Unusual structural characteristics of the *Mycobacterium tuberculosis* pentapeptide repeat protein MfpA

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Abstract. The solution structure and refolding of the *Mycobacterium tuberculosis* pentapeptide repeat protein MfpA was explored by fluorescence and circular dichroism spectroscopy. Our results show that MfpA exists in two stable structural forms which exclusively favor dimer or oligomer formation. The structural malleability of MfpA may provide a novel target for drug discovery.

Keywords: Protein structure, protein stability, drug resistance

1. Introduction

Mycobacterium tuberculosis MfpA is a member of the Pentapeptide Repeat Protein (PRP) family of proteins that confers resistance to the antibiotic fluoroquinolone. MfpA binds to DNA gyrase and inhibits its activity. Its three-dimensional structure reveals the PRP fold, a right-handed quadrilateral β helix, that mimics the size shape and electrostatic surface of B-form DNA [2]. This ‘DNA mimicry’ rationalizes inhibition of DNA gyrase by MfpA and fluoroquinolone resistance. MfpA is a dimer both in solution and in the crystal; the C-terminal α helices of MfpA form the dimer interface. The MfpA monomer is almost entirely composed of a right-handed β helix and displays a unique circular dichroism (CD) spectrum [3]. Each monomer contains three tryptophan residues that are the sole source of MfpA intrinsic fluorescence. Our studies of the structure and stability of MfpA in solution by fluorescence and CD spectroscopy suggest that its affinity for DNA gyrase depends on the flexibility of the dimer that is revealed by thermal unfolding.

2. Materials and methods

Protein preparation and instrumentation are described elsewhere [3]. The Lumry–Eyring model was applied to *study MfpA unfolding* [4]:

$$f_D = 1 - \exp \left[-\frac{1}{\nu} \int_{T_0}^T \exp \left(\frac{E_a}{R} \left(\frac{1}{T_k} - \frac{1}{T} \right) \right) dT \right], \quad (1)$$

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where f_D is the mole fraction of unfolded state, $\nu = dT/dt$ is the scanning rate, T is the absolute temperature, T_0 is the initial temperature, E_a is the activation energy, R is a gas constant and T_k is the temperature at which the rate of unfolding, $k = 1 \text{ (min}^{-1}\text{)}$.

3. Results and discussion

The secondary and tertiary structures changes observed for MfpA upon temperature unfolding occur coincidentally (Fig. 1). Numerical fitting of the CD and fluorescence data separately or globally yields comparable values of the thermodynamic parameters. The structures formed upon temperature induced denaturation are stable; cooling to physiological temperature following denaturation does not restore the initial state. Global fitting of the spectral titrations against Eq. (1) yields the thermodynamic parameters $E_a = 56.8 \pm 4.5 \text{ kcal/mole}$, $T_k = 335.7 \pm 0.8 \text{ K}$ and transition midpoint $T_m = 328.7 \pm 0.5 \text{ K}$. A ten-fold increase in light scattering is coincident with the CD and fluorescence transitions (data not shown).

The temperature dependent structural changes and aggregation observed for MfpA are irreversible. Cooling the protein from 90 to 10°C results in little CD and fluorescence spectral change; the aggregates formed at high temperature are stable. Thus, secondary, tertiary and quaternary structural changes of MfpA occur coincidentally during temperature dependent denaturation with the new MfpA structure formed at high temperatures favoring aggregation. Transition between MfpA conformers may be initiated from locally unfolded states that become accessible by thermal fluctuations occurring under physiological conditions [1]. Since MfpA mimics a distorted rather than canonical B-form DNA [2], this structural malleability may influence MfpA binding to DNA gyrase in solution and thus influence the protein's ability to confer antibiotic resistance.

Fig. 1. Temperature induced unfolding of MfpA (2 μM) in 10 mM potassium phosphate, 25 mM KCl at pH 7.6; native (f_n) and denatured (f_d) fraction of the protein are connected through relation $f_n + f_d = 1$ accordingly to two-step transition. The squares denote ellipticity at 205 nm. The circles denote fluorescence intensity at 330 nm following excitation 280 nm. Simulated curves denoted the best-fits of the transitions to Eq. (1) are shown as a dashed line for CD alone, dash-dot line for fluorescence alone and solid line for global analysis of both.

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