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Solution Structure of S100A1 Bound to the CapZ Peptide (TRTK12)

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As is typical for S100–target protein interactions, a Ca^{2+}-dependent conformational change in S100A1 is required to bind to a 12-residue peptide (TRTK12) derived from the actin-capping protein CapZ. In addition, the Ca^{2+}-binding affinity of S100A1 is found to be tightened (greater than threefold) when TRTK12 is bound. To examine the biophysical basis for these observations, we determined the solution NMR structure of TRTK12 in a complex with Ca^{2+}-loaded S100A1. When bound to S100A1, TRTK12 forms an amphipathic helix (residues N6 to S12) with several favorable hydrophobic interactions observed between W7, I10, and L11 of the peptide and a well-defined hydrophobic binding pocket in S100A1 that is only present in the Ca^{2+}-bound state. Next, the structure of S100A1–TRTK12 was compared to that of another S100A1–target complex (i.e., S100A1–RyRP12), which illustrated how the binding pocket in Ca^{2+}-S100A1 can accommodate peptide targets with varying amino acid sequences. Similarities and differences were observed when the structures of S100A1–TRTK12 and S100B–TRTK12 were compared, providing insights regarding how more than one S100 protein can interact with the same peptide target. Such comparisons, including those with other S100–target and S100–drug complexes, provide the basis for designing novel small-molecule inhibitors that could be specific for blocking one or more S100–target protein interactions.

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Introduction

S100A1 is a dimeric Ca^{2+}-binding protein (10.5 kDa per subunit) in the S100 protein family and has two EF-hands per subunit. In the N-terminus, each S100A1 subunit has a 14-residue EF-hand (EF1; residues 19–32) termed the "S100-hand" or the "pseudo-EF-hand". EF1 typically binds Ca^{2+} weakly via several backbone carbonyl ligands and a bidentate ligand from a glutamate (E32) at position 14 of the Ca^{2+}-binding domain. In the C-terminus, each S100A1 subunit has a canonical 12-residue EF-hand (EF2; residues 62–73) that coordinates Ca^{2+} in a manner identical with that of other proteins in the EF-hand superfamily, such as calmodulin and troponin C.[1] As with most dimeric S100 proteins, only EF2 of S100A1 undergoes a large structural rearrangement upon binding Ca^{2+}, and it is this conformational change that allows S100A1 to exert its biological function via interaction with and modulation of cellular targets. This conformational change in S100A1 involves the rotation of the entering helix of EF2 (helix 3) by ~90° rather than the exiting helix (helix 4) as found for most other EF-hand-containing proteins and is the defining characteristic of an "S100 Ca^{2+} switch".[2–4] Such a conformational change is unique to S100 proteins because the exiting helix (helix 4 of EF2) is typically held in place via an X-type four-helix bundle that composes the tight dimer interface, whereas in other EF-hand-containing proteins, the exiting helix is free to rotate.

In general, proteins in the S100 family are distributed in a cell-specific manner and interact with a diverse set of molecules.[5–7] For S100A1, at least 20 known protein targets have been identified, including Ca^{2+}-signaling proteins (ryanodine receptors 1 and 2, Serca2a, and phospholamban), neurotransmitter

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Abbreviations used: GFAP, glial fibrillary acidic protein; NOE, nuclear Overhauser enhancement; 2D, two-dimensional; NOESY, NOE spectroscopy; TOCSY, total correlated spectroscopy; RDC, residual dipolar coupling; Pnt, pentamidine; TAMRA, carboxytetramethylrhodamine; NDR, nuclear Dpf2-related.

release proteins (synapsins I and II), cytoskeletal and filament-associated proteins [CapZ, microtubules, intermediate filaments, tau, microfilaments, desmin, tubulin, F-actin, titin, and the glial fibrillary acidic protein (GFAP)], transcription factors and their regulators (myoDand p53), enzymes (aldolase, phosphoglucomutase, malate dehydrogenase, glycogen phosphorylase, photoreceptor guanyl cyclases, adenylate cyclases, glyceraldehyde-3-phosphate dehydrogenase, twitchin kinase, nuclear Dpf2-related (NDR) kinase, and F1 ATP synthase), and other Ca^{2+}-activated proteins (annexins V and VI, S100B, S100A4, S100P, and other S100 proteins) (reviewed in Refs. 6 and 7). It is also the case that several S100 protein family members bind to the same protein target.[6,7] For example, both S100A1 and S100B, two of the earliest discovered S100 proteins, interact in a Ca^{2+}-dependent manner with several of the same protein targets including the ryanodine receptor, microtubules, GFAP, p53, NDR kinase, phosphoglucomutase, CacyBP/Sip1, and annexin A6;[6,8–13] hence, it is possible that some S100 proteins may function redundantly. Furthermore, using phage display techniques, Ivanenkov *et al.* identified a 12-amino-acid peptide (TRTKIDWNKILS) derived from the actin-capping protein (CapZ) that bound to S100B in a Ca^{2+}-dependent manner at low micromolar concentrations.[14] Subsequently, S100A1 and several other S100 proteins (i.e., S100A1, S100A4, and S100A5) have also been shown to bind this same peptide, termed TRTK12 (Wright, N. T. & Weber, D. J., unpublished data).[5] As a result, the TRTK12 peptide is considered a general S100 protein consensus binding sequence [(K/R)(L/I)XWXXIL].

