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Structural plasticity of T4 transcription co-activator gp33 revealed by a protease-resistant unfolded state

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ABSTRACT

Gene 33 protein (gp33) is a transcriptional coactivator for late genes of the T4 bacteriophage. gp33 possesses a 5-helix bundle core, with unstructured *N*- and *C*-terminal regions that account for >50% of the protein sequence. It plays a unique role of interacting with host RNA polymerase, couples transcription with DNA replication, and plays the dual function as repressor and co-activator in phage transcription. Here, we identify protein structural plasticity as the molecular basis of the dual nature in gp33. We find that gp33 has the peculiar property of remaining protease insensitive in its urea-unfolded state. Using NMR studies with spectroscopic measurements, we propose that intra-protein interactions are replaced by protein-urea interactions in gp33. This process not only unfolds gp33 but also renders it protease-resistant. Our studies shed new light on the unique structural malleability of gp33 that might be important in its transition from a repressor to a late transcription co-activator.

1. Introduction

The T4 bacteriophage late gene transcription requires the T4 gene 55 (gp55) that acts as a sigma factor and recognizes the T4 late gene promoters [1,2]. T4 phage also produces a late gene transcription co-activator, gp33. The gp55-mediated transcription is generally repressed by gp33. However, in the presence of another phage encoded protein, gp45 (sliding clamp protein), the late gene transcription is enhanced several fold [3,4]. The *C*-terminal tails of both gp55 and gp33 interact with the sliding clamp and bring about late gene transcription. While the *C*-terminal region of gp55 is dispensable and allows RNA polymerase to achieve more than basal transcription, the gp33 *C*-terminal region is essential to achieve any transcription in the presence of gp45 [1,3]. In other words, when the gp33 *C*-terminal region is absent, transcription remains repressed. Thus gp33 has a dual function as repressor and co-activator in the transcription biology of T4 phage. It is thus of great interest to understand how one protein performs two contrasting functions.

The crystal structure of T4 gp33 bound to the flap domain of *Escherichia coli* RNA polymerase is available [5]. The structure of the ordered region of gp33 is made up of five helices, and forms the protein core (Fig. 1A). This core binds to the β-flap domain of RNA polymerase β-subunit. Thirty one residues at the *N*-terminus and 10 residues at the *C*-terminus were not visible in the electron density map, and were assumed to be disordered [5]. Other experiments also showed that ~55% of the gp33 protein remains disordered in solution [6]. Whether these unstructured regions of gp33 are relevant for its contrasting function is unclear, and calls for detailed analysis of the protein. Using biophysical studies, we now report that gp33 exhibits structural plasticity as evident from protease resistance upon denaturation with urea. Such a behavior is unique for a soluble protein, and to the best of our knowledge, has

Abbreviations: <λ>, average wavelength; BSA, bovine serum albumin; CD, circular dichroism; C_m, mid-point of chemical denaturation; f_U, unfolded fraction; GdnHCl, guanidine hydrochloride; gp33, gene 33 protein; HSQC, heteronuclear single quantum coherence; NMR, nuclear magnetic resonance; PK, proteinase K; PMSF, phenylmethane sulfonyl fluoride; SDS-PAGE, sodium dodecylsulfate – polyacrylamide gel electrophoresis.

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not been reported previously. We propose that such unique behavior of gp33 may be important for its transition from a repressor into a co-activator.

2. Materials and methods

2.1. gp33 production and purification

T4 phage gp33 gene was a kind gift from Prof. E. Peter Geiduschek, University of California at San Diego, USA. The gene was cloned in a modified pET21b vector system and expressed with an *N*-terminal His_6-tag. The single-Trp mutants (F12→W and F70→W) were generated by site-directed mutagenesis using standard protocols [7,8]. Henceforth, wild-type gp33 is referred to as WT and the two single-Trp mutants are referred to as F12W and F70W (the numbering takes into account the additional eight residues at the *N*-terminus (M-H_6-P)). *E. coli* BL21 (DE3) cells were transformed with pET21b-gp33, for protein expression. Cells were grown in LB medium at 37 °C till $OD_{600\ nm} \approx 0.8$, after which gp33 expression was induced using 1 mM IPTG for 3 h at 37 °C.

gp33 was purified in the presence of urea. Briefly, harvested cells were resuspended in lysis buffer (Table 1) for 12 h at 4 °C with rotational mixing, for complete lysis of the cells. The suspension was subjected to bath sonication at 4 °C for 5 min. The cell debris was removed by centrifugation and the supernatant was subjected to Ni-NTA pull down at 25 °C using the buffers listed in Table 1. Dialysis was carried out against Buffer C (Table 1) for refolding of gp33. All further experiments were carried out in this buffer system. ^{15}N-labeled gp33 was obtained by growing the cells in M9 minimal media with $(^{15}NH_4)_2SO_4$ as the sole nitrogen

