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FOR THE RECORD

S100B($\beta\beta$) inhibits the protein kinase C-dependent phosphorylation of a peptide derived from p53 in a Ca^{2+}-dependent manner

PAUL T. WILDER, RICHARD R. RUSTANDI, ALEXANDER C. DROHAT, AND DAVID J. WEBER

Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, Mayrland 21201

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Abstract: S100B($\beta\beta$) is a dimeric Ca^{2+}-binding protein that is known to inhibit the protein kinase C (PKC)-dependent phosphorylation of several proteins. To further characterize this inhibition, we synthesized peptides based on the PKC phosphorylation domains of p53 (residues 367–388), neuromodulin (residues 37–53), and the regulatory domain of PKC (residues 19–31), and tested them as substrates for PKC. All three peptides were shown to be good substrates for the catalytic domain of PKC. As for full-length p53 (Baudier J, Delphin C, Grunwald D, Khochbin S, Lawrence JJ. 1992. *Proc Natl Acad Sci USA 89*:11627–11631), S100B($\beta\beta$) binds the p53 peptide and inhibits its PKC-dependent phosphorylation ($IC_{50} = 10 \pm 7$ μM) in a Ca^{2+}-dependent manner. Similarly, phosphorylation of the neuromodulin peptide and the PKC regulatory domain peptide were inhibited by S100B($\beta\beta$) in the presence of Ca^{2+} ($IC_{50} = 17 \pm 5$ μM; $IC_{50} = 1 \pm 0.5$ μM, respectively). At a minimum, the C-terminal EF-hand Ca^{2+}-binding domain (residues 61–72) of each S100β subunit must be saturated to inhibit phosphorylation of the p53 peptide as determined by comparing the Ca^{2+} dependence of inhibition ($^{Ca}IC_{50} = 29.3 \pm 17.6$ μM) to the dissociation of Ca^{2+} from the C-terminal EF-hand Ca^{2+}-binding domain of S100B($\beta\beta$).

Keywords: calcium-binding protein; p53; phosphorylation; PKM; protein kinase C; S100B; S100 protein

Reprint requests to: David J. Weber, University of Maryland School of Medicine, Department of Biochemistry and Molecular Biology, 108 N. Greene St., Baltimore, Maryland 21201; e-mail, weber@noe.ab.umd.edu.

Abbreviations: PKC, protein kinase C; PKM, catalytic subunit of PKC; [Ser-25]PKC(19–31) peptide, peptide derived from the pseudo-substrate regulatory domain of PKCα (residues 19–31) with a serine at position 25 replacing the wild-type alanine (House & Kemp, 1987); PSCBD, phosphorylation site/calmodulin binding domain; TCA, trichloroacetic acid; GFAP, glial fibrillary acidic protein; MARCKS, myristoylated alanine-rich C kinase substrate; MRP, MARCKS-related proteins; Nm, neuromodulin; DTT, dithiothreitol; βME, β-mercaptoethanol; IC, inhibition constant; BCA, bicinchoninic acid; p53, tumor suppressor protein; SMP, S100 protein-modulated phosphoprotein; S100β, a subunit of dimeric S100B($\beta\beta$); S100B($\beta\beta$), dimeric S100B with noncovalent interaction at the dimer interface.

The S100 protein family is a highly conserved group of Ca^{2+}-binding proteins, and the principal member, S100B, was first discovered as a major constituent of glia (Moore, 1965). However, S100B is now known to be expressed in several tissues and cell lines including malignant tumors (Donato, 1991; Suzushima et al., 1994; Takashi et al., 1994; Zimmer et al., 1995). Although the precise mechanisms for intra- and extra-cellular functions of S100B are not well understood, processes such as neurite extension, Ca^{2+} flux, cell growth, apoptosis, energy metabolism, and protein phosphorylation are all thought to be modulated in some manner by S100B (for reviews, see Kligman & Hilt, 1988; Donato, 1991; Zimmer et al., 1995; Schafer & Heizmann, 1996).

