The Solution Structure of the Pleckstrin Homology Domain of Human SOS1

A POSSIBLE STRUCTURAL ROLE FOR THE SEQUENTIAL ASSOCIATION OF DIFFUSE B CELL LYMPHOMA AND PLECKSTRIN HOMOLOGY DOMAINS

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Jie Zheng‡, R.-H. Chen§, S. Corblan-Garcia§, Sean M. Cahill‡, Dafna Bar-Sagi§, and David Cowburn‡¶

From ‡The Rockefeller University, New York, New York 10021 and the §Department of Molecular Genetics and Microbiology, State University of New York at Stony Brook, Stony Brook, New York 11794

A large subset of pleckstrin homology (PH) domains are immediately to the C terminus of diffuse B cell lymphoma (DbH) homology (DbH) domains. DbH domains are generally considered to be GTPase-exchange factors; many are proto-oncogenes. PH domains appear to function as membrane-recruitment factors, or have specific protein-protein interactions. Since dual domain (DbH/PH) constructs are known to have significant properties in other pathways, it is possible that a defined interdomain relationship is required for DbH/PH function.

We determined the solution structure of the human SOS1 PH domain for a construct partially extended into the preceding DbH domain. There are specific structural contacts between the PH and the vestigial DbH domain. This appears to involve structural elements common to this subfamily of PH domains, and to DbH domains. The human SOS1 PH domain binds to inositol 1,4,5-triphosphate with a ~60 μM affinity. Using chemical shift titration, the binding site is identified to be essentially identical to that observed crystallographically for the inositol 1,4,5-triphosphate complex with the PH domain of phospholipase Cβ. This site may serve as an interdomain regulator of DbH or other domains’ functions.

While the overall fold of the human SOS1 PH domain is similar to other PH domains, the size and position of the intrastrand loops and the presence of an N-terminal α-helix of the vestigial DbH domain suggest that the subfamily of PH domains associated with DbH domains may be a well defined structural group in which the PH domain is a membrane recruiter and modulator.

The Ras exchanger “son of sevenless” (SOS) is a complex protein of at least four defined sequential segments (Fig. 1A). It is widely thought that PH domains are responsible for recruitment to a phospholipid membrane surface (1), and the PH domain of SOS has been implicated in the regulation of its membrane-targeting and catalytic activity (2–4). In SOS, the PH domain is immediately to the C terminus of a DbH domain, and so belongs to a set of DbH/PH dual domain-containing proteins, many of which are protooncogenes products (1, 5). DbH domains contain about 200 amino acids and function as guanine nucleotide exchange factors for Rho family members (5). DbH domains are invariably followed by a PH domain, suggesting a possible structural and functional interaction. The DbH/PH dual domain of TIAM1 is sufficient for activation of Jun N-terminal kinase, while each individual domain had little effect (6), and the PH domain of TIAM1 has been shown to be essential for its membrane recruitment (7). The PH domain of Bcl oncogene mediates its interaction with detergent-resistant cytoskeletal matrix (8).

The sequential conservation between PH domains is generally insufficient to permit homology modeling. Although several PH domains’ structures have been determined (9), the structure, at the atomic level, of a PH domain belonging to the DbH domain associated subfamily is not yet available. Here, we report a solution study of one member of this subfamily, the human SOS1 PH domain.

EXPERIMENTAL PROCEDURES

Sample Preparations—Recombinant human SOS1 PH domain was obtained from overexpression in Escherichia coli as described previously (4). Uniform 15N and 15N/13C labeling was achieved by growing the cell in MOPS-based minimal medium with 15NH4Cl and [U-13C]-D-glucose as sole nitrogen and carbon sources, respectively. The sequence of the final product is GSHMASMTGGQMQGRDP:MNEIQKNMDG-WEGKDIQGCN6F1MEGT1TRVGKHHERHIFLDLMICKS-NHGGPRLLPGASNAYRLKEKKFMKVQGRKNDKDTNEYKHAE-EHKLKDENSFAKSAEKNWMAALISKYRSTLE, consisting of (422–551) of the native sequence (10). The protein identity was confirmed by mass spectrometry.

NMR Experiments—NMR experiments were run on Bruker DMX600 or DMX500 spectrometers at 25 °C, and spectra were processed with WINNMR. Standard triple resonance and double resonance procedures were used (11). The mixing times used in NOESY experiments were 100 and 175 ms.

Secondary Structure Determination—Chemical shift value of 1H, 15N, 13Cα, 13CO, as well as Hα were used with the program CSA (12) to calculate the secondary structure. Seven β-strands and C-terminal α-helix were well defined in the calculation. In addition, the program indicated a short α-helix at the very beginning of the N terminus. The secondary structure was further confirmed by the H/D exchange effect; TIAM1, T lymphoma invasion and metastasis protein; NOESY, NOE spectroscopy.

