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Short Communication

Modulation of quaternary structure of S100 proteins by calcium ions

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ABSTRACT

It is well established that calcium binding leads to conformational changes in S100 proteins. These conformational changes are thought to activate the protein and render a protein conformation that is capable of binding other proteins. The basic quaternary structural motif of S100 proteins is a homodimer, however there is little information if higher order non-covalent oligomers are also formed and whether these oligomers are of functional relevance. To this end we performed equilibrium analytical ultracentrifugation experiments for 16 S100 proteins (S100A1, S100A2, S100A3, S100A4, S100A5, S100A6, S100A7, S100A8, S100A9, S100A10, S100A11, S100A12, S100A13, S100B, S100P, and S100Z) under reducing conditions in the absence and presence of calcium ions. We show that the addition of calcium promotes the formation of tetrameric structures which could be further enhanced under in vivo conditions where there is an additional effect of molecular crowding.

1. Introduction

It is generally suggested that the S100 protein family members are dimeric as the dimerization affinities measured for some members of this family have been found to be very high (see for example [1]). However, with new family members being discovered and taking into account the amino acid sequence identities in this family, which ranges from 20 to 60%, the question arises whether or not these generalizations can be extended across the entire S100 protein family. A potential contribution to the variation in the oligomeric structure of these proteins is that they undergo a conformational change after binding calcium [2–6]. This feature is very important for the functioning of these proteins as binding calcium exposes a region of the protein which then serves as an interacting surface for their target proteins/peptides. The consequence of this calcium induced conformational change on the quaternary structure of the S100 proteins has been reported [7–11]. The formation of higher order molecular structures in the S100 protein family is not only important from a thermodynamic point of view but also for the functionality of the S100 proteins for example, the S100B tetramer has a higher affinity for RAGE than the S100B dimer [12]. The formation of oligomers in S100 proteins can occur both under non-reducing conditions, as found in the extracellular space (e.g. [12–15]), and under reducing conditions (e.g. [8,16–18]), which is more representative of the intracellular environment. Here we present an investigation into the effect of calcium on the non-covalent quaternary structure of 16 human S100 proteins under reducing conditions using equilibrium analytical ultracentrifugation. Analytical ultracentrifugation (AUC) is the method of choice when determining the quaternary structure of proteins as forming higher order non-covalent molecular structures is a protein concentration dependent process and it is possible to sample a wide range of protein concentrations in a single experiment and in doing so, cover at least an order of magnitude range in affinity constants. The results obtained from the AUC experiments for the S100 proteins are correlated with structural information and their functional implications are also discussed.

2. Materials and methods

The S100 proteins (S100A1, S100A2, S100A3, S100A4, S100A5, S100A6, S100A7, S100A8, S100A9, S100A10, S100A11, S100A12, S100A13, S100B, S100P, and S100Z) were purified as described previously [19–22]. The equilibrium analytical ultracentrifugation (AUC) experiments were performed using a Beckman XL-A analytical ultracentrifuge using 6 sector cells at three different speeds: 22,000, 26,000 and 30,000 rpm. The radial distribution of the proteins was monitored by measuring the absorbance of 280 nm at 4 °C with the exception of S100A7 for which experiments were performed at 25 °C. To determine whether the samples had reached equilibrium, three consecutive scans, measured at 6 hour intervals, were overlaid and when there were no differences observed between these scans it was considered to be at equilibrium. For the AUC experiments performed in the absence of calcium the protein samples were dialyzed into 20 mM Tris, 0.2 mM EDTA, 1 mM DTT or 1 mM TCEP, pH 7.5. For the experiments in the presence of calcium, 5 mM calcium was added to

Abbreviations: AUC, equilibrium analytical ultracentrifugation.

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the buffer. For S100A5 and S100A8, 300 mM Na Acetate, 100 mM MES, 0.2 mM EDTA, 1 mM TCEP, pH 6.2 was used as these proteins formed higher order aggregates in the Tris buffer. As a control S100A3 was also analyzed in this buffer and it was found that the buffer had no effect on the oligomeric properties of S100A3 when compared with its behavior in the Tris buffer. For S100A7, 20 mM cacodylate, 0.2 mM EDTA, 1 mM TCEP, pH 5.5 or 6.5 was used with or without 5 mM calcium. It is important to note that all buffers contained a large excess of reducing agents (DTT or TCEP) to prevent the formation of covalent oligomers.

The experimental AUC data (absorbance versus radial position) were fitted to the following equation which describes the radial distribution of a single species at equilibrium:

$$c_r = c_{r_0} \cdot \exp\left[Mw \cdot (r^2 - r_0^2)\frac{(1-\overline{v}\cdot\rho)\cdot\omega^2}{2\cdot R\cdot T}\right] + E \quad (1)$$

where c_r is the protein concentration at radius r, c_{r0} is the concentration of monomeric protein at r_0, ω is the angular velocity, R is the gas constant equal to 8.134×10^7 erg mol^{-1} K^{-1}, T is the temperature in degrees in Kelvin, Mw is the molecular mass, $\overline{v}$ is the partial specific volume of the protein (calculated using the additivity scheme described [23]), ρ is the density of the solvent and E is the baseline offset.