The reduced homodimeric protein, S100B($\beta\beta$), is one of the best characterized proteins of the S100 family. The solution structure of reduced apo-S100B($\beta\beta$) shows that two subunits associate tightly ($K_D < 500$ pM) (Drohat et al., 1997) through extensive hydrophobic interactions to form a compact dimer with a highly charged surface (Amburgey et al., 1995; Drohat et al., 1996; Kilby et al., 1996). The general model for S100-target protein interactions is similar to that of other Ca^{2+}-binding proteins, such as calmodulin and troponin C. As for these proteins, S100B($\beta\beta$) undergoes a conformational change upon binding Ca^{2+} that promotes its interaction with a variety of target proteins (Kligman & Hilt, 1988; Drohat et al., 1996; Chaudhuri et al., 1997). For example, the Ca^{2+}-dependent binding of S100B($\beta\beta$) to microtubules (Bianchi et al., 1993), GFAP (Bianchi et al., 1994), and p53 (Baudier et al., 1992) prevents oligomerization for each of these proteins. S100B($\beta\beta$) is also known to inhibit the PKC-dependent phosphorylation of τ-protein (Baudier et al., 1987), neuromodulin (Lin et al., 1994; Sheu et al., 1994), MARCKS (Albert et al., 1984), SMP (S100 Protein-Modulated Phosphoprotein) (Patel et al., 1983), and p53 (Baudier et al., 1992; Wilder & Weber, 1996) by binding to the phosphorylation site of these PKC substrates.

To further characterize the interaction of S100B($\beta\beta$) with target proteins, we examined the interaction of S100B($\beta\beta$) with peptides derived from the tumor suppressor protein, p53, neuromodulin, and the regulatory subunit of PKC. Previously, Baudier and coworkers showed that full-length p53 is a substrate for PKC in vivo

and in vitro, and that S100B($\beta\beta$) most likely interacts at the C-terminus of p53 to inhibit phosphorylation (Baudier et al., 1992). As for full length p53, we found that a 22-residue peptide derived from the C-terminus of p53 (residues 367–388) is good substrate for PKC, and that its PKC-dependent phosphorylation is inhibited by S100B($\beta\beta$) in a Ca^{2+}-dependent manner. Thus, we show for the first time that the C-terminal domain of p53 is a site for PKC-dependent phosphorylation and S100B($\beta\beta$) binding. A preliminary abstract of this work was published previously (Wilder & Weber, 1996).

Results and discussion Synthetic peptides are often used to study the phosphorylation site of protein kinases since they usually contain the entire kinase recognition motif and give kinetic properties similar to those of the full-length substrate (Kemp & Pearson, 1991). Furthermore, a series of studies of the phosphorylation site/calmodulin binding domain (termed PSCBD peptides) on the MARCKS and MARCKS-related proteins (MRPs) indicate that peptides are excellent models for studying PKC-dependent phosphorylation kinetics and calmodulin binding (Graff et al., 1989, 1991; McIlroy et al., 1991; Verghese et al., 1994). Therefore, a peptide was first synthesized based on the putative phosphorylation site/S100B binding domain of p53 (residues 367–388) (Baudier et al., 1992) and used to study the Ca^{2+}-dependent interaction of S100B($\beta\beta$) with the p53 peptide.

The catalytic domain of PKC (PKM) was used in these studies because it lacks the regulatory domain that makes the enzyme dependent on Ca^{2+} and phospholipids (Burns et al., 1990). The p53 peptide was found to be a good substrate for PKM (K_m = 1.8 ± 0.4 μM and V_{max} = 1.6 ± 0.2 μmol/min/mg; Fig. 1, Table 1) compared to the kinetic parameters for series of known peptide substrates (K_m = 0.5–24 μM and V_{max} = 0.5–5.0 μmol/min/mg; Kemp & Pearson, 1991). Phosphorylation of S100B($\beta\beta$) was not detected under any of the assay conditions. Furthermore, S100B($\beta\beta$) was found to inhibit phosphorylation of the synthetic p53 peptide in a Ca^{2+}-dependent manner ($^{Ca}IC_{50}$ = 29.3 ± 17.6 μM; Fig. 2, Table 1). In control experiments, increasing amounts of Ca^{2+} in the absence of S100B($\beta\beta$) did not inhibit PKM-dependent phosphorylation, and likewise, the presence of S100B($\beta\beta$) in Ca^{2+}-free assays had no inhibitory effect (data not shown). A comparison of the Ca^{2+}-dependent inhibition constant ($^{Ca}IC_{50}$ = 29.3 ± 17.6 μM; Fig. 2, Table 1) to the dissociation constant of Ca^{2+} from S100β ($^{Ca}K_D$ = 35 ± 15 μM) (Baudier et al. 1986; Kligman & Hilt, 1988; Zimmer et al., 1995)[1] indicates that at least the C-terminal EF-hand of each S100β subunit must be saturated to inhibit phosphorylation. As in PKM assays, low concentrations of S100B($\beta\beta$) were able to inhibit full-length PKCα ($^{S100B}IC_{50}$ = 10 ± 7 μM; Fig. 3A, Table 1), although the Ca^{2+} dependence of this reaction could not easily be evaluated because Ca^{2+} and phospholipid vesicles are required for the activation of the full-length enzyme. Together, these data indicate that Ca^{2+} bound to the C-terminal EF-hand of each subunit in S100B($\beta\beta$) is sufficient to inhibit phosphorylation (>80%) of a synthetic peptide derived from p53.

