PIBB 生物化学与生物物理进展
Progress in Biochemistry and Biophysics
2011, 38(3): 239~247
www.pibb.ac.cn

The Ca^{2+}-Dependent Multimerization of S100 Domain in *Homo sapiens* Cornulin Protects Cells From Injury*

LI Guo-Ming[1,3], CHEN Qiang[2,3], DAI Ke-Sheng[1], ZHENG Xiao-Feng[2,3]**
([1] *School of Biological Science and Medical Engineering, Beihang University, Beijing* 100191, China;
[2] *National Laboratory of Protein Engineering and Plant Genetic Engineering, Peking University, Beijing* 100871, China
[3] *Department of Biochemistry and Molecular Biology, College of Life Sciences, Peking University, Beijing* 100871, China)

Abstract A novel type of stress proteins has been identified in mammals to defend environmental stresses and maintain tissue integrity. Cornulin (CRNN) that contains S100 EF-hand Ca^{2+}-binding motif is a stress protein highly expressed in the human esophageal squamous epithelial cells. It is downregulated in esophageal squamous cell carcinoma and functions as a modifier of deoxycholic acid (DCA) mediated cell injury. The S100 domain may be central to the function of CRNN. To further characterize the S100 domain of CRNN, the S100 domain in *Escherichia coli*, was cloned, expressed, purified and demonstrated that it was properly folded and suitable for biochemical and biophysical studies. More importantly, by nuclear magnetic resonance, gel-filtration, analytical ultracentrifugation, electrospray ionization-mass spectrometry, and Cross-linking analyses, a Ca^{2+}-dependent multimeric property of S100 domain was identified. Furthermore, in response to DCA and ethanol challenge, the multimers have stronger protective effects on cells than dimers do. These data indicate that the S100 domain is a key domain in CRNN, which functions as a survival factor through multimerization. This work helps to further understand the feature of S100 domain and its association with cell injury.

Key words cornulin (CRNN), S100 domain, Ca^{2+}, multimeric property, deoxycholic acid, cell viability
DOI: 10.3724/SP.J.1206.2010.00493

Environmental agents can influence tissue integrity, disease development, and related cancer development rate [1–2]. The cells of human esophageal squamous epithelium are under relatively unique environmental stresses including bacterial infestation, viruses, thermal stresses, and oxidizing chemicals that contribute to tissue damage and initiate diseases [3–4]. A novel type of stress proteins has been identified in mammals that can defend these environmental stresses and maintain tissue integrity[3].

Cornulin (CRNN) was originally cloned from normal esophagus and named Clone 1 open reading frame 10 (also known as squamous epithelial-induced stress protein of 53 ku (SEP53)). *CRNN* gene is located on chromosome 1q21 and encodes a 495 amino acids protein[5–6]. It is one of the stress proteins that is highly expressed in esophageal squamous epithelial cells and is characterized by the presence of EF-hand Ca^{2+}-binding motif at its N-terminus[6–7]. CRNN is a member of the S100 protein family, which is down-regulated in esophageal squamous cell carcinoma tissues [8]. Decrease of CRNN expression may result in the abnormal response of esophageal epithelium to environmental stimulation, which possibly leads to cancer [9]. Therefore, this protein presumably plays an important role in maintaining the barrier function in squamous epithelium in response to injury and

*This work was supported by grants from National Basic Research Program of China(2007CB914303, 2010CB911800), Hi-Tech Research and Development Program of China(2006AA02A314) and International Centre for Genetic Engineering and Biotechnology (ICGEB) (CRP/CHN09-01).

**Corresponding author.
Tel: 86-10-62755712, E-mail: xiaofengz@pku.edu.cn
Received: September 23, 2010 Accepted: November 23, 2010

functions as a tumor suppressor [10]. CRNN is also involved in heat shock and ethanol response and is expected to protect epithelium from damage [6, 11]. In addition, it can function as a survival factor in attenuation of deoxycholic acid (DCA) mediated cell injury[11]. The N-terminal Ca^{2+}-binding S100 domain is highly conserved and plays a key role in above functions. Deletion of the S100 domain in CRNN neutralizes its protective effects and permits Ca^{2+} elevation in cells after DCA exposure[11].

In this study, in order to further characterize the S100 domain of CRNN, we successfully cloned, expressed, purified apo human S100 domain in *Escherichia coli*(*E.coli*), and investigated its biophysical and biochemical features. Our data show that the purified S100 domain possesses a Ca^{2+}-dependent multimeric property and the multimers increase cell survival activity after DCA and ethanol injury. This work helps us to further understand the feature of S100 domain in CRNN.

1 Materials and methods

1.1 Materials and reagents

Restriction enzymes, *pfu* polymerase and T4 DNA ligase were purchased from TaKaRa (Dalian, China). The CellTiter 96® AQueous One Solution Reagent and isopropyl β-D-1-thiogalactopyranoside (IPTG) were from Promega (USA). Ni^{2+}-nitriloacetic acid resin and Sephacryl S-75 high resolution resin were obtained from Amersham Pharmacia Biotech (USA). BS^3 crosslinkers were purchased from Thermo Scitific (USA). The expression vector pET-15b and *E. coli* strains BL21 (DE3), Rosetta (DE3), BL21 (DE3) pLysS were purchased from Novagen (USA).

