Published in final edited form as:
Curr Chem Biol. 2009 May 1; 3(2): 138–145. doi:10.2174/187231309788166460.

S100A1: Structure, Function, and Therapeutic Potential

Nathan T. Wright[1], **Brian R. Cannon**[1], **Danna B. Zimmer**[2], and **David J. Weber**[*,1]

[1] Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, 108 N. Greene St., Baltimore, Maryland, 21201

[2] Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A & M University, College Station, Texas, 77845

Abstract

S100A1 is a member of the S100 family of calcium-binding proteins. As with most S100 proteins, S100A1 undergoes a large conformational change upon binding calcium as necessary to interact with numerous protein targets. Targets of S100A1 include proteins involved in calcium signaling (ryanidine receptors 1 & 2, Serca2a, phopholamban), neurotransmitter release (synapsins I & II), cytoskeletal and filament associated proteins (CapZ, microtubules, intermediate filaments, tau, mocrofilaments, desmin, tubulin, F-actin, titin, and the glial fibrillary acidic protein GFAP), transcription factors and their regulators (e.g. myoD, p53), enzymes (e.g. aldolase, phosphoglucomutase, malate dehydrogenase, glycogen phosphorylase, photoreceptor guanyl cyclases, adenylate cyclases, glyceraldehydes-3-phosphate dehydrogenase, twitchin kinase, Ndr kinase, and F1 ATP synthase), and other Ca2+-activated proteins (annexins V & VI, S100B, S100A4, S100P, and other S100 proteins). There is also a growing interest in developing inhibitors of S100A1 since they may be beneficial for treating a variety of human diseases including neurological diseases, diabetes mellitus, heart failure, and several types of cancer. The absence of significant phenotypes in S100A1 knockout mice provides some early indication that an S100A1 antagonist could have minimal side effects in normal tissues. However, development of S100A1-mediated therapies is complicated by S100A1's unusual ability to function as both an intracellular signaling molecule and as a secreted protein. Additionally, many S100A1 protein targets have only recently been identified, and so fully characterizing both these S100A1-target complexes and their resulting functions is a necessary prerequisite.

INTRODUCTION TO S100 PROTEINS

Calcium-binding proteins in the EF-hand super family typically use calcium influx as a signal for regulating varied cellular processes including apoptosis, contraction, differentiation, gene expression, and many others [1]. This review examines the structure, function, and potential therapeutic roles of S100A1, a calcium-binding protein in the S100 protein family known to play an important role in heart disease, and one that has been implicated in several neurological disorders and types of cancers [2–4].

S100 proteins are a group of dimeric Ca^{2+}-binding proteins (~10 kDa per subunit) with diverse yet highly specialized functions [3–5]. S100 proteins were originally isolated from bovine brain, and named S100 to denote their solubility in 100% saturated ammonium sulfate solution [6]. Later, it was found that the S100 fraction in brain is composed of two homologous proteins, S100B and S100A1. Since that time, more than twenty S100 proteins have been discovered

*Address correspondence to this author at the Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, 108 N. Greene St. Baltimore, MD 21201; Tel: (410) 706-4354; Fax: (410) 706-0458; dweber@umaryland.edu.

(Fig. (**1**)). At least sixteen of the S100 protein genes map to human chromosome 1q21 and are denoted S100A1-S100A16. S100 proteins that fall outside this gene locus are given single letter names (S100B, S100G, S100P, and S100Z). In addition, there are pseudogene products for S100A7 and S100A11 (S100A7P1, S100A7P2, S100A11P) as well as gene products that very closely resemble S100A7 (S100A7A and S100A7L2) [7]. This genetic organization is recapitulated in other species; S100 genes in both mouse and rat also primarily cluster together on chromosomes 3 and 2, respectively [8]. As the S100 family has expanded, so has the list of diseases associated with altered S100 expression. Specifically, S100 family members have been implicated in psoriasis, rheumatoid arthritis, cystic fibrosis, cardiomyopathy, multiple sclerosis, amylotrophic lateral sclerosis, Down's Syndrome, Alzheimer's disease and a diverse list of tumors/cancers [2,3,5,9,10]. Thus, several S100 proteins are considered to be important therapeutic targets and diagnostic/prognostic biomarkers for several of these diseases [10].

There is now general agreement that S100 proteins are expressed in a cell-specific manner and serve as modulators and integrators of calcium signaling [9]. Such functional specificity is achieved by the interaction of one or more S100 proteins with other proteins termed "target proteins", resulting in the modulation of highly specific cellular function(s) [11]. The effect of S100 binding on the activity of a protein target can be partial or an "all or nothing" effect. As found for calmodulin and other EF-hand containing proteins, the calcium binding affinity of an S100 protein can itself can be tightened by the S100-target protein interaction and/or by various cellular processes including post-translational modifications (i.e. glutothionization, phosphorylation) [12–14]. In addition, higher order oligomeric states of the core S100 dimer structure (i.e. trimer of dimers) have also been reported for several S100 proteins (i.e. S100B, S100A12) [15,16], and these various oligomeric states may influence specific protein target interactions. In a few cases, calcium-binding is not required for complex formation [17,18]. For example, the target protein binding site in S100A10 is already exposed in the apo-state, so the formation of S100A10-protein complexes does not depend on calcium [19].