§ Correspondence should be addressed: Laboratory of Physical Biochemistry, The Rockefeller University, 1230 York Ave., New York, NY 10021. Tel: 212-327-8270; Fax: 212-327-7566; E-mail: cowburn@rockefeller.edu.

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Sequential organization of SOS and its four homology segments. A, the hSOS1 sequence (10) contains a DbH domain (5), followed by a PH domain (reviewed in Shaw (1)), an intervening stretch (551–793), the CDC25 Ras-activating domain, and a proline-rich segment (PP), associated with Grb2 binding (30). The sequence expressed and structurally determined here is the gray patch. B, structure-based alignment of PH domains using pairwise superposition of the structures and direct calculation of aligned RMSDs, based on elements of secondary structure, optimized by addition of deletion of individual residue pairs. Structures are hSOS1 (this work), GRK-2/βARK-1 (D. Fushman, T. Najmabadi-Haske, S. Cahill, J. Zheng, H. LeVine III, and D. Cowburn, J. Biol. Chem., in press), dynamin (31), spectrin (18), pleckstrin (24), and PLCδ (15). The color coding corresponds to the secondary structure elements in Fig. 2 and the binding site are marked in red and underlined. C, predicted helical segments of the Dbh domain of hSOS1. D, Alignment of C terminal segment of Dbh domains, and predicted helical segments.
RESULTS AND DISCUSSION

Structure of Human SOS1 PH Domain—Attempts to express human SOS1 PH domain (443–551) in E. coli were not successful, and the protein product is insoluble. An extension to the N terminus of the PH domain (443–551) in E. coli was finally selected. In this construct, at the N terminus, there is a well defined α-helix connected to the conserved part of the structure by a flexible loop. An N-terminal α-helix was observed in the PLCδ PH domain (15), but this, however, has a different relationship to the rest of structure. The DbH domain of SOS is predicted (16) to have substantial sections of α-helix. The overall fold and surface charge of human SOS1 PH domain is shown. The topology of the fold is typical for PH domains, consisting of seven β-strands forming a β-sandwich flanked at one end by a C-terminal α-helix.

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Helical segments of the DbH domain of hSOS1, using programs DSC (17). The numbers in blocks below the sequence, labeled P_H at the left, are the deciles of the probability that the individual residue is in an α-helix. D, alignment of the C-terminal segment of multiple DbH domains, and the predicted C-terminal α-helical portion. The program CLUSTAL W (32) was used to perform the multiple alignment. Sequences are (GI = GenBank™ accession no.) CDC24 (GI1345705), DBL (GI118279), ECT2 (GI423597), FGDI1 (GI1706789), LBC (GI458210), LFC (GI1582805), LSC (GI1389756), OST (GI1083745), RasGRF (GI1083745), TIAM1 (GI897557), TIM (GI484102), and VAV (GI566213) Pfluenn/H, the decile of the probability that the individual residue in part of an α-helix (16).
in the C terminus of these DbH domains suggests that the helix, partially observed in our extended PH domain, is a constant structural part of the DbH/PH domain pair, and the defined interactions of this helix with the PH domain are also highly sequentially conserved in DbH/PH pairs (Fig. 1C). Although no structural information is available about DbH domains, secondary structure predictions (Fig. 1C) (using PHD (16) and the discrimination of protein secondary structure (17)) indicate that the DbH domain is an α-helical bundle and the last helix ends at Gln428 (hSOS1), the end of the N-terminal α-helix seen in the observed structure of the extended hSOS1 PH domain.

There is a long, disordered loop between the β3 strand and β4 strand (3/4) in the SOS PH domain. Most PH domains have a small 3/4 loop, but some loops are relatively long; there is a α-helix insert in the spectrin PH domains (18). A long 3/4 loop is a common feature among the DbH associated PH domain subfamily (19).

The SOS PH Domain Binding to Phospholipids—A general hypothesis is that PH domains recognize specifically and with high affinity anionic phospholipids, typically phosphatidylinositol-4,5-bisphosphate (PIP2). This is certainly illustrated paradigmatically for PLCδ PH domain, in which the binding of PIP2 is about 1.8 μM and IP3 is about 200 μM, and a well defined structural interaction has been characterized (20). It has been reported that hSOS1 PH domain also binds to phospholipids, especially PIP2, and some mutations of the SOS PH domain significantly reduce phospholipid binding affinity (4, 21). Fluorescence micellar binding assays (22) or vesicular assays (4) show that PIP2 as well as other phospholipids bind to the PH domain. In addition, we found, using intrinsinc fluorescence quenching in solution, that the head group of PIP2, IP3, can also bind to the PH domain. The dissociated constant for this binding is 62 ± 5 μM, which is consistent with the data obtained from NMR titration (Kd < 100 μM), and with data reported by Pawson and his co-workers (21), of about 30 μM. However, the binding data is more than 10-fold smaller than that reported by Koshiba et al. (23) obtained from NMR titration data. Nevertheless, this binding affinity is two magnitudes lower than that of the interaction between PLCδ PH domain and IP3. Other analogs have weaker binding; inositol 1,4,5,6-P4, Kd > 200 μM; inositol 3,4,5,6-P4, > 10 mM; inositol 1,3,4,5,6-P6, > 1 mM; inositol 1,3,4-P3, > 10 mM; inositol 1,2,4-P3, > 10 mM.