1.2 Construction of bacterial expression vector for recombinant S100 domain

Two primers, 5′ CGGGATCCATGCCTCAGT-TACTGC 3′ and 5′ GGAATTCCATATGAGACTCT-TGAGA 3′, were used to amplify the sequence encoding S100 domain using *CRNN* cDNA as template. The PCR product was inserted into the pET-15b vector by restriction enzyme sites *Bam*HⅠ and *Nde*Ⅰ. The resulting vector was verified by restriction digestion and sequencing from both ends of the inserted segment.

1.3 Expression of S100 domain in *E. coli*

The recombinant S100 domain was transformed into *E. coli* BL21 (DE3), Rosetta (DE3) and BL21 (DE3) pLysS strains, respectively. Cells were cultured at 37℃ overnight in 20 ml Luria-Bertani (LB) medium containing 100 mg/L ampicillin, then transferred to 1 L of fresh LB medium and incubated at 37℃ until the A_{600} reached 0.6 ~ 0.8. IPTG was added to a final concentration of 0.5 mmol/L and continuously cultured at 37℃ for 4 h. The expression of the protein was examined as follows. 1 ml of cells was collected by centrifugation and the pellet was resuspended in 50 μl ddH_2O, mixed with 10 μl 6×SDS loading buffer (0.35 mol/L Tris, pH 6.8, 10.28% SDS, 36% glycerol, 5% β-mercaptoethanol, 0.12% bromphenol blue) and denatured at 98℃ for 10 min. The samples were centrifuged at 14 000 r/min for 10 min. The supernatant was analyzed by 15% SDS-PAGE and stained by Coomassie brilliant blue R-250.

1.4 Purification of S100 domain protein

For large-scale protein purification, 2 L of culture was prepared and centrifuged at 5 000 r/min for 10 min. Cells were suspended in the binding buffer (20 mmol/L Hepes, pH 7.5, 500 mmol/L NaCl, 5 mmol/L imidazole) and ultrasonicated (on for 5 s, off for 10 s; 99 cycles). The lysate was centrifuged at 18 000 r/min for 30 min; the supernatant was filtered through a 0.22 μm filter and subsequently loaded onto a Ni^{2+} charged HiTrap Chelating HP column attached to an ÄKTA-fast protein liquid chromatography(FPLC) system. The protein was eluted by 6 bed volumes in a linear gradient of 5 ~ 500 mmol/L imidazole. The protein peak was harvested in 200 mmol/L imidazole. To further purify the protein, the concentrated elution was applied onto a gel-filtration Hi-load Superdex-75 column equilibrated with a high salt buffer containing 10 mmo/L Hepes, pH 7.5, 500 mmol/L NaCl, and 5 mmol/L EDTA to prevent ionic interactions. The peak fractions from the elution were pooled and concentrated in an Amicon Ultra centrifugal filter device, and finally dialyzed against application buffer. All purification procedures described here were performed at 4℃. The purified proteins were analyzed on 15% SDS-PAGE. The protein concentration was determined by Bradford dye assay using BSA protein standards. All further characterization was carried out in the presence of 5 mmol/L EDTA to retain it in apo form. Binding of Ca^{2+} to apo protein was achieved by dialysis against 20 mmol/L Hepes, pH 7.5, 150 mmol/L NaCl, 2 mmol/L $CaCl_2$.

1.5 Nuclear magnetic resonance spectroscopy

For nuclear magnetic resonance (NMR) experiments, the uniformly ^{15}N-labeled protein was obtained by expressing the recombinant protein in M9-minimal medium containing $^{15}NH_4Cl$ as the sole nitrogen source. The labeled protein was purified as described above. The purity of the protein was examined by SDS-PAGE to ensure a single band. 1 mmol/L of unlabeled and uniformly ^{15}N-labeled apo and Ca^{2+}-bound S100 domain samples were prepared in 10% D_2O. Samples were degassed by vacuum and nitrogen flushing shortly before each set of experiments. The NMR experiments were performed at 25 ℃ on Bruker Avance 600 MHz spectrometers equipped with four RF channels and triple-resonance probes with pulsed-field gradients. 2-dimethyl-2-silapentanesulfonic was used as the internal chemical shift reference. All NMR data were processed using the program NMRPipe and analyzed using the program NMRView[12–13].

1.6 Analytical ultracentrifugation analysis

The purified apo and Ca^{2+}-bound S100 domain samples were analyzed by analytical ultracentrifugation (AUC). The samples were centrifuged at 14 000 r/min for 10 min to remove potential aggregates formed during freezing, and then subjected to a Beckman Optima XL-Ⅰ analytical ultracentrifuge. The detection was carried out by means of an UV-visible absorbance detection system. Experiments were conducted at 20℃ using an AnTi50 eight-hole rotor and epon-charcoal standard double sector centerpieces. Absorbance scans were taken at the appropriate wavelength. The sedimentation velocity of apo and Ca^{2+}-bound S100 domain was performed at 40 000 r/min using 400 μl samples. The data were determined using programs of SEDFIT and SEDNTERP[14–15].

