Protein docking by a combined approach

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Motivation

Urokinase plasminogen activator (uPA) is a 50 kDa serine protease that has been implicated in a number of physiological and disease processes, including tumor growth and metastasis, angiogenesis, and inflammation. Many of these activities of uPA are mediated at the cell surface through a specific receptor (uPAR). uPA is a multidomain protein comprised of three domains: an N-terminal growth factor domain, a kringle domain and a serine protease domain. The growth factor and the kringle domain represent the amino-terminal fragment of uPA (ATF) that contains all molecular determinants required for binding to uPAR and the interactions of the ATF and uPA with uPAR are indistinguishable kinetically. uPAR is also a protein composed of three domains: domain DI , domain DII and domain DIII that are homologous with respect to the arrangement of disulfide bonds, but differ in their amino acid sequence.

Both uPA and uPAR are reported to interact with cell adhesion receptors of the integrin superfamily, including subfamilies beta1, beta3, and beta5, as well as the beta2 integrin subfamily expressed in cells of hematopoietic lineage. Functional uPA/uPAR/integrin association modulates various aspects of cell physiology, such as motility, mitogenic activity or adhesive phenotype. Binding of ATF to uPAR typically strengthens uPAR/integrin interactions, and could elicit the integrin-mediated signaling, but the mechanisms underlying these phenomenona are not clear. We wish to understand how uPAR and integrins interact at molecular level

Methods

The crystal structures of uPAR (PDB entries 1YWH and 2FD6) as well as the crystal structure of uPAR-ATF complex (PDB entry 2I9B) have been solved. Unfortunately all models lack a motif Gly133-Glu134-Glu135-Gly136 harboured in a loop in the domain DII of uPAR implicated in integrin signalling [Degryse et al., 2005].

The sequence of the loop harbouring the motif guided the synthesis of a peptide, named D2A,which blocks the formation of uPAR- v 3 and uPAR- 5 1 complexes, and interacts directly with at least two integrins. We determined the NMR structure of D2A. Homonuclear 2D clean TOCSY and NOESY spectra were recorded at 280 K on an Avance 400 Bruker spectrometer. From the analysis of D2A NOESY spectra 32 sequential and 6 short- and medium-range unambiguous NOEs were obtained, apart from the intraresidual ones The structure of peptide D2A was calculated and validated using CNS exploiting simulated dynamical annealing with NOEs, coupling constants and chemical shift restraints .

Comparative modelling was carried out with the program MODELLER , using the structure of uPAR (1YWH) or the structure of uPAR/ATF complex (2I9B) as templates and a file of restraints derived from NMR experiments. Models were submitted to the program SCWRL , which optimizes side-chain conformations.

Modelled uPAR and uPAR ATF where docked onto integrin in closed (PDB entry 1JV2) or opened (1TXV) conformation using FTDOCK . Then, the docking solutions are automatically ranked evaluating the binding electrostatics energy and the desolvation enerergy.

Results

NOE spectra of the synthetic peptide H2DA, derived from the sequence of uPAR and able to block its interactions with integrins reveals the presence of NHi-NHi+1 and medium-weak unambiguous beta CHi-NHi+1 cross-peaks, together with some unambiguous medium-range connectivities) which are indicative of local structure and short-range order.

We assumed that the local structure of the peptide in solution can be preserved a least in part when the peptide is nested into uPAR. We modeled the loop 139-142 of uPAR implicated in integrin signalling using NOE restraints. We were able to select a model of uPAR and one of uPAR-ATF where NMR restraints were not violated by more than 0.5 ï¿½and dihedral angles of the modelled loop 139-142 of uPAR did not fall outside the allowed regions of the Ramachandran plot .

Complexes between uPAR and integrin (closed and opened form) and uPAR/ATF and integrins (closed and opened forms) were visually analysed. We tried to reconcile data deriving from published experiments on point mutations which affect biological processes potentially triggered by the interaction of uPAR and

integrins with the complexes generated in silico.
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