International Journal of Biological Macromolecules 51 (2012) 497–503

Contents lists available at SciVerse ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

The N-terminus modulates human Caf1 activity, structural stability and aggregation

Li-Kui Feng, Yong-Bin Yan*

State Key Laboratory of Biomembrane and Membrane Biotechnology, School of Life Sciences, Tsinghua University, Beijing 100084, China

ARTICLE INFO

Article history:
Received 27 April 2012
Received in revised form 24 May 2012
Accepted 29 May 2012
Available online 6 June 2012

Keywords:
Aggregation kinetics
Homology modeling
Thermal stability

ABSTRACT

Caf1 is a deadenylase component of the CCR4-Not complex. Here we found that the removal of the N-terminus resulted in a 30% decrease in human Caf1 (hCaf1) activity, but had no significant influence on main domain structure. The removal of the N-terminus led to a decrease in the thermal stability, while the existence of the N-terminus promoted hCaf1 thermal aggregation. Homology modeling indicated that the N-terminus had a potency to form a short α-helix interacted with the main domain. Thus the N-terminus played a role in modulating hCaf1 activity, stability and aggregation.

1. Introduction

The control of mRNA turnover rate is important to the regulation of intracellular mRNA abundance for efficient translation. In eukaryotic cells, the majority of mRNAs are degraded via the deadenylation-dependent pathway, in which the shortening of the 3′-end poly(A) tail is the initial step, followed by either 5′–3′ or 3′–5′ degradation of the mRNA body [1]. Deadenylation is achieved by deadenylases, which are 3′-exoribonuclease with a high preference of poly(A) as the substrate. The well-characterized deadenylases are CCR4, Caf1/Pop2, PARN and Pan2. These deadenylases have been shown to participate in diverse physiological processes by binding with distinct partners or being recruited to different intracellular bodies [2]. Among the various deadenylases, both CCR4 and Caf1 are core components of the CCR4-Not complex, which is an evolutionary conserved platform for transcriptional control and mRNA metabolism in eukaryotes [3,4]. The CCR4-Not complex can be further recruited to the mRNA processing bodies (P-bodies), a cytoplasmic foci for mRNA decay. Previous studies have shown that deadenylation is prerequisite for the function of P-body, and both CCR4 and Caf1 have a P-body localization [5]. The P-body localization of the yeast CCR4p and Caf1p/Pop2p is modulated by the Q/N-rich region with intrinsic aggregatory potency [6,7]. However, the Q/N-rich region is not conserved in the P-body components of higher vertebrates, and the underlying assembly mechanism remains unclear.

Although the CCR4-Not complex contains two deadenylases, CCR4 is proposed to be the main deadenylase in *Saccharomyces cerevisiae* [4,8]. Nonetheless, it has been shown that Caf1/Pop2 from yeast, mouse and human beings possesses deadenylase activity in vitro [9–13], and plays an important role in deadenylation separate from the functions of CCR4 [14]. In vertebrates, there are two yeast Caf1/Pop2p homologs, named Caf1/Caf1a/CNOT7 and Pop2/Caf1b/CALIF/CNOT8, respectively [15,16]. The human Caf1 (hCaf1) has been shown to interact with the antiproliferative proteins Tob/BTG family protein [13,17] and arginine methyltransferase 1 (PRMT1) [18]. In *Arabidopsis*, Caf1 is involved in environmental stress responses [19]. These observations suggest that Caf1 plays a crucial role in intracellular regulations and responses to environmental stresses.

The crystal structures of Caf1/Pop2 from *Sacchromyces cerevisiae*, *Schizosaccharomyces pombe* and *Homo sapiens* indicate that Caf1 shares a conserved structure across species, and belongs to the DEDD 3′-exonuclease superfamily [17,20,21]. Caf1 is a monomeric single-domain protein with a kidney shape. The overall structure of Caf1 contains a six-strand β-sheet flanked by ∼13 α-helices (Fig. 1A). The active site is located at a highly negatively charged cavity, and the four conserved acidic amino acid residues DEDD coordinate with two divalent metal ions to facilitate the catalysis via the two-metal-ion mechanism [21]. In the crystal structure of hCaf1-Tob complex, the N-terminus (residues 1–10) and

Abbreviations: ANS, 1-anilinonaphtalene-8-sulfonate; CD, circular dichroism; DTT, dithiothreitol; hCaf1, human Caf1; IPTG, isopropyl-1-thio-β-D-galactopyranoside; SDS, sodium dodecyl sulfate; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; SEC, size-exclusion chromatography.