Baudier and co-workers recognized that the C-terminal domain of p53 resembles the PKCα phosphorylation site found on myristoylated alanine-rich C kinase substrate (MARCKS) protein (Baudier et al., 1992). Similar to p53, the PKC-dependent phosphorylation of the MARCKS protein is inhibited by S100B($\beta\beta$) in a Ca^{2+}-dependent manner (Albert et al., 1984). Furthermore, a mixture of S100A1 and S100B binds PKC substrates such as neuromodulin and neurogranin and inhibits their PKC-dependent phosphorylation (Baudier et al., 1995; Sheu et al., 1995). In fact, we synthesized a peptide based on the phosphorylation domain of neuromodulin (residues 37–53) and found that its PKC-dependent phosphorylation is also inhibited by S100B($\beta\beta$) as a function of Ca^{2+} ($^{S100B}IC_{50}$ = 17 ± 5 μM; Fig. 3B, Table 1). Also, a well-characterized PKC substrate derived from the regulatory domain of PKC itself is inhibited by S100B($\beta\beta$) in a similar manner as that observed for the p53 peptide ($^{S100B}IC_{50}$ = 1.0 ± 0.5 μM; Fig. 3C, Table 1). Dimlich and co-workers discovered a putative

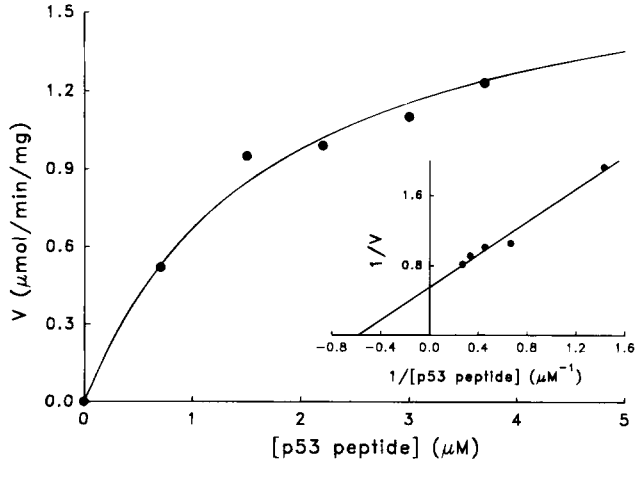

Fig. 1. Kinetics of the PKC-dependent phosphorylation of a peptide derived from the C-terminal domain of p53 (residues 367–388). (Inset) Lineweaver-Burke plot of the kinetic data used to determine values for K_m and V_{max} (Table 1). Conditions are described in Materials and methods.

Table 1. *Kinetic parameters of the PKC-dependent phosphorylation reaction*

Substrate	K_m (μM)	V_{max} (μmol/min/mg)	$^{S100B}IC_{50}$ (μM)
p53 peptide[a]	1.8 ± 0.4	1.6 ± 0.2	10 ± 7[b]
Nm peptide[c]	1.3 ± 0.4[d]	0.7 ± 0.2	17 ± 5
[Ser-25]PKC(19–31)[e]	0.3 ± 0.1	1.0 ± 0.2	1 ± 0.5

[a]The peptide was synthesized based on residues 367–388 of human p53.
[b]The S100B($\beta\beta$)-dependent inhibition was shown to be Ca^{2+} dependent ($^{Ca}IC_{50}$ = 29.3 ± 17.6 μM; Fig. 2).
[c]The peptide was synthesized based on residues 37–53 of neuromodulin.
[d]Some sigmoidal nature was observed for the kinetics of this peptide; therefore, the value is a half-maximum velocity ($S_{0.5}$), as previously reported for other peptide substrates (Schleiff et al., 1996).
[e]This peptide was purchased from Gibco BRL, Inc., and two of the kinetic parameters listed here (K_m and V_{max}) are similar to values determined previously (House & Kemp, 1987). The peptide consists of residues 19–31 from the regulatory domain of PKC except that the alanine residue at position 25 was changed to serine (A25S) (House & Kemp, 1987).