Since the identification of the first S100A1/B target protein, fructose 1,6-bisphosphate aldolase [20], target proteins have been discovered for nearly all of the S100 family members [9,11, 21]. For S100A1 and S100B, the two earliest discovered members, more than twenty target proteins have been described for each (Table 1) [4,5,22]. As the list of target proteins for individual family members has increased, it has become clear that several target proteins are regulated by more than one S100 protein. Furthermore, S100 proteins can form heterodimers with other family members, providing a large number of S100 heterodimer combinations and possible biological outcomes. Thus, one must consider cellular location, protein levels, the physical state of a specific S100 protein and its protein target, as well as the possibility for functional redundancy amongst family members when studying the role of an S100 protein. The fact that S100 proteins modulate numerous cellular processes and that more than one S100 protein can affect a protein target is consistent with the subtle phenotypes observed when a single S100 protein is knocked out in mice [23–25]. Nonetheless, when an S100 protein is over expressed, as found in several disease states, restoration of normal cellular function can be achieved by inhibiting the S100 protein that is adversely elevated [26–29].

S100 STRUCTURE AND DRUG DESIGN

The three-dimensional structures for several S100 proteins have been solved using X-ray crystallography and NMR spectroscopy techniques, which have contributed to drug design efforts [19,30–42]. With calbindin D9K as an exception, S100 proteins in the Ca^{2+}-free, Ca^{2+}-bound, and target protein bound states are structural homologues with extensive hydrophobic interactions between helices 1, 1′, 4, and 4′ that form a noncovalent X-type four helix bundle at the dimer interface. As a result of this fold, dissociation of an S100 protein dimer occurs only in the sub nanomolar range, which is well below the concentration of a

typical S100 protein in the cell (i.e. micromolar levels) [43]. Each subunit of an S100 protein has two helix-loop-helix EF-hand calcium-binding domains connected by a linker region termed the hinge region (Fig. (**2**)). The EF-hand in the N-terminal region of the protein (residues 19-32 in S100A1) is 14 amino acids in length and called the pseudo or S100 EF-hand. The second EF-hand is in the C-terminal region of the protein (residues 62-73 in S100A1), and is a canonical 12-residue EF-hand that matches exactly the consensus sequence for calcium-binding domains found in calmodulin (CaM), troponin C, and other members of the EF-hand superfamily [41,44]. The typical EF-hand relies on specific side chain oxygen atoms for calcium coordination, whereas the pseudo EF-hand coordinates calcium primarily via backbone carbonyl oxygen atoms with only the last residue in the EF-hand, a glutamic acid residue, forming a bidentate ligand from its two side chain carboxylate oxygen atoms to calcium. Multiple studies now indicate that upon binding calcium, only the canonical EF-hand undergoes a large conformational change involving helix 3 (the entering helix), which is close to parallel with helix 4 (the exiting helix) in the apo-state but almost perpendicular to helix 4 in the Ca^{2+}-bound state (Fig. (**3**)). This reorientation of helix 3 upon binding calcium is a unique characteristic of S100 proteins as compared to other EF-hand containing proteins (i.e. CaM, TnC etc) for which the exiting helix (helix 4) moves. It should also be mentioned that the extent of the helix 3 reorientation differs between S100 family members due primarily to the position of helix 3 relative to helix 4 in the apo state; S100A1 and S100B experience an almost 90 degree reorientation of helix 3 upon calcium-binding, while S100A4 has a less drastic change, and S100P experiences virtually no change at all [32,33,45]. The pseudo EF-hand does not undergo such a conformational change upon calcium-binding (in S100A1, S100B, S100A6, etc); however, when examined closely, the exiting helix (helix 2) typically twists slightly to accommodate the large conformational change that occurs in the canonical EF-hand [41].

The Ca^{2+}-dependent conformational change is acknowledged to be the activation trigger for most S100-target protein interactions [11,32,46–48]. Specifically, a large hydrophobic pocket that is buried in the apo state is typically exposed upon binding calcium (Fig. (**3**)) [32,41]. For all S100 proteins characterized so far, the target protein binding site consists of residues from the hinge region, helix 3, and helix 4, and residues in these areas of the protein have the lowest homology among S100 family members [18]. Thus, the diversity in the putative target protein binding site of the various S100 proteins allows for target-specific binding based on the topology and chemical makeup of the hydrophobic pocket. However, while each S100 target binding site is unique, the pockets are similar enough, in some cases, to elicit overlapping target protein interactions.