To examine in detail how two S100 proteins can bind to the same protein target, we have determined the solution structure of S100A1–TRTK12 and compared it to the previously determined structure of S100B–TRTK12.[15] NMR chemical shift perturbation data were suggestive that TRTK12 interacts with the hydrophobic pocket that is common to both Ca^{2+}-S100A1 and Ca^{2+}-S100B.[4,5] However, when these two structures were compared here at atomic resolution, it was found that the specific positioning of the TRTK12 peptide in the target binding pocket was not conserved in S100A1 and S100B and that interactions between the peptide and the two S100 protein side chains were considerably different. This structure also leads to salient insights into the necessary attributes of productive S100A1–target protein interactions, some of which may be common in several S100–target protein interactions, including those for S100B.

Results

Chemical shift and NOE assignments for the S100A1–TRTK12 complex

The first step in solving the high-resolution structure of S100A1–TRTK12 (24 kDa) by NMR was to unambiguously assign the resonances and nuclear Overhauser enhancement (NOE) correlations for the complex, using data from a series of heteronuclear multidimensional NMR experiments. The ^{1}H, ^{13}C, and ^{15}N chemical shift assignments for all observable backbone and side-chain resonances of ^{13}C,^{15}N-labeled S100A1 bound to TRTK12 were completed *a priori* using standard NMR through-bond experiments as described in Wright *et al.*[5] Unambiguous resonance and NOE assignments for protons of the unlabeled TRTK12 peptide bound to ^{13}C,^{15}N-labeled S100A1 were then made using two-dimensional (2D) ^{12}C-filtered spectra [NOE spectroscopy (NOESY) and total correlated spectroscopy (TOCSY) in H_2O and D_2O], as previously described for other protein–peptide complexes.[15–18] Representative NOE data from a region of a 2D ^{12}C-filtered NOESY collected in D_2O is illustrated (Fig. 1a), which show NOE correlations for bound TRTK12 between $I10_{\delta}$ and other protons of I10 ($I10_{\alpha}$ and $I10_{\gamma 2}$) as well as to protons of K9 ($K9_{\beta,\gamma,\delta}$) and W7 ($W7_{\delta,\xi}$). That W7 was proximal to I10 also provided an early indication that the TRTK12 peptide was helical when bound to Ca^{2+}-S100A1 (Fig. 1a). In addition, proton resonances for I10 and W7 of TRTK12 (i.e., $I10_{\gamma 2}$, $I10_{\delta}$, and $W7_{\delta,\xi}$) were found to be proximal to the β-protons of C85 of ^{13}C,^{15}N-labeled S100B in a 3D ^{13}C-edited, ^{12}C-filtered NOESY experiment (Fig. 1b). Intermolecular NOE data such as these were critically important for the structure determination of the S100A1–TRTK12 complex as well as for validating proton assignments on unlabeled TRTK12 bound to S100A1 (Fig. 1b). In summary, the observable ^{1}H resonances of TRTK12 together with the ^{1}H, ^{13}C, and ^{15}N resonances of S100A1 in the S100A1–TRTK12 complex were assigned unambiguously and deposited into the BioMagResBank (BMRB) database† under BMRB accession number 16050.

NOE assignments were made using data from 3D ^{15}N-edited NOESY, 3D ^{13}C-edited NOESY, 4D ^{15}N,^{13}C-edited NOESY, and 4D ^{13}C,^{13}C-edited NOESY experiments (Fig. 1c). As found in all other dimeric S100 protein structures, it was clear from NOE data that helices 1 and 4 were an integral part of the S100A1 dimer interface in the S100A1–TRTK12 complex.[19] For example, early in the NOE assignment and structure determination process, several NOE correlations were observed between residues at the N- and C-termini of helix 1 (i.e., $L4_{\delta 1}$ to $F15_{HN}$ and several others). Because of the physical impossibility of having two residues at opposite ends of a helix being proximal in space, such NOE correlations were assigned as intersubunit between helices 1 and 1′ of the S100A1 dimer. Similarly, the assignment of intermolecular NOEs could be made for residues at the N- and C-termini of helices 4 and 4′ due to the antiparallel alignment of these helices (i.e., $F71H_N$ to $V83_{g1}$, and several others). As expected, such NOE data for S100A1 in the S100A1–TRTK12 complex were fully consistent

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