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Structural insight into the interaction of ADP-ribose with the PARP WWE domains

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ABSTRACT

The WWE domain is often identified in proteins associated with ubiquitination or poly-ADP-ribosylation. Structural information about WWE domains has been obtained for the ubiquitination-related proteins, such as Deltex and RNF146, but not yet for the poly-ADP-ribose polymerases (PARPs). Here we determined the solution structures of the WWE domains from PARP11 and PARP14, and compared them with that of the RNF146 WWE domain. NMR perturbation experiments revealed the specific differences in their ADP-ribose recognition modes that correlated with their individual biological activities. The present structural information sheds light on the ADP-ribose recognition modes by the PARP WWE domains.

1. Introduction

The WWE domain was termed according to its conserved Trp and Glu residues, and has been found in two functional subfamilies [1]. One is associated with ubiquitination and the other with poly ADP-ribose polymerases (PARPs). The first structural information about a WWE domain was obtained for the Deltex protein, a ubiquitin E3 ligase targeting the ankyrin repeats of the Notch receptor [2]. Each of the two tandemly-linked Deltex WWE domains adopts a β1–β2–α1–β3–β4–β5–β6 topology, and they intra-molecularly interact with each other to stabilize their binding to the ankyrin repeats on the surface of these domains.

In addition, a recent X-ray crystallographic study revealed that the WWE domain of the RNF146 protein, which is a ubiquitin E3 ligase for poly-ADP-ribosylated axin, preferentially binds to the iso-ADP-ribose moiety (Fig. 1A) on the top of the β-barrel structure [3]. Thus, structural information about the WWE domains that are involved in ubiquitination has been reported so far. However, no structural information is available for the WWE domains from PARPs, and the binding preference of the PARP WWE domains to ADP-ribose moieties was not revealed in previous surface plasmon resonance (SPR) experiments with several WWE domains and a poly ADP-ribose chain [3].

The binding preferences of the WWE domains of PARPs for ADP-ribose derivatives are interesting subjects, because only the ADP-ribose moiety appears at the terminus of the mono ADP-ribosylated chain, instead of the iso-ADP-ribose moiety (Fig. 1A), and it could be the enzymatic target of some PARPs [4]. Here, we report the solution structures of the WWE domains of the PARP subfamily members PARP11 and PARP14, and we compared the new structures with that of RNF146 (Fig. 1B) to reveal the differences in their interactions with derivatives of ADP-ribose.

2. Materials and methods

2.1. Protein sample preparation

The protein sample used for the NMR experiments were constructed for the WWE domains of mouse RNF146, human PARP11 and mouse PARP14 (corresponding to residues 83–179, 15–105 and 1542–1618, respectively).

Abbreviations: HSQC, heteronuclear single quantum coherence; NOE, nuclear Overhauser enhancement; NOESY, NOE spectroscopy; PARP, poly-ADP-ribose polymerase; ITC, isothermal titration calorimetry

