Hypothesis

A model for target protein binding to calcium-activated S100 dimers

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Abstract S100 proteins are a family of dimeric calcium-binding proteins implicated in several cancers and neurological diseases. Calbindin D_{9k} is an unusual monomeric member of the S100 family. A calbindin D_{9k} mutant containing a novel calcium-induced helix is characterized. Based on sequence comparison, this helix could be a component of other S100 proteins and a factor in target protein binding. The origin of structural differences between three reported apo S100 dimer structures is verified. We conclude that the differences are a result of modeling rather than a function of different target binding properties. A mechanism for target protein binding is suggested.

Key words: S100; Calcium-binding protein; Protein structure; Target protein binding

1. Introduction

EF-hand proteins contain paired helix-loop-helix motifs that comprise a four-helix bundle module with a short β-sheet between the typically cooperatively binding calcium-binding loops (see Fig. 1) [1,2]. Structural biology, and NMR in particular, has played an important role in characterizing the many functions displayed within this superfamily of proteins [3,4]. For example, calcium binding to the sensor proteins calmodulin or troponin C, two of the best known members of this superfamily, causes large structural changes and exposure of the hydrophobic core resulting in an ability to bind target proteins. This is not the case for calbindin D_{9k} for which calcium binding induces very little structural change and for which no target proteins are known [5]. It is instead thought to act as a calcium buffer or transport protein [6].

S100 proteins form an important sub-class of EF-hand proteins that are typically small, about 90 residues (one EF-hand module), and dimeric [2,7]. Many S100 proteins display structural changes and exposure of hydrophobic surfaces upon calcium binding, reminiscent of the sensor properties of calmodulin, and interact with a number of identified target proteins [2,7]. Calbindin D_{9k} is also a member of the S100 family, despite the lack of sensor properties. The mechanism for the interaction between S100 and target proteins is of importance as a large biological and medical interest revolves around the numerous diseases with which abnormal levels of S100 proteins are associated, for example several cancers and Alzheimer's disease [2,7]. This paper investigates a possible target binding mechanism for S100 proteins.

2. Discussion

2.1. A calcium-induced helix in the P43MG mutant of calbindin D_{9k}

The P43MG calbindin D_{9k} mutant contains an additional residue in the linker between the two EF-hands, previously believed to be without structure, and has a calcium-binding affinity greater than that of native calbindin D_{9k} [8]. NMR data attribute this increase to a calcium-induced helix at the start of the linker region and close to the mutation point (Fig. 2). A comparison of HN chemical shifts indicates similar structures for the apo forms of P43G and P43MG while a difference in structure is suggested by the calcium-bound data. The NMR data for P43G in the apo and calcium-bound states show that the linker region contains no ordered structure [9,10], although high-resolution structures suggest some ill-defined helical character [5,11]. The lack of structure is supported by backbone relaxation data of P43G in both states that show the linker region to be highly mobile. The calcium-bound form of P43MG contains a unique short helix of at least four residues (S38–K41) on the basis of HN CSI, CαH CSI and $^3J_{HNH\alpha}$ values.

NOE evidence indicates that the calcium-induced helix of P43MG is maintained through the burial of two residues (L39 and L40) into the base of the hydrophobic core formed by the four-helix bundle [8]. We note that a hydrophobic triad (F36, L39 and L40 in calbindin D_{9k}) is conserved for many of the S100 proteins (Fig. 3). The analogous residue of F36 in both S100B assignments adopts an extended conformation [12,13] similar to that observed for calbindin D_{9k} structures [5,11]. Does the calcium-induced helix of P43MG play a more general role in other S100 proteins?

