Specificity and Zn\(^{2+}\) Enhancement of the S100B Binding Epitope TRTK-12*

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The calcium-binding protein S100B (an S100 dimer composed of two S100\(\beta\) monomers) is proposed to act as a calcium-sensory protein through interactions with a variety of proteins. While the nature of the exact targets for S100B has yet to be defined, random bacteriophage peptide mapping experiments have elucidated a calcium-sensitive “epitope” (TRTK-12) for S100B recognition. In this work, interactions of TRTK-12 with S100B have been shown to be calcium-sensitive. In addition, the interactions are enhanced by zinc binding to S100B, resulting in an approximate 5-fold decrease in the TRTK-12/S100B dissociation constant. Moreover, Zn\(^{2+}\) binding alone has little effect. TRTK-12 showed little evidence for binding to another S100 protein, S100A11 or to a peptide derived from the N terminus of S100B, indicating both a level of specificity for TRTK-12 recognition by S100B and that the N-terminal region of S100B is probably not involved in protein-protein interactions. NMR spectroscopy revealed residues most responsive to TRTK-12 binding that could be mapped to the surface of the three-dimensional structure of calcium-saturated S100B, revealing a common region indicative of a binding site.

The S100s are a group of proteins that belong to the EF-hand calcium-binding protein family (1–3). This family includes such mechanistically well understood proteins as the calcium-dependent muscle sensor troponin-C, the ubiquitous enzyme regulator calmodulin, and the visual signaling molecule recoverin. Signaling by these molecules is controlled through calcium binding to the EF-hand protein and subsequent induction of a conformational change that modifies protein-protein interactions with a target protein. For example, troponin-C undergoes a calcium-induced conformational change, allowing a strengthening of its interactions with a second member of the troponin complex, troponin-I (4, 5). An analogous mechanism has been proposed for several S100 proteins, allowing them to control such diverse processes as protein phosphorylation, cytoskeletal protein assembly, neurite outgrowth, and cell cycle regulation through a variety of calcium-sensitive interactions with other proteins (3, 6, 7).

S100B (an S100 dimer composed of two S100\(\beta\) monomers) is one member of the S100 protein family for which several potential cellular targets have been identified. For example, polymerization of cellular architecture molecules such as glial fibrillary acidic protein and tubulin can be inhibited through a calcium-dependent interaction with S100B (8–10). Alternatively, S100B can inhibit the phosphorylation of proteins such as myristoylated alanine-rich C kinase substrate (11), the Alzheimer protein Tau (12, 13), and p53 (14, 15) through interaction with these proteins rather than with the kinase responsible. This action is different from that of the S100 protein S100A12, which has been shown to inhibit phosphorylation of the myosin protein kinase twitchin through interaction with its regulatory region (16) in a fashion reminiscent of calmodulin’s action with myosin light chain kinase (17).

In order to clarify potential target proteins, a bacteriophage random peptide library has recently been used to define a recognition sequence for S100B (18). These studies showed that a 12-residue sequence containing the consensus motif (K/R)(L/V)XXWXXL was sufficient to bind to S100B in a calcium-sensitive manner. Further studies have shown this peptide (TRTK-12) successfully competes with other proteins such as glial fibrillary acidic protein and CapZ for calcium-sensitive S100B binding (10, 18). Similar approaches have identified several 15-residue sequences, analogous to the sequences for myosin light chain kinase, melittin, and mastoparan, as representative samples of the calcium-dependent target proteins for calmodulin (19). A clear distinction exists between these binding “epitopes” for S100B and calmodulin as the sequences of TRTK-12 and the calmodulin peptides are not related by alignment. However there is a similarity in composition in that both peptides have a preponderance of hydrophobic and basic residues.

Recently, NMR spectroscopy and x-ray crystallography have been used to determine the three-dimensional structures of calcium-saturated human (20), rat (21), and bovine S100B (22). The structures revealed the S100B dimer has two symmetric monomers each comprising two EF-hand calcium binding sites. The N-terminal EF-hand is formed from helices I and II where helix I (and I’ from the other monomer) are integral to the maintenance of the dimer. Likewise the C-terminal calcium binding site (site II) is composed of helices III and IV where helix IV (and IV’) interact at the dimer interface. Calcium binding to S100B has a minimal effect on the conformation of the N-terminal site I but has a pronounced effect on the canonical C-terminal EF-hand. This results in a reorientation of helix III with respect to other helices in the protein (20–23). Further, it has been observed that a hydrophobic region composed of several residues near the C terminus of helix IV and in the linker between sites I and II is present on the surface of Ca\(^{2+}\)-S100B\(^{1}\) (20). Based on this structural data, these regions