1.7 Electrospray ionization-mass spectrometry analysis

Molecular mass was determined by Electrospray ionization-mass spectrometry (ESI-MS). The purified Ca^{2+}-bound protein samples were exchanged into 10% acetonitrile with 0.1% formic acid using a 10 ku MWCO spin concentrator. The data were processed by Masslynx mass spectrometry software.

1.8 BS^3 cross-linking analysis

The BS^3 was dissolved in water and then added into the purified apo and Ca^{2+}-bound protein (0.5 mmol/L) to a final concentration of 1 mmol/L. The reaction mixture was incubated on ice for 2 h. The reaction was quenched by adding 1 mol/L Tris-HCl (pH 7.5) to a final concentration of 20～50 mmol/L and incubated at room temperature for 15 min. The samples were then analyzed by 15% SDS-PAGE and stained by Coomassie brilliant blue R-250.

1.9 Cell culture and assay for cell viability

HEK 293T cells were grown in IMDM medium (Gibco) containing 10% (*v*/*v*) fetal calf serum (Hyclone) at 37℃ in 5% CO_2. Cells were seeded in 96-well culture plates (5×10^3 cells in 0.1 ml IMDM/well) and grown for 48 h in IMDM supplemented with 10% fetal calf serum. Cells viability was quantified by absorbance at 490 nm using a CellTiter 96® AQueous One Solution Reagent after incubation cells with 500 μmol/L DCA, or 0.5%～2% ethanol and S100 domain species for 20 h. The protect effect of S100 domain species on cells was also examined under DCA+ethanol-induced condition for 16 h. All data are expressed as $x \pm s$. Statistical analysis was performed using one-way analysis of variance, followed by Student's *t*-test.

2 Results and discussion

2.1 S100 domain is conserved among human S100 family proteins

Comparison of S100 domain in CRNN with other human S100 family proteins revealed that this domain is conserved. S100 domain has 44%～51% identity with trichohyalin, hornerin, filaggrin, and repetin, whereas it shares 30%～35% identity with other S100 proteins. As shown in Figure 1, some amino acids are strictly conserved or highly similar among the S100 proteins, these residues participate in binding Ca^{2+} or maintaining structural stability in known structures of S100 proteins[16–20]. Therefore, we could deduce that the conserved residues in S100 domain of CRNN, such as Tyr18, Leu28, Glu32, Lys34, Glu40, Leu61, Asp62 and Glu73, play the same roles. More importantly, Phe15, Phe71 and Phe74/Tyr74 are highly conserved throughout the S100 proteins, which form a central hydrophobic core together with several other residues. The three conserved residues that are substituted for polar residues would have drastic effects on the structural stability of S100 proteins[21].

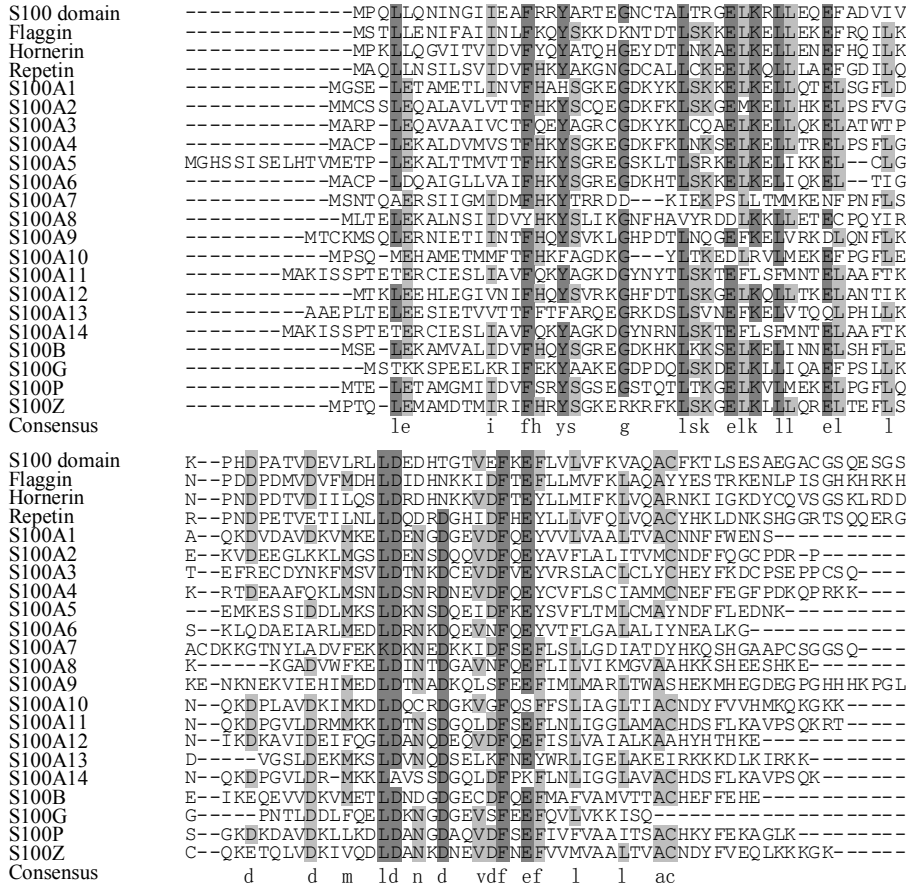

Fig. 1 Sequence alignment of S100 domain in CRNN with known human S100 family proteins

Residues are colored according to the degree of substitution at each position: dark gray, highly similar; light gray, less similar; white, not conserved.

