Crowding, molecular volume and plasticity: An assessment involving crystallography, NMR and simulations

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The discrepancy between the X-ray and NMR structures of *Mycobacterium tuberculosis* peptidyl-tRNA hydrolase in relation to the functionally important plasticity of the molecule led to molecular dynamics simulations. The X-ray and the NMR studies along with the simulations indicated an inverse correlation between crowding and molecular volume. A detailed comparison of proteins for which X-ray and the NMR structures appears to confirm this correlation. In consonance with the reported results of the investigations in cellular compartments and aqueous solution, the comparison indicates that the crowding results in compaction of the molecule as well as change in its shape, which could specifically involve regions of the molecule important in function. Crowding could thus influence the action of proteins through modulation of the functionally important plasticity of the molecule.

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1. Introduction

The effect of macromolecular crowding on the shape, folding and action of proteins has received considerable attention in recent years (Ellis and Minton 2003; Zhou *et al*. 2008; Gershenson and Gierasch 2011; Pernilla 2011). This issue is of substantial biological significance as close to half the volume of a typical cell is occupied by biomolecules, and therefore macromolecules like proteins function in a crowded environment in biological systems (Ellis and Minton 2003). This situation is not factored into most of the solution and computational studies on the structure and function of proteins, although there have been a couple of attempts to do so (Ai *et al*. 2006; Roque *et al*. 2007; Homouz *et al*. 2009). Perhaps the only extensive studies which are carried out in an overcrowded environment involve those pursued in crystals where typically the protein molecules occupy 50% of the volume. Admittedly, in crystals a protein molecule is usually surrounded by molecules of the same kind while the environment is much more heterogeneous inside the cell. Furthermore, the mobility of molecules in crystals is much more limited than in the cell. However, the level of overcrowding is comparable in the two cases. It has also been demonstrated that crystal contacts are different in nature from interactions at interfaces involved in specific assemblies Bahadur *et al.* (2003)). Therefore, the situation in protein crystals provides a handle, imperfect though it might be, for approaching the problem. The differences in protein structures derived using crystallography and NMR are particularly interesting in this context. Such differences observed in eubacterial peptidyl-tRNA hydrolase are the genesis of the work reported here.

Peptidyl-tRNA hydrolase (Pth), which catalyses the hydrolysis of stalled peptidyl-tRNA during protein synthesis, is an essential enzyme in eubacteria. Premature stalling of translation, caused by a variety of events, leads to the dropping off of peptidyl-tRNA from the ribosome. Accumulation of peptidyl-tRNA is toxic to the cell. Pth cleaves the ester bond between tRNA and the peptide, thus preventing this toxicity and also releasing tRNA for further use. The enzyme from *Mycobacterium tuberculosis* (*Mt*Pth), is a 191-residue monomeric protein. We deter-

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