

**1H, 13C and 15N resonance assignments of S114A mutant of UVI31+ from Chlamydomonas reinhardtii**

Himanshu Singh · Vandana Raghavan · Manish Shukla · Basuthkar J. Rao · Kandala V. R. Chary

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**Abstract**  Almost complete sequence specific 1H, 13C and 15N resonance assignments of S114A mutant of UVI31+ from *Chlamydomonas reinhardtii* are reported. The cDNA of S114A mutant of UVI31+ was cloned from a eukaryotic green algae (*C. reinhardtii*) and overexpressed in *E.coli* from where the protein was purified to homogeneity. The point mutation S114A in UVI31+ reduces its DNA endonuclease activity substantially as compared with its wild type. As a prelude to the structural characterization of S114A mutant of UVI31+, we report here complete sequence-specific 1H, 13C and 15N NMR assignments.

**Keywords**  NMR · Resonance assignments · S114A · UVI31+ · *Chlamydomonas reinhardtii*

**Biological context**

Apoptosis in higher multi-cellular organisms plays an important role in homeostasis, development and defence; however its significance in unicellular organisms remains unclear. *Chlamydomonas reinhardtii*, a unicellular green alga, has been shown to undergo apoptosis in response to UV-C irradiation (Kim et al. 1997; Moharikar et al. 2006). In order to understand the process of UV mediated apoptosis in *C. reinhardtii*, we undertook in silico global genome analysis and found a protein, which was identified as UVI31+, one amongst several UV inducible transcripts/proteins and a novel β-lactamase (Moharikar et al. 2006, Rout et al. 2010). UVI31+ protein is also endowed with endonuclease activity, in vitro. Cell biological regulation reveals interesting localization changes of UVI31+ protein in *Chlamydomonas reinhardtii* chloroplast compartment (Shukla et al. 2012).

*Chlamydomonas reinhardtii* is about 10 μm in diameter that swims with two flagella. They have a cell wall made up of hydroxyproline-rich glycoproteins, a large cup-shaped chloroplast, a large pyrenoid, and an “eyespot” that senses light (Moharikar et al. 2006). It undergoes apoptosis in response to UV-C irradiation (Moharikar et al. 2007). It shows classical hall-marks of animal cell apoptosis and hence can be used as a model system for studying its molecular mechanism in a plant-like environment. Certain candidate molecules were recently identified as to be either UV-regulated or involved in apoptosis. They include apoptosis protease activating factor (APAF), a caspase-3 like protein and a defender against apoptotic death (*dad1*). All of them exhibited a distinct activation pattern correlating with onset of death following UV irradiation (Moharikar et al. 2006, 2007). One of the putative candidate molecules that is yet to be characterized is the aforementioned UV inducible gene, *uv31+,* the focus of the present study.

*Uvi31+* was originally isolated from *Schizosaccharomyces pombe* (Lee et al. 1994), whose expression was unaltered by other DNA damaging or cytotoxic agents. Interestingly, *uvi31+* showed no significant sequence homology to the known DNA repair genes. It was observed that *uvi31+* transcript increases during normal cell cycle in G1 phase before septation and also during diauxic shift (Kim et al. 1997). A null mutant of *uvi31+* from *S. pombe*
showed sensitivity to UV-light, defects in septation and cytokinesis during the resumption of cell division following UV damage-induced cell cycle arrest (Kim et al. 2002).

The uvi31+ protein has structural homology with the BolA protein of *Escherichia coli*, which was identified by its ability to induce round cell morphology when over-expressed in cells and also following a general stress response (Aldea et al. 1988; Santos et al. 1999; Huynen et al. 2005). Recently, it has been found that uvi31+ exhibited endonuclease activity (Shukla et al. 2012). However, uvi31+ has no structural homology with the known endonucleases. The mutation at S114A site at uvi31+ reduced its endonuclease activity as derived from biochemical analysis (yet to be published). And it has also been speculated that the Ser residue (S114) may be responsible for this activity. With this in the backdrop, we have mutated Ser114 to Ala114 and set out to structurally characterize the mutant of UVI31+ (named as S114Auv31+) by NMR spectroscopy, to find out whether S114 is involved in endonuclease activity. Towards this goal, we report almost complete sequence specific 1H, 13C and 15N NMR assignments of S114A mutant of UVI31+.