With high-resolution three dimensional structural data available for numerous S100 proteins, several groups have begun to search for inhibitors that are specific for one or more S100 proteins. In particular, large efforts are currently ongoing to develop inhibitors of S100B [49–51] and S100A4 [52] due to their involvement in numerous cancers [9]. In these drug design studies, the high resolution structures have been used for screening large databases of small molecules using computer aided drug design approaches [53] and/or by experimental fragment based approaches [51]. Several lead compounds, identified with these methods, have been further evaluated via biochemical and biological screens and the drug-S100 protein interactions examined via structural biology techniques [50]. The hope is to take the most promising of these leads and use synthetic chemistry approaches to optimize binding and specificity [53]. Similar approaches have more recently been initiated for the development of S100A1 inhibitors.

ELEVATED S100A1 LEVELS IN NEUROLOGICAL DISORDERS: A ROLE FOR S100A1 ANTAGONISTS

There is a growing interest in developing inhibitors of S100A1 since they may be beneficial for treating a variety of human diseases including neurological diseases, diabetes mellitus, heart failure, and several types of cancer [3,54–56]. The absence of significant phenotypes in S100A1 knockout mice provides some early indication that an S100A1 antagonist could have minimal side effects in normal tissues [23,57]. However, development of S100A1-mediated therapies is complicated by S100A1's unusual immunological/biochemical properties and its ability to function as both an intracellular signaling molecule and as a secreted protein. Additionally, many S100A1 protein targets have only recently been identified, and so fully characterizing both these S100A1-target complexes and their resulting functions is a prerequisite to completing S100A1 drug design efforts. Of the approximately twenty tissues where S100A1 is expressed, most studies involving S100A1 function have been completed in muscle or brain, where it is highly abundant, so they will be considered further here.

In the brain, increased S100A1 expression contributes to pathologies related to neurological diseases, and as a result efforts to inhibit S100A1 and reverse such processes are underway. There is reason to believe such inhibition studies could prove to be medicinally relevant; neuronal PC12 cells that do not express S100A1 are more resistant to Aβ-induced cell death than cells that express normal levels of S100A1 [3], indicating that S100A1 antagonists could have potential for treating Alzheimer's disease. In addition, extracellular S100A1 is cytotoxic to PC12 cells (Zimmer, unpublished observations;[58,59]) and injection of anti-S100A1 antibodies was found to lessen learning and memory deficits [60]. Other S100A1-regulated intracellular processes are also consistent with S100A1 augmentation of Alzheimer's disease pathology including S100A1-linked altered amyloid precursor protein expression, destabilization of calcium homeostasis, S100A1-mediated increased cell growth, decreased dendritic arborization, and decreased tubulin polymerization/stability [3,61]. Together, such studies provide incentive to develop S100A1 antagonists, so that this protein can be regulated in both its intra- and extra-cellular locations. Such a molecule and/or an antibody may also have use as a new scientific reagent for studying S100A1 function as well as for the development of new therapeutics.

Using methods similar to those for identifying small molecule inhibitors of S100B, our group has recently found that a U.S. Food and Drug Administration approved antimicrobial medication, pentamidine (Pnt), inhibits S100A1-target interactions (Fig. (**4**)). Another group has also identified several antiallergic drugs including olopatadine, amlexanox, and cromolyn that interact with different residues lining the S100A1 the target protein binding site [62]. Additionally, Nakatani *et al.* have shown that the beta blocker propanolol binds to multiple S100 proteins, including S100A1, with low micromolar affinity [63]. Currently none of these drugs have been tested for *in vivo* therapeutic efficacy, but efforts are underway to examine whether they hold promise as therapeutics for treatments of neurological disorders such as Alzheimer's disease. While these inhibitors of S100A1 represent new lead compounds, our group and others are continuing drug development efforts with the goal of obtaining novel high affinity molecules that specifically inhibit S100A1 and have beneficial effects *in vivo*.

DIMINISHED S100A1 LEVELS IN CARDIAC AND SKELETAL MUSCLE MYOPATHIES: A POTENTIAL NEED FOR S100A1 GENE THERAPY

S100A1's involvement in muscle contraction was first suspected when it was discovered that cardiac S100A1 levels decrease in patients with heart failure [64]. In an effort to elucidate the function of S100A1 in both heart and skeletal muscle, multiple studies have since demonstrated

that removing S100A1 decreases the fractional cell shortening of myocytes as a result of functionally impaired ryanodine receptor (RyR) [57,65–67]. S100A1 co-localizes with RyR in both heart and skeletal muscle (RyR1, RyR2 respectively) and decreases RyR calcium transients via a Ca^{2+}-dependent S100A1-RyR interaction [57,68]. Importantly, S100A1 competes directly with CaM for the same binding site on RyR1 and RyR2 [57,69]. Because Ca^{2+}-CaM binding to RyR inhibits RyR-induced calcium release in both skeletal and cardiac myocytes [70], the simplest model for S100A1-dependent activation of RyR involves competing away a well-established RyR inhibitor (i.e. CaM). Alternatively, it cannot be ruled out that the S100A1-RyR interaction stabilizes active RyR and that CaM-dependent inhibition of the RyR occurs by CaM displacing S100A1 [57].

