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The Intertwining of Structure and Function: Proposed Helix-Swapping of the SH2 Domain of Grb7, A Regulatory Protein Implicated in Cancer Progression and Inflammation

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Abstract

Grb7 is a multidomain intracellular signaling protein that links activated tyrosine kinases with downstream signaling targets. Best known for its regulatory role in cell migration and tumor metastasis, Grb7 also regulates inflammation by coupling NF-kappaB-inducing kinase with erbB/EGFR family receptors. The "adaptor" role of Grb7 in these processes depends upon binding to membrane-associated tyrosine kinases through its C-terminal SH2 domain. The Grb7-SH2 domain shares structural and functional similarity with the SH2 domain of Grb2, a constituent of the MAP kinase pathway. Both domains show unusual affinity for cyclic (*beta*-turn) ligands. The Grb2-SH2 domain also shows distinctive self-association behavior, forming intertwined ("swapped") dimers. While Grb7 and its SH2 domain are each known to dimerize, the mechanisms and functional significance of this self-association are incompletely understood. Additional residues in the Grb7-SH2 domain effectively lengthen its "EF loop" and render the domain a good candidate for swapped dimerization, through exchange of a C-terminal helix. We propose the existence of a swapped dimeric form of the Grb7-SH2 domain and offer a structural model derived through novel application of nuclear magnetic resonance-derived restraints for homology model refinement.

Keywords

domain swapping; swapped; unswapped; NMR refinement; homology model; Grb2; FAK; EGFR; erbB2; receptor tyrosine kinase

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Grb7 is a five-domain intracellular protein that couples membrane-associated tyrosine kinases with downstream signaling partners. The C-terminal SH2 domain (Fig. 1) mediates physical interaction of Grb7 with activated tyrosine kinases through recognition of a phosphorylated tyrosine motif. Grb7 is primarily known as a promoter of tumor progression through regulation of cell migration signaling mediated by the FAK (focal adhesion kinase) and the EphB1 receptor tyrosine kinase.[1] As such, its overexpression in tumor tissues is prognostic of high metastatic potential.[2–6] Through a distinct pathway, Grb7 overexpression promotes inflammation by coupling NIK (nuclear factor-kappaB-inducing kinase) with growth factor receptors in the erbB/EGFR family, thereby stimulating activation of NF-κB.[7] Moreover, improper regulation of Grb7 phosphorylation may promote apoptotic escape, contributing to eosinophilia in patients with atopic dermatitis.[8] Grb7 has additionally been

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identified as a potential component of the T-cell activation pathway[9] and as a likely autoimmune target in rheumatoid arthritis.[10]

The Grb7-SH2 domain shares distinctive structural and functional features with the SH2 domain of the adaptor protein Grb2, known for its role in the MAP kinase/ERK pathway.[11] The SH2 domains of Grb7 and Grb2 show similar protein recognition behavior, binding partners in the unusual "*beta*-turn" (or cyclic) conformation and preferring an unusual phosphotyrosine motif (pYXN, where X is any amino acid).[12–15] Both Grb7 and Grb2 self-associate to form dimers. Further, the SH2 domains of both proteins are known to dimerize,[16–21] although most SH2 domains are monomeric.[17] Crystallographic studies of the Grb2-SH2 domain have shown intertwined ("swapped") dimers, in which the component monomers exchange a C-terminal helix and form two hybrid "functional monomers."[19–21] We have come to suspect that the Grb7-SH2 domain may also engage in swapped dimerization, and we propose here a structural model constructed through homology modeling coupled with nuclear magnetic resonance (NMR)-derived restraints.

Although a recent crystallographic study of the Grb7-SH2 domain found unswapped dimers,[18] this finding does not exclude the possibility that a swapped dimer could also exist. Some proteins are known to dimerize in both swapped and unswapped forms, including the well-documented case of bovine seminal ribonuclease.[22–23] While the ligand-free Grb7-SH2 domain is predominantly dimeric,[18] the relationship of its dimerization to Grb7 function remains unclear. Furthermore, the possible existence of a domain-swapped form has not been explored in the literature.

We recently carried out molecular dynamics simulations of the Grb7-SH2 domain monomer, in complex with the erbB2 receptor peptide pY1139. (The solution structure of the Grb7-SH2/pY1139 complex was solved previously by our group.[13]) Several independent explicit solvent simulations showed destructuring of the domain's C-terminal tertiary fold (Fig. 2B). Although the secondary structures remained largely intact, they lost the fixed relative positions associated with the canonical SH2 domain architecture and became more dynamic with respect to the well-folded N-terminal region. Affected structures include the D'E and EF *beta*-sheets, as well as the *alpha*-B helix and the BG loop (using SH2 domain nomenclature established by Eck et al.[24]). The destructuring occurred in few-nanosecond simulations at 300 K with a 2-femtosecond time step, but was not seen in a 21-nanosecond simulation at 300 K with a 1-femtosecond time step and with lower average values for total and potential energy (Fig. 2A; energy data not shown). The Grb7-SH2 domain's propensity for conformational flexibility in the C-terminal region is supported by a corresponding sparsity of NMR-derived distance restraints, particularly in the D'E and EF-loop region.

