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Network of long-range concerted chemical shift displacements upon ligand binding to human angiogenin

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Abstract: Molecular recognition models of both induced fit and conformational selection rely on coupled networks of flexible residues and/or structural rearrangements to promote protein function. While the atomic details of these motional events still remain elusive, members of the pancreatic ribonuclease superfamily were previously shown to depend on subtle conformational heterogeneity for optimal catalytic function. Human angiogenin, a structural homologue of bovine pancreatic RNase A, induces blood vessel formation and relies on a weak yet functionally mandatory ribonucleolytic activity to promote neovascularization. Here, we use the NMR chemical shift projection analysis (CHESPA) to clarify the mechanism of ligand binding in human angiogenin, further providing information on long-range intramolecular residue networks potentially involved in the function of this enzyme. We identify two main clusters of residue networks displaying correlated linear chemical shift trajectories upon binding of substrate fragments to the purine- and pyrimidine-specific subsites of the catalytic cleft. A large correlated residue network clusters in the region corresponding to the V_1 domain, a site generally associated with the angiogenic response and structural stability of the enzyme. Another correlated network (residues 40–42) negatively affects the catalytic activity but also increases the angiogenic activity. ^{15}N-CPMG relaxation dispersion experiments could not reveal the existence of millisecond timescale conformational exchange in this enzyme, a lack of flexibility supported by the very low-binding affinities and catalytic activity of angiogenin. Altogether, the current report potentially highlights the existence of long-range dynamic reorganization of the structure upon distinct subsite binding events in human angiogenin.

Keywords: allostery; Carr-Purcell-Meiboom-Gill; chemical shift projection analysis; NMR; protein dynamics; relaxation dispersion; ribonuclease