The small GTP-binding protein RhoA participates in regulation of actin cytoskeleton reorganization and cell adhesion through specific effector proteins. Rho-binding domains of these effector proteins have been classified into at least two motifs, Class 1 characterized by a polybasic region followed by a leucine-zipper-like region and Class 2 by a putative coiled-coil forming sequences. To understand the molecular mechanisms of the Rho effector recognition, we have determined the crystal structure of the Rho-binding domain of Rho-kinase, which has a Class 2 Rho-binding motif, and compared the structure with the previously solved structure (1) of a Class 1 motif, human protein kinase N (PKN) bound to RhoA. The Rho-binding domain of Rho-kinase has appeared to form a dimer featuring a parallel coiled-coil with two long consecutive helices extending to about 97 Å. This structure is dissimilar to that of the PKN Rho-binding domain, which features a short α-helix and two long α-helices forming an anti-parallel coiled-coil fold, and to those of other binding motifs for small GTP-binding proteins including the other Rho-family members, Rac and Cdc42 (2).

References

Keywords: G PROTEIN EFFECTOR RHOA

The cytoplasmic tyrosine kinase Csk plays pivotal roles in cell signaling by phosphorylating an inhibitory tyrosine residue on the C-terminal tail of the Src family kinases (SFKs). The enzyme consists of an SH1, an SH2, and a kinase domain (KD) in this order. It has been reported the inhibited forms of the SFKs take compact structures in which the SH2 domain binds to the phosphorylated C-terminal tail. Though Csk and SFKs have similar domain ordering (SH3-SH2-KD), Csk lacks regulatory tyrosine residues which is in SFKs. Thus it was suspected that the domain arrangement of Csk is distinct from that of SFKs.

We have determined the crystal structure of full-length Csk at 2.5 Å resolution. As expected the structures of the three domains resemble those of the SFKs. However the disposition of these structural elements is quite different. Especially the SH2 domain, which interacts with the C-terminal lobe of the KD in the SFKs faces outward the molecule and exclusively contacts with the N-terminal lobe in Csk. In addition two highly-conserved domain linkers make extensive hydrophobic interaction with the N-lobe. It is known that Csk is recruited to a membrane microdomain, so-called lipid Rafts and directly activated by a transmembrane phosphoprotein Cbp/PAG. To elucidate the structural basis of this phenomenon, the atomic details of the association of these two proteins are now under investigation.

Keywords: CSK SRC SRC HOMOLOGY

The endothelial-specific receptor tyrosine kinase Tie2 (tyrosine kinase with immunoglobulin and EGF-like domains 2) plays a key role in the development of the embryonic vasculature and tumor angiogenesis. The extracellular region of Tie2 contains two Ig-like, three EGF-like and three fibronectin type III domains. To characterize the domains involved in ligand binding, we constructed various deletion mutants of the extracellular region. We report here the crystal structure of the immunoglobulin (Ig)-like domain of Tie2 (Tie2-D5; amino acids No. 345-443). The crystal structure shows that the Tie2-D5 is a β-sandwich. The superposition with other Ig-like domains mostly comes from the relative orientation between two β-sheets. The bulky side chain of W422 between strand A and B may fix the direction of β-sheet. W422 and several hydrophobic residues also generate the large cavity. W422 is one of the twenty residues, which comprise the V frame set, a key framework in many Ig-like domains. Most of receptor tyrosine kinases have I1 set Ig-like domains including telokin, FGK receptor, VEGF receptor and several cell adhesion molecules result in large deviations. These differences mostly come from the relative orientation between two β-sheets. The bulky side chain of W422 between strand A and B may fix the direction of β-sheet. W422 and several hydrophobic residues also generate the large cavity. W422 is one of the twenty residues, which comprise the V frame set, a key framework in many Ig-like domains. Most of receptor tyrosine kinases have I1 set Ig-like domains for the ligand-binding or receptor oligomerization. Considering these observations, the relative orientation of two β-sheets and the resulting large cavity may be involved in the function of Tie2-D5.

Keywords: RECEPTOR KINASE IMMUNOGLOBULIN