Hypothesis

Self-complementary motifs (SCM) in α-crystallin small heat shock proteins

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Abstract Small heat shock proteins (sHsp) have been implicated in many cell processes involving the dynamics of protein–protein interactions. Two unusual sequences containing self-complementary motifs (SCM) have been identified within the conserved α-crystallin domain of sHsps. When two SCMs are aligned in an anti-parallel direction (N to C and C to N), the charged or polar residues form either salt bridges or hydrogen bonds while the non-polar residues participate in hydrophobic interactions. When aligned in reverse order, the residues of these motifs in α-crystallin subunits form either hydrophobic and/or polar interactions. Homology based molecular modeling of the C-terminal domain of α-crystallin subunits using the crystal structure of MjHSP16.5 suggests that SCM1 and 2 participate in stabilizing secondary structure and subunit interactions. Also there is overwhelming evidence that these motifs are important in the chaperone-like activity of α-crystallin subunits. These sequences are conserved and appear to be characteristic of the entire sHsp superfamily. Similar motifs are also present in the Hsp70 family and the immunoglobulin superfamily.

Key words: Small heat shock protein; α-Crystallin; Chaperone activity; Molecular modeling; Amino acid sequence motif

1. Introduction

As a major protein in all vertebrate lenses, α-crystallin has a central role in controlling the protein–protein interactions in fiber cells that maintain lens transparency. Under pathological conditions, it is a major component of light scattering elements that result in lens opacification, the medical condition of cataract. In its role as a chaperone-like protein, α-crystallin can respond to stressful conditions by sequestering dysfunctional proteins thus preventing abnormal polypeptide aggregation [1,2]. Interest in α-crystallin has been heightened by identification of its αB subunits in many cell types and its accumulation in a wide variety of degenerative, infectious and neoplastic neurological diseases ([3] and references therein). Its subunits, αA and αB, coaggregate with other small heat shock proteins (sHsps) [4,5] and share significant sequence homology of ~100 amino acids within the C-terminal domain [6]. In addition, αB has been identified as a sHsp [7]. This class of sHsp has been implicated in many cell processes such as transcription, growth, differentiation and cytoskeletal protein dynamics as well as chaperone-like activity. Many of these processes involve protein–protein interactions during the physiological stress associated with differentiation and/or pathological stress associated with disease.

In the present study, we have identified two unusual self-complementary sequences within the highly conserved α-crystallin domain shared by sHsps. This ~100 amino acid sequence is within the C-terminal domain which includes both exons 2 and 3 of α-crystallin subunits. When two self-complementary sequences are aligned in an anti-parallel direction (N to C and C to N), the charged or polar residues form either salt bridges or hydrogen bonds while the non-polar residues participate in hydrophobic interactions. Because of this unique property, each sequence constitutes a self-complementary amino acid motif (SCM) that has the potential for stabilizing subunit structure and/or inducing subunit interaction. Although the individual amino acids in α-crystallin SCM1 and 2 are not highly conserved in distantly related sHsps, the self-complementary nature of the sequence is conserved. The importance of these sequences is discussed in the context of experimental results.

2. Recognition of two SCM in sHsps

In α-crystallin subunits, SCM1 contains 13 (αA; 75–87) and 17 (αB; 77–93) amino acid residues and SCM2 contains eight residues (αA, 99–106; αB, 103–110) (Fig. 1). SCM1 contains both complementary hydrophobic and hydrophilic residues. In contrast, SCM2 in α-crystallin subunits contains only polar residues. Since Hsp27 shares sequence and predicted secondary structure homology within the α-crystallin domain [8] and is known to associate with αB in other tissues [5], similar complementary sequences in Hsp27 are also presented (Fig. 1). A database search for homologous sequences revealed that similar sequences and structure are found in the Hsp70 family and the immunoglobulin superfamily. We have also noted that SCMs exist in amyloidogenic Aβ and insulin [9]. Several lines of evidence point to a crucial role of these sequences in the formation of crossed-β fibrils [10–13]. Another self-complementary sequence of 16 alternating hydrophobic and hydrophilic residues has been identified in zoutin, a yeast protein that binds left-handed Z-DNA [14]. This complemen-