[1]The dissociation of Ca^{2+} from the S100B($\beta\beta$)-Ca^{2+}-p53 peptide complex ($^{Ca}K_2$ = 20.4 ± 3.1 μM) (Rustandi et al., 1998) also indicates that at least the C-terminal EF-hand (residues 61–72) of each S100β subunit must be saturated in order to inhibit phosphorylation.

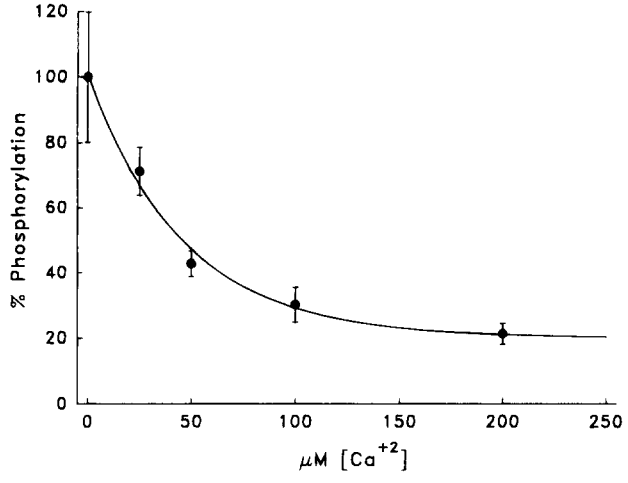

Fig. 2. Ca^{2+}-dependence of S100B($\beta\beta$) inhibiting ($^{Ca}IC_{50}$ = 29.3 ± 17.6 μM) p53 peptide phosphorylation. The catalytic domain of PKCα (PKM) was used because it is not regulated by Ca^{2+} or phospholipids (House & Kemp, 1987; Burns et al., 1990). Other conditions are described in Materials and methods.

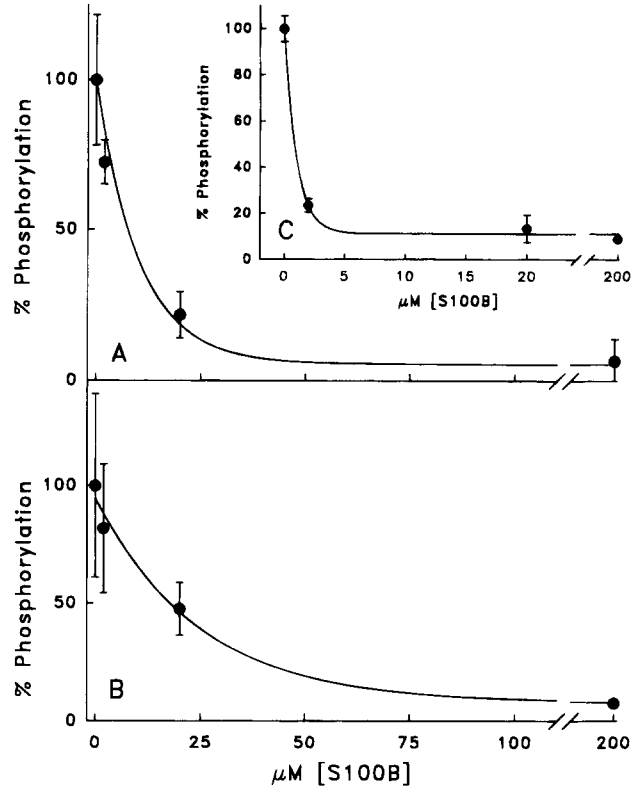

Fig. 3. S100B($\beta\beta$)-dependent inhibition of PKC-dependent phosphorylation of the (**A**) p53 peptide, (**B**) neuromodulin peptide, and (**C**) the peptide derived from the regulatory subunit of PKC. Conditions are described in Materials and methods.