2.2 Expression and purification of S100 domain

We designed primers to amplify the nucleotide sequence encoding for the N-terminal residues of CRNN, harbouring the S100 domain. The C-terminus primer corresponding to a hydrophilic area of CRNN was selected in order to obtain soluble recombinant protein. The DNA fragment encoding S100 domain of CRNN was cloned into expression vector pET-15b and confirmed by restriction enzyme digestion and DNA sequencing. The protein expression of S100 domain was examined in *E. coli* BL21 (DE3), Rosetta (DE3) and BL21 (DE3) pLysS strains, respectively. Cells were collected and analyzed by SDS-PAGE. The his-tagged recombinant protein (122 residues) with the predicted molecular mass was highly expressed in Rosetta (DE3) after IPTG induction (Figure 2a). Analysis of the sequence coding for S100 domain revealed a number of rare codons. The *E. coli* Rosetta (DE3) can provide the lacking tRNAs corresponding to these *E. coli* rare codons[22]. Therefore, using this strain as expression host significantly improved the expression yield of S100 domain compared with other strains tested.

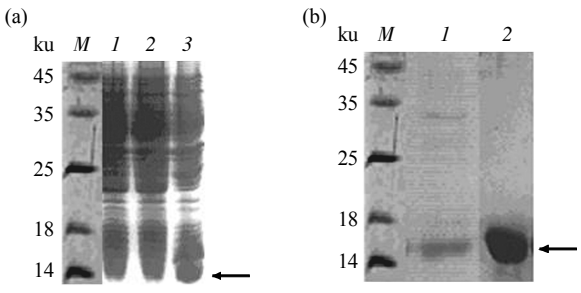

Fig. 2 Expression and purification of S100 domain

(a) Comparison of S100 domain expression level in different *E. coli* host strains. *1*: BL21 (DE3); *2*: BL21 (DE3) pLysS; *3*: Rosetta (DE3); *M*: protein molecular mass markers. (b) Purification of recombinant S100 domain. *M*: Protein molecular mass markers; *1*: The protein after Ni^{2+} affinity column purification; *2*: The protein after S-75 gel-filtration column purification.

After two-step purification by Ni^{2+} affinity chromatography and S-75 gel-filtration chromatography, the recombinant protein was purified to near homogenous (Figure 2b). The final yield of apo protein is 10 mg/L. The purified protein was stable after storage at 4℃ for one month.

2.3 Conformational changes occur in S100 domain upon Ca^{2+} binding

Analysis of the dispersion of NMR resonance signals is a good indicator of folded protein. One-dimensional ^1H NMR spectra of apo S100 domain exhibited characterization of a well-folded protein featuring good resonance dispersions in the regions of the methyl protons, α-protons, and amide protons (data not shown). Two-dimensional ^1H-^{15}N HSQC spectra showed a good dispersion of backbone amides, indicating that this protein is correctly folded(Figure 3a). Moreover, we examined the effects of Ca^{2+} on S100 domain by employing NMR-based assays. Indeed, significant chemical shift changes were observed in the ^1H-^{15}N HSQC spectrum of the Ca^{2+}-bound form compared with that of the apo protein (Figure 3b). The NMR data show that S100 domain could bind Ca^{2+} and conformational changes occur upon Ca^{2+} binding.

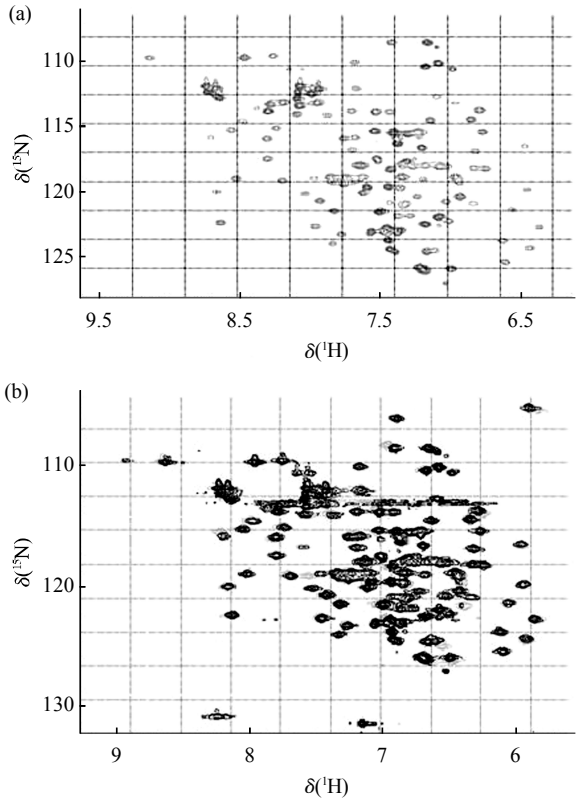

Fig. 3 2D ^1H-^{15}N HSQC NMR spectra of S100 domain

(a) 2D ^1H-^{15}N HSQC NMR spectrum used uniformly ^{15}N labeled apo S100 domain. (b) Comparison of 2D ^1H-^{15}N HSQC NMR spectra of apo and Ca^{2+}-bound S100 domain. Spectrum of uniformly ^{15}N labeled apo and Ca^{2+}-bound S100 domain is presented in gray and black, respectively.

