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Biophysical characterization of the structural change of Nopp140, an intrinsically disordered protein, in the interaction with CK2α

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ABSTRACT

Nucleolar phosphoprotein 140 (Nopp140) is a nucleolar protein, more than 80% of which is disordered. Previous studies have shown that the C-terminal region of Nopp140 (residues 568–596) interacts with protein kinase CK2α, and inhibits the catalytic activity of CK2. Although the region of Nopp140 responsible for the interaction with CK2α was identified, the structural features and the effect of this interaction on the structure of Nopp140 have not been defined due to the difficulty of structural characterization of disordered protein. In this study, the disordered feature of Nopp140 and the effect of CK2α on the structure of Nopp140 were examined using single-molecule fluorescence resonance energy transfer (smFRET) and electron paramagnetic resonance (EPR). The interaction with CK2α was increased conformational rigidity of the CK2α-interacting region of Nopp140 (Nopp140C), suggesting that the disordered and flexible conformation of Nopp140C became more rigid conformation as it binds to CK2α. In addition, site specific spin labeling and EPR analysis confirmed that the residues 574–589 of Nopp140 are critical for binding to CK2α. Similar technical approaches can be applied to analyze the conformational changes in other IDPs during their interactions with binding partners.

1. Introduction

Nucleolar phosphoprotein p140 (Nopp140) is a nucleolar protein of mammalian cells that contains 710 amino acids and is involved in nucleolus biogenesis during cell division. It interacts with a variety of proteins, such as protein kinase CK2 alpha subunit (CK2α), RNA polymerase I, p80 coilin, NAP57 and snRNPs that are involved in the synthesis and assembly of ribosomes [1]. The cellular function of Nopp140 has been studied using gene silencing and the interaction partners have been identified, but efforts to determine the 3-dimensional structure of Nopp140 have not been extensively reported. Sequence analysis of Nopp140 suggest that Nopp140 has a distinct structural feature: more than 80% of the sequence was predicted to be composed of random coil [2]. Extensive sequence analysis of Nopp140 using several disordered region prediction algorithms indicated that Nopp140 is one of the intrinsically disordered proteins (IDPs) that consist mostly of disordered structure [1]. The high sensitivity of Nopp140 to proteases, random coil features found in circular dichroism (CD) spectra, and its hydrodynamic properties further confirmed that Nopp140 is a member of the IDPs [2].

Nopp140 has a unique characteristic among IDPs in which majority of the sequences are disordered and lacks a well-folded domain. It contains only a few short stretches of potential helix that are located at the N- and C-termini (Fig. 1A). So far, only a small number of IDPs that consist almost exclusively disordered regions have been reported. One of these, U1-70k, has the disordered regions that represent 60% of the whole sequence. Crystal structures of the protein complex containing U1-70k showed that U1-70k wraps around the core domain of U1 snRNP and interacts with several components of U1 snRNP particle [3]. The structure of this complex demonstrated how an IDP interacts with several protein components, stabilizes the complex structure, and its own disordered regions.

