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Crystal Structure of the Urokinase Receptor in a Ligand-Free Form

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The urokinase receptor urokinase-type plasminogen activator receptor (uPAR) is a surface receptor capable of not only focalizing urokinase-type plasminogen activator (uPA)-mediated fibrinolysis to the pericellular microenvironment but also promoting cell migration and chemotaxis. Consistent with this multifunctional role, uPAR binds several extracellular ligands, including uPA and vitronectin. Structural studies suggest that uPAR possesses structural flexibility. It is, however, not clear whether this flexibility is an inherent property of the uPAR structure *per se* or whether it is induced upon ligand binding. The crystal structure of human uPAR in its ligand-free state would clarify this issue, but such information remains unfortunately elusive. We now report the crystal structures of a stabilized, human uPAR (H47C/N259C) in its ligand-free form to 2.4 Å and in complex with amino-terminal fragment (ATF) to 3.2 Å. The structure of uPAR$^{H47C/N259C}$ in complex with ATF resembles the wild-type uPAR·ATF complex, demonstrating that these mutations do not perturb the uPA binding properties of uPAR. The present structure of uPAR$^{H47C/N259C}$ provides the first structural definition of uPAR in its ligand-free form, which represents one of the biologically active conformations of uPAR as defined by extensive biochemical studies. The domain boundary between uPAR DI–DII domains is more flexible than the DII–DIII domain boundary. Two important structural features are highlighted by the present uPAR structure. First, the DI–DIII domain boundary may face the cell membrane. Second, loop 130–140 of uPAR plays a dynamic role during ligand loading/unloading. Together, these studies provide new insights into uPAR structure–function relationships, emphasizing the importance of the inter-domain dynamics of this modular receptor.

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Abbreviations used: uPAR, urokinase-type plasminogen activator receptor; uPA, urokinase-type plasminogen activator; ATF, amino-terminal fragment; PDB, Protein Data Bank; suPAR, soluble uPAR; GPI, glycosylphosphatidylinositol.

Introduction

The urokinase-type plasminogen activator receptor (uPAR) is a glycolipid-anchored membrane protein consisting of three Ly6/uPAR/α-neurotoxin-like (LU) domains (designated DI, DII, and DIII). uPAR binds the serine protease urokinase-type plasminogen activator (uPA) or its zymogen (pro-uPA) with very high affinity and specificity.[1–3] This interaction is mediated by the amino-terminal fragment (ATF, residues 1–143) of uPA[4,5] and localizes uPA-mediated plasminogen activation at cell surfaces both *in vitro*[6] and *in vivo*.[7,8] This cell surface proteolytic activity leads to fibrin clearance,[9] extracellular matrix remodeling,[10,11] growth factor activation,[12] the initiation of intracellular signaling,[3,13] neutrophil infiltration,[14–16] and phagocytotic clearance of apoptotic cells.[17,18] In line with these pleiotropic biological effects, uPAR is claimed to interact with a range of protein ligands, including uPA, vitronectin,[19–21] several integrins,[22,23] G-protein-coupled receptor,[24,25] epidermal growth factor receptor,[25] low-density lipoprotein receptor-related protein,[26] high-molecular-weight kininogen,[27,28] factor XII,[29,30] and others. Among these ligands, only uPA and vitronectin are thoroughly characterized in their interactions with uPAR from a biochemical as well as a structural perspective. The functional epitopes on uPAR for both these ligands have been identified by systematic alanine scanning mutagenesis,[31–33] and we have in addition reported the crystal structure of a ternary complex between soluble uPAR (suPAR), ATF, and the somatomedin B domain (SMB; residues 1–44) of vitronectin.[34] This structure confirmed the binding epitopes that were identified through previous biochemical studies and showed that uPAR can accommodate both ATF and SMB of vitronectin without generating steric clashes in the tri-molecular complex. ATF binds at the central pocket of uPAR formed by all three domains, whereas SMB binds at the outer side on the interface between DI and DII. Structurally, the binding of SMB to uPAR does not perturb the structure of the binary uPAR·ATF complex.

The molecular mechanism by which such a relatively small receptor (283 amino acids) recognizes an array of protein ligands is largely unknown. A certain level of conformational flexibility in uPAR is evident from the structural studies on the uPAR–ligand interactions. In the uPAR·ATF complex,[5] all three LU domains of uPAR assemble to form a central pocket, which accommodates the growth factor domain (GFD) module of uPA. The three domains arrange in a triangular way, leading to direct contact between DI and DIII. Such direct DI–DIII contacts are intriguingly lost in the uPAR·peptide structure[4] due to about ~30° rotation of the central DIII β-sheet (when DI's were superimposed). This rotation of the DIII β-sheet is also observed in the murine uPAR·ATF structure.[35] Such conformational flexibility may represent one mechanism by which uPAR accommodates multiple protein ligands. However, it is not clear whether this flexibility is an inherent property of the uPAR structure *per se* or whether the flexibility is related to ligand binding. A crystal structure of human uPAR in its ligand-free conformation would clarify this issue. Such information is still lacking, presumably due to the fact that the suPAR is prone to aggregate in the absence of its uPA ligand, and such conformational heterogeneity generally imposes severe barriers on structural studies. For this and other reasons, we have recently generated a stabilized form of human uPAR (H47C/N259C) by engineering an inter-domain disulfide bond to cross-linking DI and DII.[36] Here we determine the crystal structure of a complex between this soluble $uPAR^{H47C/N259C}$ mutant and ATF and demonstrated that the engineered disulfide bond does not perturb the overall structure of uPAR. This mutant has a homogeneous conformation in solution, which enabled its crystallization and allowed us to determine the crystal structure of ligand-free uPAR at 2.4 Å. This is the first snapshot image of uPAR in its ligand-free form, providing the first insights into the conformational flexibility of this receptor. From the structural dissection, we now show that the DI–DII domain boundary is more flexible than the DII–DIII domain boundary, which may account for the previously noted variability in the displacement of the DI–DIII boundary dependent on which ligand engages the central binding cavity. We also demonstrate that loop 130–140 linking strands βIIC and βIID plays a dynamic role in regulating ligand loading/unloading into the central uPAR cavity.

Results and Discussion

Strategy for the crystallization of ligand-free uPAR

suPAR, lacking the glycosylphosphatidylinositol (GPI) anchor, binds its *bona fide* protease ligand (uPA) with an affinity identical with that of the full-length wild-type uPAR. Recombinant suPAR expressed by *Drosophila* S2 cells exhibits a macroheterogeneity in its glycosylation profile comparable to that of wild-type uPAR, but the attached carbohydrate chains are generally smaller and more homogenous.[37,38] This recombinant suPAR is accordingly optimally suited for structural studies of the uPAR–ligand interaction. One-step affinity purifications on either an antagonist peptide column[39] or an uPA column[40] seems at present the most well suited purification protocols for recombinant suPAR with a view to subsequent X-ray