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1 The abbreviations used are: Ca\(^{2+}\), S100B, S100\(\beta\), and S100A11,
have been proposed to form a possible recognition site for calcium-sensitive protein-protein interactions in S100B. The composition of this site, primarily hydrophobic and acidic residues, is reminiscent of the protein recognition surface in calmodulin (17). In addition, the amino acid sequences in the C-terminal helix and linker show the least sequence similarity among the S100 protein family, suggesting a rationale for target protein specificity (3, 24, 25).

Several groups have reported that some of the S100 proteins including S100B, S100A12, S100A6, S100A11, calgranulin C, and S100A3 are not only calcium-binding proteins but also bind Zn\(^{2+}\) with high affinity (26–30). Further, zinc binding has the pronounced effect of increasing calcium affinity in S100B (26) and calgranulin C (29) by at least 10-fold. Such an observation is unique in the EF-hand calcium-binding protein family and may indicate a new mode for calcium regulation and signaling in the S100 proteins. The impact of these observations was recently demonstrated for the giant protein kinase twitchin, in the S100 proteins. The impact of these observations may indicate a new mode for calcium regulation and signaling.

**EXPERIMENTAL PROCEDURES**

**Materials**—[^15N]Phenylalanine, (^13)NAlanine, Tris-d11, CH\(_3\)COONa, d\(_5\), and deuterium oxide were obtained from Isotec Inc. (Miamisburg, OH). Calcium chloride, magnesium chloride, and zinc chloride were all purumetric grade from Alfa-Aesar (Mississauga, Canada). All other chemicals used were of the highest purity commercially available. The auxotrophic strain DL39 AvtA::Tn5 (31) was kindly donated by Dr. L. McIntosh (University of British Columbia).

Recombinant human S100B was expressed in Escherichia coli (strain N99) and purified to homogeneity as described previously (32). The backbone amides of alanine and phenylalanine residues of S100B were selectively ^[15]N-labeled and purified as described previously (33).

**Fluorescence Spectroscopy**—Spectra were obtained using a Hitachi F-4010 fluorescence spectrophotometer equipped with a stirred cell holder. Tryptophan fluorescence was excited at 295 nm and emission-scanned from 305 to 450 nm using an emission band pass of 5 nm. The addition of two equivalents of apo-S100B to TRTK-12 solution had little effect on the tryptophan fluorescence of TRTK-12, which was used to identify potential sites for TRTK-12 interaction with Ca\(^{2+}\)-S100B and correlate this with the identification of a potential biological target.

Interactions of TRTK-12 with S100B—The intrinsic fluorescence spectrum of TRTK-12 is shown in Fig. 1A. The tryptophan displays an emission maximum at 354 nm for the single Trp\(^2\) in the sequence. This wavelength of emission is consistent with the tryptophan located in a polar aqueous environment. For interaction with S100B, this tryptophan provided a convenient marker, since S100B itself contains no tryptophan residues. The addition of two equivalents of apo-S100B to TRTK-12 resulted in a minimal change in the TRTK-12 tryptophan fluorescence, indicating that any interaction between the apo-S100B calcium-binding protein and TRTK-12 is very weak. The addition of physiological levels of Mg\(^{2+}\) (5 mM) to the apo-S100B/TRTK-12 solution had little effect on the tryptophan fluorescence of TRTK-12 despite previous observations that Mg\(^{2+}\) may bind to S100B. In contrast, the addition of saturating amounts of calcium to the apo-S100B/TRTK-12 solution resulted in a 40% enhancement and a blue shift to 332.8 nm for tryptophan fluorescence. These observations are consistent with previous results showing that TRTK-12 is able to interact with the calcium form of S100B (18, 37). Further, the blue shift indicates the tryptophan residue moves to a more nonpolar environment. S100B has been shown to tightly bind 2 mol of Zn\(^{2+}\) per monomer, and binding of this metal causes a significant change in the surface hydrophobicity of the protein (26). As shown in Fig. 1A, binding of Zn\(^{2+}\) to apo-S100B resulted in little enhancement or shift of the TRTK-12 fluorescence. These observations indicate that calcium is the primary metal responsible for TRTK-12 binding to S100B.

