FtsZ' molecule, are sufficient to confer GTPase and Mg^{2+}-dependent, GTP-induced and GTP-specific polymerization activities on the molecule *in vitro*.

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