consensus sequence (K/R)(L/I)XWXXIL for peptides that bind S100B($\beta\beta$), and found that a peptide homologous to the actin capping protein, CapZ, has the highest affinity among the peptides they screened (Ivanenkov et al., 1995). Most of these S100-target peptides contain a substantial portion of this consensus sequence (Table 2); however, few other sequence homologies are found. Nonetheless, another common characteristic is that all of the peptides contain several Arg or Lys residues (Table 2), which is also the case when sequences for substrates of PKC are compared (House et al., 1987; Kemp & Pearson, 1991).

Based on the sequence homology between the C-terminus of p53 and CapZ, it is not surprising that S100B($\beta\beta$) binds the p53 peptide with a relatively high affinity.[2] Thus, the Ca^{2+}-dependent inhibition of p53 peptide phosphorylation (IC_{50} = 10 ± 7 μM) observed in this study is likely the result of a direct interaction between S100B($\beta\beta$) and the p53 peptide. Perhaps a general mechanism for regulating signal transduction is emerging because S100 proteins are found to inhibit several PKC substrates in a Ca^{2+}-dependent manner. Calmodulin is also known to inhibit the PKC-dependent phosphorylation of MARCKS and MARCKS-related proteins (MRPs) by directly binding the protein substrate (Verghese et al., 1994; Smith et al., 1997). Thus, S100 proteins and calmodulin may provide a pre-emptive control mechanism in signal transduction that is ultimately regulated by intracellular calcium ion concentration.

Materials and methods *Materials:* All chemical reagents were ACS grade or higher and purchased from Sigma unless otherwise indicated. All buffers were passed through Chelex-100 resin (Bio-Rad) to remove trace metals.

S100B($\beta\beta$) and p53-derived peptide preparations: Recombinant S100B($\beta\beta$) was overexpressed in *E. coli* strain HMS174(DE3) transformed with an expression plasmid containing the rat S100β gene, and protein was prepared and purified (>99%) under reducing conditions as described previously (Amburgey et al., 1995; Drohat et al., 1996). The S100β subunit concentration was determined using a BCA protein assay with S100B($\beta\beta$) of known concentration as the standard (Amburgey et al., 1995; Drohat et al., 1996, 1997). The concentration of standard S100β used in the BCA assay was determined by amino acid analysis (Analytical Biotechnology Services, Boston, Massachusetts).

A peptide, acetyl-SHLKSKKGQSTSRHKKLMFKTE-am, derived from human p53 (residues 367–388) was synthesized using solid-phase peptide synthesis (Biopolymer Laboratory, University of Maryland School of Medicine at Baltimore, Maryland) with its N- and C-termini acetylated (acetyl-) and amidated (-am), respectively. The p53 peptide was stored as a lyophilized powder, and dissolved in 1 mM Tris-d_{11}-HCl, pH 7.6 prior to use. The purity (>99%) of the p53 peptide was determined using HPLC, and its concentration and composition were confirmed by amino acid analysis (Analytical Biotechnology Services, Boston, Massachusetts). Another substrate of PKC was purchased from Gibco-BRL ([Ser-25]PKC(19–31) peptide). This peptide was derived from the PKCα pseudo-substrate regulatory domain residues 19–31 with a serine at position 25 replacing the wild-type alanine (House & Kemp, 1987). The neuromodulin (Nm) peptide, acetyl-KIQASFRGHITRKKLKG-am, corresponding to residues 37–53

[2]When F385 of the p53 peptide is mutated to a tryptophan, it has a higher homology to the consensus sequence for S100B binding (Table 2) (Ivanenkov et al., 1995). As expected, the F385W mutation increases the peptides affinity for S100B($\beta\beta$) by as much as fourfold (K_3 = 6.4 ± 1.1 μM versus $K_3 \leq$ 23.5 ± 6.6 μM for the wild-type p53 peptide) (Rustandi et al., 1998).