Given the helix-swapping behavior of the Grb2-SH2 domain, we suspect that the partially destructured Grb7-SH2 domain represents an intermediate in the formation of a helix-swapped dimer. Our results seem to conform to a model recently proposed by Malevanets et al. regarding the general mechanism of swapped dimer formation. Namely, our partially destructured monomer could represent a "molten globule-like" state, which poises the monomer to engage in helix swapping with another monomer.[25]

To generate a structural model of this putative swapped dimeric form of the Grb7-SH2 domain, we have used homology modeling, followed by refinement with NMR-derived distance and angle restraints. The model was initially constructed with SWISS-MODEL software,[26] using a swapped Grb2-SH2 domain dimer (2H5K) as a template.[21] We manually docked the pY1139 ligand onto each monomer of the model via superposition of the monomeric NMR structure for the Grb7-SH2/pY1139 complex. Subsequently, we refined the model with molecular dynamics simulated annealing using AMBER 9 software[27–30]

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with the *ff99SB* force field[29,31] and with implicit solvent (modified[32] generalized Born model[33]).

Distance and dihedral angle restraints derived from NMR data for the Grb7-SH2/pY1139 complex[13] were applied during the simulated annealing experiments. All distance restraints were defined as ambiguous, with pairs of atoms either within the same monomer or across monomers able to satisfy each restraint. Six iterations of 32-pico-second molecular dynamics simulated annealing from 1200 to 0 K were performed. The resulting refined model of the helix-swapped Grb7-SH2 domain in complex with the pY1139 peptide is shown in Figure 3. Statistics demonstrating the model quality are provided in Table 1.

It is noteworthy that the so-called EF loop of the Grb7 family SH2 domains contains a four-amino acid insertion that is absent in other SH2 domains. The EF loop serves as the “hinge loop” structure[34] that mediates domain swapping in the Grb2-SH2 domain.[19–21] We suggest that the EF loop insertion may promote swapped dimerization in the Grb7 family SH2 domains, as hinge loop lengthening has been observed to facilitate domain swapping in other proteins.[35]

In our model, the functional monomers show a “head-to-head” orientation (Fig. 3C), which contrasts with the “head-to-tail” orientation of the crystallographic (unswapped) dimer but resembles the Grb2-SH2 domain dimer orientation.[19–21] However, there appears to be significant potential for rotation of the functional monomers with respect to one another. Namely, the application of six rounds of molecular dynamics simulated annealing to a modified homology model, with the functional monomers in a head-to-tail starting orientation, resulted in a head-to-tail model with only slightly less favorable restraint energy than the head-to-head model featured here (alternate model data not shown).

Although our group previously found the Grb7-SH2 domain to be largely monomeric when bound to the pY1139 peptide (based on measured molecular tumbling times),[16] we have used NMR-derived restraints obtained through study of the Grb7-SH2/pY1139 complex to help us create an approximate swapped dimer model. We should expect the functional monomers of a swapped Grb7-SH2 domain dimer to be highly similar in structure to the monomeric domain, as is the case for the Grb2-SH2 domain and for many other proteins.[21,32] The functional monomers of our model overlay well (RMSD around 1.5 Å) with the Grb7-SH2 domain monomer refined from the previously published Grb7-SH2/pY1139 NMR structure using molecular dynamics simulated annealing in generalized Born solvent (unpublished data). Our success in producing a model that fits the NMR-derived restraints strengthens our hypothesis that the Grb7-SH2 domain can exist in a swapped dimer form. Further computational modeling has enabled us to suggest elsewhere (publication forthcoming) a thermodynamic argument as to why swapped dimers are not observed in a previously described dimerization-deficient Grb7-SH2 domain mutant.[17]