To determine whether TRTK-12 fluorescence and binding to Ca\(^{2+}\)-S100B could be enhanced or reduced by other metal ions, we examined the additional influence of Mg\(^{2+}\) and Zn\(^{2+}\) on tryptophan fluorescence (Fig. 1B). In the presence of Ca\(^{2+}\)-S100B the addition of 5 mM Mg\(^{2+}\) had a small negative effect on the fluorescence intensity of TRTK-12. This finding was similar to the observed small effect in Fig. 1A, where the addition of Mg\(^{2+}\) alone had only a weak effect on Trp\(^2\) fluorescence. In contrast, the addition of Zn\(^{2+}\) to the Ca\(^{2+}\)-S100B solution resulted in an approximate 10% increase in tryptophan fluorescence with no further change in the emission maximum. These results are consistent with Zn\(^{2+}\) binding to S100B and enhancement of the interaction of TRTK-12 with Ca\(^{2+}\)-S100B. The addition of 5 mM Zn\(^{2+}\) to this sample resulted in a small decrease in tryptophan fluorescence similar to that observed in the absence of Zn\(^{2+}\).

While the above work and other studies have indicated that TRTK-12 interacts with S100B in a calcium-sensitive manner,
the specificity of TRTK-12 for other S100 proteins has not been examined. We investigated this using an S100 protein that has some sequence differences from S100B, especially in the linker and C-terminal regions. S100A11 is an S100 protein (also called S100C) originally isolated from cardiac muscle (34), which, like S100B, has been shown to bind both Ca$^{2+}$ and Zn$^{2+}$ (38). Fig. 1C shows the tryptophan fluorescence spectra obtained for the addition of Ca$^{2+}$-S100B and Ca$^{2+}$-S100A11 to a TRTK-12 solution. The spectra show the characteristic shift in TRTK-12 fluorescence in the presence of Ca$^{2+}$-S100B. However, the addition of Ca$^{2+}$-S100A11 yielded little change in the TRTK-12 fluorescence emission wavelength or amplitude, indicating that Ca$^{2+}$-S100A11 does not perturb TRTK-12 fluorescence. This result is consistent with little or no interaction between TRTK-12 and Ca$^{2+}$-S100A11.

In an effort to localize the region of Ca$^{2+}$-S100B that interacts with TRTK-12, we studied the effect of a 46-residue peptide comprising a single EF-hand from the N-terminal region of S100B (hs1bI) on TRTK-12 fluorescence. This peptide has been shown to be mostly $\alpha$-helical by circular dichroism spectroscopy, similar to S100B, and is able to form a tetramer analogous in structure to the arrangement of the four EF-hand motifs in the S100B dimer (39). As shown in Fig. 1D, the addition of hs1bI in the presence of calcium resulted in little change in TRTK-12 fluorescence compared with TRTK-12 alone. This observation indicates that the N terminus of S100B alone is not sufficient to interact with TRTK-12.

**TRTK-12 Affinity for S100B**—The interaction of TRTK-12 with Ca$^{2+}$-S100B was measured by following the tryptophan fluorescence emission at 332.8 nm. Since the previous section showed that TRTK-12 does not interact with apo-S100B, it was important to ensure that Ca$^{2+}$-S100B was the major populated species during these titrations. To address this, two titrations of TRTK-12 with S100B were done in the presence of differing amounts of excess calcium (1 and 10 mM). Under these conditions and given the calcium dissociation for S100B ($K_d \approx 7-200$ μM) (40), Ca$^{2+}$-S100B should be the dominant species. Thus, the interaction of TRTK-12 should not be affected by the apo-S100B to Ca$^{2+}$-S100B equilibrium. Fig. 2 shows three representative titrations of TRTK-12 with Ca$^{2+}$-S100B. Fig. 2 shows that TRTK-12 fluorescence at 332.8 nm increases as a function of added Ca$^{2+}$-S100B, a result of TRTK-12 binding to Ca$^{2+}$-S100B.