Table 2. *Sequence alignment of peptide substrates of PKC*[a]

Position																	1	2	3	4	5	6	7	8					
S100 binding[b]																	K	L	X	W	X	X	I	L					
p53 peptide[c]	*S*	H	L	**K**	S	**K**	**K**	G	Q	S	*T*	*S*	**R**	H	**K**		K	L	M	F	K	*T*	E						
Nm peptide[d]			**K**	I	Q	A	*S*	F	**R**	G	H	I	*T*	**R**	**K**		K	L	K	G									
PKC peptide[e]																	R	F	A	**R**	K	G	*S*	L	**R**	Q	**K**	N	V
CapZ[f]													T	**R**	T		K	I	D	W	N	K	I	L					

[a]Lysine and arginine residues are shown in bold; sites (or potential sites) for phosphorylation are shown in italics, and residues that match the S100B binding sequence are underlined.

[b]The S100B-binding domain was determined by Dimlich and co-workers (Ivanenkov et al., 1995) by screening a peptide library, and the CapZ peptide was found to be the tightest binding peptide of those screened. The consensus sequence has either a lysine or an arginine residue at the first position and either a leucine or an isoleucine residue at the second position.

[c]The p53 peptide was synthesized based on residues 367–388 of human p53.

[d]The Nm peptide was synthesized based on residues 37–53 of neuromodulin.

[e]The PKC peptide was purchased from Gibco BRL Inc. It is based on residues 19–31 from the regulatory domain of PKC; however, a serine replaces the alanine residue at position 25 (House & Kemp, 1987).

[f]The CapZ peptide is based on residues 265–276 of CapZα1 and CapZα2 from the heterodimeric actin capping protein (Ivanenkov et al., 1995).

of the full-length protein was synthesized and purified in the same manner as the p53 peptide.

PKC and PKM phosphorylation assays: The PKCα-dependent phosphorylation of the p53 peptide was done as previously described (Roskoski, 1983; Kemp & Pearson, 1991). The ability of S100B($\beta\beta$) to inhibit phosphorylation was monitored by adding varying amounts (2–200 μM) of S100B($\beta\beta$) to the PKCα assay mixture. The final reaction volume was 150 μL (pH 7.5) containing 10 mM magnesium acetate, 20 mM Tris-HCl (Bio-Rad), 750 μM $CaCl_2$, 2 μM p53 peptide, 50 μg/mL leupeptin, 100 μM ATP-Tris (containing 0.5–1 μCi ^{32}P-γ-ATP (Dupont-NEN)), 10 μg L-phosphatidyl-L-serine, 2 μg 1,2-dioleoyl-sn-glycerol, 0.01 μg PKCα (Gibco-BRL) (>700 nmol/min/mg assay by Gibco-BRL using the Gibco-BRL PKCα synthetic peptide substrate), and 0–200 μM S100B($\beta\beta$). In each experiment, four separate control assays were completed without ATP, PKCα, phospholipids, and substrate, respectively, and all assays were done in triplicate. Lipid vesicles were prepared immediately before use by sonication. The assay was started by the addition of enzyme, and run for 10 min at 37 °C. Reactions were terminated by the addition of 25% TCA (75 μL) and centrifuged for 2 min. Next, the supernatant (75 μL) was spotted onto P81 phosphocellulose paper (Whatman) and washed three times with 4 mL of 75 mM phosphoric acid. All samples were counted for 10 min using a Packard (CT) Tri-Carb Model 1600TR Liquid Scintillation Counter.

Phosphorylation of the p53 peptide was also assayed with a form of PKC that lacks the Ca^{2+}-dependent regulatory domain (PKM) (House & Kemp, 1987). This enabled us to study the Ca^{2+}-dependence of inhibition by S100B($\beta\beta$) because the isolated catalytic domain, PKM, does not require Ca^{2+} or phospholipids for activation. Conditions for phosphorylation of human p53 synthetic peptide by PKM differed slightly from the standard PKCα assay. The final reaction volume was 150 μL (pH 7.5) containing 2 mM magnesium acetate, 20 mM Tris-HCl, 2 μM p53 synthetic peptide, 50 μg/mL leupeptin, 100 μM ATP-Tris (containing 0.5–1 μCi ^{32}P-γ-ATP), 0.01 μg PKM (Signal Transduction Inc., San Diego, California) (>820 nmol/min/mg assayed using a H1 histone substrate by Signal Transduction Inc.), with either 0 or 20 μM S100B($\beta\beta$), and with 0–400 μM $CaCl_2$. Each assay had three control experiments run without ATP, PKM, and substrate, respectively. The dependence of peptide concentration was monitored using assays under similar conditions with the p53 peptide concentration varying from 0 to 3.7 μM. Similarly, peptide titrations were completed with the Nm and [Ser-25]-PKC(19–31) peptides. As for PKC assays, the reactions were started by the addition of enzyme, run for 10 min at 37 °C, stopped with 25% TCA (75 μL), centrifuged for 2 min, and the supernatant (75 μL) was spotted onto P81 phosphocellulose paper. The filters were washed three times with 4 mL of 75 mM phosphoric acid, and then counted. All assays with PKM were completed in triplicate.