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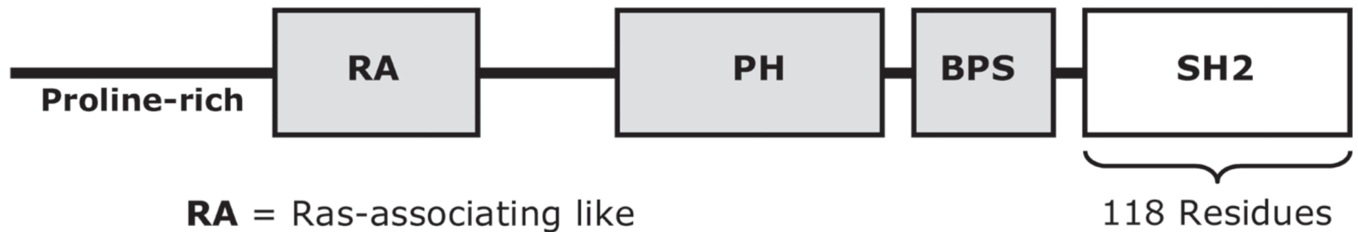

RA = Ras-associating like
PH = Pleckstrin homology
BPS = Between Pleckstrin and Src
SH2 = Src homology-2

FIGURE 1.
Domain topology of the Grb7 protein, highlighting the C-terminal SH2 domain.

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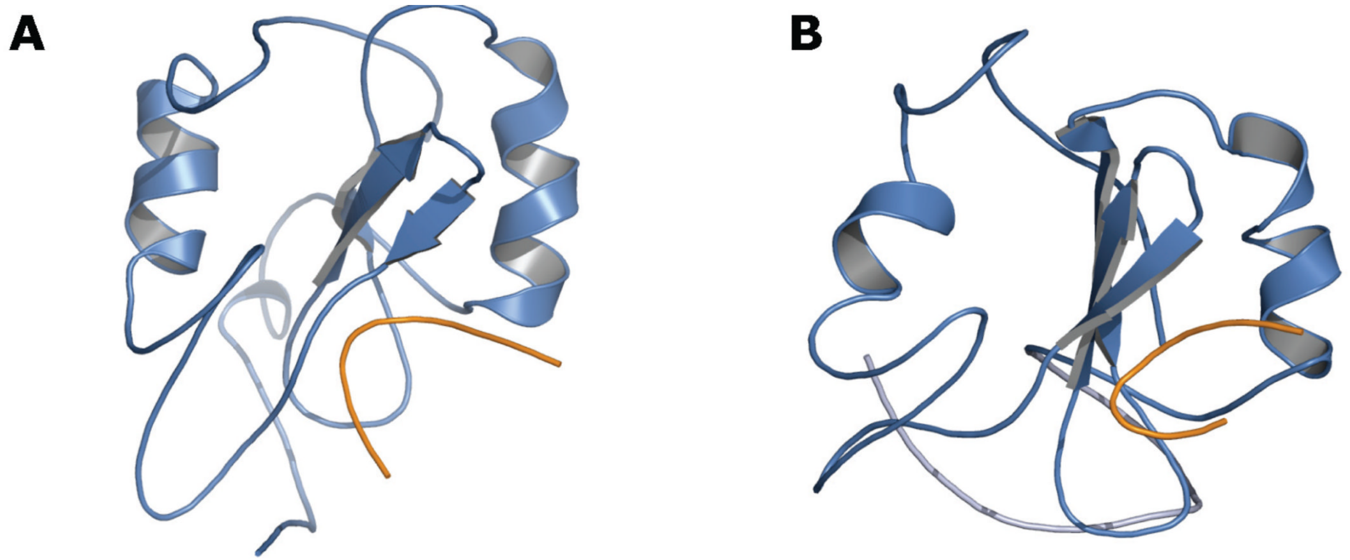

FIGURE 2.
Molecular dynamics snapshots of the Grb7-SH2 domain (blue) in complex with the erbB2 receptor peptide pY1139 (orange). A, Integrity of the canonical SH2 domain fold following 21 nanoseconds of unrestrained explicit solvent molecular dynamics with a 1-femtosecond time step. B, Destructuring of the domain's C-terminal region during explicit solvent simulations with a 2-femtosecond time step and with higher average total and potential energy than the simulation represented in panel A. The image shown is a snapshot taken after 11 nanoseconds of unrestrained dynamics at 300 K.

