A 20-amino-acid autonomous RNA-binding domain contained in an enoyl-CoA hydratase

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A+U-rich elements in the 3' untranslated region of mRNA species coding for lymphokines and early response genes play a pivotal role in the control of their rapid turnover. In a search for corresponding trans-acting factors, we have previously affinity-purified and cloned a human 32-kDa A+U-binding protein, termed AUH. AUH exhibited dual activities, namely A+U-specific RNA-binding and catalytic activity as enoyl-CoA hydratase. In this report we map the RNA-binding site by analysis of a series of deletion and substitution recombinant proteins. Ultraviolet cross-linking experiments demonstrated that the deletion of a 20-amino-acid segment, Lys109-Ile128, abolished more than 80% of the relative RNA-binding activity. This segment conferred RNA-binding activity when fused to maltose binding protein. Binding of this fusion protein to A+U-rich RNA was significantly competed by an AUUUA cluster and poly(U), followed by poly(G), but not by poly(A) nor poly(C). Furthermore, RNA binding of the fusion protein was competed by a synthetic peptide corresponding to Lys109-Ile128. Circular dichroic measurement indicated formation of a specific complex between this peptide and poly(U) but not with poly(A). The identified 20 amino acids therefore constitute an automonous RNA-binding domain, distinct from the RNA-recognition motifs of the family of ribonucleoproteins or NAD/RNA-binding sites in dehydrogenases found in hitherto reported A+U-binding proteins. Replacement of Arg125 in this motif with Glu reduced binding twofold, indicating this residue is integral to the binding function. Deletion of other parts of the protein did not impair RNA binding to any significant extent. By contrast, the hydratase activity of AUH required an intact three-dimensional conformation, as most mutations downstream of Ser68 impaired enzymatic activity.
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