To map out the binding site of IP3 on the surface of the PH domain, chemical shift perturbation titration experiments (22), were performed. The amount of shift perturbation upon IP3 binding is shown in Fig. 3A using the width and color of the protein’s backbone trace. Most of the backbone 15N and 1H chemical shifts are unchanged between the free and bound forms, demonstrating that the structure of hSOS1 domain is not grossly affected by IP3 binding. However, a few resonances show substantial chemical shift changes, indicating the binding site of the IP3, formed mostly by positively charged residues, including Lys456, Arg459, Lys472, and Arg469.

This binding site is consistent with two very recent independent mutagenesis studies (4, 21). Pawson and his co-workers (21) have shown that the R459A mutation reduces PIP2 binding by 50-fold. In our chemical shift perturbation studies, Arg469 has the third largest chemical shift perturbation upon IP3 binding.

The IP3 Binding Site Is Very Similar to That of PLCδ PH Domain—This IP3 binding site on the SOS PH domain surface is apparently virtually identical to that of the PLCδ PH domain, with some subtle differences between them (Fig. 3). This binding site is also similar to the binding site of IP3 to the N-terminal pleckstrin PH domain (24, 25). In the case of PLCδ, the whole 3/4 loop bends toward to the bound IP3 and most of the residues on that loop have contacts with the bound IP3. In the SOS PH domain, only the residues on the edges of the 3/4 loop, e.g. Lys472 and Arg469, have significant chemical shift perturbation upon the IP3 binding. These differences may reflect the origin of the different binding affinities of the two domains toward the phospholipids and their head groups. The SOS PH domain, and almost all other PH domains which have been studied so far, binds to IP3, the head group of PIP2, with much lower affinity than to vesicles containing PIP2. The role of the phosphatidyl inositolide binding to SOS is unclear, and one study (4) indicates that PH domain is not localized to the membrane by PIP2. The case of PLCδ is the only exception identified so far where the PH domain can form a more stable complex with IP3, than with vesicular PIP2. This difference may indicate that the interactions between PH domains and inositol...
phosphate have different physiological functions, and this is now clearly indicated for the modulation of AKR type 8 virus homologous protooncogene via its PH domain by phosphatidylinositol 3,4,5-P₃ (26, 27). In the case of PLC-δ PH domain (15), the interaction of the PH domain with PIP₂ brings the enzyme to the membrane and allows processive substrate hydrolysis by the enzyme. The product of this hydrolysis, IP₃, competes with the membrane and allows processive substrate hydrolysis by the enzyme. For all other PH domains, no physiological function has been defined for the interactions between IP₃ and PH domains (1).

The Binding of Phospholipids May Mediate the Interaction between DbH and PH Domain—Although IP₃ binds to SOS PH domain with relatively weak affinity, it binds to the PH domain specifically on a well defined site similar to that of PLC-δ, but different from those of other PH domains (22, 28, 29). This interaction provides additional binding affinity and specificity to the SOS PH domain binding of PIP₂-containing membrane. This membrane anchoring ability is not apparently significantly reduced by a mutation within the IP₃ binding site(4); possibly the interaction between the head group of PIP₂ and the SOS PH domain may mediate the interactions of this PH domain with other proteins. The IP₃ binding site is very close to the 3/4 loop and the N-terminal α-helix; the N-terminal α-helix and IP₃ binding site sandwich the β-4 strand (Fig. 2D). The binding will certainly affect the conformation(s) of the 3/4 loop in the whole SOS molecule although we did not observe apparent changes in the 15N-HMQC-NOESY when the IP₃ was absent to the PH domain. It is possible that the binding of the higher affinity phosphatidylinositol inositides may mediate the interactions between the PH and DbH domains. Additional mutagenesis studies may test this.

The structure of human SOS PH domain represent the first structure of those PH domains which are in the subfamily associated with DbH domains. The uniquely long 3/4 loop is likely to interact with DbH domain. The SOS PH domain binds to phospholipid in a portion of the structure similar to that in PLC-δ PH domain. The spatial proximity of the IP₃ binding site and the possible DbH domain contact provides the possibility that the binding of phospholipid may affect the interaction between the PH and DbH domains. These features may be significant for other PH domains in this DbH domain-associated subfamily.

After the preparation of this article, a solution structure of the closely related mouse SOS1 (mSOS1) PH domain was published (23). As noted above, the mSOS1 PH domain is reported to have significantly lower affinity for IP₃. The general details of secondary structure and tertiary fold are apparently identical for the mSOS1 and hSOS1 PH domain.

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