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Structural landscape of the proline-rich domain of Sos1 nucleotide exchange factor

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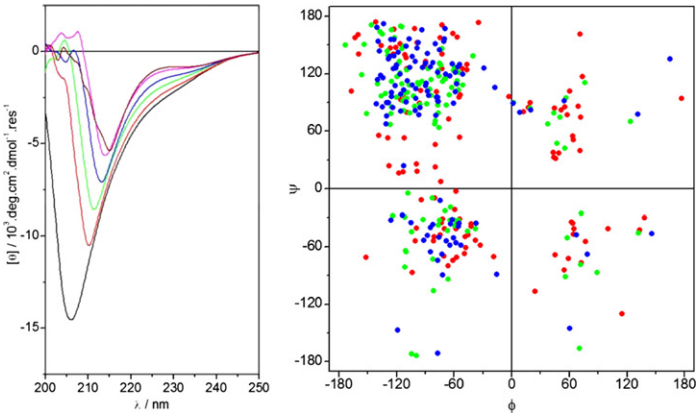
HIGHLIGHTS

- PR domain of Sos1 is structurally disordered.
- PR domain adopts an extended random coil-like conformation.
- PR domain displays a highly dynamic conformational basin.
- Chemically-denatured state of the PR domain harbors polyproline II helices.
- Chemical denaturants have little or no effect on the size of PR domain.

GRAPHICAL ABSTRACT



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ABSTRACT

Despite its key role in mediating a plethora of cellular signaling cascades pertinent to health and disease, little is known about the structural landscape of the proline-rich (PR) domain of Sos1 guanine nucleotide exchange factor. Herein, using a battery of biophysical tools, we provide evidence that the PR domain of Sos1 is structurally disordered and adopts an extended random coil-like conformation in solution. Of particular interest is the observation that while chemical denaturation of PR domain results in the formation of a significant amount of polyproline II (PPII) helices, it has little or negligible effect on its overall size as measured by its hydrodynamic radius. Our data also show that the PR domain displays a highly dynamic conformational basin in agreement with the knowledge that the intrinsically unstructured proteins rapidly interconvert between an ensemble of conformations. Collectively, our study provides new insights into the conformational equilibrium of a key signaling molecule with important consequences on its physiological function.

Abbreviations: Abi1, Abl interactor 1; CD, Circular dichroism; Cdc25, Cell cycle division 25; DH, Dbl homology; DLS, Dynamic light scattering; EGFR, Epidermal growth factor receptor; Eps8, EGFR kinase substrate 8; Grb2, Growth factor receptor binder 2; GuHCl, Guanidine hydrochloride; HF, Histone fold; LIC, Ligation-independent cloning; MAP, Mitogen-activated protein; MD, Molecular dynamics; PH, Pleckstrin homology; PPII, Polyproline II (helix); PR, Proline-rich; REM, Ras exchange motif; RTK, Receptor tyrosine kinase; SAXS, Small-angle X-ray scattering; SEC, Size-exclusion chromatography; SH3, Src homology 3; Sos1, Son of sevenless 1; TMAO, Trimethylamine N-oxide.

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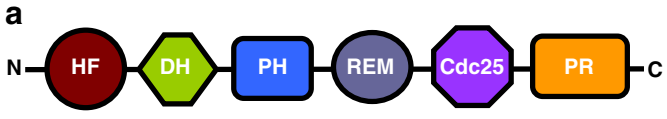
1. Introduction

Sos1 guanine nucleotide exchange factor, comprised of the HF-DH-PH-REM-Cdc25-PR signaling cassette (Fig. 1a), activates Ras and Rac GTPases that relay external signals from receptor tyrosine kinases (RTKs) such as EGFR at the cell surface to downstream effectors such as transcription factors within the nucleus [1–7]. Notably, both Ras and Rac are tethered to the inner membrane surface via prenylation and act as molecular switches by virtue of their ability to cycle between active GTP-bound and inactive GDP-bound states. How does Sos1 activate Ras and Rac?

In the cytoplasm of quiescent cells, Sos1 exists in two functional pools in complex with Grb2 and Abi1 signaling adaptors—this association is mediated via the binding of the proline-rich (PR) domain of Sos1 to SH3 domains within Grb2 and Abi1. Upon stimulation of RTKs with growth factors and hormones, the Sos1–Grb2 complex becomes recruited to the inner membrane surface from the cytoplasm in a phosphotyrosine-dependent manner. Such translocation facilitates the Cdc25 domain of Sos1 to trigger GTP-GDP exchange within Ras and, in so doing, switches on a key signaling circuit that involves the activation of downstream MAP kinase cascade central to cellular growth and proliferation [8,9]. On the other hand, Sos1–Abi1 complex is recruited to actin filaments found within membrane ruffles in an Eps8-dependent manner. Given that Rac preferentially localizes to the confined areas of membrane ruffles, the recruitment of Sos1–Abi complex to the actin cytoskeleton network aids the DH domain of Sos1 to catalyze GTP–GDP exchange within Rac and, in so doing, plays a key role in actin remodeling central to cell invasion and migration [10,11].