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**FIG. 1.** Tryptophan fluorescence spectra of 1.05 μM TRTK-12 in 50 mM Tris, 50 mM KCl at pH 7.2. A shows TRTK-12 alone (a), and with 1.02 μM apo-S100B (b), 1.02 μM S100B and 5 mM MgCl$_2$ (c), 1.02 μM S100B and 1 mM CaCl$_2$ (d), and 1.02 μM S100B and 20.4 μM ZnCl$_2$ (e). B shows TRTK-12 with 1.02 μM S100B and 1 mM CaCl$_2$ (a) and with 20.4 μM ZnCl$_2$ (b), 5 mM MgCl$_2$ (c), or 20.4 μM ZnCl$_2$ and 5 mM MgCl$_2$ (d). C shows TRTK-12 alone (a) and with 1.02 μM S100B and 1 mM CaCl$_2$ (b) or with 1.18 μM S100A11 and 1 mM CaCl$_2$ (c). D shows TRTK-12 alone (a) and with 1.02 μM S100B and 1 mM CaCl$_2$ (b) or 1.0 μM hs1bI and 1 mM CaCl$_2$ (c). In all cases, fluorescence is expressed in relative units, and background buffer has been subtracted.
Zn$^{2+}$ Enhancement of TRTK-12 Binding to S100B

S100B. There is excellent agreement between the two curves, indicating that Cu$^{2+}$-S100B must be the predominant form of the protein at both calcium concentrations and that the interaction of TRTK-12 with Ca$^{2+}$-S100B is the major event being monitored by these titrations. The shape of the curves does not reveal the stoichiometry of TRTK-12 binding to Ca$^{2+}$-S100B, which has been suggested to be either 1 or 2 molecules of TRTK-12/S100B dimer (i.e. 1 molecule of TRTK-12/dimer or 1 molecule of TRTK-12 for each S100B monomer) (37). Indeed, fitting of the above data for either a single TRTK-12 or two TRTK-12 molecules binding to Ca$^{2+}$-S100B yielded very similar results. However, examination of titration data in the presence of Zn$^{2+}$ or as monitored by NMR spectroscopy (see below) clearly indicated a stoichiometry of 1 TRTK-12 molecule/Ca$^{2+}$-S100B monomer. A further titration was done using a 40% more dilute TRTK-12 solution. As expected, the change in fluorescence was correspondingly lower, and a subtler hyperbolic curve was obtained. From these data, a dissociation constant of 0.91 ± 0.17 μM for TRTK-12 binding to Ca$^{2+}$-S100B was determined.

Fluorescence experiments shown in Fig. 1 indicated that tryptophan emission of TRTK-12 was enhanced in the presence of both Zn$^{2+}$ and Ca$^{2+}$ compared with Ca$^{2+}$ only. To determine whether this was a direct result of increased affinity caused by Zn$^{2+}$, titrations to determine the binding affinity of TRTK-12 for Ca$^{2+}$-S100B were done in the presence of Zn$^{2+}$. As with the previous calcium titration experiments, a Zn$^{2+}$ concentration of 20-fold the S100B concentration was chosen based on the reported dissociation constants of S100B for Zn$^{2+}$. The data plotted in Fig. 2 show that Zn$^{2+}$ binding to S100B in addition to Ca$^{2+}$ increased the response of TRTK-12 toward S100B. From these data, a stoichiometry of 1:1 TRTK-12:S100B monomer is clearly evident. Iterative curve fitting of this data yielded a dissociation constant of 0.18 ± 0.01 for TRTK-12 binding to Zn$^{2+}$/Ca$^{2+}$-S100B, an approximate 5-fold tighter binding than to Ca$^{2+}$-S100B alone.

Regions of S100B Affected by TRTK-12 Binding—Fluorescence studies showed that the TRTK-12 tryptophan fluorescence was affected very little in the presence of apo-S100B. This was reinforced by $^1$H-$^1$N HSQC spectra of apo-S100B in the absence and presence of TRTK-12, which indicated the backbone resonances of apo-S100B are not influenced by equimolar amounts of TRTK (data not shown). Qualitatively, this would indicate that the affinity of TRTK-12 for apo-S100B is conservatively 10-fold larger than the apo-S100B concentration (1 mM S100B).