When the single species model did not satisfactorily fit the data, as judged by inspection of the residuals, and/or fitted molecular mass, then the next more complex model was used which describes a monomer–dimer or a dimer–tetramer equilibrium. For the S100 proteins, the following two scenarios were considered. Scheme 1 describes a reversible association of two monomers (M) forming a dimer (M_2) with an association constant $K_{a,12}$ and a dissociation constant $K_{d,12}$. Scheme 2 describes a reversible association of the M_2 forming a tetramer (M_4) with an association constant $K_{a,24}$ and a dissociation constant $K_{d,24}$.

$$2M \underset{K_{d,12}}{\overset{K_{a,12}}{\leftrightarrow}} M_2 \qquad \text{Scheme 1}$$

$$2M_2 \underset{K_{d,24}}{\overset{K_{a,24}}{\leftrightarrow}} M_4 \qquad \text{Scheme 2}$$

The K_a and K_d of these two schemes describe the same concentration dependent process of two reactants forming one product where the only difference is the molecular mass of the products and reactants. For the two equilibrium reactions shown in Scheme 1 and Scheme 2, the following equation was used to fit the data:

$$c_r = c_{r_o} \cdot \exp\left[Mw \cdot (r^2 - r_0^2)\frac{(1-\overline{v}\cdot\rho)\cdot\omega^2}{2\cdot R\cdot T}\right] + \frac{c_{r_o}^2}{K_d}\exp\left[2\cdot Mw \cdot (r^2 - r_0^2)\frac{(1-\overline{v}\cdot\rho)\cdot\omega^2}{2\cdot R\cdot T}\right] + E \quad (2)$$

where K_d is the dissociation constant (which could be $K_{d,12}$ or $K_{d,24}$ describing a monomer–dimer equilibrium or a dimer–tetramer equilibrium, respectively). This model is used if the fitted Mw using Eq. (1) is less than that expected for a dimer. In this case the Mw is kept constant and equal to the molecular mass of the monomer calculated from amino acid composition which allows a better estimate of the association constant. Similarly, if the fitted Mw using Eq. (1) is greater by more than 5% than that expected for a dimer, then Eq. (2) is used to fit the data as a dimer–tetramer equilibrium where again the Mw is kept constant in the fitting routine, however this time its value is that of a dimer. The extinction coefficient was assumed to be independent of its association state, e.g. the extinction coefficient for the dimer would be twice that of the monomer. All fits were done using nonlinear regression software (NLREG) and in-house written scripts.

3. Results and discussion

Table 1 shows the results of the analysis of AUC data for 16 different human S100 proteins in the absence (0.2 mM EDTA) or presence (5 mM) of Ca^{2+}-ions. It is apparent that despite the high sequence homology [2–5], S100 proteins show very different tendencies for their oligomerization states ranging from a monomer–dimer equilibrium (e.g. S100A6 and S100A12) to a monodispersed dimer (e.g. S100B, S100Z, S100A10, S100A13) to a dimer–tetramer equilibrium (e.g. S100A2, S100A5, S100A7, S100A8, S100A11, and S100A12).

The equilibrium AUC data analysis is usually performed based on the Occam's razor principle (the simplest explanation or strategy tends to be the best one). This is particularly relevant as models of increased complexity are used to fit the experimental AUC data. For the acceptance of the model three criteria were used: distribution of the residuals, deviation of the fitted molecular mass from the theoretical mass and molecular symmetry. To demonstrate this approach for the analysis of the results of equilibrium AUC for S100

Table 1
Summary of the results of sedimentation equilibrium data obtained from the AUC experiments.

