NMR Spectroscopy Shows that Proteins as Simple as RNA Can Form Stable and Specifically Folded Structures

D.V. Laurents[1], J.P. López Alonso[1], M.A. Pardo Cea[1], J. López de la Osa[1], A. Chakrabartty[2], C. González Ibáñez[1]

[1]Instituto de Química Física "Rocasolano", C.S.I.C., 28006 Madrid, Spain
[2]Departments of Medical Biophysics and Biochemistry, University of Toronto, M5G-2M9 Toronto, Canada

We have used NMR spectroscopy to determine the stability and structure of a peptide family called KIA7 composed chiefly of just three residues; namely Lys, Ile and Ala. The sequence of this peptide family is: acetyl-AKAAAAAIKAIAAIIKAGGϕ-amide, where ϕ is I, H, Y, F, or W. Some results for KIA7I, F and Y have been reported.[1] NMR spectra (1D ^{1}H, 2D ^{1}H TOCSY, 2D ^{1}H NOESY and 2D ^{1}H-^{13}C HSQC) were recorded on a Bruker 800 MHz AV spectrometer equipped with a cryoprobe. Measurements were made at 5 ºC in 200 mM NaCl, 50 μM DSS and 10 mM NaAc/HAc pH 5.0. After spectral assignment, about 1500 distance constraints derived from NOE signals of each peptide and the computer programs DYANA and AMBER were used to calculate and refine the structures of KIA7H, KIA7Y, KIA7F and KIA7W. The results show that KIA7Y, KIA7F and KIA7W tetramerize and adopt four helix X-bundle structures. Folding occurs on the ms timescale. The twelve Lys of the tetramer are solvent-exposed, while Ala and Ile side chains pack to form a well ordered hydrophobic core. Although KIA7H is denatured under the conditions mentioned above, it folds mildly alkaline conditions where the imidazole ring is neutral. The KIA7H structure is highly similar to those of KIA7Y and KIA7F. The side chain of the C-terminal residue caps the hydrophobic core and makes key contributions to the tetramers' stability and structure. Indeed KIA7I, with the smallest side chain, shows some characteristics of a molten globule and others of a well-folded protein. Interestingly, KIA7I can bind to benzene and phenol as detected by intermolecular NOEs. KIA7H is marginally stable. KIA7Y and KIA7F have stabilities comparable to those of small natural proteins: ΔG_{HX} = 2.0 kcal/mol, ΔG^{o} = 20 kcal/mol. KIA7W is more stable: ΔG_{HX} = 4.0 kcal/mol. In KIA7W, the packing of the bulky indole rings alters the orientation of the helices. The stability and specificity of these structures arises from a severely limited set of amino acids and just a few types of stabilizing interactions. This indicates that for proteins, a sequence composition as simple as RNA's can direct the formation of a complex structure. Both KIA7W and KIA7H interact with RNA and seem to accelerate RNA degradation. The KIA7 peptides are composed chiefly of amino acids which are thought to have been common on the Prebiotic Earth. Therefore, an interesting though speculative implication for these findings is that well folded proteins could have arisen earlier than is generally accepted and might have co-inhabited the RNA World.

[1] López de la Osa, J; Bateman, D.A.; Ho, S.; González, C.; Chakrabartty, A.; Laurents, D.V., *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 14941-14946.

This work has been carried out with financial aid of grant CTQ2004-08275 from the Spanish Ministry of Education and Science, grant NIRG-04-1083 from the Alzheimer's Association and a grant from the National Science and Engineering Research Council of Canada.