* Corresponding author at: School of Life Sciences, Tsinghua University, Beijing 100084, PR China. Tel.: +86 10 6278 3477; fax: +86 10 6277 1597.
E-mail addresses: ybyan@tsinghua.edu.cn, ybyan@139.com (Y.-B. Yan).

0141-8130/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijbiomac.2012.05.032

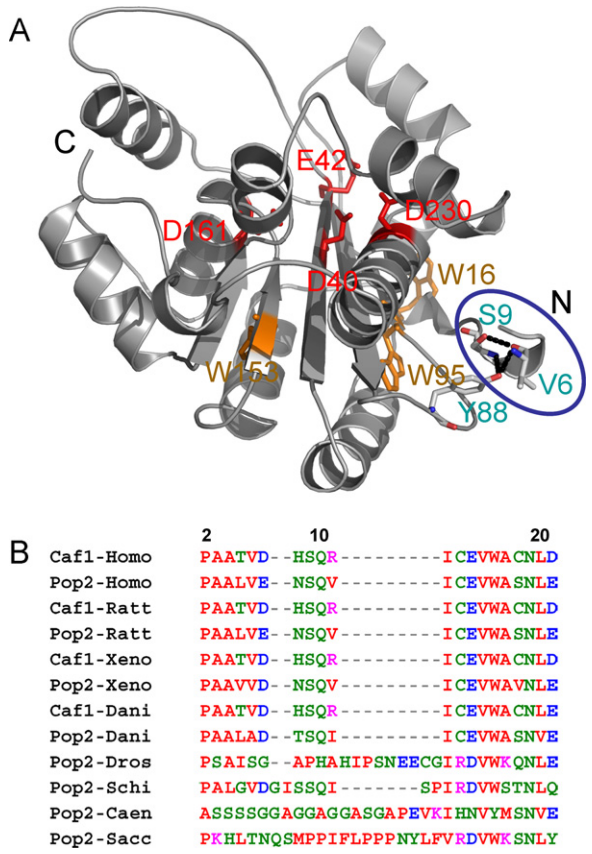

Fig. 1. Structure and sequence alignment of the N-terminus of Caf1. (A) Structure of hCaf1 by homology modeling using the crystal structure of *S. cerevisiae* (PDB ID: 1UOC) as the template. The modeled structure of the main domain was similar to the crystal structure of hCaf1 in the Tob-hCaf1 complex (data not shown) [17]. The potential hydrogen bonding between the N-terminus and the loop connecting β3 and β4 are presented as dotted lines. The positions of the three Trp residues and four acidic residues in the active site are highlighted in orange and red, respectively. N and C denote the N- and C-terminus, respectively. (B) Sequence alignment of the N-terminus of Caf1 performed by the online software MAFFT (http://www.ebi.ac.uk/Tools/msa/mafft/). The sequences used for alignment are: *Homo sapiens* Caf1 (AAH07315), *Homo sapiens* Pop2 (AAH17366), *Rattus norvegicus* Caf1 (AAI67766), *Rattus norvegicus* Pop2 (AAH85856), *Xenopus laevis* Caf1 (NP_001089689), *Xenopus laevis* Pop2 (NP_001080119), *Danio rerio* Caf1 (NP_001070723), *Danio rerio* Pop2 (AAI53628), *Drosphila melanogaster* Pop2 (ACZ94700), *Saccharomyces cerevisiae* Pop2 (CAA96333), *Schizosaccharomyces pombe* Pop2 (CAA21420) and *Caenorhabditis elegens* Pop2 (Q17345).

C-terminus (residues 263–285) are disordered in the electron density map although the full-length hCaf1 was used for crystallization of the complex [17]. Sequence alignment indicates that the N-terminus of Caf1 is partially conserved in vertebrates, but not in invertebrates (Fig. 1B). In this research, we investigated the role of the N-terminus in hCaf1 activity, structure, stability and aggregation behavior by mutational analysis. The results showed that the N-terminus affected hCaf1 activity but not the structural features. The deletion of the N-terminus destabilized the protein against thermal denaturation, while slowed down hCaf1 thermal aggregation. These results suggested that the N-terminus of hCaf1 is not fully disordered, and has a role in modulating hCaf1 structural stability and functions by interacting with the main domain.