In previous work, we determined that calcium binding to S100B results in excessive line broadening, making studies of the Ca$^{2+}$-S100B species more difficult than apo-S100B. We had shown that this line broadening was a result of aggregation of Ca$^{2+}$-S100B in the absence of a target protein such as TRTK-12 (32). To reduce the possibility of Ca$^{2+}$-S100B aggregation in this work, we studied the interaction of TRTK-12 with Ca$^{2+}$-S100B by the addition of incremental amounts of Ca$^{2+}$ to apo-S100B/TRTK-12 solutions. This yielded identical results compared with direct TRTK-12 addition to Ca$^{2+}$-S100B. As shown in Fig. 4A, the $^1$H-$^1$N HSQC spectrum of Ca$^{2+}$-S100B and Ca$^{2+}$-S100B/TRTK-12 show some significant differences. In particular, the largest changes in the N and NH chemical shifts (weighted average >0.25 ppm) were noted for residues Asp$^{12}$, Ser$^{41}$, Phe$^{43}$, Ile$^{47}$, Val$^{52}$, Val$^{66}$, Thr$^{99}$, Asp$^{101}$, Ala$^{128}$, Cys$^{84}$, Phe$^{87}$, Phe$^{88}$, and His$^{99}$. In the case of alanine and phenylalanine residues, these assignments were confirmed using a specifically $^{15}$N-labeled sample (Fig. 4B). Interestingly, we observed little change in the position of Ile$^{13}$ upon binding of TRTK-12. This was consistent with observations for binding of a p53 peptide to S100B (41) but different from a previous study of TRTK-12 binding to bovine S100B, where Ile$^{13}$ was observed to undergo the largest shift of any residue (37).

**DISCUSSION**

The focus of this work was to determine how the interaction of the TRTK-12 binding epitope for S100B varies with metal ion coordination and whether this binding showed a degree of specificity for S100B. These aspects of S100B interaction have not been dealt with in previous studies with TRTK-12. In addition, we have used our data to probe a region on the Ca$^{2+}$-S100B three-dimensional structure that may be responsible for peptide interaction.

The affinity of TRTK-12 for Ca$^{2+}$-S100B is approximately 1 μM. This value is higher than previously measured in the absence of added salts (0.15 μM) and may reflect a sensitivity of...
peptide binding to ionic strength which is known to have a significant negative influence on S100B calcium affinity. The magnitude of the TRTK-12 dissociation constant is consistent with those found for other calcium-binding protein-peptide complexes such as TnI peptides binding to skeletal muscle troponin C (24 μM) (4) and caldesmon peptides binding to calmodulin (1 μM) (42, 43). The interaction of TRTK-12 with S100B is Ca\(^{2+}\)-specific, since neither Mg\(^{2+}\) nor Zn\(^{2+}\) binding to peptide complexes such as TnI peptides binding to skeletal muscle troponin C (24 μM) (4) and caldesmon peptides binding to calmodulin (1 μM) (42, 43). The interaction of TRTK-12 with S100B is Ca\(^{2+}\)-specific, since neither Mg\(^{2+}\) nor Zn\(^{2+}\) binding to
S100B could stimulate peptide binding. The finding that Zn\(^{2+}\) binding alone to S100B is unable to promote TRTK-12 binding is in contrast to results using the hydrophobic probe TNS, where a large increase in TNS fluorescence is observed in the presence of Zn\(^{2+}\)-S100B (26, 27, 44). This observation occurs as a result of the interaction of TNS with the Zn\(^{2+}\) form of S100B. Since TNS is known to be a probe for hydrophobic surface rather than a specific binding site, these differences indicate that the hydrophobic surface exposed in S100B by Zn\(^{2+}\) binding is not specific for TRTK-12 binding. In turn, this probably indicates that the protein does not adopt a proper conformation in the Zn\(^{2+}\) form to allow target protein binding.

The most dramatic effect on peptide binding to S100B is in the presence of both Zn\(^{2+}\) and Ca\(^{2+}\), where an increase in peptide affinity of about 5-fold is noted compared with the presence of Ca\(^{2+}\) only. This observation is consistent with results for S100A1\(_p\), where a 30-fold increase in twitchin kinase activity was observed upon the addition of Zn\(^{2+}\) to the calcium form of the protein (16). Together with previous observations that S100B is able to bind two Zn\(^{2+}\) ions per monomer (26, 44) this indicates that binding of TRTK-12 is enhanced by Zn\(^{2+}\) binding to S100B in the presence of Ca\(^{2+}\) only. Since TRTK-12 does not appear to bind to Zn\(^{2+}\)-S100B as judged by the present experiments, it would appear the calcium binding to S100B is critical. This indicates that Zn\(^{2+}\) plays more of a structural role in proteins such as S100B and S100A\(_p\) rather than a regulatory role. Similar proposals have been suggested for the S100 proteins S100A3 (30) and calgranulin C (29). Consistent with this idea, the affinity of Zn\(^{2+}\) for S100B (10\(^{-7}\) M) is near the physiological intracellular concentrations of free Zn\(^{2+}\), which are thought to be in the micromolar range. It has been previously proposed that Zn\(^{2+}\) can act as a structural ion in other S100 proteins based on the observation of a His-X\(_{3,4}\)-His pattern found near the C terminus (30). This pattern is similar to that observed for Zn\(^{2+}\)-catalytic sites that frequently display a His-X\(_{3,4}\)-His motif (45). It is intriguing to note that the three-dimensional structure of human Ca\(^{2+}\)-S100B shows that one possible Zn\(^{2+}\) binding site might include residues His\(_{35}\) and His\(_{80}\) based on their proximity in the structure (20). This region ultimately appears to be important for peptide binding, since several changes in chemical shift and exposed surface area are noted for residues in the C terminus.