S100A1 is important in regulating myocyte calcium cycling not only though modulating the activity of RyR, but also by influencing SERCA/PLB and protein kinase A (PKA) activity [71–75]. In support of this statement, S100A1 gene transfer increases cardiac muscle SERCA activity ~30%, and reciprocally S100A1 knockout mice show a ~30% decrease in calcium reuptake following an action potential in skeletal muscle fibers ([76], unpublished observations). The molecular mechanism underlying this phenomenon is still unknown. S100A1 also binds to the regulatory subunit of PKA IIb, thereby increasing PKA activity and increasing PKA-dependant L-type calcium channel activation in cardiomyocytes [77]. However, another group reports that S100A1 does not affect either the L-type calcium channel or the sodium-calcium exchanger [78]. Therefore, additional work needs to be done to clarify the role of S100A1 with regard to PKA activation.

While most attention has centered on S100A1's role in systolic (contracting) cytoplasmic calcium levels, there is also evidence that S100A1 affects diastolic (resting) calcium regulation. Volkers *et al.* have shown a functional consequence of decreased calcium spark activity in cells overexpressing S100A1, suggesting that S100A1 decreases calcium leaks in cardiomyocytes [75]. Besides modulating calcium levels in contracting and resting muscle, S100A1 may have other functions in the heart. For example, electrocardiograms done on S100A1-deficient mice display long QT, QTc, and ST intervals, indicating a prolonged cardiac repolarization period [79]. A lack of S100A1 also leads to ventricular conduction defects. At this time, there is little known regarding the molecular basis for most of these cardiac phenotypes; however, they warrant further investigation nonetheless.

The ability of S100A1 to restore cell shortening in cardiomyocytes has made this protein an exciting prospect for therapeutics. One hallmark of failing cardiomyocytes is a down-regulation of S100A1, and thus methods of reversing this trend could potentially be useful in a wide spectrum of human heart and muscle diseases [80]. Towards this goal, several groups, most notably those of Patrick Most and Walter Koch, have developed gene therapy methods for introducing S100A1 into diseased or damaged cardiomyocytes. In some promising experiments, S100A1 delivered by an adenoviral-based vector system was shown to restore diminished calcium transients and reverse contractile dysfunction in a rat heart failure model [71]. A related study found that S100A1 administered after cardiac infarction in rats restored cardiac contractile ability through stabilization of calcium levels [65]. Traditionally, a major problem of gene therapy has been an inability to sustain prolonged stable protein expression in the tissue of interest; however, long-term (at least 10 weeks) cardiac-targeted S100A1 expression via adeno-associated viral transfer has been shown in rats, and such expression restored cardiac function by over 30% during this time period [81,82]. This level of restoration of function for the entire organ is similar to what others have seen in studies on individual cells. The fact that S100A1 addition has a dramatic effect on animals undergoing heart failure, combined with rapid advances in the therapeutic testing of S100A1, foretells an increase in the number of gene therapy studies using S100A1 vectors.

CONCLUSIONS REGARDING THERAPEUTIC STRATEGIES INVOLVING S100A1

Changes in S100A1 expression have been reported in end stage heart failure [64], diabetes (54), ischemia [83,84], and chronic pulmonary hypertension [85], and it is generally recognized that decreased S100A1 levels contribute to the downhill clinical course of heart failure. One of the first indications of S100A1's beneficial effects on heart function was a report that S100A1 knockout mice are unable to adapt to acute and hemodynamic stresses [23]. In fact, increased S100A1 abrogates heart failure [67,86] and has been investigated as a mechanism for strengthening engineered cardiac grafts prior to implantation [87]. It has also been suggested that cardioprotective drugs eventually exert their effects through reversing the down-regulation of S100A1 expression [63,65], and the use of gene therapy to deliver S100A1 to patients with failing hearts has the potential to be a promising future treatment [65,81]. While S100A1 is becoming better understood in striated muscle and is generally thought of as an enhancer of contractile performance, it will be important to resolve some important questions. For example, it is not yet clear whether intracellular and/or extracellular S100A1 is responsible for the activation of MAPK signaling pathways in myocytes as reported [72]. It would also be prudent to know whether S100A1 binds specifically to the RAGE receptor *in vivo* and whether this S100A1-receptor interaction affects cardiac output in normal and/or diabetic patients [88].

On the other hand, many S100 protein family members, including S100A1, are up-regulated in several other disease states including Alzheimer's disease, Down's Syndrome, diabetes, and in several cancers [3,89,90]. Therefore, inhibiting S100 protein function via small molecule inhibitors holds promise for several therapeutic purposes. S100A1's contribution to Alzheimer's disease pathology, including altered APP expression, destabilization of calcium homeostasis, and increased sensitivity to Aβ toxicity [3], makes it a promising new protein to target via rational drug design. The use of inhibitors that block both S100A1 and S100B function simultaneously may be particularly effective for Alzheimer's disease since there is evidence that both of these proteins contribute to neuritic plaque formation. Thus, the FDA approved drug pentamidine may prove to be an ideal lead, since this compound has now been shown to bind both S100A1 and S100B (Fig. (**4**)).