2.4 S100 domain is multimerized in the presence of Ca^{2+}

Most of S100 proteins have a strong tendency to dimerize, but some of them could multimerize[23–27]. To explore whether the S100 domain has multimeric property, the concentrated apo and Ca^{2+}-bound S100 domain were loaded onto S-75 gel-filtration column and eluted with the buffer containing 20 mmol/L Hepes, pH 7.5, 150 mmol/L NaCl with 5 mmol/L EDTA or 2 mmol/L $CaCl_2$. The results suggest that the apo S100 domain is mainly present as dimers. However, a significant improvement in the multimerization state of the Ca^{2+}-bound form was observed compared with the apo form. In the presence of Ca^{2+}, about 35% of the protein was found as multimers and 65% as dimers (data not shown). This indicates that multimerization of S100 domain is affected by the presence of Ca^{2+}.

AUC is a technique used to characterize auto-association processes of proteins in solution, thus reflecting their native states. Determination of sedimentation velocity can provide information concerning the protein's molecular mass. We therefore compared the molecular mass of the apo and Ca^{2+}-bound S100 domain according to their sedimentation coefficients. The results demonstrate that S100 domain is mostly dimerized without Ca^{2+} (Figure 4a) and can form multimers in the presence of Ca^{2+} (Figure 4b).

ESI-MS analysis was performed to further confirm the multimerization of Ca^{2+}-bound S100 domain. The samples were prepared and subjected to ESI-MS analysis as described in **Materials and methods**. The data show that Ca^{2+}-bound S100 domain exists in three forms, dimers (28 397.744 u), trimers (42 460.778 u) and tetramers(56 614.371 u)(Figure 4c).

Addition of a crosslinker to the protein is used to test the direct self association. We found that Ca^{2+}-bound S100 domain was partially multimerized (Figure 4d, lane *2*). However, apo S100 domain had no multimerization, only dimers were observed(Figure 4d, lane *3*). The presence of monomers under denaturing conditions indicated an incomplete cross-linking reaction. These results also corroborate that Ca^{2+} appears to induce formation of multimers.

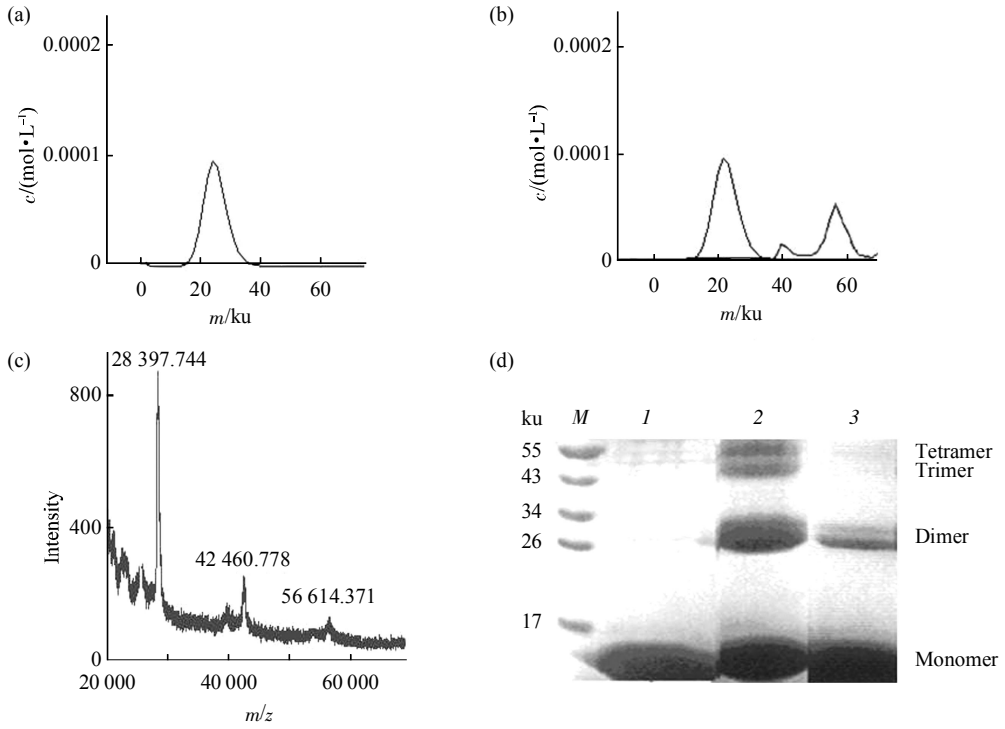

Fig. 4 S100 domain is multimerized in the presence of Ca^{2+}

(a, b) AUC studies of apo (a) and Ca^{2+}-bound (b) S100 domain. (c) ESI-MS analysis of Ca^{2+}-bound S100 domain. (d) Cross-linking analysis of S100 domain. *M*: Protein molecular mass markers; *1*: Control; *2*: Purified Ca^{2+}-bound S100 domain; *3*: Purified apo S100 domain.

