Expression, purification and NMR analysis of the RING finger domain from the human RBBP6 protein

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RBBP6 is a 230 kDa human protein that has been shown to bind both p53 and Rb in vivo [1, 2]. We have recently shown that the protein contains a conserved N-terminal ubiquitin-like domain (unpublished data), which is potentially significant in view of the fact that RBBP6 also contains a conserved RING finger, a domain commonly found in proteins involved in the ubiquitination pathway. The RING finger from RBBP6 is atypical in that it contains a C4C4 motif rather than that more common C3HC4 motif. The structures of only two other C4C4 RING proteins have been determined so far, both using heteronuclear NMR [3].

We report here on the recombinant expression and purification of the RING finger from human RBBP6, and the backbone assignment using heteronuclear NMR. An 11.6 kDa fragment containing the RING finger was expressed in bacteria as a GST-fusion, and found to be soluble. Bacteria were grown in minimal media supplemented with 15N-ammonium chloride and 13C glucose to ensure that the expressed protein was double-labelled for heteronuclear NMR analysis. The protein was purified using a combination of glutathione agarose chromatography, anion exchange and gel filtration. Mass spectrometry confirmed that the expressed protein incorporated two-bound zinc ions, as is expected, which led us to believe that the protein was natively folded. A complete set of heteronuclear NMR data was collected at 700 and 900 MHz, from which full assignment of the backbone resonances has been achieved. These assignments will now be used as the starting point for determination of the structure of the domain.

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