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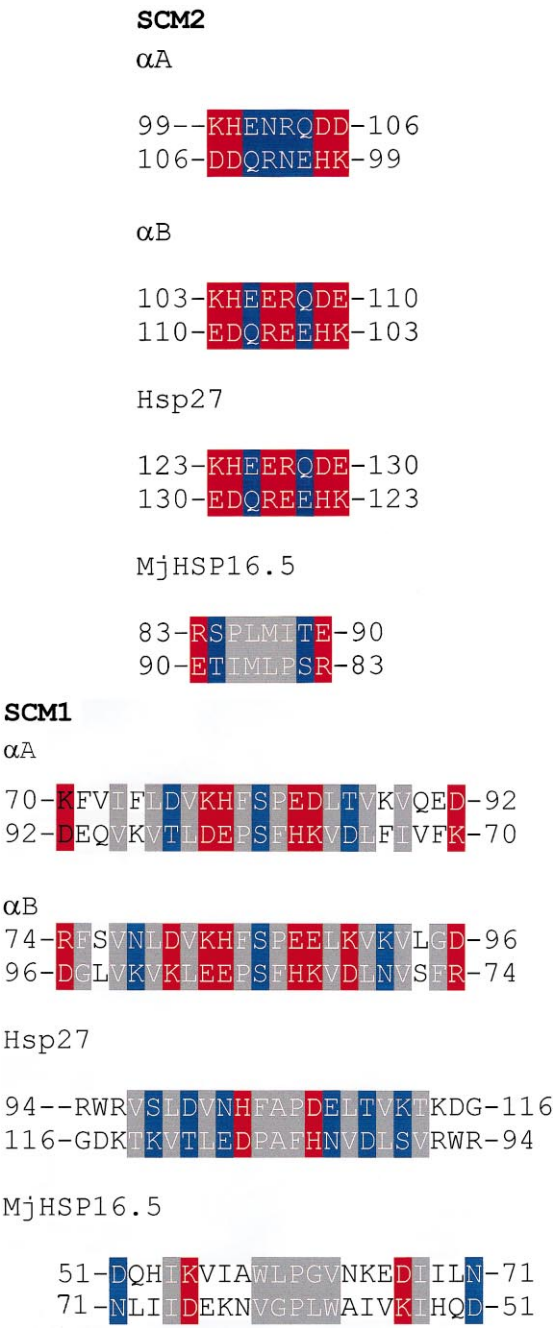

Fig. 1. The SCM1 and 2 in α-crystallin small heat shock proteins. In this figure each sequence belonging to SCM1 and 2 from αA, αB, human Hsp27 and MjHSP16.5 is aligned in the reverse order. The residues capable of forming ion pairs are shaded red, the hydrogen bonds blue. The hydrophobic residues are shaded gray. Note that hydrophobic residues dominate the SCM1 in MJHSP16.5.

tary peptide spontaneously forms a macroscopic membrane composed of β-sheet secondary structure. Similar to amyloid protein structure, the membrane is impervious to dissolution in acidic or alkaline solutions or upon the addition of guanidine hydrochloride, sodium dodecyl sulfate/urea or a variety of proteolytic enzymes [15]