S100 proteins are characterized by the presence of two Ca^{2+}-binding motifs of the EF-hand type interconnected by a hinge region. In each Ca^{2+}-binding motif, a Ca^{2+}-binding loop is flanked by α-helices, resulting in a helix-loop-helix arrangement [21]. At present there is no information about the structure of S100 domain in CRNN, but we deduced that it might share a similar structure with other S100 proteins due to the high sequence similarity. Our data presented here show that the S100 domain has a Ca^{2+}-dependent multimeric property and multimer formation is more favored when Ca^{2+}-binding motif is occupied. In combination with some previous reports on the Ca^{2+}-induced conformational changes of S100 proteins that have been well characterized by NMR and X-ray crystallographic studies [16–20], we therefore speculate that dimeric S100 domain undergoes a significant conformational changes upon Ca^{2+} binding and these conformational changes alter the structure sufficiently to allow it to further form noncovalent multimers.

2.5 Multimeric S100 domain attenuates DCA and ethanol-induced cell injury

According to previous reports, DCA is a significant constituent of gastric fluid and can stimulate DNA damage, cell injury, and mediate increases in intracellular free Ca^{2+} as well. It is often used as a model damaging agent[11]. CRNN functions as a survival factor after DCA-induced cell injury and deletion of S100 domain neutralizes the protective effects[11]. Some S100 proteins show biological activity *in vitro* and can directly affect cells [28]. To determine the role of S100 domain multimerization in cellular functions, we treated 293T cells with same concentration (100 mg/L) of dimers and multimers of S100 domain isolated from gel-filtration chromatography under normal or DCA-induced stressed conditions. Cells were treated with 500 μmol/L DCA and 100 mg/L S100 domain species for 20 h, and then the cell viability was examined. In the presence of DCA up to a concentration of 500 μmol/L, a significant decrease of cell viability was obtained in 293T cells as expected. Compared to the cells only treated with DCA, addition of dimers or multimers in DCA-treated cells showed an obvious increase of cell viability, but the effect of multimers was much stronger than dimers (Figure 5a). While under normal physiological

condition, accession of exogenous dimeric or multimeric form of the protein showed no visible protection on cell proliferation (Figure 5b). In addition, ethanol exposure is an often occurred stress for epithelial cells, we therefore examined the protective effects of S100 domain species under ethanol-induced and ethanol+DCA-induced conditions, respectively. Our results showed that ethanol may be an associated risk factor for tissue injure, however, it alone at up to 2% (v/v) does not affect viability in cells for 20 h(Figure 5c). On the other hand, the combined action of ethanol with 500 μmol/L DCA is more toxic than DCA alone for 16 h, and multimers reduced the toxic effect than dimers (Figure 5d). Taken together, these data further suggest that CRNN is a cellular stress-response protein, and the multimerization state of S100 domain strongly affects its function.

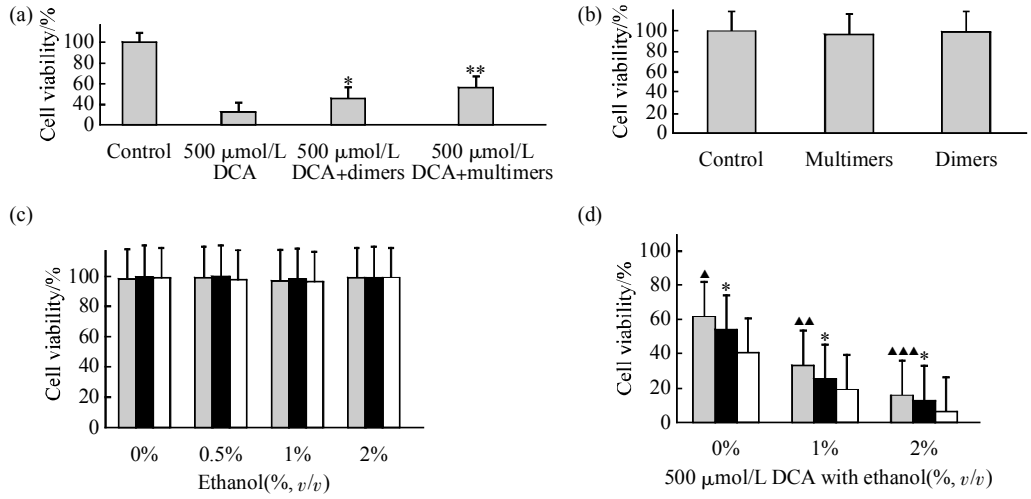

Fig. 5 Effects of multimers and dimers of S100 domain on cell viability under normal or stressed conditions