In summary, S100 proteins represent a large family of calcium-binding proteins in higher vertebrates. As the therapeutic potential of S100A1 in muscle becomes clearer, so does the pressing need to design safe and effective modes of increasing muscle S100A1 potency. Conversely, inhibiting brain S100A1 may lead to potential tools to combat neuro-degenerative diseases. Thus, the field currently faces a conundrum: how can we inhibit S100A1 function in the brain without impairing its function in heart and vice versa to achieve the proper physiological balance? In addition to standard dosing methodologies, other potential methods are being developed to address this fundamental question. Currently, tissue-specific gene therapy and the development of rationally-based drugs with tissue-specific delivery techniques could improve therapies that will need to thread the needle of tissue- and target- specificity.

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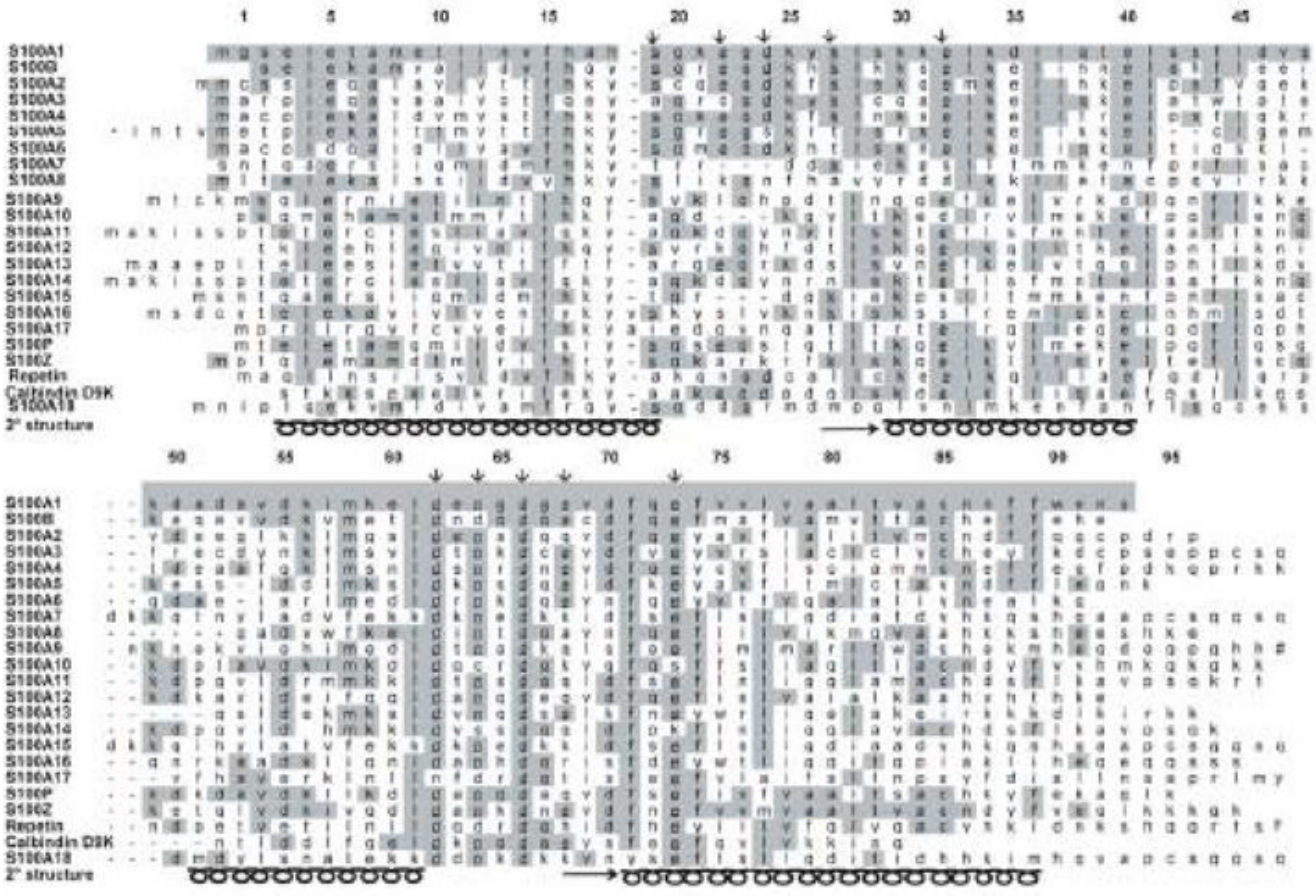

Fig. 1.

Sequence homology of the S100 family of proteins. S100 proteins are small calcium-binding proteins, with two helix-loop-helix domains. Residues in gray share sequence homology to S100B, and residues marked with an arrow are involved in calcium coordination.