The measured affinity of TRTK-12 for Ca\(^{2+}\)-S100B represents at least a 5-fold increase in affinity compared with that for a 23-residue peptide from the tumor suppressor protein, p53 (residues 367–388) (41). Further, Zn\(^{2+}\) binding to S100B did not have a notable effect on p53 peptide binding. These observations indicate there are some clear differences between these two peptide species. As indicated by Wilder et al. (15), the p53 peptide studied previously fits some of the TRTK-12 consensus sequence but lacks residues Ile\(_{10}\) and Leu\(_{11}\). Further, sequence analysis of nine unique p53 sequences from the PIR data base reveals that all forms of p53 do not resemble the TRTK-12 binding motif for these last two residues.\(^2\) This may explain the decreased affinity of p53 for S100B. It is intriguing that second structure prediction methods show that TRTK-12 should form an amphipathic \(\alpha\)-helix between residues Lys\(_4\) and Leu\(_{11}\). If this structure exists in the S100B-protein complex, it would place residues Thr\(_3\), Trp\(_1\), Leu\(_{10}\), and Ile\(_{11}\) on one side of the helix, allowing integral contact of Leu\(_{10}\) and Ile\(_{11}\) with S100B. On the surface, this would provide differences between the interactions of TRTK-12 with Ca\(^{2+}\)-S100B compared with p53.

The results presented in this work allow an initial overview of the TRTK-12 interaction with Ca\(^{2+}\)-S100B previously attempted in the absence of a Ca\(^{2+}\)-S100B three-dimensional structure (37). Residues in the N terminus (Asp\(_{12}\)), linker and N terminus of helix III (Ser\(_{39}\), Phe\(_{43}\), Ile\(_{47}\), Val\(_{53}\)) and C terminus (Ala\(_{78}\), Ala\(_{86}\), Cys\(_{84}\) and Phe\(_{89}\)) undergo the most significant changes in chemical shift upon TRTK-12 binding. Since TRTK-12 has little interaction with the N-terminal peptide, hs1bI, from S100B this would indicate that the linker and C terminus are more important. Using the structure-activity relationship (46), it can be proposed that residues change chemical shift as a direct result of interaction with TRTK-12. Fig. 5 shows the structure-activity relationship for Ca\(^{2+}\)-S100B based on changes in chemical shift. The S100B structure clearly shows these residues comprise a large localized hydrophobic surface on the protein. Further, several residues including Phe\(_{43}\), Ala\(_{86}\), Phe\(_{87}\), and Phe\(_{89}\) are included in or near this region and increase their accessible surface area more than 20% upon calcium binding (20). These observations are consistent with a region for interaction of TRTK-12 with S100B.

S100B has been proposed to interact with a large number of potential target proteins including the head domain of glial fibrillary acidic protein that bears some sequence similarity to TRTK-12 (10). Most recently, studies have probed this interaction with p53 (15, 41), guanylate cyclase, and p80 (47, 48). During the course of the current work, a series of synthetic peptides were used to map the regions of S100B that stimulate guanylate cyclase activity and phosphorylation of p80. In agreement with our current findings, these studies indicated that the C-terminal region of S100B encompassing residues Thr\(_{81}\)-Glu\(_{91}\) was most effective for guanylate cyclase activation. It was also apparent that residues Leu\(_{52}\)-Leu\(_{60}\) could elicit a similar response. This region is just N-terminal to residue Ser\(_{64}\) observed here. Interestingly, our peptide hs1bI (residues 1–46) does not bind to TRTK-12, perhaps suggesting a weaker contribution from the linker region when TRTK-12 is bound and a stronger contribution for guanylate cyclase. Supporting this possibility, it has also been noted that guanylate cyclase does not contain a region similar in sequence to TRTK-12, so this may indicate a fine difference between interaction of S100B and its target proteins.
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