THE RRM1-2 TANDEM DOMAINS OF HUR BEHAVE AS INDEPENDENT MODULES AND UNDERGO CONFORMATIONAL EXCHANGE IN SOLUTION

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Introduction: Human antigen R (HuR) is a translation-promoting factor that regulates gene expression post-transcriptionally by stabilizing mRNA transcripts. HuR is a multi-domain protein composed of three RNA Recognition Motifs (RRMs). The two N-terminal RRM1 and RRM2 domains are disposed in tandem and selectively recognize AU-rich elements in the 3'-UTR of mRNAs. Here, we characterized the conformational dynamics of HuR RRM1-2 domain by Nuclear Magnetic Resonance. Material and Methods: HuR RRM1-2 was expressed and purified to homogeneity. ¹⁵N- and/or ¹⁵N/¹³C-labeled RRM1-2 samples were used for NMR experiments. NMR spectra were recorded at 25°C on a Bruker 800 MHz spectrometer. Results and Discussion: Nearly all backbone resonances of HuR RRM1-2 were assigned, with the exception of Met1, Ile152, and Ile179. We noted a set of extra 20 resonances that could not be assigned, representing a second minor conformation of RRM1-2 in solution. We were able to directly transfer the resonance assignment of HuR RRM1 domain to the NMR spectra of RRM1-2, indicating that the RRM1 and RRM2 domains behave as independent structural modules in the absence of mRNA. Consistent with this idea, the \( \Delta C\alpha - \Delta C\beta \) values calculated for free RRM1 and RRM1-2 are highly superimposable. ¹⁵N auto correlated relaxation parameters showed that the N-terminal intrinsically disordered region (residues 1-18) and the RRM1-RRM2 connecting region (residues 95-106) are highly flexible. Moreover, HuR RRM1-2 chemical shifts were dependent on protein concentration, suggesting that RRM1-2 homodimerizes in solution. Based on chemical shift perturbation analysis, homodimerization is mediated by RRM1 loop 3 and occurs with a ~5 \( \mu \)M binding affinity. Conclusions: Our NMR results indicate that HuR RRM1-2 behave as independent modules and undergo conformational exchange in the absence of mRNA. Moreover, we identified RRM1 loop3 as the main homodimerization site in RRM1-2. We are performing NMR relaxation dispersion experiments to determine the kinetic constant and population distribution of RRM1-2 different conformers.