Three-Dimensional Domain Swapping Changes the Folding Mechanism of the Forkhead Domain of FoxP1

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Resumen

The forkhead family of transcription factors (Fox) controls gene transcription during key processes such as regulation of metabolism, embryogenesis, and immunity. Structurally, Fox proteins feature a conserved DNA-binding domain known as forkhead. Interestingly, solved forkhead structures of members from the P subfamily (FoxP) show that they can oligomerize by three-dimensional domain swapping, whereby structural elements are exchanged between adjacent subunits, leading to an intertwined dimer. Recent evidence has largely stressed the biological relevance of domain swapping in FoxP, as several disease-causing mutations have been related to impairment of this process. Here, we explore the equilibrium folding and binding mechanism of the forkhead domain of wild-type FoxP1, and of two mutants that hinder DNA-binding (R53H) and domain swapping (A39P), using size-exclusion chromatography, circular dichroism, and hydrogen-deuterium exchange mass spectrometry. Our results show that domain swapping of FoxP1 occurs at micromolar protein concentrations within hours of incubation and is energetically favored, in contrast to classical domain-swapping proteins. Also, DNA-binding mutations do not significantly affect domain swapping. Remarkably, equilibrium unfolding of dimeric FoxP1 follows a three-state N-2 <-> 2I <-> 2U folding mechanism in which dimer dissociation into a monomeric intermediate precedes protein unfolding, in contrast to the typical two-state model described for most domain-swapping proteins, whereas the A39P mutant follows a two-state N <-> U folding mechanism consistent with the second transition observed for dimeric FoxP1. Also, the free-energy change of the N <-> U in A39P FoxP1 is similar to 2 kcal.mol(-1) larger than the I <-> U transition of both wild-type and R53H FoxP1. Finally, hydrogen-deuterium exchange mass spectrometry reveals that the intermediate strongly resembles the native state. Our results suggest that domain swapping in FoxP1 is at least partially linked to monomer folding stability and follows an unusual three-state folding mechanism, which might proceed via transient structural changes rather than requiring complete protein unfolding as do most domain-swapping proteins.

Palabras clave

KeyWords Plus:LANGUAGE DISORDER; MOLTEN-GLOBULE; CYANOVIRIN-N; DNA-BINDING; PROTEIN-L; DIMER; GENE; STATE;EVOLUTION; DYNAMICS

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