Interestingly, Sos1 can also be recruited to the inner membrane surface via the binding of its PH domain to phosphatidic acid, a component of phospholipids, in response to RTK stimulation with growth factors and hormones [12]. Accordingly, recruitment of Sos1 to the inner membrane surface in a PH-dependent manner provides an alternative route for the activation of Ras. However, unlike the dispensability of Sos1–Grb2 complex for the activation of Ras, the Sos1–Abi1 complex is believed to be absolutely required for the activation of Rac. Importantly, the HF and REM domains within Sos1 play a regulatory role and fine tune the activity of Sos1 [13,14]. Briefly, the binding of Ras-GTP to REM domain serves as an allosteric switch to further stimulate the catalytic activity of Cdc25 domain. In contrast, the HF domain—comprised of a tandem copy of histone folds—associates in an intramolecular manner with the PH domain and, in so doing, down-regulates the PH-dependent activation of Ras.

In an attempt to understand the physical basis of how Sos1 activates Ras and Rac GTPases, the crystal structure of a Sos1 construct containing all contiguous domains but the C-terminal PR domain was recently solved to high resolution [15]. However, structural insights into the ability of the PR domain to adopt a well-defined conformation, or lack thereof, would further our understanding of how Sos1 mediates RTK signaling. Herein, using a battery of biophysical tools, we provide evidence that the PR domain of Sos1 is structurally disordered and adopts an extended random coil-like conformation in solution. Given that many intrinsically unstructured proteins undergo folding in the presence of their cognate ligands [16–22], the possibility that the PR domain may also adopt a well-defined conformation upon binding to its ligands cannot be ruled out.



b

MHHHHHHSSGLVPRGSGMKETAAAKFERQHMDSPDLGTDDDDKIW
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QPTSKAYSPRYSISDRTSISDPPESPPLLPPREPVRTPDVFSSSP
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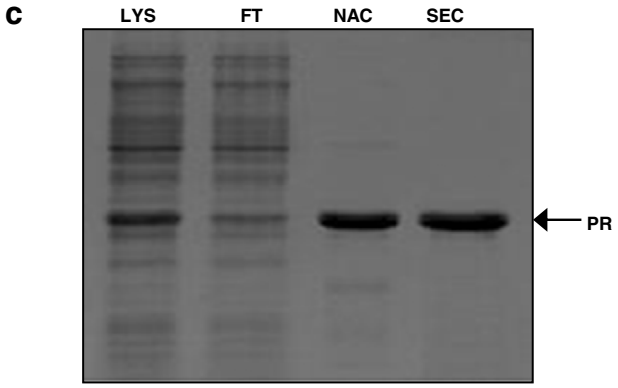

Fig. 1. Purification and characterization of the PR domain of Sos1. (a) Within Sos1 (residues 1–1333), the PR domain lies at the extreme C-terminal end. Other domains within Sos1 are HF (histone fold), DH (Dbl homology), PH (pleckstrin homology), REM (Ras exchange motif) and Cdc25 (cell division cycle 25). (b) Complete amino acid sequence of the recombinant PR domain (residues 1141–1300). The non-native amino acid residues introduced during cloning at both the N- and C-termini of the PR domain are underlined for clarity. (c) SDS-PAGE analysis of the PR domain. Briefly, total bacterial lysate (LYS) was loaded onto a Ni-NTA column, the flow-through (FT) was collected and after elution from the Ni-NTA affinity chromatography (NAC) column, the recombinant protein was further purified to apparent homogeneity by size-exclusion chromatography (SEC).

2. Materials and methods

2.1. Sample preparation

The PR domain (residues 1141–1300) of human Sos1 was cloned into pET30 bacterial expression vector with an N-terminal sequence containing an His-tag (HHHHHH) and an enterokinase cleavage site (DDDDK) using Novagen LIC technology (Fig. 1a and b). Additionally, a tryptophan (W) residue was added to both the N- and C-termini of the PR domain to aid in the quantification of protein concentration using spectrophotometry. The recombinant protein was expressed in *Escherichia coli* BL21* (DE3) bacterial strain and purified on a Ni-NTA affinity column followed by size-exclusion chromatography (SEC) on a Hiload Superdex 200 column using standard procedures as described previously [23]. Final yield was typically between 5 and 10 mg protein of apparent homogeneity, as judged by SDS-PAGE analysis (Fig. 1c), per liter of bacterial culture. Notably, the molar mass of the PR domain was estimated to be around 26 kD on the basis of SDS-PAGE analysis. This is in an excellent agreement with the theoretical molar mass of 22 kD calculated from its amino acid sequence alone. Protein concentration was determined by the fluorescence-based Quant-It assay (Invitrogen) and spectrophotometrically on the basis of an extinction coefficient of 13,980 M^{-1} cm^{-1} calculated for the recombinant PR domain using the online software ProtParam at ExPasy Server [24]. Results from both methods were in an excellent agreement. The PR domain was dialyzed into a buffer of 50 mM sodium phosphate at pH 8.0 in the presence or absence of appropriate concentrations of urea or GuHCl prior to the conduct of all biophysical measurements described hereinafter. All measurements were repeated at least three times. It should be noted that the treatment of recombinant PR domain with enterokinase substantially reduced the yield of the protein due to partial digestion. Accordingly, all experiments reported herein were conducted on the recombinant PR domain containing non-native residues at both the N- and C-termini (Fig. 1b). Importantly, while control experiments were also carried out on the cleaved construct to check that the non-native