Protein	Calculated dimer *Mw*	Ca^{2+} free apparent *Mw*	Ca^{2+} free $K_{d1,2}$ (μM)	Ca^{2+} free $K_{d2,4}$ (μM)	5 mM Ca^{2+} apparent *Mw*	5 mM Ca^{2+} $K_{d1,2}$ (μM)	5 mM Ca^{2+} $K_{d2,4}$ (μM)
S100A1	20.862	22.4 ± 0.3			23.8 ± 0.2		30 ± 6
S100A2	22.234	24.1 ± 0.2		700 ± 140	33.4 ± 0.2		50 ± 10
S100A3	23.428	24.0 ± 0.2			33.4 ± 0.1		30 ± 6
S100A4	23.458	23.1 ± 0.1			24.8 ± 0.2		
S100A5	21.490	25.3 ± 0.3		75 ± 15	26.1 ± 0.3		70 ± 15
S100A6	20.360	17.7 ± 0.2	30 ± 6		20.4 ± 0.3		
S100A7	22.916	26.1 ± 0.3		280 ± 60	26.5 ± 0.2		280 ± 60
S100A8	21.670	34.9 ± 0.3		6 ± 2	34.3 ± 0.2		8 ± 2
S100A9	26.492	26.4 ± 0.2			30.7 ± 0.3		170 ± 30
S100A10	22.376	23.9 ± 0.2			23.7 ± 0.1		
S100A11	23.482	30.0 ± 0.2		240 ± 50	40.6 ± 0.4		25 ± 5
S100A12	21.152	18.1 ± 0.4	70 ± 15		17.4 ± 0.1	160 ± 30	
S100A13	22.682	21.3 ± 0.2			23.9 ± 0.3		
S100B	21.428	20.1 ± 0.3			22.5 ± 0.2		
S100P	20.800	21.4 ± 0.2			28.8 ± 0.3		160 ± 30
S100Z[a]	22.860	22.1 ± 0.1			22.1 ± 0.1		

[a]S100Z was analyzed previously in our laboratory and the data was taken from [21].

proteins, we will discuss in more detail several representative examples.

From Table 1 it can be seen that only S100A6 and S100A12 were found to have a monomer–dimer equilibrium in the absence of calcium with $K_{d,12}$s of 30 ± 6 and 70 ± 15 μM, respectively. In the presence of calcium S100A12 maintains a monomer–dimer equilibrium, with the same $K_{d,12}$ (see Table 1). However, when calcium is present, the S100A6 protein behaves as a dimer (see Fig. 1). In fact, the fit of the data to a monomer–dimer equilibrium for S100A6 in the presence of calcium returns the value of $K_{d,12} = 0$. As a monomer–dimer equilibrium is observed only for S100A6 and S100A12, it can be concluded that the affinity for dimer formation for the other S100 proteins is relatively high and that the concentrations used in these experiments significantly exceed the dissociation constant for monomer–dimer formation. Indeed, large-zone chromatography [1] for the S100B protein shows that the dimerization constant for this protein is on the order of 2–4 pM, much lower than the micromolar concentrations accessible in AUC.

Fig. 2 shows an example of the AUC profiles for the S100P protein in the presence and absence of calcium ions. In the apo-form, S100P profiles can be fit well to a single species model with the molecular mass of a dimer. Addition of calcium ions leads to a clear change in the AUC profiles — they become steeper at higher radial positions which is consistent with the increase in the molecular mass of species (Fig. 2). Analysis of the profiles of S100P in calcium using single species model gives a mass of 29 kDa, much larger that molecular mass of a dimer (20.8 kDa). One can argue that this corresponds to a molecular mass of a trimer which has a theoretical mass of 31.2 kDa. However, a trimer does not seem to be a viable model based on the available three-dimensional structures of S100 proteins. Indeed numerous structures available for S100 proteins show that the basic structural unit is a symmetrical dimer with a two-fold axis of rotation (see for example [2–6,24]). Consequently, the next higher order complex would be a tetramer, which would exclude the possibility of trimer formation. The data was then fitted to dimer–tetramer model (Eq. (2)), keeping the molecular mass constant to that of a dimer, and it was found that the $K_{d,24}$ for the S100P protein in the presence of calcium is 160 ± 30 μM.

One important general trend that can be seen from the data in Table 1 is that the presence of calcium changes the oligomerization state of the S100 proteins from having either a monomer–dimer equilibrium, or being dimeric in the absence of calcium, to being purely dimeric or having a dimer–tetramer equilibrium. For example, S100A11 shows a dimer–tetramer equilibrium both in the presence and absence of calcium. However, in the presence of calcium, the $K_{d,24}$ is an order of magnitude lower than in the absence of calcium. This suggests that the conformational changes that occur after binding calcium alter the structure sufficiently to allow them to form tetramers. This change in structure however does not seem to weaken the dimer interface and drive equilibrium toward formation of monomeric species. Overall, this agrees with the observation that S100 proteins function as dimeric proteins, observed using NMR or X-ray crystallography, even though these methods require significantly higher protein concentrations [2,5,6]. It is important to note that S100A10, which does not have the ability to bind calcium, remains dimeric when calcium is added. This protein serves as an important control to determine the general effects of calcium on the oligomeric structure of the S100 proteins.

The observed oligomerization equilibria for S100P proteins obtained using AUC compares well to the biochemical data obtained using mostly chemical cross-linking, and in some cases other analytical methods such as gel-filtration chromatography. For the S100A3 protein it has been shown using gel-filtration chromatography [8] that S100A3 is dimeric in the presence of EGTA, and addition of Ca^{2+} leads to the formation of tetramers. This agrees well with the observations presented here (Table 1).

