Structural determinants of the regulatory action exerted by the amino-terminal region of hSos1 on the Ras-GEF activity

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Introduction

The information carried by the aminoacidic sequence can be used by different bioinformatic methods, in order to predict the 3D structure of a protein or its domains. The tools for sequence alignment permit to individuate homologous regions among proteins, and this represent the basis for a homology modeling procedure. The algorithms of secondary structure prediction use chemical, physical and statistical parameters to recognize if a region of sequence could assume a specific secondary structure. Fold recognition servers can test if a protein sequence is compatible with one of the known folds in the PDB. If these different tools give rise to homogeneous responses, it is possible to predict with good reliability the fold of a protein or single domains of unknown structure.
hSos1 is a multidomain protein involved in the activation of the Ras signaling by catalyzing guanine nucleotide exchange on Ras [1]. The Ras-GEF domain of hSos1 (Sos-Cat) is flanked by amino- and carboxyl-terminal regions, which are able to inhibit hSos1 activity towards Ras. To investigate the structural determinants of this inhibition, it is necessary to know the structural features of the involved domains.
The carboxyl terminus of hSos1 contains a proline rich domain with consensus sequences for binding to the SH3 domains, while the amino-terminal region of hSos1 includes three domains: Histone domain, Dbl Homology domain (DH) and Pleckstrin homology domain (PH). The Histone domain is involved in the inhibition of the Ras-GEF activity of hSos1. It can also bind the PH domain, while it cannot interact with the DH domain [2]. The DH domain is implicated in the inhibition of the Ras-GEF activity of hSos1, possibly through direct interaction with Sos-Cat [1]. The PH domain is able to interact with the DH domain [3]; the crystal structure of the PH-DH complex is available [4].
We have focused on the intra-molecular interactions that occur among these domains in the activation/inhibition of hSos1 by means of computational tools, like the low-resolution protein-protein docking [5]. The essence of the procedure is the reduction of protein structures to digitized images on a three-dimensional grid. The structural elements smaller than the step of the grid (e.g., atom-size) are not present in the docking. This feature permits to reduce the negative effect of structural changes upon complex formation on docking calculation.

Results

The region of hSos1 containing residues 90-190 is clearly homologous to the sequence of histone H2a [2], while the segment upstream was not reported to share any homology with proteins of known structure. We have studied the segment 1-89 by means of fold recognition. The results we obtained indicated that this region is compatible with the structure of histone H2b; a subsequent analysis based on secondary structure prediction servers reinforced this hypothesis. Moreover, sequence alignment between histone H2b and hSos1 put in evidence a remote similarity, and the conservation of a pattern of hydrophobic residues, typical of histones.
Therefore, we supposed that the region 1-190 of hSos1 could assume a fold very similar to the structure of the H2a-H2b dimer. We obtained a model of this domain by means of homology modeling, using the histone dimer

H2a-H2b of *Gallus gallus* as a template. Subsequent publication of the crystal structure of the Histone domain of hSos1 confirmed our prediction [6]. The structural differences between our model and the PDB record were very low, thus confirming the quality of the alignment on which the model was based.
The application of the low resolution docking revealed that the PH domain could bind the Histone domain in the same region of surface that is implicated in the interaction with the DH domain.
Experiments of functional complementation of *cdc25* temperature sensitive yeast mutant showed that hSos1 full length and a mutant with a deletion in the PH domain are not active on Ras, while the deletion of the DH domain or the Histone domain brings the protein to a fully active state. However, a mutant of hSos1 lacking the carboxyl-terminal domain is partially active on Ras. Per se these data indicate that both the Histone domain and the DH domain constraint the Ras-GEF domain, in cooperation with the carboxyl-terminal domain. The PH domain seems not to be involved in the inhibition.
The computational data above reported provide an intriguing model for the interpretation of the experimental results suggesting that the mechanism of inhibition of hSos1 exerted by the amino-terminal region is based on the network of competitive interaction inter-played by the Histone, DH and PH domains.
In order to validate these hypothesis, we are now developing, by means of the low resolution docking, a computational analysis on the surface compatibility allowing the interactions between the different domains of hSos1. In addition, we are performing a study on the constraints imposed by the linker regions between the domains, in order to obtain a reliable 3D model of this protein.

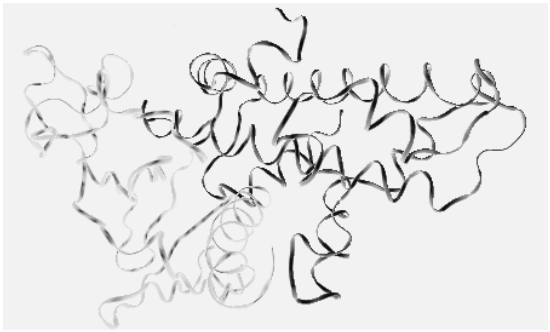

Fig.1 Results of docking applied to the PH and Histone domains

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