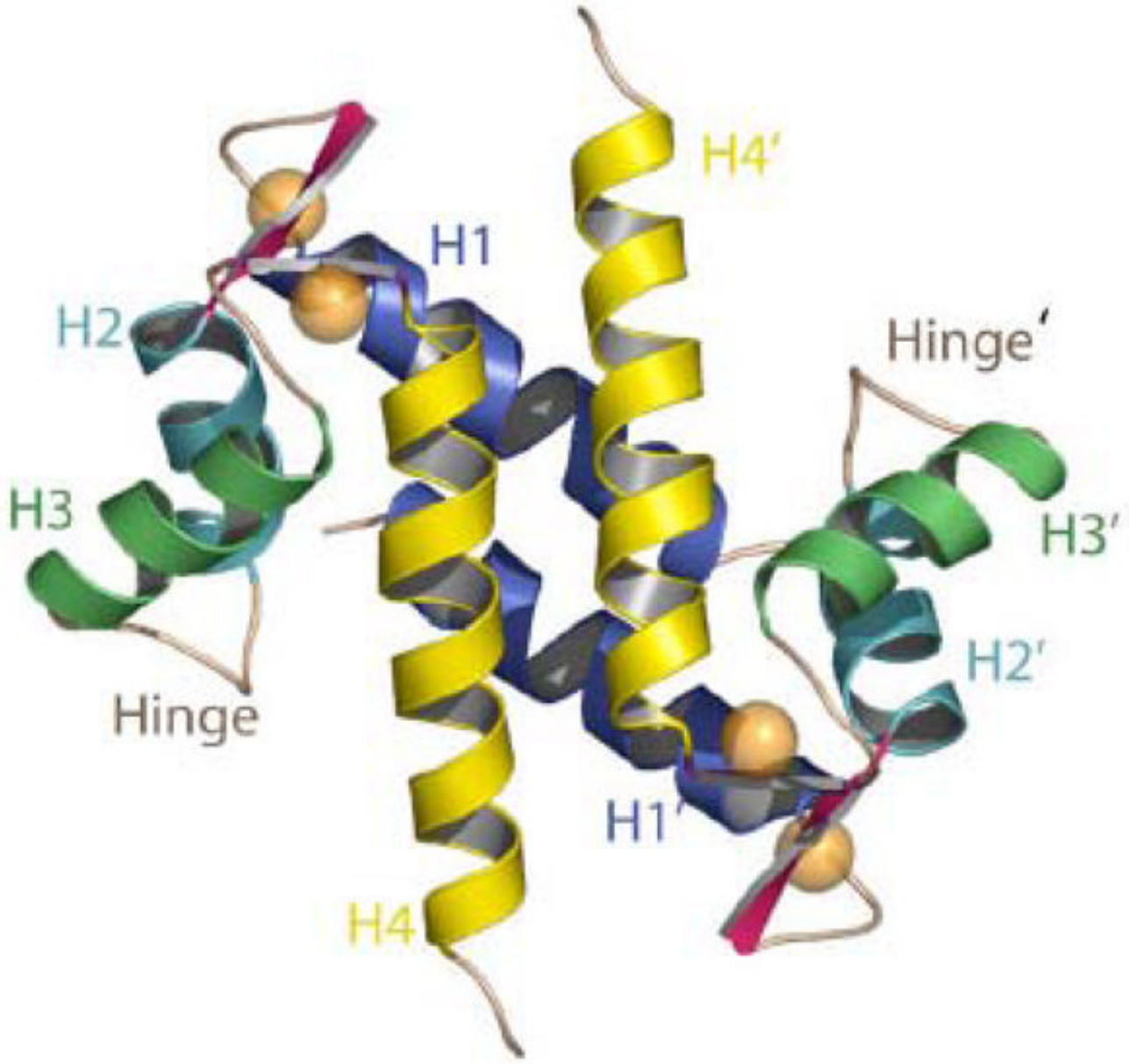

Fig. 2.

Ribbon diagram of Ca^{2+}-S100A1, a typical S100 protein. The X-type four-helix bundle, at the dimer interface of S100A1, has an antiparallel alignment of helices H1 and H1′ (in blue) and H4 and H4′ (in yellow), respectively. A short antiparallel sheet between the pseudo (pink) and typical (purple) EF-hands is shown using arrows. The pseudo-EF hand is formed by the flexible loop between helix I (blue) and helix II (aqua). The typical EF-hand is formed by the flexible loop between helix 3 (green) and helix 4 (yellow). The two EF-hands are connected via the 'hinge' region. Taken from [41].