For the S100A4 protein, size exclusion chromatography in the absence and presence of Ca^{2+} only shows the existence of stable dimers [25]. In addition Tarabykina et al. [26] obtained a very tight dimerization dissociation constant (2 μM) in the apo-form of S100A4. They did not report the numerical results in the presence of Ca^{2+} but noted that calcium binding promoted dimerization. More recently chemical cross-linking of S100A4 in the presence of calcium also showed presence of stable dimers and no indication of tetramer formation [17]. All these observations are consistent with the data presented in the Table 1.

SDS-PAGE analysis of the cross-linking of S100A6 [18], shows a higher concentration of dimers in the presence of 1 mM Ca^{2+} than in the presence of 2 mM EGTA, again in qualitative agreement with our data that suggests a monomer–dimer equilibrium in the absence of calcium and the presence of dimers in the presence of 5 mM of calcium ions. The difference in calcium concentration might be a major reason for only obtaining a qualitative agreement between the data in Table 1 and the cross-linking experiments of [18].

SDS-PAGE analysis of cross-linking of apo-forms of the S100A8 and S100A9 proteins showed that while S100A9 shows only cross-linked dimers, S100A8 shows both cross-linked dimers and tetramers [27]. Both these observations are consistent with the AUC analysis

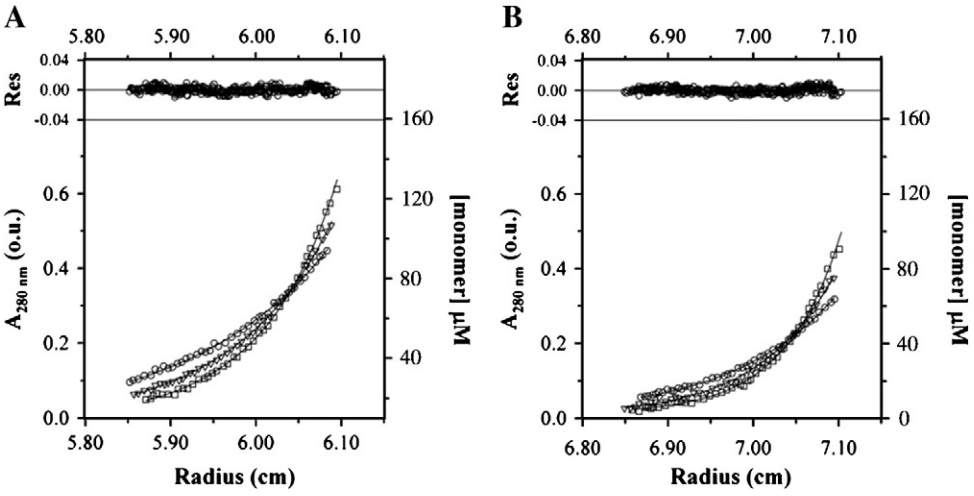

Fig. 1. Radial distribution of S100A6 monitored using the absorbance at 280 nm in the absence (Panel A) and presence of 5 mM Ca^{2+} (Panel B). For clarity, only every fifth data point is shown as symbols at 22,000 (circles), 26,000 (triangles) and 30,000 rpm (squares). The solid lines represent the fits of the data to Eq. (2) (in Panel A) and to Eq. (1) (in Panel B). Top panels show the residuals for each fit. See Table 1 for details.

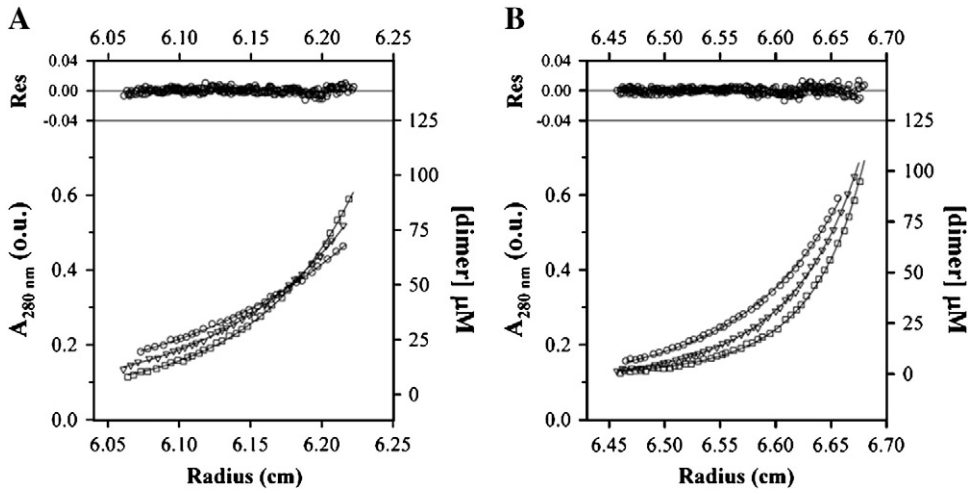

Fig. 2. Radial distribution of S100P monitored using the absorbance at 280 nm in the absence (Panel A) and presence of 5 mM Ca^{2+} (Panel B). For clarity, only every fifth data point is shown as symbols at 22,000 (circles), 26,000 (triangles) and 30,000 rpm (squares). The solid lines represent the fits of the data to Eq. (2) (in Panel A) and to Eq. (1) (in Panel B). Top panels show the residuals for each fit. See Table 1 for details.

