Resonance assignment, solution structure and H/D exchange of eosinophil cationic protein

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Eosinophil cationic protein (ECP)/ribonuclease-3 is a member of the RNase A superfamily involved in inflammatory processes mediated by eosinophils. ECP is bactericidal, helminthotoxic, and cytotoxic to tracheal epithelium cells and to several mammalian cell lines, although its RNase activity is low. Human ECP, found in the large specific granules of eosinophils, is a single polypeptide of 133 residues and a molecular mass of 15.5 kDa, with a high positive charge (19 arginines and 1 lysine compared to only 6 aspartates). In previous studies (Protein Sci. 2006, **15**: 2816:2827), we analyzed the thermal unfolding and stability of ECP by a variety of experimental techniques (4^{th} derivative UV, CD, DSC and FTIR), where we found that ECP is highly stable ($T_{1/2} \approx 72$ºC), and that the thermal unfolding of the protein is a nonreversible process.

Here, we have assigned practically all resonances of ^{1}H, backbone $^{13}C'$ and proton-bearing nuclei of ^{13}C and ^{15}N of uniformly labelled ^{13}C-^{15}N-ECP. A human synthetic gene was expressed in the *E. coli* BL21 (DE3) strain in a minimal medium containing $^{15}NH_4Cl$ and u-$^{13}C_6$-D-glucose to obtain recombinant ^{13}C-^{15}N-ECP. A series of 2-D and 3-D homo- and hetero-nuclear spectra were obtained at 800 MHz for the purposes of assignment and structure calculation. The program CYANA-2 was used for the latter objective, using as constraints: distances derived from observed NOEs in 2D-NOESY in $^{1}H_2O$, 2D-NOESY in $^{2}H_2O$ and 3D-^{15}N-HSQC-NOESY, together with torsion angles derived from application of the program TALOS. The pairwise RMSD of the 20 lowest energy models of the three-dimensional structure (residues 4-133) was of 0.60Å for the backbone nuclei and 1.30Å for all heavy atoms. The resulting structure is very similar to those determined by X-ray crystallography, and to the solution structure of bovine pancreatic RNase A, the paradigm of the superfamily, although some small differences are notable. In a preliminary study, the exchange rates H/D of amide protons have been measured. A group of 16 amide ^{1}Hs are resistant to exchange at pH 6 and 35ºC after some 120 hours, in agreement with the high stability of the protein. They are located in helix α1, helix α2, strand β-1, strand β-2, strand β-3, strand β-4 and strand β-5. It is of note that no proton in helix α-3 is protected against exchange. These data, obtained under conditions where unfolding is reversible, are used to place limits on the free energy of the conformational stability. The resonance assignment will be employed to obtain further information on the unfolding process and to study the structural determinants of ECP cytotoxic activity. To understand the ECP RNase catalytic mechanism, complexes with RNA substrate analogues will be characterized. Moreover, we will study the protein binding to unique bacteria cell wall components with the aim of determining the molecular basis of the ECP antimicrobial activity.