Structural Analysis of the Growth Arrest and DNA Damage Protein (Gadd45α) by NMR

R. Sánchez[1], D. Pantoja-Uceda[2], J. Prieto[3], D. Torres[3], R. Campos-Olivas[3] y F. J. Blanco[1]

[1]Structural Biology Unit, CIC Biogune, Technological Park of Bizkaia, Ed. 800,
E-48160 Derio (Email: rsanchez@cicbiogune.es)
[2]Molecular Structure and Spectroscopy Department, NMR, Instituto de Química-Física
Rocasolano, Serrano 119, E-28006, Madrid
[3]Structural Biology and Biocomputing Programme, Spanish National Cancer Center
(CNIO), Melchor Fernández Almagro 3, E-28029, Madrid

Gadd45α (Growth arrest and DNA damage α) is one of the three members of the family of Gadd45 human proteins, originally identified from genes induced after cell growth arrest and DNA damage[1]. Gadd45α is an acidic protein of 18.3 kDa localized predominantly in the nucleus. Its biological function is unclear, although it seems to be involved in growth control, maintenance of genomic stability, DNA repair, cell cycle control and apoptosis[2].

After unsuccessful attempts by other groups to crystallize this protein, we decided to address its structural analysis and interactions with other proteins (Aurora A and PCNA) by NMR. In order to reach this goal, we have overexpressed and purified Gadd45α protein uniformly labelled (^{15}N and ^{13}C/^{15}N). We have also obtained Gadd45α samples selectively ^{15}N labelled in the amino acids leucine, isoleucine, valine and phenylalanine/tyrosine to facilitate the assignment of overlapping resonances. By means of ^{1}H-^{15}N-HSQC spectra we determined the best conditions for NMR analysis of this protein, which shows a tendency to aggregate. We have found that at concentrations < 200 μM the protein is predominantly monomeric, as determined by analytical ultracentrifugation.

By means of triple resonance experiments, we have assigned 91% and 86% of the Gadd45α backbone and side chains resonances, respectively. From the backbone chemical shifts, the elements of the secondary structure of the protein (5 α-helices and 5 β-strands) and two random coil regions have been identified. These two segments (the first N-terminal 15 residues, and the loop residues 103-122) are flexible in solution, as confirmed by heteronuclear NOE {^{1}H}-^{15}N measurements.

The marginal stability of the protein, its tendency to aggregation and the low concentration of the samples posed great difficulties for its structural analysis by NMR. Nevertheless, from 3D and 4D NOESY experiments ^{13}C and/or ^{15}N edited, and 2D NOESY spectra registered at 900MHz with a cryoprobe, we have obtained a set of distance and angular constraints, that define a global fold consisting of a central mixed 5 parallel-antiparallel β-sheet with the 5 helices distributed on both sides of the β-sheet.

[1]. Fornace, Jr, A.J.; Alamo, I.; Hollander, MC, *PNAS*, **1988**, *85*, 8800-8804
[2]. Hollander, M.C.; Fornace Jr, A.J., *Oncogene*, **2002**, *21*, 6228-6233.