3. Structure of SCM motifs

In the absence of a 3D structure of α-crystallin, we utilized the reported crystal structure of a sHsp, MjHSP16.5, from *Methanococcus jannaschii*, a hyper-thermophilic Archaeon [16] for homology modeling of SCM1 and 2. There is sequence and predicted secondary structural homology within the segments that contain SCM1 and 2 of αA (66–106), αB (70–110) and MjHSP16.5 (amino acids 49–92). Therefore it is reasonable to use the crystal structure of MjHSP16.5 for homology based molecular modeling. The results show that SCM1 (αA, 75–87; αB, 77–93) and SCM2 (αA, 99–106; αB, 103–110) are positioned to play a prominent role in the stabilization of a compact β-sandwich folding unit of the monomer and in dimerization of α-crystallin subunits. 3D models of the αA subunit segments containing SCM1 and SCM2 are shown in Fig. 2A. It is evident from our 3D model of αA and the crystal structure of MjHSP16.5 [16] that hydrophobic residues of SCM1 participate in the formation of the hydrophobic core that is buried within a β-sandwich. The interactions within αA-crystallin monomers that stabilize this β-sandwich are L75–V87, V77–L85 and F80–P82 (Fig. 2A). Interactions that stabilize topologically equivalent β-sheets 3 and 4 in the α-crystallin domain of MjHSP16.5 are also hydrophobic and include A58, L60, V63 and I68 [16]. αA dimers are stabilized by ion pairs among D76–K78 and H79 (Fig. 2B). Whereas, in MjHSP16.5, hydrophobic amino acid interactions contribute to dimerization, W59–P61, G62 and F42 (Fig. 2C).

SCM2 in α-crystallin is completely hydrophilic, whereas in MjHSP16.5, the corresponding sequence contains both hydrophobic and hydrophilic complementary residues (Fig. 1). The residues of SCM2 in the MjHSP16.5 oligomer form three sides of the eight triangular 'windows' [16] and are also present at the interface of the monomers in six square 'windows'. The similar 3D structure of this segment of α-crystallin subunits and MjHSP16.5 suggests that the polar residues of SCM2 of αA are also on the subunit surface and, therefore, are likely to have a role in subunit interaction and chaperone-like function during physiological and/or pathological stress.

4. Functional importance of SCM1 and SCM2

A conserved ~100 amino acids in the C-terminal domain of sHsps is referred to as the α-crystallin domain [17]. This suggests a conserved structure and function. In addition, SCM2 is within a very highly conserved region referred to as HCR1 [18]. Both SCM1 and 2 are in the N-terminal region of the α-crystallin domain (66–106, αA). The crystal structure of MjHSP16.5 homology molecular modeling reported here and our previous secondary structure predictions for α-crystallin subunits and Class 1 plant sHsps [19] by the PHD method of Rost and Sander [20] indicate that this segment has a conserved β-sheet structure. These results are also in accord with the experimentally deduced secondary structure in site directed spin-labeling studies of this region [21]. Our 3D structures show that charged amino residues of SCM1 and 2 are exposed on the subunit surface and, therefore, are available for subunit interactions and chaperone-like activity.

Chaperone binding sites for several proteins have been localized in SCM1 [22,23]. Subsequently, Sharma et al. have also presented evidence that the sequence, K70–K88, can serve as a mini-chaperone by preventing the light scattering associated with protein denaturation [23]. This segment includes SCM1 of αA (75–87) (Fig. 1). Although other sites