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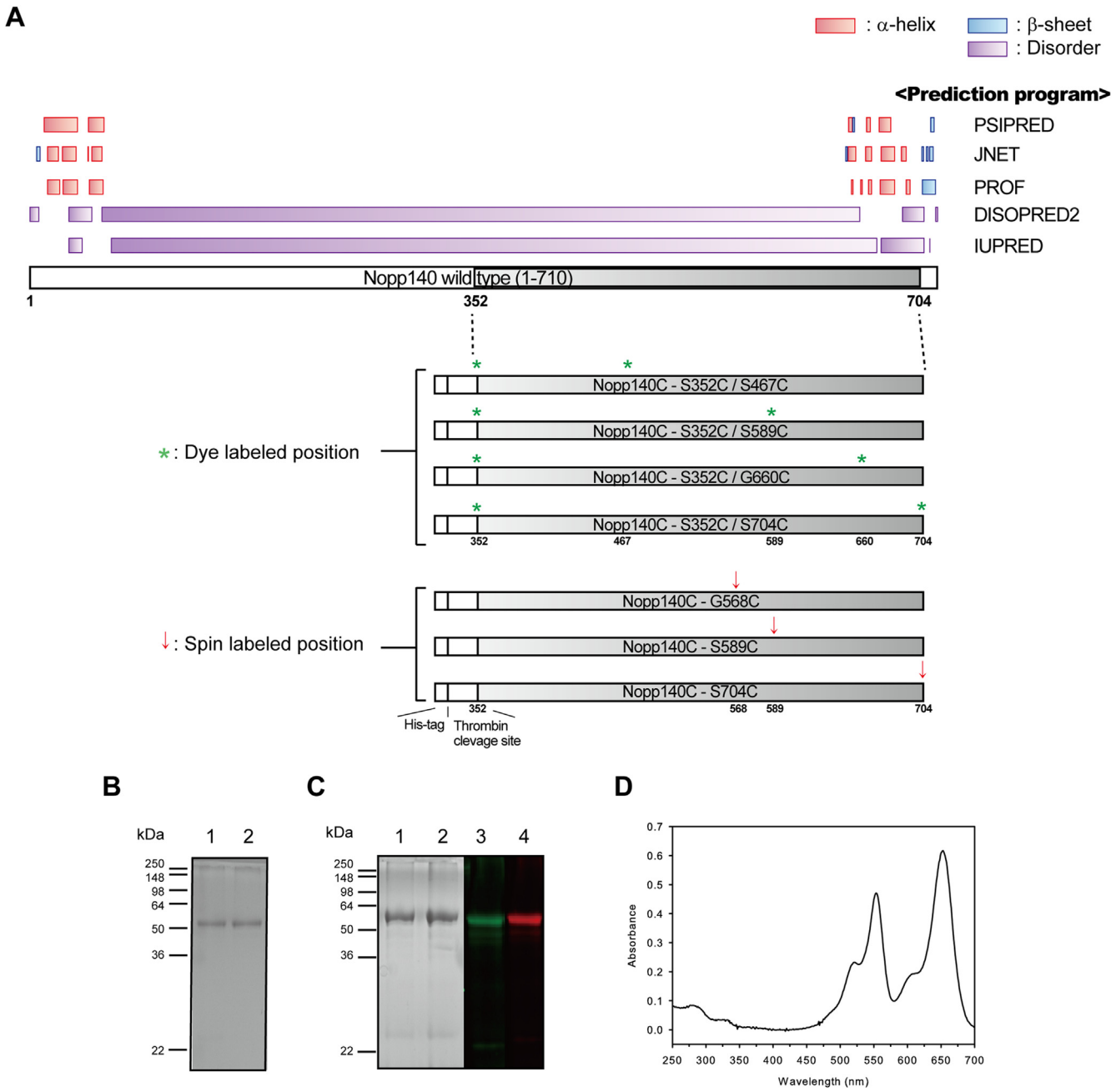

Fig. 1. (A) Schematic representation of Nopp140 and Nopp140Cs. Secondary structure regions of Nopp140 were predicted with the Quick2D bioinformatics toolkit (http://toolkit.tuebingen.mpg.de/quick2_d). Fluorescent dye labeled positions (S352C/S467C, S352/S589C, S352C/G660C, and S352C/S704C) are indicated with green asterisk symbol (*). EPR spins labeled positions (G568C, S589C, and S704C) are indicated with red arrows (↓). (B) SDS-PAGE analysis of MTSSL labeled Nopp140C (lane 1: before labeling, lane 2: after labeling) (C) SDS-PAGE analysis of Cy3-and Cy5-labeled Nopp140C. Images of lane 3 and lane 4 were obtained by fluorescence gel documentation. (lane 1: before labeling, lane 2: after labeling, lane 3: green laser excited Nopp140 C-terminus fragment, lane 4: red laser excited Nopp140 C-terminus fragment). (D) Representative absorbance spectrum of Cy3 and Cy5 labeled Nopp140C. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The interaction between Nopp140 and the catalytic subunit of CK2 (CK2α) has been extensively studied. The kinase active of CK2 is suppressed by several cellular factor(s), and this suppression is relieved by IP_6 [4]. Nopp140 was shown to be one of the cellular suppressors of CK2α, and the C-terminal region of Nopp140 (residues 568–596) contained the binding site for CK2α [5]. Structural analysis of CK2α and IP_6, together with mutational studies, further confirmed the competitive interactions between Nopp140 and IP_6 for CK2α [5]. Although the interaction site of Nopp140 to CK2α was identified, the structural features that were involved, and the effect of the interaction on the overall structure of Nopp140 still remain unknown.

The dynamic motion of IDP has been studied using spectroscopic methods such as nuclear magnetic resonance (NMR) and EPR. These methods can determine the overall flexibility of a region of interest [6–8]. Moreover, single-molecule fluorescence resonance energy transfer (smFRET) between two fluorescence probes labeled at different positions of α-synuclein had been successfully applied to determine the conformational changes in the protein under different environments [9]. In this study, we examined the

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