(a) S100 multimers can attenuate DCA-induced cell injury. Cells without or with multimers and dimers were treated with 500 μmol/L DCA for 20 h (n=4). Cells without any treatment were used as a control. *P < 0.001 vs. 500 μmol/L DCA, **P < 0.001 vs. 500 μmol/L DCA+dimers. (b) Effect of S100 dimers and multimers on cell viability after 20 h without DCA treatment (n=4). The control was the same as above.(c) Cells without (as a control) or with multimers and dimers were treated with increasing concentrations of ethanol for 20 h (n=4). □: Dimers; ■: Multimers; □: Control. (d) Cells without (as a control) or with multimers and dimers were treated with combination of 500 μmol/L DCA and increasing concentrations of ethanol for 16 h (n=4). *P < 0.001 vs. control, ▲P < 0.003, ▲▲P < 0.001 or ▲▲▲P < 0.004 vs. dimers. All data were reported as $\bar{x} \pm s$. P values were determined by Student's t test. □: Multimers; ■: Dimers; □: Control.

The noncovalent multimers of S100 proteins are found to play a major role in extracellular and intracellular functions. For example, it is shown that only multimeric S100A4 has neurite sprouting activity [23]. The Ca^{2+}-induced S100A8/A9 tetramer promotes the formation of microtubules and the Ca^{2+}-induced S100A12 hexamer may interact with RAGE [24-25, 29]. Because DCA can induce release of intracellular Ca^{2+} and cell injury[11] and multimerization of CRNN S100 domain is Ca^{2+}-dependent (Figure 4), the highly conserved S100 domain may sense cellular Ca^{2+} perturbation and subsequently form multimers to further protect cells from injury.

In conclusion, we cloned, expressed, purified and preliminarily characterized human S100 domain of CRNN. We identified the multimerization of the S100 domain in response to the changes of Ca^{2+} and found that it was able to attenuate DCA and ethanol induced injury. To further understand the mechanism of this effect would be an interested topic to be ascertained, which will help us to unravel the physiological function of CRNN.

Acknowledgements We sincerely thank Dr. LIU Zhi-Hua for gifting CRNN cDNA. We are grateful to LI Hong-Wei and Prof. JIN Chang-Wen at Beijing NMR centre for NMR analyses and BI Shuang-Yu for AUC analysis.

References

[1] Dixon A J, Dixon B F. Ultraviolet radiation from welding and possible risk of skin and ocular malignancy. Med J Aust, 2004,

181(3): 155-157

[2] Brody J G, Rudel R A, Michels K B, *et al*. Environmental pollutants, diet, physical activity, body size, and breast cancer: where do we stand in research to identify opportunities for prevention?. Cancer, 2007, **109**(12 Suppl): 2627-2634

[3] Bulger E M, Helton W S. Nutrient antioxidants in gastrointestinal diseases. Gastroenterol Clin North A, 1998, **27**(2): 403-419

[4] Goldstein J L, Watkins J L, Greager J A, *et al*. The esophageal mucosal resistance: structure and function of an unique gastrointestinal epithelial barrier. J Lab Clin Med, 1994, **123**(5): 653-659

[5] Xu Z, Wang M R, Xu X, *et al*. Novel human esophagus-specific gene c1orf10: cDNA cloning, gene structure, and frequent loss of expression in esophageal cancer. Genomics, 2000, **69**(3): 322-330

[6] Yagui-Beltran A, Craig A L, Lawrie L, *et al*. The human oesophageal squamous epithelium exhibits a novel type of heat shock protein response. Eur J Biochem, 2001, **268**(20): 5343-5355

[7] Contzler R, Favre B, Huber M, *et al*. Cornulin, a new member of the "fused gene" family, is expressed during epidermal differentiation. J Invest Dermatol, 2005, **124** (5): 990-997

[8] Imai F L, Uzawa K, Nimura Y, *et al*. Chromosome 1 open reading frame 10 (C1orf10) gene is frequently down-regulated and inhibits cell proliferation in oral squamous cell carcinoma. Int J Biochem Cell Biol, 2005, **37**(8): 1641-1655

[9] Luo A, Kong J, Hu G, *et al*. Discovery of Ca^{2+}-relevant and differentiation associated genes downregulated in esophageal squamous cell carcinoma using cDNA microarray. Oncogene, 2004, **23**(6): 1291-1299

[10] Zhang W, Chen X, Luo A, *et al*. Genetic variants of C1orf10 and risk of esophageal squamous cell carcinoma in a Chinese population. Cancer Sci, 2009, **100**(9): 1695-1700

[11] Darragh J, Hunter M, Pohler E, *et al*. The calcium-binding domain of the stress protein SEP53 is required for survival in response to deoxycholic acidmediated injury. FEBS, 2006, **273**(9): 1930-1947

[12] Delaglio F, Grzesiek S, Vuister G W, *et al*. NMRPipe: a multidimensional spectral processing system based on UNIX pipes. J Biomol NMR, 1995, **6**(3): 277-293

[13] Johnson B A, Blevins R A. NMRView: a computer program for the visualization and analysis of NMR data. J Biomol NMR, 1994, **4**(3): 603-614

[14] Schuck P, Rossmanith P. Determination of the sedimentation coefficient distribution by least-squares boundary modeling. Biopolymers, 2000, **54**(5): 328-341

