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Comparative folding analyses of unknotted versus trefoil-knotted ornithine transcarbamylases suggest stabilizing effects of protein knots

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ABSTRACT

Ornithine transcarbamylases (OTCs) are conserved enzymes involved in arginine biosynthesis in microbes and the urea cycle in mammals. Recent bioinformatics analyses identified two unique OTC variants, N-succinyl-L-ornithine transcarbamylase from *Bacteroides fragilis* (*Bf*SOTC) and N-acetyl-L-ornithine transcarbamylase from *Xanthomonas campestris* (*Xc*AOTC). These two variants diverged from other OTCs during evolution despite sharing the common tertiary and quaternary structures, with the exception that the substrate recognition motifs are topologically knotted. The OTC family therefore offers a unique opportunity for investigating the importance of protein knots in biological functions and folding stabilities. Using hydrogen-deuterium exchange-coupled mass spectrometry, we compared the native dynamics of *Bf*SOTC and *Xc*AOTC with respect to the unknotted ornithine transcarbamylase from *Escherichia coli* (*Ec*OTC). Our results suggest that, in addition to substrate specificity, the knotted structures in *Xc*AOTC and *Bf*SOTC may play an important role in stabilizing the folding dynamics, particularly around the knotted structural elements.

1. Introduction

Knotted proteins entwine themselves in their backbone to form a knot that makes them topologically constrained [1–3]. The first example of a knotted protein, carbonic anhydrase, was described in 1977 by Richardson [4] and it was later characterized more systematically and mathematically in 1994 by Mansfield [5]. Since then, more than 1000 knotted protein structures have been identified in the protein data bank [6]. These knotted proteins were classified according to their topologies, which include trefoil knots, figure-eight knots, Gordian knots, and Stevedore's knot [2,7].

Several knotted motifs are conserved throughout evolution, some are located near the catalytic sites and these evolutionary conservations suggest functional roles of protein knots [8,9]. Recent studies have demonstrated that knotted proteins exhibit mechanostabilities against vectorial unfolding [10,11]. Nonetheless, there are few examples of protein families that have both knotted and unknotted protein members to allow pair-wise comparisons to ascribe functional attributions of protein knots. In this regard, *Xc*AOTC and *Bf*SOTC are unique, evolutionarily divergent, and contain trefoil knotted structures while the rest of the OTC family members are unknotted (Fig. 1 and Supplementary Fig. S1) [12,13].

Here we chose three members within the OTC superfamily that belong to the OTCace_N clan [14], namely the unknotted *Ec*OTC, the knotted *Bf*SOTC, and the knotted *Xc*AOTC to carry out comparative analyses of their folding dynamics in the context of topological knots. These proteins are homotrimeric and the individual protomers share a high degree of structural similarity with the exception of the knot-promoting motifs, however they share limited sequence similarity (Fig. 1). The individual monomer possesses a carbamyl phosphate (CP) binding domain and a substrate-binding (SSB) domain. The CP domains of these proteins are structurally similar whereas the SSB domain in the unknotted *Ec*OTC protein is distinct from those of *Bf*SOTC and *Xc*AOTC in the knot core region. The entanglement of the 240s region of *Bf*SOTC and *Xc*AOTC proteins with the proline-rich (Pro) region results in the formation of knot [15,16], while the 240s region hangs over the Pro region in *Ec*OTC to make it unknotted [12,13,17]. According to KnotProt [6], *Bf*SOTC and *Xc*AOTC are two of the most deeply knotted proteins in

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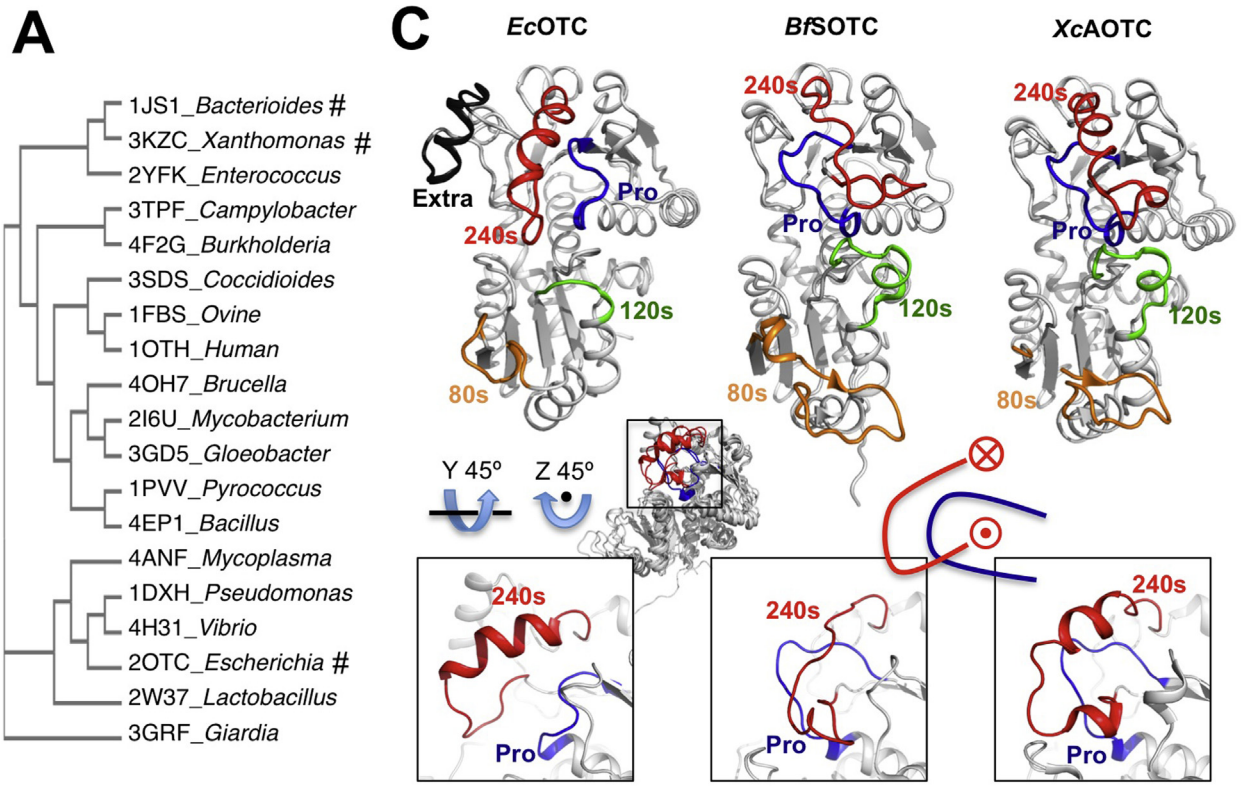