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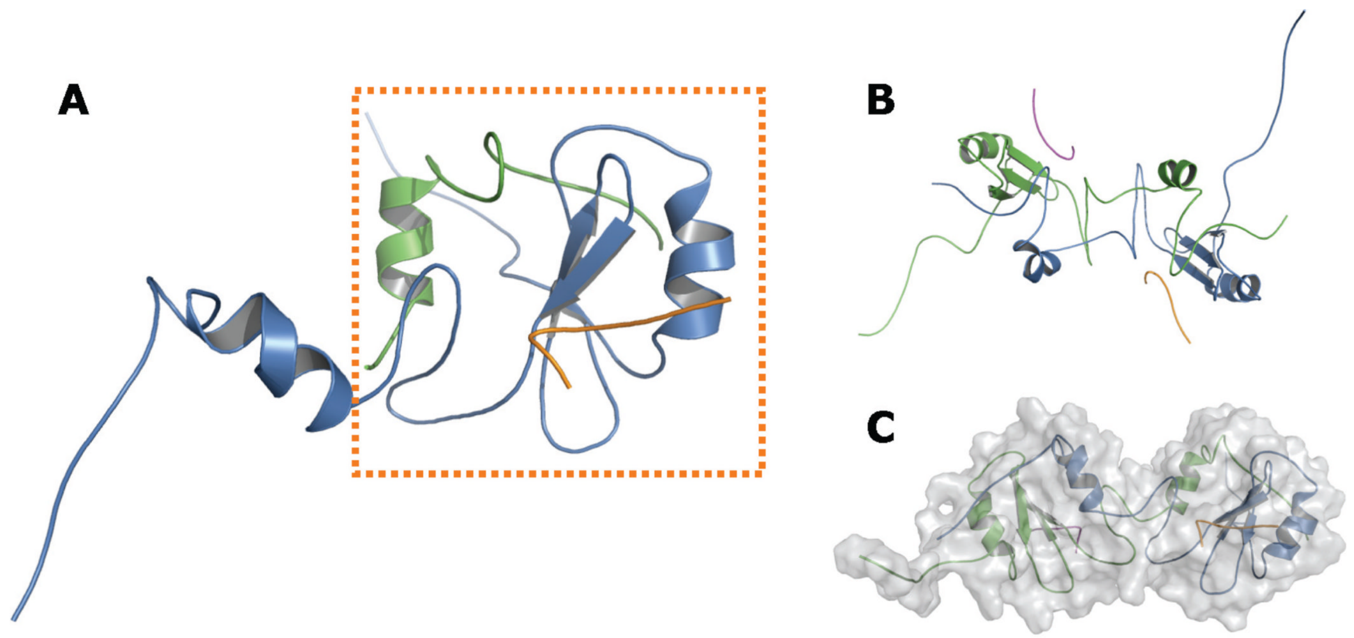

FIGURE 3.

Proposed helix-swapped Grb7-SH2 domain model. Panels A to C show various views of the Grb7-SH2 domain / pY1139 peptide complex following refinement using NMR-derived restraints. Each monomer and each ligand is shown in a distinct color. A, Side view of a single hybrid Grb7-SH2 domain "functional monomer," shown in the dashed box. The N-terminal segment of the functional monomer is composed of approximately 85 residues from a single Grb7-SH2 domain chain (blue), ending with the unique Grb7-SH2 domain sequence MD-DGQ. The C-terminal segment is made up of approximately 33 residues from a second Grb7-SH2 domain chain (green), beginning with the sequence T'R'F'T', which immediately follows M'D'D'G'Q' in the second chain. The remaining residues of the chain are hidden for clarity. B, View along the axis of symmetry. The model shows C2 symmetry, although no symmetry was imposed in the refinement protocol. C, "Head-to-head" orientation of the two functional monomers, with the molecular surface shaded in gray. Figures rendered with PyMOL.[36]

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TABLE 1

Assessment Statistics for the Grb7-SH2/pY1139 Swapped Dimer Model[13,26,37]

Violation statistics[a]	
Per chain average number of	
Distance restraint violations > 0.5 Å	0.5
Distance restraint violations > 0.2 Å	20
Dihedral angle restraint violations > 5°	8
Maximum distance restraint violation	0.54 Å
Maximum dihedral angle restraint violation	10.7°
RMSD from idealized covalent geometry[b]	
Covalent bonds	0.036 Å
Angles	3.6°
PROCHECK-NMR Ramachandran plot statistics[b]	
(excluding glycine, proline, phospho-tyrosine, and end residues)	
Residues in most favored regions	64.0 %
Residues in additional allowed regions	33.2 %
Residues in generously allowed regions	2.8 %
Residues in disallowed regions	0.0 %
WHAT IF statistics[c]	
Z-scores, indicating the number of standard deviations from the expected value for well-refined X-ray structures:	
Second-generation packing quality	−3.85
Ramachandran plot appearance	−0.67
chi-1/chi-2 rotamer normality	−0.72
Backbone conformation	−5.35
RMS Z-scores, expected to fall around 1.0:	
Bond lengths	1.93
Bond angles	1.88
Inside/outside residue distribution	1.23

[a]Violation statistics for the original ensemble as reported previously.[1]

[b]Statistics acquired using the ADIT validation server of the RCSB protein data bank (http://deposit.pdb.org/validate/).

[c]WHAT_CHECK (WHAT IF) assessment performed using the Swiss Model Workspace.[2–3]