presented here, i.e. apo-S100A9 does not form tetramers while apo-S100A8 does (Table 1).

Chemical cross-linking of the S100A11 protein shows the presence of tetramers both in the presence and absence of Ca^{2+}-ions [16]. However, comparison of band intensities suggests a higher population of tetramers in the presence of 1 mM Ca^{2+} than in the presence of 4 mM EGTA. These results are in good agreement with the AUC data presented here, i.e. S100A11 is in a dimer–tetramer equilibrium with a $K_{d,24}$ dissociation constant lower in the presence of calcium ions. Marlatt et al. [28] have shown the presence of dimers of apo-S100A11 in high concentrations of salt. However, the electrospray mass spectroscopy used to monitor the oligomerization state of apo-S100A11 in this case was not extended to a higher molecular mass, so it is not clear whether tetramers were present.

For the S100A12 protein, chemical cross-linking shows the presence of dimers and monomers with no appreciable amount of higher order oligomers [29]. This observation is in qualitative agreement with the data presented in Table 1.

Becker et al. reported that in the absence of Ca^{2+} only the dimer of the S100P protein could be detected on the gel [30]. Similar results were obtained using analytical ultracentrifugation [7] with the dimer of S100P observed in the presence of EDTA and an indication for the presence of tetramers in the presence of 5 mM Ca^{2+}. Optical biosensor analysis of S100P dimerization showed that in the presence of non-saturating (0.5 mM) Ca^{2+}, the dimerization constant is 20–50 higher than in 50 μM EGTA [31,32]. It should be noted however that the data analysis by optical biosensor is model dependent and cannot provide information about stoichiometry of the complex being formed and thus does not exclude the presence of tetramers in the presence of calcium.

For S100B, Drohat et al. [1] used large-zone analytical gel-filtration chromatography and concluded that S100B both in the presence and absence of Ca^{2+} exists largely as a dimer. This method allowed estimates of the dimerization constant for S100B to be in picomolar range. These findings were further confirmed by the work of Landar et al. that used light-scattering and analytical ultracentrifugation experiments [33]. The presence of dimers for S100B under physiological salt concentrations was also shown by Marlatt et al. [28] using electrospray mass spectroscopy. It should be noted that these authors also reported that under very low salt conditions (~50 μM) S100B exists predominantly as a monomer.

Overall, the results of the AUC analysis presented here compare well with the biochemical data obtained using various methods on different S100 proteins. They suggest that some S100 proteins are affected by the presence of calcium leading to an increase in the oligomerization state and, in particular, to a dimer–tetramer equilibrium. This leads to the question of what are the potential sequence and/or structural determinants for this effect? To answer this question we analyzed the three-dimensional structures of S100

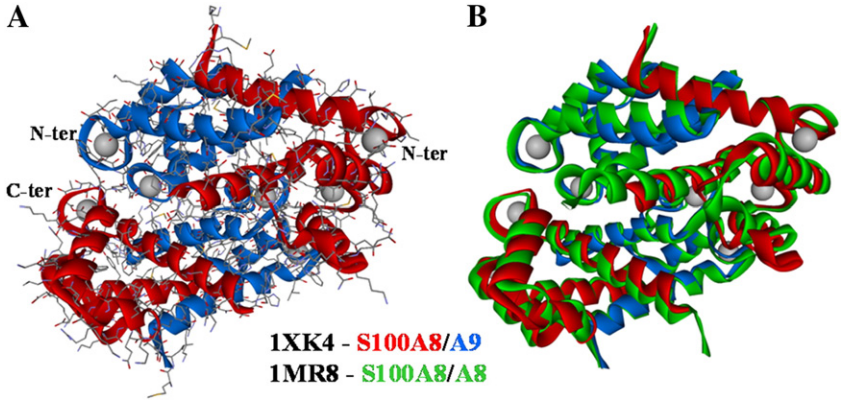

Fig. 3. Panel A: Cartoon representation of the tetramer structure of S100A8/S100A9 (1XK4) as reported by [9]. Panel B: Structural alignment of S100A8/S100A9 tetramer with the structure of S100A8 tetramer obtained by applying the crystal symmetry to 1MR8 [35]. Solid spheres represent Ca^{2+}-ions. N-ter and C-ter identify N and C-terminal calcium binding loops, respectively.

