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Intrinsically disordered regions stabilize the helical form of the C-terminal domain of RfaH: A molecular dynamics study

Sangni Xun [a], Fan Jiang [a,*], Yun-Dong Wu [a,b,*]

[a] Laboratory of Computational Chemistry and Drug Design, Laboratory of Chemical Genomics, Peking University Shenzhen Graduate School, Shenzhen 518055, China
[b] College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China

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ABSTRACT

RfaH protein functions as both transcription anti-terminator and translation enhancer in bacteria. Recent studies have shown that the C-terminal domain (CTD) is an α-helical hairpin (two-helix bundle) in full-length RfaH, despite the intrinsically favored β-barrel structure. Here, we carried out μs-timescale molecular dynamics (MD) simulations for the wild-type (WT) RfaH, its E48S mutant and an established model without the intrinsically disordered region (IDR1) linking the CTD and the N-terminal domain (NTD). Our simulations showed that the WT can be well stabilized by our RSFF1 force field, while the E48S mutant and the model without IDR1 undergo considerable structural change, which is in good agreement with experimental observations. The IDR1 plays important roles in stabilizing the hydrophobic environment near the crucial E48–R138 salt-bridge as well as in tethering α4 helix in CTD to α3 helix in NTD. In the absence of the IDR1, destabilization of key interdomain contacts and unfolding of the CTD α5 helix were observed in the simulation. In addition, the intrinsically disordered tail of the CTD (IDR2) is also of great significance to stabilize the bound conformation of CTD. These findings provide important implications for consideration of simulations in revealing the functions of residues invisible in a crystal structure.

1. Introduction

RfaH from bacteria is a transcription factor belongs to the NusG protein family.[1,2] Most NusG-like proteins contain one or more freely linked C-terminal domain(s) (CTD) folded in β-barrel-like structure. By contrast, the structure of the CTD in RfaH is quite unique (Fig. 1). As the crystal structure in Figure 1B shows, it folds as an α-helical hairpin (two-helix bundle) and bounds with the N-terminal domain (NTD) of the protein.[2–4] RfaH also has a special biological function in activating the expression of horizontally transferred genes,[5] some of which relate to the virulence of bacteria.[6] As Figure 1C shows, RfaH binds to RNA polymerase (RNAP) at the operon polarity suppressor (*ops*) site[5,7,8] and inhibits Rho-dependent termination via modification of RNAP and exclusion of the Rho cofactor NusG.[2,8,9] Spontaneously, the CTD of RfaH binds with S10 of the ribosome to facilitate the translation process.[10] Thus, RfaH plays a role in both transcription and translation.[11] The dual roles depend on a large structural transformation of CTD as shown in Figure 1B and C.[10,12,13] Initially in the closed state, the CTD is in an α-helix hairpin (PDBID: 2OUG) bound with NTD and masking its RNAP binding site.[14] Interaction of RfaH with its DNA target *ops* triggers the dissociation of CTD and turns RfaH into open state. In this open state, when away from the NTD, the CTD immediately refolds to a β-barrel with five β-strands (PDBID: 2LCL).[10] The binding between RNAP and the NTD in the open-state RfaH engages in transcription. The CTD-β contacts with S10 subunit of ribosome and sequentially promotes translation.[2]

The α to β transformation of CTD origins from its intrinsic structural preference. As Figure 1B shows, in vitro experiments indicated that the CTD fold as a β-barrel when isolated in water.[10] In full-length RfaH, the disruption of a salt-bridge between E48 in the NTD and R138 in the CTD by E48S substitution resulted in a 1:1 equilibrium between α-helix and β-sheet conformations for the CTD in solution.[10] Therefore, the E48–R138 salt-bridge plays an important role in stabilizing CTD-α. Also, cleavage of the IDR1 linker by TEV protease resulted an α to β transformation of the CTD, suggesting that the linker connecting the two domains is crucial for the interdomain binding. Therefore, CTD-α only exists in the closed state in which the CTD is tightly interacts with the NTD. CTD-β corresponds to the open state when the NTD dissociates from the CTD. The large conformational change of RfaH is quite unique, leading to new paradigm of a transformer

* Corresponding authors.
E-mail addresses: jiangfan@pku.edu.cn (F. Jiang), wuyd@pkusz.edu.cn (Y.-D. Wu).