2.2. S100 dimer structures

We decided to investigate a possible target protein binding mechanism in S100 proteins using the published apo structures of calcyclin (S100A6) [14,15], rat S100B (r-S100B) [12] and bovine S100B (b-S100B) [13], and the large amount of data for calbindin D_{9k} [5,6,8,9,11]. The apo S100 dimer structures illustrate monomeric subunits sharing a high structural identity with calbindin D_{9k} for much of the well-defined portions of the structures, namely helices I, II and IV, and the first calcium-binding loop [15]. However, various structural differences exist in the linker region between helices II and III, the orientation of helix III and the conformation of the

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Abbreviations: CSI, chemical shift index; NOE, nuclear Overhauser effect spectroscopy crosspeak; P43G, a calbindin D_{9k} mutant; P43MG, a calbindin D_{9k} mutant where residue P43 is replaced with the sequence M43G43a

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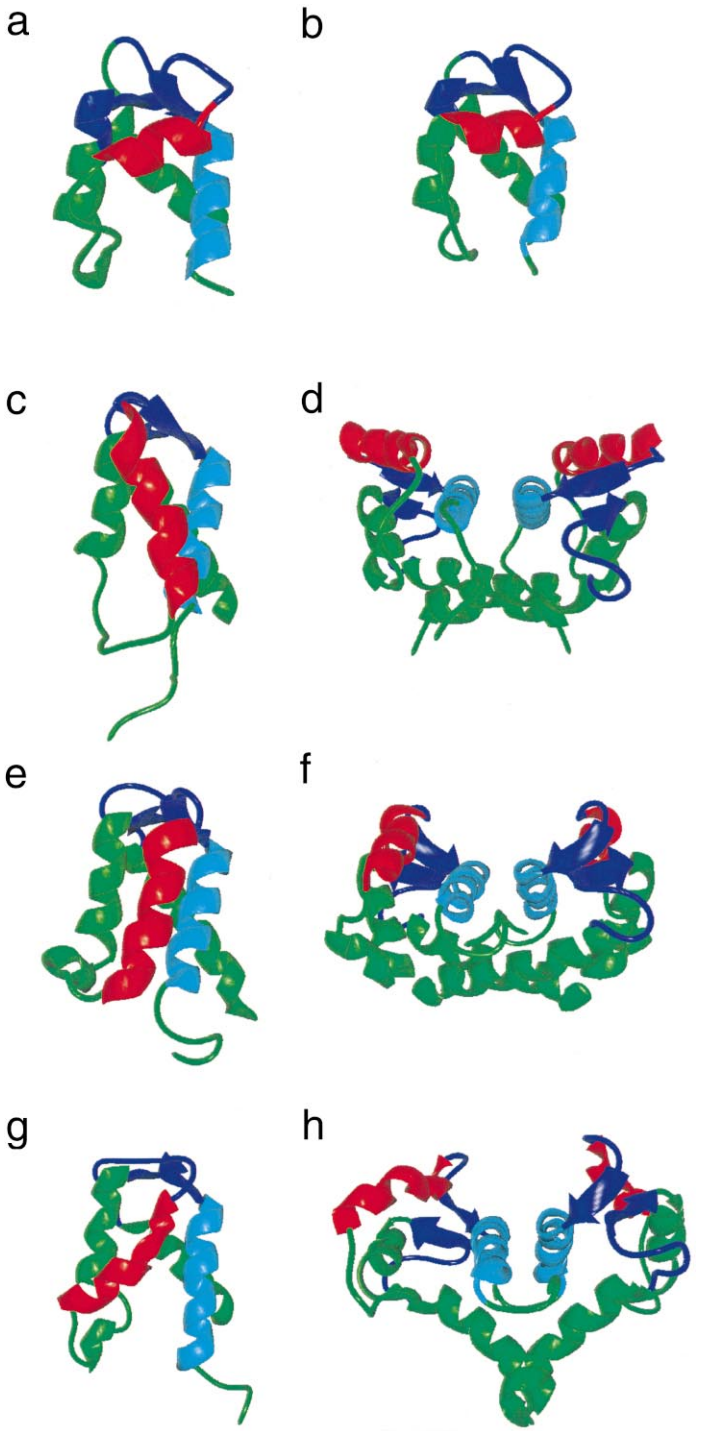

