Structural characterization of natively unfolded USrc by NMR and SAXS

Yolanda Pérez[1], Margarida Gairí[2], Pau Bernadó[1], and Miquel Pons[1,3]

[1] Institute for Research in Biomedicine, Parc Científic de Barcelona, 08028 Barcelona, Spain.
[2] Unitat de RMN d'Alt Camp, Serveis de Suport a la Recerca, Universitat de Barcelona, 08028 Barcelona, Spain
[3] Departament de Química Orgànica, Universitat de Barcelona, 08028 Barcelona, Spain.

The N-terminal region of human c-Src kinase, comprising the SH4 and unique domains (1-84, USrc), is a natively unfolded region for which the available information is still scarce. We present here the structural characterization of natively unstructured USrc by SAXS and high resolution NMR techniques[1]: (i) ^{15}N residual dipolar couplings (RDCs), (ii) paramagnetic relaxation enhacement (PREs) from nitroxide spin labels attached to Cys mutants (A2C, A59C and A87C) to identify long-range contacts and (iii) chemical shift analysis (CSA) for H^N, N^H, Cα, Cβ and C' atoms to characterize secondary structure propensity. SAXS and CSA results indicated that the USrc behaves essentially as a random coil. Both techniques are less sensitive to the presence of low populated conformations with small degrees of structure than RDCs. RDCs suggest that in the 60-75 region there is a small population of helical structure (at both acid and neutral pH) that disappear upon addition of 4 M urea.

Until now, there are very few studies about the function of this disordered region. Considering that the N-terminal regions of c-Src family kinases are highly divergent and that most of their direct activities can result from the interaction with binding partners, probably their different sequences are important to the specificity on molecular recognition processes. Previous investigations have identified NADH dehydrogenase subunit 2 (ND2) as a c-Src unique domain interacting protein.[2] Our present results shows that residues 312-329 of ND2 interact directly with residues 4-7 of c-Src. Residues 47-49 of c-Src interact with other c-Src molecules in vitro suggesting that is also a relevant region for protein-protein interactions involving the unique domain.[3]

Disordered regions are important as sites of post-translational modifications. For USrc it has been described different sites of serine/threonine phosphosrylation.[4,5] We have studied the structural effects of phosphorylation at Ser17 by NMR. Our results suggest that phosphorylation only modifies the conformational sampling locally.

[1] Bernadó, P.; Blanchard, L.; Timmins, P.; Marion, D.; Ruigrow, R.W.H.; Blackedge, M., *Proceedings of the National Academy of Sciences USA*, **2005**, *102*, 17002-17007.
[2] Gingrich, J.R., *et al*., *Proceedings of the National Academy of Sciences USA*, **2004**, *101(16)*, 6237-6242.
[3] Pérez, Y.; Gairí, M.; Bernadó, P.; Pons, M., *Journal of Molecular Biology*, Sent, in revision.
[4] Walker, F.; deBlaquiere, J.; Burgess, A.W., *The Journal of Biological Chemistry*, **2003**, *268*, 19552-19558.
[5] Shenoy, S.; Chackalaparampil, I.; Bagrodia, S.; Lin, P.; Shalloway, D., *Proceedings of the National Academy of Sciences USA*, **1992**, *89*, 7237-7241.

This work has been carried out with financial aid of Spanish Ministry of Education and Science, EMBL-Hamburg outstation and EU-NMR.