2. Materials and methods

2.1. Chemicals

Dithiothreitol (DTT) and RNasin were purchased from Promega. Tris, methylene blue, polyadenylic acid potassium salt, isopropyl-1-thio-β-D-galactopyranoside (IPTG) and sodium dodecyl sulfate (SDS) were purchased from Sigma–Aldrich. All other chemicals were purchased from local products of analytical grade. The N-terminal peptide (^{1}MPAATVDHSQR^{11}) was synthesized by SBS Genetech and verified by HPLC and MS.

2.2. Protein expression and purification

The gene of wild type (WT) hCaf1 was cloned and constructed into the pET28a expression vector. The mutants H8N, R11V and Δ11 were constructed using the following primers synthesized by Invitrogen: the H8N, R11V and Δ11 were constructed using the following primers synthesized by Invitrogen: H8N Forward primer, 5′-CATGGCTAGCATGCCAGCGGCAACTGTAGATAAT-3′; R11V Forward primer, 5′-CATGCTAGCATGCCAGCGGCAACTGTAGATCATAGCCAAGTAATTTG-3′; Δ11 Forward primer, 5′-CATCGCTAGCATTTGTGAAGTTTGGGCTTGCAACTTG-3′; Reverse primer for the three mutants, 5′-GCTAAGCTTTCATGACTGCTTGTTGGCTTCCTC-3′. The recombinant proteins were overexpressed in *Escherichia coli* and purified as described previously [12]. In brief, the His-tagged proteins were purified by a 5 ml HisTrap HP column and a Superdex 200 column sequentially. The His-tag at the N-terminus was not cleaved in this research since the tag did not affect the properties of the recombinant proteins. The proteins were diluted in buffer A, which contained 20 mM Tris–HCl, pH 8.0, 100 mM KCl, 0.2 mM EDTA, 0.5 mM DTT, 1.5 mM $MgCl_2$ and 20% (v/v) glycerol. Protein concentration was determined by the Bradford method [22], and the protein concentration used in this study was 6 μM unless otherwise indicated.

2.3. Enzyme assay

The deadenylase activity was measured using the standard methylene blue method [23] with some modifications. In brief, the reaction buffer contained 20 mM Tris–HCl (pH 7.0), 100 mM KCl, 0.5 mM DTT, 0.2 mM EDTA, 0.1% BSA, 10% (v/v) glycerol and 0.1 U of RNasin. After 60 min reaction in dark at 37 °C, the absorbance at 662 nm was measured at 25 °C on an Ultraspec 4300 pro UV/visible spectrophotometer. All activity data of hCaf1 and mutants were measured at least three repetitions.

2.4. Homology modeling of hCaf1b

Since the N-terminal 10 residues are invisible in the crystal structure of hCaf1 in the Tob-hCaf1 complex [17], the potential structural features of hCaf1 N-terminus was obtained by automated protein structure homology modeling of hCaf1 using the online software SWISS-MODEL [24]. The X-ray crystal structure of *S. cerevisiae* (PDB ID: 1UOC) [20] was used as the template. The modeled structure of hCaf1b was manipulated and rendered using PyMol (http://www.pymol.org/).

2.5. Spectroscopy

Far-UV circular dichroism (CD) spectra were obtained on a Jasco-715 spectrophotometer (Jasco) using a 1 mm path-length cell and a resolution of 0.2 nm. Fluorescence spectra were collected on an F-2500 spectrofluorometer (Hitachi) using a 10 mm pathlength cuvette. Intrinsic Trp fluorescence was excited at 295 nm and emitted in the wavelength range of 300–400 nm. Parameter *A*, which reflects the position and shape of the intrinsic fluorescence spectrum [25], was derived by dividing the intensity at 320 nm by that at 365 nm (I_{320}/I_{365}). ANS extrinsic fluorescence was excited at 380 nm and emitted in the range of 400–600 nm. The ANS fluorescence samples were prepared by incubating the protein solutions