**Methods and results**

Cloning, over-expression and purification of UVI31+ were carried out as described earlier (Rout et al. 2010). 13C6-glucose or/and 15NH4Cl (Cambridge Isotopes Inc.) were used as the sole source of carbon or/and nitrogen. The purification of the protein was achieved by Ni2+-NTA (Ni2+- nitrilotriacetate) agarose (Qiagen, Hilgen, Germany). His6-tagged S114A mutant of UVI31+ was eluted with 250 mM imidazole in 50 mM sodium phosphate (pH 7.6), 100 mM NaCl. The eluted fractions were dialyzed overnight against 50 mM sodium phosphate (pH 6.4), 100 mM NaCl.

For NMR experiments, protein samples were prepared in a mixed solvent of 90 % H2O and 10 % 2H2O containing 50 mM sodium phosphate (pH 6.4) and 100 mM NaCl. The protein concentrations were 0.8 mM. NMR experiments were recorded on a Bruker Avance 800 MHz NMR spectrometer equipped with a 5 mm triple-resonance cryogenic probe. Experiments recorded at 298 K with uniformly 15N-labeled-S114A mutant of UVI31+ included sensitivity-enhanced 2D [15N–1H]–HSQC using water-flipback for minimizing water saturation (Bax et al. 1991). The 3D experiments recorded with doubly (13C and 15N) labeled samples were, HNCO, HN (CA)CO, CBCANH, and CBCA(CO)NH (Bax et al. 1991; Bax and Grzesiek 1993; Wuthrich et al. 1986), essentially for the assignment of backbone resonances. For the assignment of the side chain 1H and 13C resonances, we recorded were assigned using 3D C(CO)NH and 3D H(CCO)NH (Montelione et al. 1992) and 13C- and 15N-edited NOESY spectra (Muhandiram et al. 1993). NMR data processing was carried out using Bruker Topsin 3.1 software and analyzed with TATAPRO (Atreya et al. 2000, 2002) and CARA (Keller 2002). 1H chemical shifts were referenced with respect to the external standard 2,2-dimethyl-2-silapentene-5-sulfonates (DSS), while 15N and 13C chemical shifts were...
Sequence specific resonance assignments of S114A mutant of UV131+, which is free of Cys and Trp, could be carried out for nearly all \(^{1}H\), \(^{13}C\) and \(^{15}N\) spins using a suite of 3D NMR experiments mentioned in Methods. The \(^{1}H\) and \(^{15}N\) assignments thus derived are shown in the 2D \(^{15}N–^{1}H\)–HSQC (Fig. 1). A total of 117 (1Hi, 13Ci and 15Ni) correlations revealed that the protein is in a well folded state. The secondary structure elements (Fig. 2) of the protein have been characterized using the well known empirical relations of \(^{13}C\) chemical shifts (\(\Delta C_{\text{a}}–\Delta C_{\text{p}}\)) (Spera and Bax 1991; Wishart et al. 1995; Barnwal et al. 2008). The CSI plot (Fig. 2) reveals the presence of both \(\alpha\)-helical and \(\beta\)-strand segments separated by short stretches of unstructured elements. Comparison of [\(^{15}N–^{1}H\)]–HSQC spectra of (S114A)-UV131+ with that of wild type-uvi31+, both recorded under similar experimental conditions reveal subtle spectral changes between them, though most of the \(^{15}N–^{1}H\) peaks are unperturbed, suggesting that the overall structural topology of the protein may remain same with substantial conformational changes in the structure particularly near and around the S114A point mutation. Complete 3D structural characterization of the mutant UV131+ will throw more light on the intricate structural changes caused by the point mutation. This work is in progress.

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