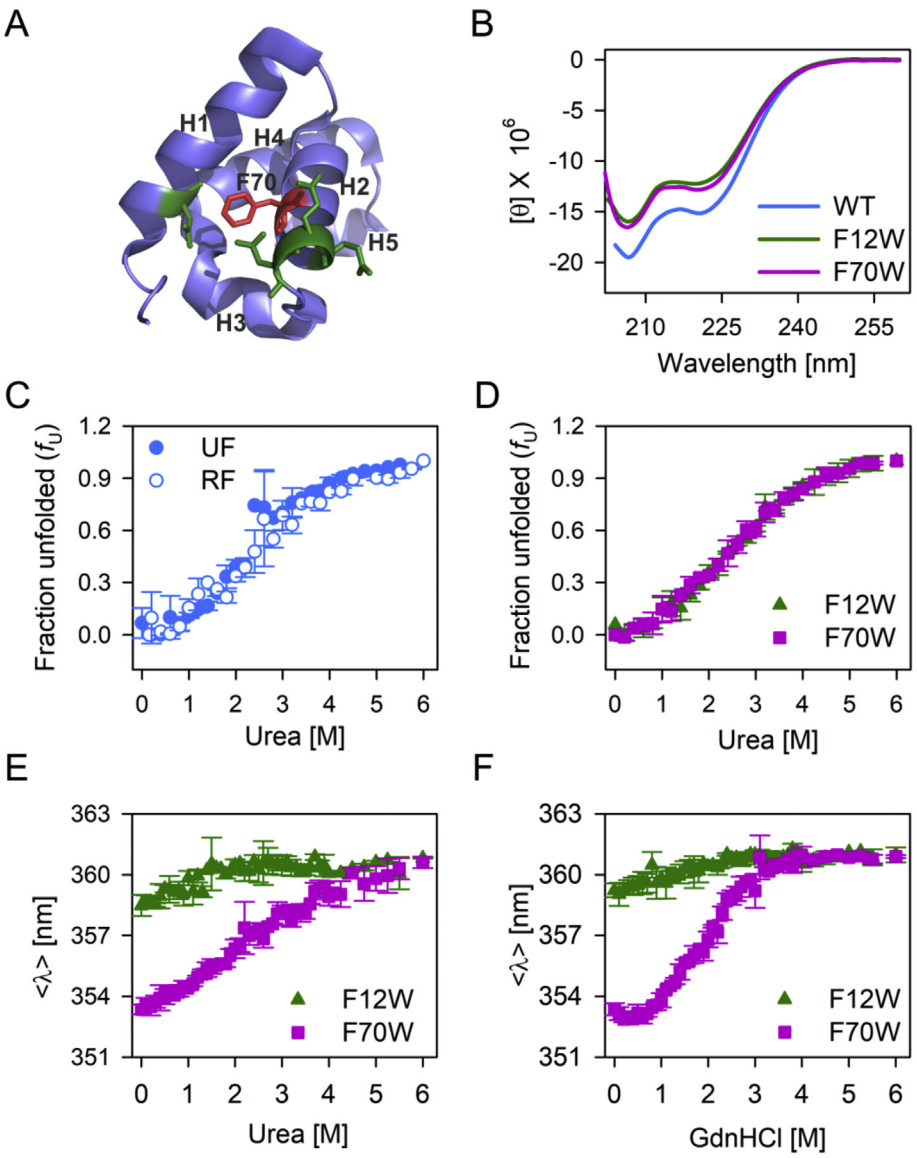

Fig. 1. gp33 core shows two-state global unfolding. (A) Cartoon representation of gp33 core (PDB ID: 3TBI). The five helices (H1H5) are numbered. Residues in the 5 Å vicinity of Phe70 (red) that form strong interactions with Phe are shown in green. Phe12 forms a part of the *N*-terminal unstructured region and is not seen in the crystal structure. (B) Far-UV CD profiles of gp33 WT and its single-Trp mutants. (C) Unfolding (UF, filled symbols) and refolding (RF, open symbols) processes for WT gp33 monitored using far-UV CD at 222 nm are normalized from 0 to 1 and shown as fraction unfolded (f_U). (D–F) Unfolding profiles for the single-Trp mutants monitored using far-UV CD (D) and fluorescence ((E), urea; (F), GdnHCl). Errors denote standard deviations from two independent experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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