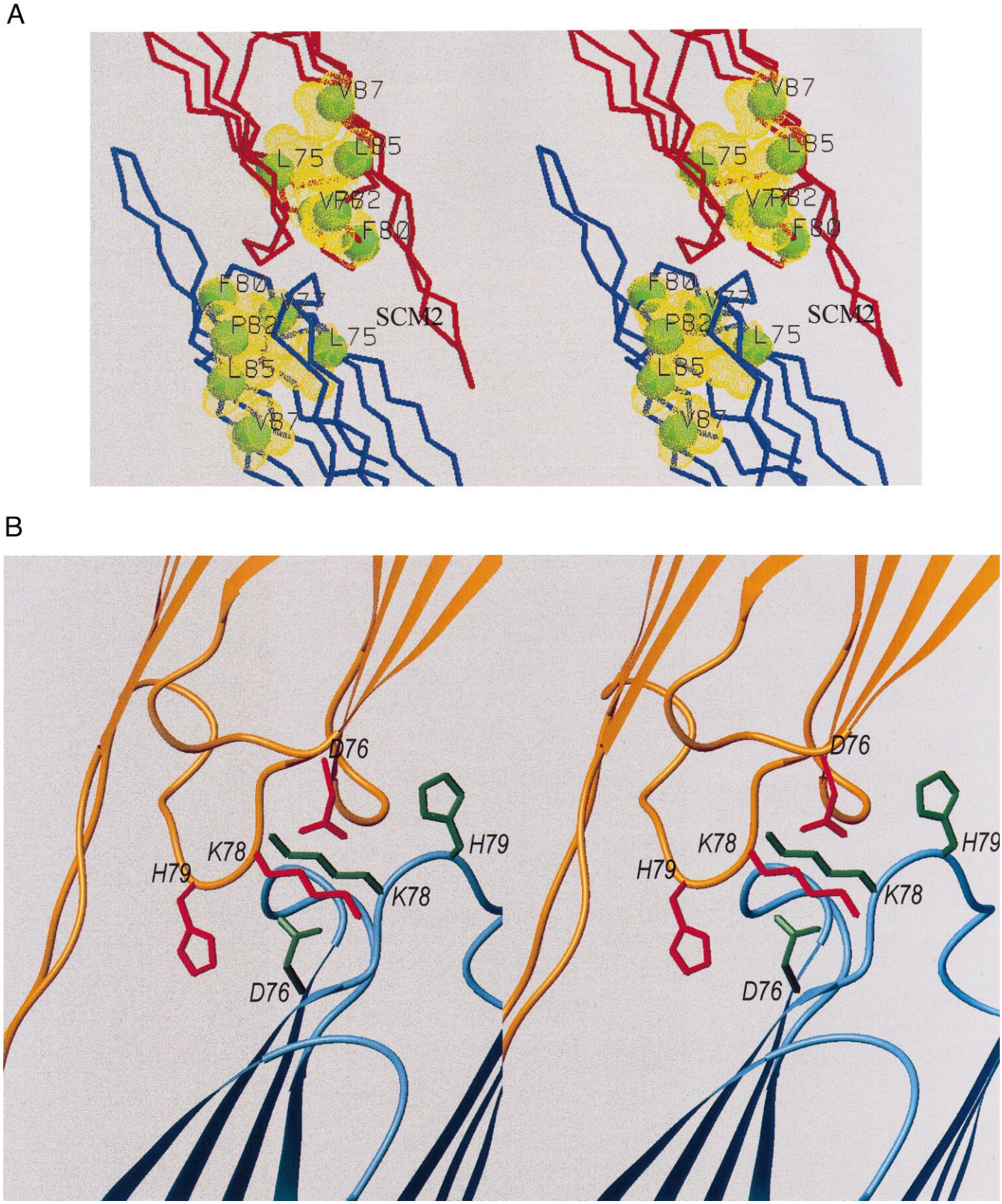

Fig. 2. The position of SCM1 and 2 in α-crystallin sHsps. A: Stereo view of hydrophobic residues in SCM1 that stabilize a β-sandwich in αA. The Cα atoms of the hydrophobic residues are depicted as green balls. The van der Waals space occupied by hydrophobic residues is represented by yellow dots. In B and C, for clarity, the two monomers of the dimers are colored separately (dark orange and blue). B: Stereo view depicting the position of the labeled αA polar residues (rendered as sticks) that are complementary for dimerization of the monomers. The position of SCM1 and 2 is also indicated. In C, the hydrophobic residues topologically equivalent to polar residues shown in B (rendered as sticks) that participate in the dimerization of MjHSP16.5 in SCM1 are shown.