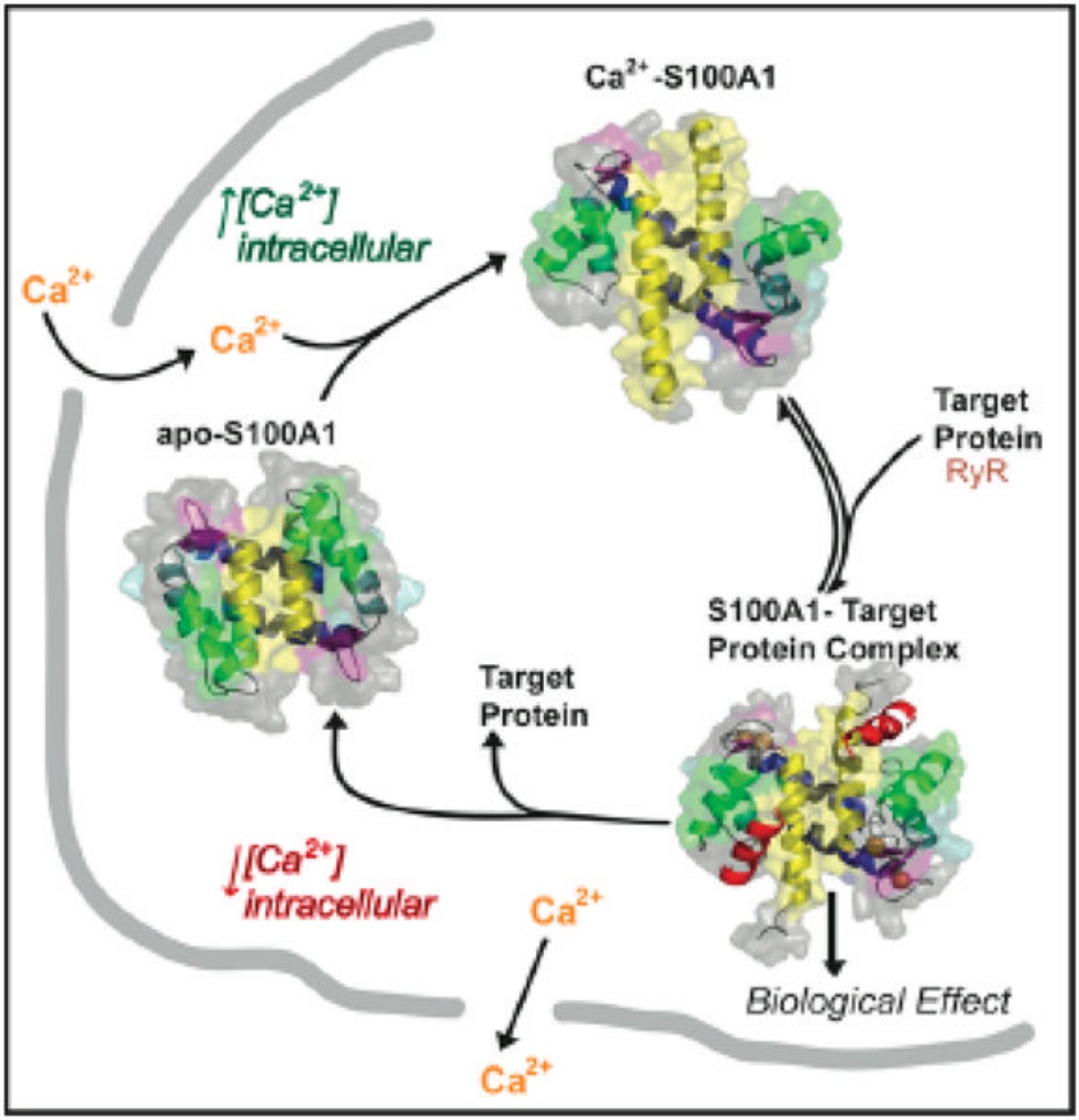

Fig. 3.

S100A1 mode of action demonstrating the Ca^{2+}-dependent conformational change that is necessary for S100A1 to interact with its target molecules. In the apo-form of the protein, the hydrophobic cleft is buried, occluding the target peptide binding residues. These same residues are exposed upon the addition of calcium.