Fig. 1. Ribbon representations of the (a) apo [5] and (b) calcium-bound [11] forms of P43G calbindin D_{9k}; (c, d) views of an apo r-S100 monomer subunit and the dimer [12]; the same views of the (e, f) apo b-S100B [13] and (g, h) calcyclin [14] dimers. Color scheme: helices I and II, linker region and C-terminal residues, green; calcium-binding loops, blue; helix III, red; and helix IV, cyan. The linker region, helix III and the second calcium-binding loop face the front (panels a–c, e and g) and the view is rotated by 90° (panels d, f and h) for end-on representations of the putative target-binding clefts. Protein coordinates were obtained from the Brookhaven Data Bank, accession codes 1clb for apo calbindin D_{9k}, 2bcb for calcium-bound calbindin D_{9k}, 1sym for r-S100B, 1cfp for b-S100B and 1cnp for calcyclin. This figure was prepared using MIDAS [19] to overlay the monomeric units with apo calbindin D_{9k} (residues 3–14, 25–35 and 63–72): calcium-bound calbindin D_{9k} (residues as for apo), S100B (residues 7–18, 29–39 and 70–79) and calcyclin (residues 9–20, 31–41 and 70–79). In all cases RMSD values for the match increased significantly upon addition of helix III residues to the match criteria.

second calcium-binding loop (Fig. 1). We have tried to rationalize these differences that might otherwise be considered biologically, and/or functionally, relevant and related to different target-binding functions.

The calcyclin structure is of lowest resolution and no evidence is given to support the orientation of helix III and its adjoining loop regions [14,15]. One reason that the calcyclin structure does not resemble that of r-S100B could be that

helix III adopts a calcium-bound conformation in the absence of an adequate number of long-range distance restraints. Several other features in the helix III region of the apo calcyclin structure resemble those expected for the putative calcium-bound conformation, as described below.

While the b-S100B structure is of adequate resolution, features of the linker region can be called into question. A comparison of amide and CαH chemical shifts of b-S100B with r-S100B suggests that both S100B structures should contain the same secondary structure elements (Fig. 2). We note that the N-terminal anchoring point of helix III is a poorly defined region of the b-S100B structure (Figure 1 of reference [13]) and a solvent-exposed hydrophobic residue (I47) is present in the linker region. Evidence for a linker helix [16], absent in the r-S100B structure [12], is not as strong as for P43MG [8].

The r-S100B structure [12] is the one in which we have most confidence for the correct orientation of helix III from the published data. The orientation of helix III in r-S100B is unusual when compared to calbindin D_{9k} and other apo EF-hand structures (Fig. 1). It severely constrains the adjoining loops by extending the linker region between helix II and helix III, and by distorting the second calcium-binding loop (this latter feature is also seen in the b-S100B structure [13]) through a different C-terminal anchoring point of helix III when compared to calbindin D_{9k} (Fig. 1).

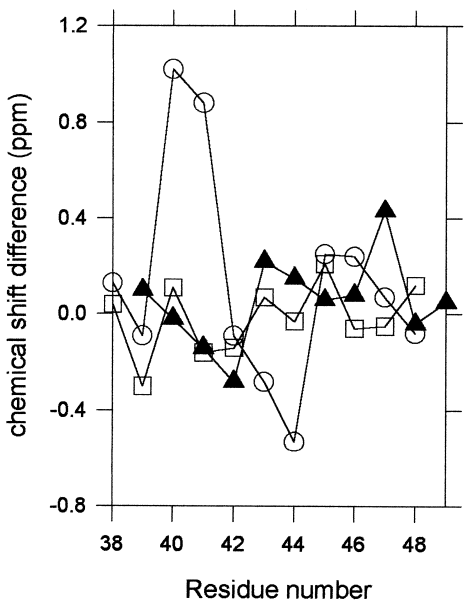

Fig. 2. Plots of chemical shift difference (Δδ) versus residue numbers in the linker region of EF-hand homologues. The close similarity of the backbone ^{1}H chemical shifts (r-S100B and b-S100B values) suggest that the two S100B homologues share the same secondary structure (filled triangles). Maximum differences for several residues that result in up to three different distinct residues are plotted and the real differences might be smaller. The multiple resonances are due to partial formylation of the N-terminus that neighbors the linker region [20]. Two calbindin mutants (P43MG and P43G values) in their apo state (open squares) also share similar structures, but not in their calcium-bound states (open circles) which have several large differences at positions 40 and 41 corresponding to a new calcium-induced helix. P43G (apo) shifts were obtained from reference [10], P43G (calcium-bound) from reference [9] and P43MG (apo and calcium-bound) from reference [8]. r-S100B chemical shifts were obtained from BioMagResBank and b-S100B chemical shifts from references [13] and [16].