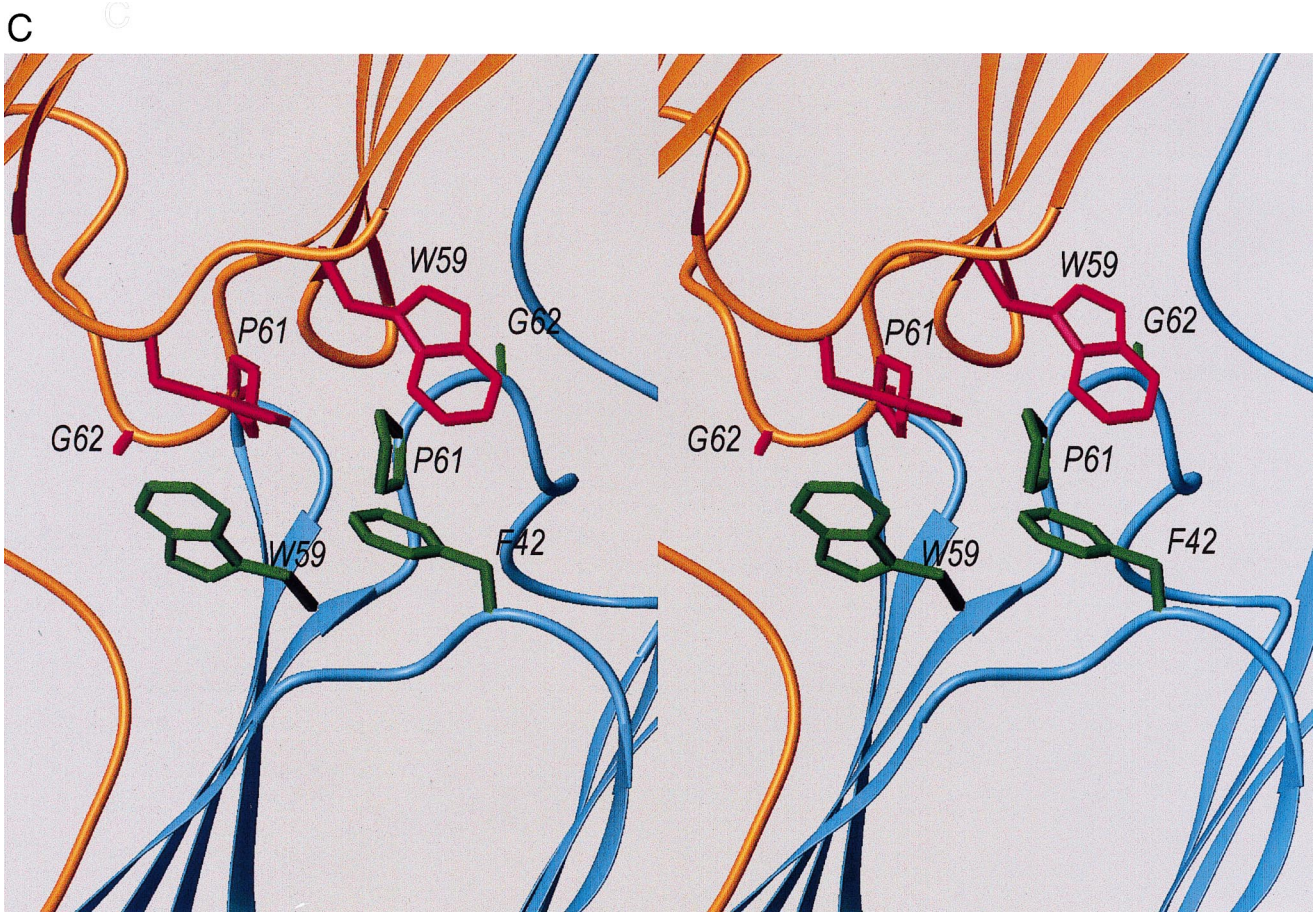

Fig. 2 (*continued*).

within sHsps have been identified as chaperone binding sites, only K70–K88, prevents protein aggregation [23]. Our molecular modeling shows that complementary charged amino acids in this functional unit are also involved in subunit interactions (D76–K78, H79). Experimental evidence for a highly ordered structure within the sequence 75–90 is data showing that at 22°C, the D–H exchange of amide hydrogens is slowest in this segment [24]. Increased temperature produces an increased D–H exchange denoting a structural alteration, which is accompanied by an enhanced chaperone-like activity. The hydrophobic sequence 71–75 is also subject to increased exposure on heating [25]. In summary, SCM1 has three very important functions, (i) the stabilization of the hydrophobic core of the β-sheet sandwich, (ii) subunit–subunit interactions and (iii) during heat stress, an altered structure resulting in increased exposure of chaperone binding sites.