[15] Schuck P, Perugini M A, Conzales N R, *et al*. Size-distribution analysis of proteins by analytical ultracentrifugation: strategies and application to model systems. Biophysical J, 2002, **82**(2): 1096-1111

[16] Zhang H, Wang G, Ding Y, *et al*. The crystal structure at 2Å resolution of the Ca^{2+}-binding protein S100P. J Mol Biol, 2003, **325**(4): 785-794

[17] Bertini I, Das Gupta S, Hu X, *et al*. Solution structure and dynamics of S100A5 in the apo and Ca^{2+}-bound states. J Biol Inorg Chem, 2009, **14**(7): 1097-1107

[18] Smith S P, Shaw G S. A novel calcium-sensitive switch revealed by the structure of human S100B in the calcium-bound form. Structure, 1998, **6**(2): 211-222

[19] Inman K G, Yang R, Rustandi R R, *et al*. Solution NMR structure of S100B bound to the high-affinity target peptide TRTK-12. J Mol Biol, 2002, **324**(5): 1003-1014

[20] Wright N T, Varney K M, Ellis K C, *et al*. The three-dimensional solution structure of Ca^{2+}-bound S100A1 as determined by NMR spectroscopy. J Mol Biol, 2005, **353**(2): 410-426

[21] Heizmann C W, Fritz G, Schafer B W. S100 proteins: structure, functions and pathology. Front Biosci, 2002, **7**(1): 1356-1368

[22] Kane J F. Effects of rare codon clusters on high-level expression of heterologous proteins in *Escherichia coli*. Curr Opin Biotechnol, 1995, **6**(5): 494-500

[23] Kiryushko D, Novitskaya V, Soroka V, *et al*. Molecular mechanisms of Ca^{2+} signaling in neurons induced by the S100A4 protein. Mol Cell Biol, 2006, **26**(9): 3625-3638

[24] Korndorfer I P, Brueckner F, Skerra A. 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. J Mol Biol, 2007, **370**(5): 887-898

[25] Xie J, Burz D S, He W, *et al*. Hexameric Calgranulin C (S100A12) binds to the receptor for advanced Glycated end products (RAGE) using symmetric hydrophobic target-binding patches. J Biol Chem, 2007, **282**(6): 4218-4231

[26] Gribenko A V, Makhatadze G I. Oligomerization and divalent ion binding properties of the S100P protein: a Ca^{2+}/Mg^{2+}-switch model. J Mol Biol, 1998, **283**(3): 679-694

[27] Chang N, Sutherland C, Hesse E, *et al*. Identification of a novel interaction between the Ca^{2+}-binding protein S100A11 and the Ca^{2+}- and phospholipid-binding protein annexin A6. Am J Physiol Cell Physiol, 2007, **292**(4): 1417-1430

[28] Donato R. Intracellular and extracellular roles of S100 proteins. Microsc Res Tech, 2003, **60**(6): 540-551

[29] Leukert N, Vogl T, Strupat K, *et al*. Calcium-dependent tetramer formation of S100A8 and S100A9 is essential for biological activity. J Mol Biol, 2006, **359**(4): 961-972

人类 Cornulin 蛋白 S100 结构域钙依赖的多聚化能有效减少细胞的损伤 *

李国明[1, 3]　陈　强[2, 3]　戴克胜[1]　郑晓峰[2, 3]**

([1]北京航空航天大学生物与医学工程学院，北京 100191；[2]北京大学植物基因与蛋白质工程国家重点实验室，北京 100871；
[3]北京大学生命科学院生物化学与分子生物学系，北京 100871)

摘要　在哺乳动物中发现一类新的能够抵制环境压力和保持组织完整性的应激蛋白．含有 S100 钙结合结构域的 Cornulin (CRNN)是这类蛋白质之一，它在人类食管鳞状上皮细胞中高表达，而在食管鳞状上皮细胞癌中却低表达，它能抑制脱氧胆酸诱导的细胞损伤．S100 结构域在 CRNN 的功能上具有重要作用．为了进一步探讨 CRNN S100 结构域的生物学特性，克隆、表达、纯化了该结构域，证明其折叠正确，适合用于生物物理和生物化学特性的研究．更为重要的是，通过核磁共振、凝胶过滤层析、超速离心、质谱和蛋白质交联分析，发现 S100 结构域具有钙依赖的多聚性质，而多聚体的形成更有利于保护细胞免受脱氧胆酸和乙醇的损伤．上述结果表明，S100 结构域是 CRNN 发挥功能的关键结构域，它可以通过多聚化更好地保护细胞．该工作将进一步揭示 S100 结构域的生物学功能．

关键词　Cornulin(CRNN)，S100 结构域，钙，多聚性质，脱氧胆酸，细胞存活
学科分类号　Q2，Q5，Q6，Q7　　**DOI**: 10.3724/SP.J.1206.2010.00493

---

* 国家高技术研究发展计划(863)(2006AA02A314), 国家重点基础研究发展计划(973)(2007CB914303, 2010CB911800)资助项目和 ICGEB 国际合作项目(CRP/CHN09-01).

** 通讯联系人.
Tel: 010-62755712, E-mail: xiaofengz@pku.edu.cn

收稿日期：2010-09-23，接受日期：2010-11-23