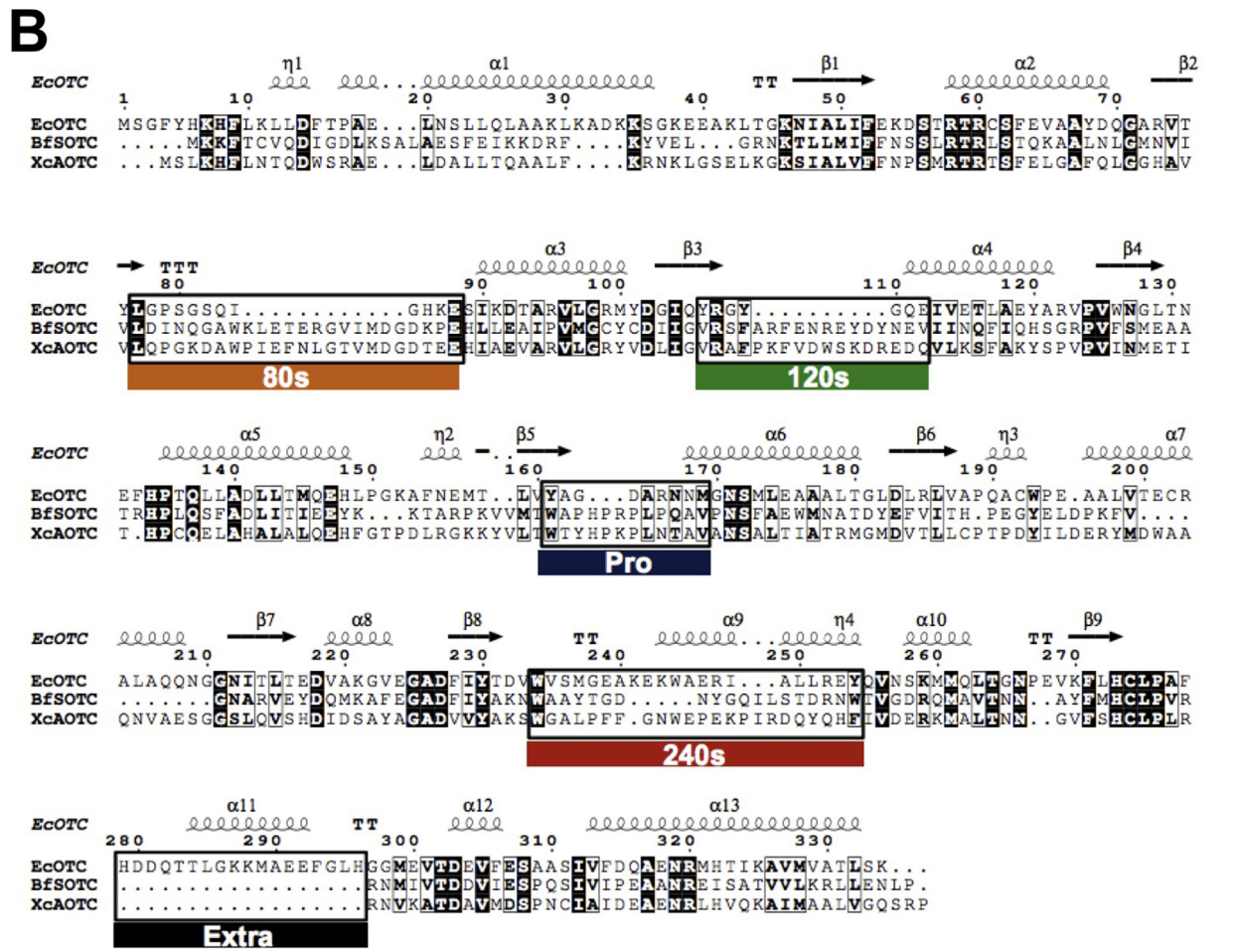

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