(a)		
consensus [1]		Eh--h h----
S100A1 (S100α)	39-	ELSGF LDAQK
S100A2 (S100L)	40-	ELPSF VGEKV
S100A3 (S100E)	40-	ELATW TPTEF
S100A4 (CAPL)	40-	ELPSF LGKRT
S100A7 (psoriasin)	37-	NFPNF LSACD
S100A8 (calgran A)	40-	ECPQY IRKKG
S100A9 (calgran B)	43-	DLQNF LKKEN
S100A10 (p11)	36-	EFPGF LENQK
S100A12 (calgran C)	39-	ELANT IKNIK
S100A13	36-	QLPHL LKDVG
S100B (S100β)	38-	ELSHF LEEIK
S100D (Calbindin D_{9k})	38-	EFPSL LKGPN
P43MG b-Calbindin D_{9k}	37-	*EFPSL LKGMG*
S100E (S100P)	39-	ELPGF LQSGK
TRICHOHYALIN	39-	EFGAV LRRPH
PROFILAGGRIN	39-	EFRQI LKNPD
(b)		
S100A5 (S100D)	58-	ELCLG EMKES
S100A6 (calcyclin)	40-	ELTIG SKLQD
S100A11 (S100C)	45-	ELAAF TKNQK
(c)		
troponin C [2]	46-	MLGQN PTPEE
troponin C [3]	122-	ATGET ITEDD
calmodulin [2]	38-	SLGQN PTEAE
calmodulin [3]	111-	NLGEK LTDEE

[1] E = glutamic acid and h = hydrophobic residues (typically L or F – G, I, V, W and Y are less common).
[2] loop between EF-hands II and III
[3] loop between EF-hands VI and VII

Fig. 3. Simple sequence alignment of the first 10 residues from the putative linker regions of human S100 proteins and other EF-hand protein examples. A well-conserved glutamate residue is shaded (dark gray) with a hydrophobic triad of residues (light gray) in this sequence-variable region of the protein. a: The majority of S100 proteins, including the multi-modular proteins tricohyalin and profilaggrin, conform to this rule and have the same regular-helical propensity as the P43MG mutant of bovine calbindin D_{9k} (italics). b: A minority of S100 proteins, including calcyclin, do not contain a full hydrophobic triad but may form tighter 3_{10} turns in this region. c: The hydrophobic triad is not observed for calmodulin or troponin C.

2.3. Target binding by S100 dimers

The base of the putative target-binding site, suggested by several groups, contains helix IV from each monomer paired in an anti-parallel orientation in the dimer structure with helix III from each monomer subunit forming its sides (Fig. 1) [12–14]. Fig. 1d illustrates that the potential binding cleft of r-S100B is shallow with the two helices III skewed across the helices IV – a direct consequence of the different helix III orientation. The most important residues that are involved in putative target binding are those at the C-terminus of helix IV [17]. These hydrophobic residues are effectively hidden from the base of the putative binding cleft of r-S100B by residues in helix III and the linker region [12] but partly exposed in the other two dimer structures. Therefore, only the r-S100B structure appears to conform to the results of Smith et al. which indicate that the C-terminal residues are buried in the apo form and exposed in the calcium-bound form [18]. Moreover, this suggests that the calcyclin and b-S100B structures might contain local calcium-bound conformations in the C-terminus region of helix IV.