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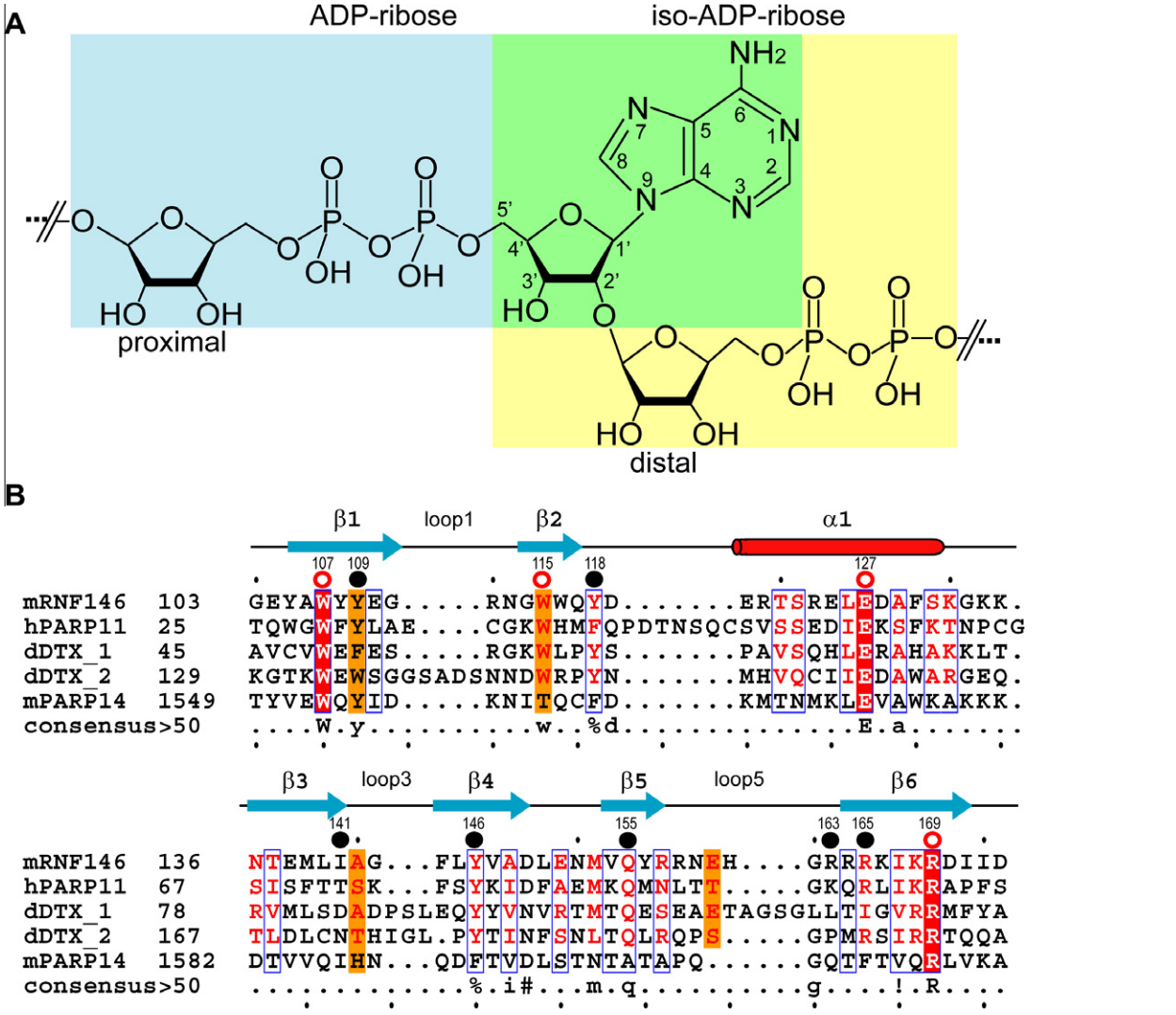

Fig. 1. (A) Structures of the ADP-ribose and iso-ADP-ribose moieties in the poly-ADP-ribose chain. (B) Structure-based sequence alignment of WWE domains. Secondary structure elements of the RNF146 WWE domain are shown on the top. The highly conserved Trp and Glu residues are marked by open red circles for the RNF146 WWE domain. The closed black circles indicate the amino acid residues involved in the recognition of the ADP-ribose moiety.

These WWE domains were synthesized by a cell-free protein synthesis system [5,6]. For the structure determination, a sample of 1.1 mM uniformly ^{13}C- and ^{15}N-labeled protein was prepared in 20 mM ^{2}H-Tris–HCl buffer, containing 100 mM NaCl, 1 mM dithiothreitol (DTT), and 0.02 % (w/v) NaN_3, with the addition of D_2O to 10% v/v, at pH 7.0. ADP-ribose, ADP, ATP, adenine, and NAD+ were purchased from Sigma.

2.2. NMR spectroscopy and resonance assignments

NMR experiments were performed at 25 °C on 600 and 800 MHz spectrometers (Bruker DRX600 and AV800), equipped with *xyz*-pulsed field gradients. Backbone and side chain assignments were obtained by standard triple resonance experiments [7]. All assignments were checked for consistency with 3D ^{15}N- and ^{13}C-edited NOESY-HSQC spectra. 3D NOESY spectra were recorded with mixing times of 80 ms. For the assignment of ATP in complex with the RNF146 WWE domain, 2D filtered NOESY spectra with mixing times of 80 and 150 ms, and 2D filtered TOCSY spectra were used [8]. The NMR data were processed with the program NMRPipe [9]. Spectra were analyzed with the programs NMRView [10], KUJIRA [11], and SPARKY (T. D. Goddard and D. G. Kneller, SPARKY 3, University of California, San Francisco).

2.3. Structure calculations

The three-dimensional structures were determined by combined automated NOESY cross peak assignment and structure calculation with torsion angle dynamics, implemented in the CYANA program [12,13]. Restraints for the backbone torsion angles ϕ and ψ were determined by a chemical shift database analysis with the program TALOS [14]. For the determination of the three-dimensional structures of the RNF146 WWE domain-ATP complex, NMR measurements were performed with mixtures of the RNF146 WWE domain and ATP at molar ratios of 1:0.5, 1:1, and 1:2. The intermolecular protein-ATP NOEs were assigned automatically and inspected manually, using the 3D NOESY-HSQC spectra and the 2D filtered NOESY spectra with mixing times of 80 and 150 ms, respectively. Structure calculations were performed in the same manner as for the free WWE domains. The 20 structures from the CYANA calculation were subjected to restrained energy refinement with the program AMBER9, using the Generalized Born