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References

Albert KA, Wu WC-S, Nairn AC, Greengard P. 1984. Inhibition by calmodulin of calcium/phospholipid-dependent protein phosphorylation. *Proc Natl Acad Sci USA 81*:3622–3625.

Amburgey JC, Abildgaard F, Starich MR, Shah S, Hilt DC, Weber DJ. 1995. ^{1}H, ^{13}C, ^{15}N NMR assignments and solution secondary structure of rat Apo-S100β. *J Biomol NMR 6*:171–179.

Baudier J, Bergere E, Bertacchi N, Weintraub H, Gagnon J, Garin J. 1995. Interaction of myogenic bHLH transcription factors with calcium-binding calmodulin and S100a($\alpha\alpha$) proteins. *Biochemistry 34*:7834–7846.

Baudier J, Delphin C, Grundwald D, Khochbin S, Lawrence JJ. 1992. Characterization of the tumor suppressor protein p53 as a protein kinase C substrate and a S100B-binding protein. *Proc Natl Acad Sci USA 89*:11627–11631.

Baudier J, Mochly-Rosen D, Newton A, Lee S-H, Koshland DE, Cole RD. 1987. Comparison of S100B protein with calmodulin: Interactions with melittin and microtubule-associated τ proteins and inhibition of phosphorylation of τ proteins by protein kinase C. *Biochemistry 26*:2886–2893.

Baudier J, Glasser N, Gerard D. 1986. Ions binding to S100 proteins. *J Biol Chem 261*:8192–8203.

Bianchi R, Giambanco I, Donato R. 1993. S-100 Protein, but not calmodulin, binds to glial fibrillary acidic protein and inhibits its polymerization in a Ca^{+2}-dependent manner. *J Biol Chem 268*:12669–12674.

Bianchi R, Verzini M, Garbuglia M, Giambanco I, Donato R. 1994. Mechanism

of S100 protein-dependent inhibition of glial fibrillary acidic protein (GFAP) polymerization. *Biochim Biophys Acta 1223*:354–360.

Burns DJ, Bloomenthal J, Lee M-H, Beel RM. 1990. Expression of the a, bII, and g PKC isozymes in the baculovirus insect cell expression system. *J Biol Chem 265*:12044–12051.

Chaudhuri D, Horrocks WW, Amburgey JC, Weber DJ. 1997. Characterization of lanthanide ion binding to the EF hand protein S100β by luminescence spectroscopy. *Biochemistry 36*:9674–9680.

Donato R. 1991. Perspectives in S-100 protein biology. *Cell Calcium 12*:713–726.

Drohat AC, Amburgey JC, Abildgaard F, Starich MR, Baldisseri D, Weber DJ. 1996. Solution structure of rat Apo-S100B($\beta\beta$) as determined by NMR spectroscopy. *Biochemistry 35*:11577–11588.

Drohat AC, Nenortas E, Beckett D, Weber DJ. 1997. Oligomerization state of S100B($\beta\beta$) at nanomolar concentration determined by large-zone analytical gel filtration chromatography. *Protein Sci 6*:1577–1582.

Graff JM, Rajan RR, R. RR, Nairn AC, Blackshear PJ. 1991. Protein kinase C substrate and inhibitor characteristics of peptides derived from the myristoylated alanine-rich C kinase substrate (MARCKS) protein phosphorylation site domain. *J Biol Chem 266*:14390–14398.

Graff JM, Young TN, Johnson JD, Blackshear PJ. 1989. Phosphorylation-regulated calmodulin binding to prominent cellular substrate for protein kinase C. *J Biol Chem 264*:21818–21823.

House C, Kemp BE. 1987. Protein kinase C contains a pseudosubstrate prototope in its regulatory domain. *Science 238*:1726–1728.