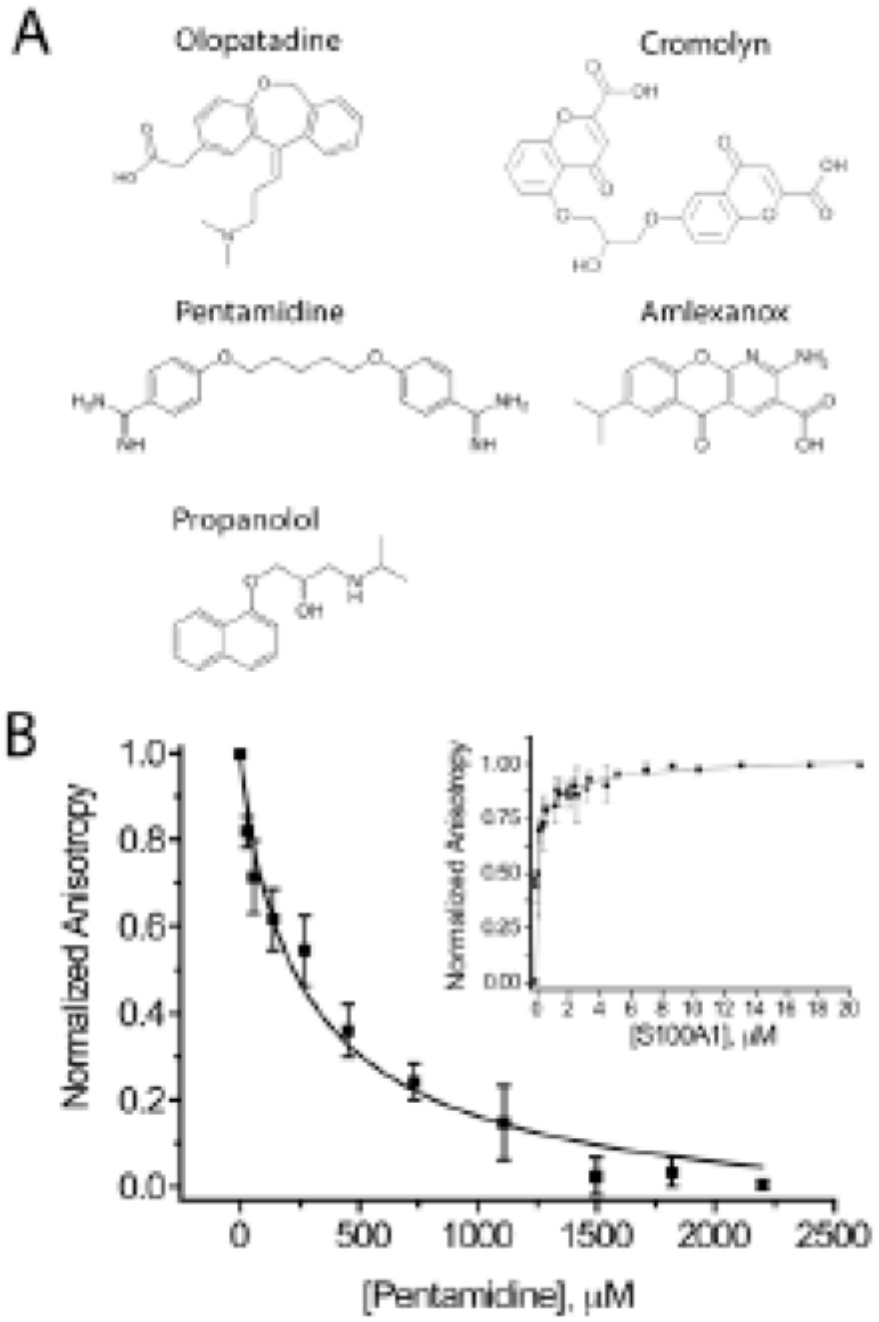

Fig. 4.

S100A1-binding small molecules (A) chemical structures and names of S100A1 inhibitors, as reported in [62,63] and here. (B) Competition experiment where increasing amounts of pentamidine (Pnt) disrupts the S100A1 interaction with a TAMRA-labeled RyRP12 peptide, as measured by fluorescence anisotropy. The conditions are: 5mM HEPES pH 7.5, 25 mM NaCl, 1 mM $CaCl_2$, 1 mM DTT, 50 nM TAMRA-RyRP12, 25 °C. The pentamidine kickoff experiment was conducted at 2 mM [S100A1]. The RyRP12 K_D for S100A1 is 139 ± 19 nM, and using equations for calculating K_D from the K_I, reported in [106], the dissociation constant (K_D) of Pnt from the Pnt-Ca^{2+}-S100A1 complex is 20 ± 4 mM.

Table 1

S100A1 Target Proteins and Functions

S100A1 Target	Biological Function of S100A1	Ref.
Ryanodine Receptor	Increases SR calcium release	[57,91]
SERCA/PLB	Increases calcium reuptake	[74]
PKA-R(IIb)	Influences L-type calcium channel activation	[77]
Tubulin	Increases microtubule stability	[61,92,93]
GFAP	Blocks intermediate filament assemblies	[92,94]
Synapsin I	Inhibits F-actin bundling	[95]
RAGE receptor	Growth factor	[96]
PEVK	Passive tension modulator	[97]
CapZ	Unknown	[41]
Phosphoglucomutase	inhibits phosphoglucomutase activity	[17]
Membrane-associated guanylate cyclase	Stimulates guanylate cyclase	[98]
Annexin IV	Regulates annexin function	[94,99]
Twitchin	Increases kinase activity	[100]
Myo D	Inhibits myoD function/phosphorylation	[101]
Other S100 proteins	Unknown	[40,45,102]
β-adrenergic receptor	Involved in calcium cycling	[82]
PKC	PKC signaling alteration	[103]
HSP 70	Member of the Hsp70/Hsp90 chaperone complex	[62]
HSP 90	Member of the Hsp70/Hsp90 chaperone complex	[62]
FKBP52	Member of the Hsp70/Hsp90 chaperone complex	[62]
CyP40	Member of the Hsp70/Hsp90 chaperone complex	[62]
Aldolase	Possible chaperone/folding partner	[62]
citrate synthase	Possible chaperone/folding partner	[62]
glyceraldehyde-3-phos-phate dehydrogenase	Possible chaperone/folding partner	[62]
Rhodanese	Possible chaperone/folding partner	[62]
ERK1/2	Anti-apoptotic effector	[104]
CacyBP/SIP	Modulates ubiquitylation	[105]
p53	Inhibits cell repair	[92]