proteins, in particular, the X-ray structures of S100A8/S100A9 (pdb 1XK4 [9]) where it was reported that a tetramer was formed by heterodimers of S100A8/A9 (see Fig. 3A). There is an inherent difficulty in identifying higher order molecular structures from crystallographic studies, which is a well known problem (see e.g. [34]). The issue in many cases is that it is difficult to distinguish crystal contacts from true oligomer formation. For example, the reported crystal structure of S100A8 (pdb 1MR8 [35]) has been used to classify this protein as a dimer. However, by rebuilding the molecule using crystal symmetry one can show that S100A8 forms tetramers that are nearly identical to the tetramers formed by the S100A8/A9 heterodimer (see Fig. 3B). The dimers assume an antiparallel orientation and more importantly, the tetramer interface is formed by the interdigitation of one C-terminal EF-hand loop of one dimer between the N- and C-terminal EF-hand loops of the other dimer. The formation of tetramers will therefore depend strongly on the calcium ligation state of the EF-hands and suggests that tetramer formation will be largely favored when both sites are occupied. It is known that the N-terminal calcium binding site in S100 proteins is weaker than the C-terminal site [7,21].

In the context of the data available presently for calcium binding affinities, these observations suggest that calcium-mediated tetramer formation probably does not have functional relevance. The reported binding constants for the high affinity C-terminal binding sites are in micromolar range and vary between 0.1 μM (S100A5, S100P, and S100Z) and 100 μM (S100A4 and S100A6). The binding constants for the low affinity N-terminal binding site are at least two–three orders of magnitude lower (e.g. in millimolar range) and range between 0.1 mM (e.g. S100P, S100A13) and 10 mM (S100Z). It can be argued that these binding constants are outside the biologically relevant concentrations of calcium ions, which could in turn suggest that the tetrameric state is not functionally important. However, it is possible that for the cases where the tetramer has a higher affinity for the target protein, for example S100B and RAGE, these favorable protein-protein interactions could drive the dimer–tetramer equilibrium towards the formation of tetramer. In addition, it has been shown recently that the affinity for calcium can increase upon ligand binding [36] which suggests a linkage between calcium binding, function and tetramerization.

Not only is the calcium concentration in the cell an important determinant for the biological relevance of tetramerization but so is the concentration of the protein itself. Of the proteins that show tetramerization in the absence of calcium, i.e. S100A2, S100A5, S100A7, S100A8 and S100A11, only S100A8 has a low micromolar dissociation constant for the tetramer–dimer equilibrium. The rest of the proteins have 10-100 times higher dissociation constants (Table 1). Considering that the highest reported concentration for an S100 protein in the cell is ~5 μM [37], all these S100 proteins, except S100A8, will have more than 96% of their population in the dimeric form. It should be noted that these values are based purely on the relatively dilute reducing conditions used in the AUC experiments which can be significantly different from the in vivo conditions, particularly in the context of molecular crowding. In fact, it has been shown that the affinity can increase by approximately two orders of magnitude [38] when molecular crowding is taken into account which, in the case of the S100 proteins, can make their tetramerization more biologically relevant. One also has to keep in mind that other divalent ions such as Mg^{2+} or Zn^{2+} might be able to trigger changes in the oligomerization state of S100 proteins [7,39,40] possibly through a different mechanism as their binding sites are different from the calcium binding sites.