Although interactions among the more hydrophobic N-terminal domains are required for chaperone activity [26], the formation of dimers and tetramers among CαA monomers or the multimers of either CαB or Hsp27 establish that subunit–subunit interactions occur at sites within the α-crystallin domain as well [27]. A study of random mutations in the C-terminal domain of αB (CαB) [28] revealed that CαB interacts with itself, αA, αB and Hsp27 in a similar fashion. Similarity in the binding sites would explain the similar effects of binding and non-binding CαB mutants. Although it is difficult to determine the cumulative effect of up to 15 mutations, the mutations in non-binding CαB within the sequences containing SCM1 and 2 results in a loss of negative charge. Conversely, the gain of negative charge is typical of the binding CαB mutants. The authors conclude that electrostatic interactions are important in the attraction among mutants and wild type sHsps. Both SCM1 and 2 contain numerous solvent exposed charged amino acids with the potential to form ion pairs within a subunit and/or salt bridges between subunits. Therefore, the electrostatic subunit interactions and their response to stress can modify structure and surface area exposure and, thereby, modulate the functional state of sHsps. For example, uncoupling of the subunit–subunit interactive site of SCM1, D76–K78, H79, within the chaperone binding site in αA (70–88) may also serve to modulate chaperone activity.

The spin labeling experimental protocol to gain insight into subunit–subunit interactions within the α-crystallin domain required single mutations of each amino acid with cysteine. The effects of each mutation were monitored by measuring chaperone activity and molecular mass [21,29]. The perceived increase in molecular mass can be attributed to changes in the hydrodynamic radius of the subunit needed to accommodate the molar volume of the mutant. In residues 121–155, only D136 and G137 mutants showed increased chaperone activity of 190 and 350% and a 13 and 70% increase in molecular mass [21]. In contrast, similar mutations within sequence 60–108 produced substantial alterations in both parameters only within SCM1 and 2 [29]. In the mutations of amino acids

within 76–80, there were significant increases in both chaperone activity, ranging from 300 to 800%, and molecular mass, from 0.72 to 0.92 MDa (under the conditions of these experiments WT is ~0.67). According to our molecular modeling, D76, K78 and H79 participate in salt bridges between subunits while F80 stabilizes the hydrophobic core within a β-sheet sandwich. The other major region of increased chaperone activity and molecular mass was within SCM2, namely H100, D105 and D106. The increase in chaperone activity for these amino acids is ~240%. The increase in molecular mass ranges from the normal ~0.67 to 0.86 to 0.91 MDa. This very highly conserved sequence is on the subunit surface. Cysteine mutations within SCM1 and 2 produce the most significant alterations in αA structure and chaperone activity. This demonstrates the importance of these sequences to both structure and function of sHsps.

5. Summary

In summary, we have discovered two SCM, SCM1 and SCM2, in α-crystallin and related sHsps. The N-terminal region of the highly conserved α-crystallin domain containing SCM1 and 2 constitutes a functional unit involving both structural stabilization and, under stress, structural modification and chaperone-like activity. Significant variations in the surface area exposure of α-crystallin are most likely due to equilibria between formation and dissolution of the dimers and/or tetramers induced by subunit interactions among C-terminal domains [28]. Greater surface area exposure is expected to enhance chaperone-like activity. A proposed 'open' micellar structure of α-crystallin quaternary structure [18,30,31] is devoid of dimers and tetramers. Therefore, it represents maximum surface exposure. Modulation of the subunit–subunit interactions within the α-crystallin domain is most likely involved in subunit exchange, level of chaperone activity, polydispersity and the variability of α-crystallin quaternary structure.

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