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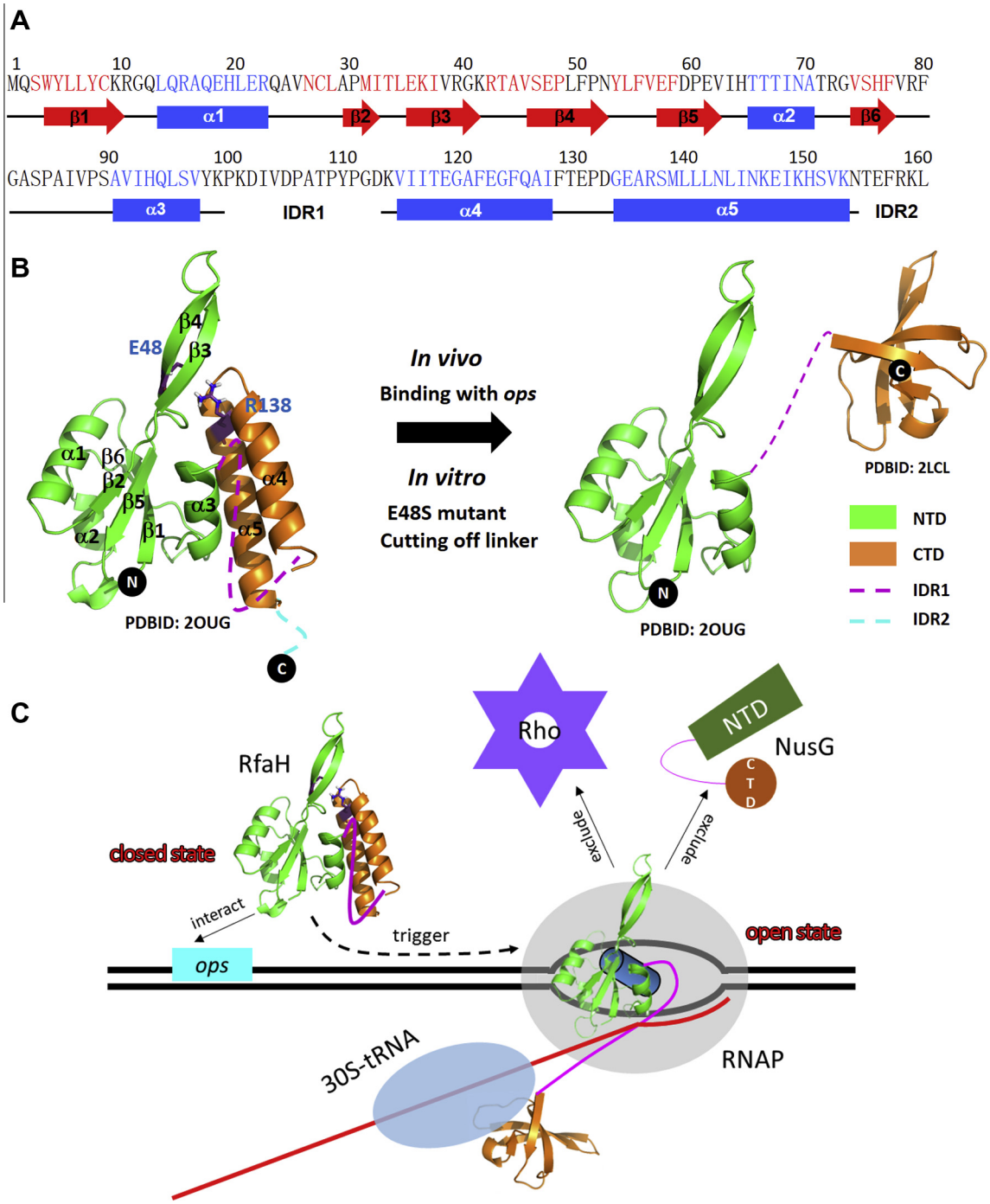

Figure 1. The full length RfaH from *E. coil*. (A) Sequence with its secondary structures (blue: helix, red: sheet) in crystal (PDB ID: 2OUG, closed state) given below. (B) Transition from the closed state (left) to the open state (right). The experimental structures of its N-terminal domain (NTD) and C-terminal domain (CTD) are shown in different colors. The NMR structure of isolated CTD (PDB ID: 2LCL) is used for the open state. The intrinsically disordered regions (IDR1 and IDR2) are invisible in the crystal structure. (C) Model for the dual roles of RfaH in vivo.

protein.[15,16] Other examples of structural rearrangements that lead to different functions have been found previously, including lymphotactin[17] and Mad2[18], but they are less dramatic than RfaH.

The above significant conformational change from CDT-α in the closed state to CDT-β in the open state attracted several theoretical studies. On the isolated CTD, Li et al. carried out steered MD simulations in implicit solvent and built up an α to β transition network with Markov State model analysis.[12] Xiong et al. used MD simulations of coarse-grained off-lattice model to study the folding and allostery in the full-length RfaH. Results show that α to β transition can be approximately described by a two-state model and three parallel pathways.[19] Ramírez-Sarmiento et al. built up a dual basin model that biased the full-length RfaH to fold from α to β state, which stresses the requirement of disruption of interdomain contacts to trigger the α to β transformation.[20] Most recently, Jeevan et al. investigated α to β transformation of CTD in the full-length RfaH using targeted and steered MD to illustrate the role of interdomain interactions,[21] and found that interdomain contacts may be the main barrier in the transformation.