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References

[1] A.C. Drohat, E. Nenortas, D. Beckett, D.J. Weber, Oligomerization state of S100B at nanomolar concentration determined by large-zone analytical gel filtration chromatography, Protein Science 6 (1997) 1577–1582.
[2] D.B. Zimmer, P. Wright Sadosky, D.J. Weber, Molecular mechanisms of S100-target protein interactions, Microscopy Research and Technique 60 (2003) 552–559.
[3] C.W. Heizmann, G. Fritz, B.W. Schafer, S100 proteins: structure, functions and pathology, Frontiers in Bioscience 7 (2002) d1356–1368.
[4] R. Donato, S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles, International Journal of Biochemistry and Cell Biology 33 (2001) 637–668.
[5] S. Bhattacharya, C.G. Bunick, W.J. Chazin, Target selectivity in EF-hand calcium binding proteins, Biochimica et Biophysica Acta 1742 (2004) 69–79.
[6] L. Santamaria-Kisiel, A.C. Rintala-Dempsey, G.S. Shaw, Calcium-dependent and -independent interactions of the S100 protein family, Biochemical Journal 396 (2006) 201–214.
[7] A.V. Gribenko, G.I. Makhatadze, Oligomerization and divalent ion binding properties of the S100P protein: a Ca2+/Mg2+-switch model, Journal of Molecular Biology 283 (1998) 679–694.
[8] K. Kizawa, H. Takahara, H. Troxler, P. Kleinert, U. Mochida, C.W. Heizmann, Specific citrullination causes assembly of a globular S100A3 homotetramer: a putative Ca2+ modulator matures human hair cuticle, The Journal of Biological Chemistry 283 (2008) 5004–5013.
[9] I.P. Korndorfer, F. Brueckner, A. Skerra, The crystal structure of the human (S100A8/S100A9)2 heterotetramer, calprotectin, illustrates how conformational changes of interacting alpha-helices can determine specific association of two EF-hand proteins, Journal of Molecular Biology 370 (2007) 887–898.
[10] N. Leukert, T. Vogl, K. Strupat, R. Reichelt, C. Sorg, J. Roth, Calcium-dependent tetramer formation of S100A8 and S100A9 is essential for biological activity, Journal of Molecular Biology 359 (2006) 961–972.
[11] T. Vogl, N. Leukert, K. Barczyk, K. Strupat, J. Roth, Biophysical characterization of S100A8 and S100A9 in the absence and presence of bivalent cations, Biochim. Biophys. Acta 1763 (2006) 1298–1306.
[12] T. Ostendorp, E. Leclerc, A. Galichet, M. Koch, N. Demling, B. Weigle, C.W. Heizmann, P.M. Kroneck, G. Fritz, Structural and functional insights into RAGE activation by multimeric S100B, The EMBO Journal 26 (2007) 3868–3878.
[13] W. Ma, S.E. Lee, J. Guo, W. Qu, B.I. Hudson, A.M. Schmidt, G.R. Barile, RAGE ligand upregulation of VEGF secretion in ARPE-19 cells, Investigative Ophthalmology & Visual Science 48 (2007) 1355–1361.
[14] D. Kiryushko, V. Novitskaya, V. Soroka, J. Klingelhofer, E. Lukanidin, V. Berezin, E. Bock, Molecular mechanisms of Ca(2+) signaling in neurons induced by the S100A4 protein, Molecular and Cellular Biology 26 (2006) 3625–3638.
[15] J. Xie, D.S. Burz, W. He, I.B. Bronstein, I. Lednev, A. Shekhtman, Hexameric calgranulin C (S100A12) binds to the receptor for advanced glycated end products (RAGE) using symmetric hydrophobic target-binding patches, The Journal of Biological Chemistry 282 (2007) 4218–4231.
[16] N. Chang, C. Sutherland, E. Hesse, R. Winkfein, W.B. Wiehler, M. Pho, C. Veillette, S. Li, D.P. Wilson, E. Kiss, M.P. Walsh, Identification of a novel interaction between the Ca(2+)-binding protein S100A11 and the Ca(2+)- and phospholipid-binding protein annexin A6, American Journal of Physiology - Cell Physiology 292 (2007) C1417–1430.
[17] V.N. Malashkevich, N.G. Dulyaninova, U.A. Ramagopal, M.A. Liriano, K.M. Varney, D. Knight, M. Brenowitz, D.J. Weber, S.C. Almo, A.R. Bresnick, Phenothiazines inhibit S100A4 function by inducing protein oligomerization, Proceedings of the National Academy of Sciences of the USA 107 (2010) 8605–8610.
[18] M. Nowotny, M. Spiechowicz, B. Jastrzebska, A. Filipek, K. Kitagawa, J. Kuznicki, Calcium-regulated interaction of Sgt1 with S100A6 (calcyclin) and other S100 proteins, The Journal of Biological Chemistry 278 (2003) 26923–26928.
[19] W.W. Streicher, M.M. Lopez, G.I. Makhatadze, Annexin I and annexin II N-terminal peptides binding to S100 protein family members: specificity and thermodynamic characterization, Biochemistry 48 (2009) 2788–2798.
[20] A.V. Gribenko, M. Guzman-Casado, M.M. Lopez, G.I. Makhatadze, Conformational and thermodynamic properties of peptide binding to the human S100P protein, Protein Science 11 (2002) 1367–1375.
[21] A.V. Gribenko, J.E. Hopper, G.I. Makhatadze, Molecular characterization and tissue distribution of a novel member of the S100 family of EF-hand proteins, Biochemistry 40 (2001) 15538–15548.
[22] A. Gribenko, M.M. Lopez, J.M. Richardson III, G.I. Makhatadze, Cloning, over-expression, purification, and spectroscopic characterization of human S100P, Protein Science 7 (1998) 211–215.
[23] G.I. Makhatadze, V.N. Medvedkin, P.L. Privalov, Partial molar volumes of polypeptides and their constituent groups in aqueous solution over a broad temperature range, Biopolymers 30 (1990) 1001–1010.
[24] Y.C. Lee, D.E. Volk, V. Thiviyanathan, Q. Kleerekoper, A.V. Gribenko, S. Zhang, D.G. Gorenstein, G.I. Makhatadze, B.A. Luxon, NMR structure of the Apo-S100P protein, Journal of Biomolecular NMR 29 (2004) 399–402.
[25] T.M. Ismail, S. Zhang, D.G. Fernig, S. Gross, M.L. Martin-Fernandez, V. See, K. Tozawa, C.J. Tynan, G. Wang, M.C. Wilkinson, P.S. Rudland, R. Barraclough, Self-association of calcium-binding protein S100A4 and metastasis, The Journal of Biological Chemistry 285 (2010) 914–922.

