Solution Structure of Hirsutellin A: an example of an economic ribotoxin

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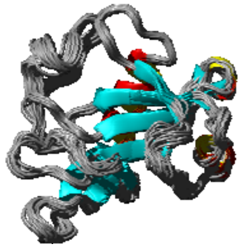
Hirsutellin A (HtA) is a 130 residues extracellular protein produced by the invertebrate fungal pathogen *Hirsutella thompsonii*. This protein displays biological properties similar to those of the ribosome-inactivating proteins (RIPs) of the α-sarcin family and, as these ribotoxins, is highly basic, possesses cytotoxic activity and inhibits protein synthesis and protein translation via site-specific cleavage of ribosomal RNA[1,2]. The sequence alignment with microbial RNases and ribotoxins suggests that the common structural core is conserved in HtA being the most significant difference the length of the loops connecting the α-helical and the β-sheet regions[3] which are thought to be involved in specific activities and cytotoxicity[4,5].

The focus of our work is on understanding the structural requirements for the specific activities of these proteins. The identification of the structural segments or motifs contributing to the different biological behaviours is of great interest as it would open the door to engineering proteins with novel properties. In this context, the three-dimensional structure of HtA in aqueous solution has been determined by NMR. The structure is well determined (pairwise RMSD = 0.98 Å for all backbone atoms), and the global fold is similar to that reported for cytotoxins. However differences in the conformation and length of the different loops can be appreciated. As HtA has all the specific properties of a cytotoxin in spite of its short sequence it can be considered a more evolved and economic ribotoxin that can play the same roles as the larger members with less players. The differences shown in the relative position of the catalytic residues in the active site will be discussed.



NMR solution structure of HtA

[1] Martínez-Ruiz, A.; Martínez del Pozo, Á; Lacadena, J.; Oñaderra, M.; Gavilanes, J. G., *J. Invertebr. Pathol*., **1999**, *74*, 96-7.
[2] Liu, J.-C.; Boucias, D.G.; Pendland, J.C.; Liu, J.-Z.; Maruniak, J., *J. Invertebr. Pathol.,* **1996**, *67*, 224-8.
[3] Herrero-Galán, E; Lacadena, J.; Martínez del Pozo, Á.; Boucias, D.G.; Olmo, N.; Oñaderra, M.; Gavilanes, J.G., *Prot. Sci.,* **2008***, in press.*
[4] Pérez-Cañadillas, J.; Santoro, J.; Campos-Olivas, R.; Lacadena, J.; Martínez del Pozo, Á.; Gavilanes, J.G., Rico, M.; Bruix, M., *J. Mol. Biol.*, **2000**, *299*, 1061-73.
[5] García-Mayoral, M.F.; García-Ortega, L.; Lillo, P.; Santoro, J.; Martínez del Pozo, Á.; Gavilanes, J.G.; Rico, M.; Bruix, M., *Prot. Sci.,* **2004**, *13*, 1000-10

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