House C, Richard EH, Wettenhal REH, Kemp BE. 1987. The influence of basic residues on the substrate specificity of protein kinase C. *J Biol Chem 262*:772–777.

Ivanenkov VV, Jamieson GAJ, Gruenstein E, Dimlich RVW. 1995. Characterization of S100B binding epitopes. Identification of a novel target, the actin capping protein, CapZ. *J Biol Chem 270*:14651–14658.

Kemp BE, Pearson RB. 1991. Design and use of peptide substrates for protein kinases. *Methods Enzymol 200*:121–134.

Kilby PM, Van Eldik LJ, Roberts GCK. 1996. The solution structure of the bovine S100B protein dimer in the calcium-free state. *Structure 4*:1041–1052.

Kligman D, Hilt D. 1988. The S100 protein family. *Trends Biochem Sci 13*:437–443.

Lin L-H, Van Eldik LJ, Osheroff N, Nordon JJ. 1994. Inhibition of protein kinase C- and casein kinase II-mediated phosphorylation of GAP-43 by S100β. *Mol Brain Res 25*:297–304.

McIlroy BK, Walters JD, Blackshear PJ, Johnson JD. 1991. Phosphorylation-dependent binding of a synthetic MARCKS peptide to calmodulin. *J Biol Chem 266*:4959–4964.

Moore B. 1965. A soluble protein characteristic of the nervous system. *Biochem Biophys Res Commun 19*:739–744.

Patel J, Marangos PJ, Heydorn WE, Chang G, Verma A, Jacobowitz D. 1983. S-100-Mediated inhibition of brain protein phosphorylation. *J Neurochem 41*:1040–1045.

Roskoski R, Jr. 1983. Assays of protein kinase. *Methods Enzymol 99*:3–6.

Rustandi RR, Drohat AD, Baldisseri DM, Wilder PT, Weber DJ. 1998. The Ca^{+2}-dependent interaction of S100B($\beta\beta$) with a peptide derived from p53. *Biochemistry*, in press.

Schafer BW, Heizmann CW. 1996. The S100 family of EF-hand calcium-binding proteins: Functions and pathology. *Trends Biochem Sci 21*:134–140.

Schleiff E, Schmitz A, McIlhinney RAJ, Manenti S, Vergeres G. 1996. Myristoylation does not modulate the properties of MARCKS-related protein (MRP) in solution. *J Biol Chem 271*:26794–26802.

Sheu F-S, Azmitia EC, Marshak DR, Parker PK, Routtenberg A. 1994. Glial-derived S100b protein selectively inhibits recombinant β protein kinase C (PKC) phosphorylation of neuron-specific protein F1/GAP43. *Mol Brain Res 21*:62–66.

Sheu F-S, Huang FL, Huang K-P. 1995. Differential responses of protein kinase C substrates (MARCKS, neuromodulin, and neurogranin) phosphorylation to calmodulin and S100. *Arch Biochem Biophys 316*:335–342.

Smith SP, Barber KR, Shaw GS. 1997. Use of a differentially modified N-terminal methionine to analyze tertiary structure and conformational changes in human S100b. *Protein Sci 6*:1110–1113.

Suzushima H, N. A, Hattori T, Takatasuki K. 1994. Adult T-cell leukemia derived from S100 beta positive double-negative (CD4− CD8−) T cells. *Leuk Lymphoma 13*:257–262.

Takashi M, Sakata T, Nakano Y, Yamada Y, Miyake K, Kato K. 1994. Elevated concentrations of the beta-subunit of S100 protein in renal cell tumors in rats. *Urol Res 22*:251–255.

Verghese GM, Johnson JD, Vasulka C, Haupt DM, Stumpo DJ, Blackshear PJ. 1994. Protein kinase C-mediated phosphorylation and calmodulin binding of recombinant myristoylated alanine-rich C kinase substrate (MARCKS) and MARCKS-related protein. *J Biol Chem 269*:9361–9367.

Wilder PT, Weber DJ. 1996. S100β Inhibition of PKCα and PKM phosphorylation of a synthetic peptide derived from p53. *Biophys J 70*:A62.

Zimmer DB, Cornwall EH, Landar A, Song W. 1995. The S100 protein family: History, function, and expression. *Brain Res Bull 37*:417–429.