[26] S. Tarabykina, D.J. Scott, P. Herzyk, T.J. Hill, J.R. Tame, M. Kriajevska, D. Lafitte, P.J. Derrick, G.G. Dodson, N.J. Maitland, E.M. Lukanidin, I.B. Bronstein, The dimerization interface of the metastasis-associated protein S100A4 (Mts1): in vivo and in vitro studies, The Journal of Biological Chemistry 276 (2001) 24212–24222.
[27] M.J. Hunter, W.J. Chazin, High level expression and dimer characterization of the S100 EF-hand proteins, migration inhibitory factor-related proteins 8 and 14, The Journal of Biological Chemistry 273 (1998) 12427–12435.
[28] N.M. Marlatt, B.L. Boys, L. Konermann, G.S. Shaw, Formation of monomeric S100B and S100A11 proteins at low ionic strength, Biochemistry 48 (2009) 1954–1963.
[29] T. Hatakeyama, M. Okada, S. Shimamoto, Y. Kubota, R. Kobayashi, Identification of intracellular target proteins of the calcium-signaling protein S100A12, European Journal of Biochemistry 271 (2004) 3765–3775.
[30] T. Becker, V. Gerke, E. Kube, K. Weber, S100P, a novel Ca(2+)-binding protein from human placenta. cDNA cloning, recombinant protein expression and Ca2+ binding properties, European Journal of Biochemistry 207 (1992) 541–547.
[31] G. Wang, S. Zhang, D.G. Fernig, D. Spiller, M. Martin-Fernandez, H. Zhang, Y. Ding, Z. Rao, P.S. Rudland, R. Barraclough, Heterodimeric interaction and interfaces of S100A1 and S100P, Biochemical Journal 382 (2004) 375–383.
[32] H. Zhang, G. Wang, Y. Ding, Z. Wang, R. Barraclough, P.S. Rudland, D.G. Fernig, Z. Rao, The crystal structure at 2A resolution of the Ca2+-binding protein S100P, Journal of Molecular Biology 325 (2003) 785–794.
[33] A. Landar, T.L. Hall, E.H. Cornwall, J.J. Correia, A.C. Drohat, D.J. Weber, D.B. Zimmer, The role of cysteine residues in S100B dimerization and regulation of target protein activity, Biochimica et Biophysica Acta 1343 (1997) 117–129.
[34] Q. Xu, A.A. Canutescu, G. Wang, M. Shapovalov, Z. Obradovic, R.L. Dunbrack Jr., Statistical analysis of interface similarity in crystals of homologous proteins, Journal of Molecular Biology 381 (2008) 487–507.
[35] K. Ishikawa, A. Nakagawa, I. Tanaka, M. Suzuki, J. Nishihira, The structure of human MRP8, a member of the S100 calcium-binding protein family, by MAD phasing at 1.9 A resolution, Acta Crystallographica Section D: Biological Crystallography 56 (2000) 559–566.
[36] T.H. Charpentier, L.E. Thompson, M.A. Liriano, K.M. Varney, P.T. Wilder, E. Pozharski, E.A. Toth, D.J. Weber, The effects of CapZ peptide (TRTK-12) binding to S100B-Ca2+ as examined by NMR and X-ray crystallography, Journal of Molecular Biology 396 (2010) 1227–1243.
[37] G. Fano, S. Biocca, S. Fulle, M.A. Mariggio, S. Belia, P. Calissano, The S-100: a protein family in search of a function, Progress in Neurobiology 46 (1995) 71–82.
[38] T.C. Jarvis, D.M. Ring, S.S. Daube, P.H. von Hippel, "Macromolecular crowding": thermodynamic consequences for protein–protein interactions within the T4 DNA replication complex, The Journal of Biological chemistry 265 (1990) 15160–15167.
[39] R.S. Mani, J.G. Shelling, B.D. Sykes, C.M. Kay, Spectral studies on the calcium binding properties of bovine brain S-100b protein, Biochemistry 22 (1983) 1734–1740.
[40] O.V. Moroz, K.S. Wilson, I.B. Bronstein, The role of zinc in the S100 proteins: insights from the X-ray structures, Amino Acids (2010), doi:10.1007/s00726-010-0540-4.