Calcium binding to the two S100B structures requires a large structural rearrangement of the second calcium-binding loop and this will affect the adjacent secondary structure elements – namely the short stretch of β-sheet and helix III. As the relative positions of the β-sheet and its calcium-binding ligands are already fixed in conformations expected for the calcium-bound form then a change in loop conformation is more likely to influence the unusual orientation of helix III, acting through the position of its C-terminus. This may drive the helix III orientation towards one more typical of other EF-hand proteins with a concomitant change in the N-terminal anchoring point of helix III. Calcium binding to calbindin D_{9k} affects the same structural elements [5], albeit on a smaller scale, suggesting that it has a limited and deactivated ancestral calcium sensor response.

Native calbindin D_{9k} is believed to have a shorter linker region than other S100 proteins and this may explain why a stable helix has not been observed before. Sequence alignments have not indicated the correlation of Fig. 3 in the past (e.g. see Figure 2 of [14]), due to the variable length of the linker region. A linker helix is not compatible with the extended linker conformation in the r-S100B structure but becomes feasible if the calcium-bound structure adopts a helix III orientation similar to calbindin D_{9k}. A calcium-induced linker helix would define the helix III N-terminal anchoring point and stabilize nearby residues in the C-terminus of helix IV.

The helix III orientation resulting from these changes would resemble that seen in calcium-bound calbindin D_{9k} and calcyclin (Fig. 1a,b,g). The C-terminal residues of helix IV are made available in the binding site by the structural changes of the linker region and helix III (Fig. 1h). This model does not require large changes in the rest of the dimeric structure. The elegant comparison of the calcyclin, S100B and calbindin D_{9k} structures illustrates that these proteins share a common hydrophobic core [15]. The effect of calcium binding on calbindin D_{9k} is well characterized – it does not involve large structural changes for helices I, II and IV [5]. If similar small changes occur on calcium binding to other S100 proteins then this would allow them to retain their dimeric structure as most of the dimeric interactions involve helices I and IV.

We believe that calcium binding to S100 proteins will result in structural changes in the linker, helix III and second calcium-binding loop regions. These changes should be sufficient to result in new characteristics for the potential binding cleft. Our model suggests a reorientation of helix III that leads to a binding cleft more closely resembling that of calcyclin, which possesses a deeper and narrower cleft than r-S100B (although these properties may, in part, be a function of the calcyclin dimer interface that is not so well-defined). In the calcyclin model the C-terminal residues are more exposed in the bottom of the cleft as a result of the contrasting helix III orientation. This model is supported by other recent studies on the S100B system which suggest that residues in the C-terminal region become solvent-exposed upon calcium binding [18]. Therefore, our model is consistent in this respect. A more subtle point is that the smaller, either ancestral or residual, calcium response of calbindin D_{9k} [5] fits this model. As the potential target-binding site is already partially formed in the apo S100 structures then only a relatively small calcium-induced structural change is required to allow target binding – this is in contrast to calmodulin where the target-binding site is not apparent in its apo structure and on which the current target protein mechanism for S100 proteins is based because of their similar sensor properties [7].

3. Conclusion

The authors of this paper support the work by Weber and coworkers that places helix III of r-S100B in a novel orientation compared to other EF-hand proteins [12]. The experimental techniques employed are as good as, or, as in the case of definition of the dimer interface, superior to those used in the other S100 dimer studies [13,14]. Calcium-bound conformations of S100 dimers will be available soon and a full elucidation of the S100 target protein mechanism requires accurate and precise structures of both the apo and calcium-bound forms. The subtleties of the mechanism, such as the proposed stabilizing calcium-induced helix, would otherwise be difficult to deduce. Careful analysis allows us to propose a model for calcium binding to S100 dimers based on predicted structural changes to the linker, helix III, and the second calcium-binding loop of r-S100B and on the body of work available for calbindin D_{9k}. Little structural change is expected in the rest of the dimer and we anticipate that the common dimer interface of the apo S100 structures will be retained in their calcium-bound forms. The region of major structural change lies on one face of the dimer with the target protein interaction surface made available in the calcium-bound state of the S100 dimers.

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