Ultrastructural Distribution of the S100A1 Ca^{2+}-Binding Protein in the Human Heart

B. MACO[1], A. MANDINOVA[2], M. B. DÜRRENBERGER[2], B. W. SCHÄFER[3], B. UHRÍK[1], C. W. HEIZMANN[3]

[1]*Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia,* [2]*Maurice E. Müller-Institute, Biocentrum, University of Basel, Basel, Switzerland,* [3]*Division of Clinical Chemistry and Biochemistry, Department of Pediatrics, University of Zurich, Zurich, Switzerland*

Summary

Impaired calcium homeostasis and altered expression of Ca^{2+}-binding proteins are associated with cardiomyopathies, myocardial hypertrophy, infarction or ischemia. S100A1 protein with its modulatory effect on different target proteins has been proposed as one of potential candidates which could participate in these pathological processes. The exact localization of S100A1 in human heart cells on the ultrastructural level accompanied with biochemical determination of its target proteins may help clarify the role of S100A1 in heart muscle. In the present study the distribution of the S100A1 protein using postembedding (Lowicryl K4M) immunocytochemical method in human heart muscle has been determined quantitatively, relating number of antigen sites to the unit area of a respective structural component. S100A1 antigen sites have been detected in elements of sarcoplasmic reticulum (SR), in myofibrils at all levels of sarcomere and in mitochondria, the density of immunolabeling at Z-lines being about 3 times and at SR more than 5 times higher than immunolabeling of remaining structural components. The presence of the S100A1 in SR and myofibrils may be related to the known target proteins for S100A1 at these sites.

Key words

Introduction

Calcium ions as second messengers play an important role in the regulation of large number of cellular processes such as muscle contraction, secretion, cell division, cell cycle progression, energy production, proliferation, gene transcription and differentiation (Berridge 1997). The key molecules to transduce Ca^{2+} signaling are Ca^{2+}-binding proteins mainly belonging to the large EF-hand protein superfamily (Heizmann and Hunziker 1991, Kawasaki *et al.* 1998).

Since the first isolation of S100 proteins from bovine brain (Moore 1965) they have become the largest subfamily of EF-hand Ca^{2+}-binding proteins (Zimmer *et al.* 1995, Schäfer and Heizmann 1996, Heizmann and Cox 1998, Donato 1999). S100 proteins are acidic proteins of low molecular weight (10-12 kDa) and are

---

characterized by two distinct EF-hand domains with different affinities for Ca^{2+}. To date, 19 different proteins which display various degree of amino acid sequence homology, have been assigned to the S100 protein family (Donato 1999). A new S100 nomenclature is based on the clustered organization of at least 13 S100 genes on human chromosome 1q21 (Engelkamp *et al.* 1993, Heizmann and Schäfer 2001) and mouse chromosome 3, respectively (Ridinger *et al.* 1998).

S100 proteins show tissue- and cell-specific expression, a characteristic they share with most other EF-hand Ca^{2+}-binding proteins but not with the multifunctional and ubiquitously expressed calmodulin (Crivici and Ikura 1995). S100 proteins mainly exert their modulatory effect through Ca^{2+}-regulated interactions with intracellular specific target proteins.

Although both S100A1 and S100B were originally assumed to be neuronal specific proteins, it is now clear that S100A1 is specifically expressed in slow-twitch skeletal and heart muscles (for review see Heizmann and Cox 1998, Donato 1999).

Ultrastructural localization studies of the S100A1 in skeletal (Haimoto and Kato 1987, Maco *et al.* 1997, 1999, 2000a) and heart muscle cells (Haimoto and Kato 1988) have been done mostly in rodents and have localized S100A1 in myofibrils associated with actin and myosin filaments and with Z-lines, in the sarcoplasmic reticulum, in mitochondria, along the sarcolemma, and in fascia adherens of the intercalated discs.

Expression and localization of S100 proteins in human tissues may differ from that in rodent tissues (Engelkamp *et al.* 1992). However, no corresponding ultrastructural studies have been performed in human heart. Changes in expression of S100A1 in human cardiomyopathy (Remppis *et al.* 1996) and a right ventricular upregulation of S100A1 in chronic pulmonary hypertension (Ehlermann *et al.* 2000) indicate that more attention should be paid to the role of S100A1 and probably also of other calcium binding proteins in cardiac diseases. In addition, the increased level of S100A1 in the serum after myocardial infarction with the possibility to differentiate between infarction and angina pectoris (Usui *et al.* 1990) suggests a potential diagnostic value of this protein.

The importance of a distinct subcellular location of S100A1 for the cell function under normal and pathologic conditions would be more evident if quantitative data on the distribution of antigens in relation to structure would be available. In the present ultrastructural study the distribution of S100A1 protein using immunocytochemical method in human heart was determined quantitatively, relating number of antigen sites to the unit area of a respective structural component.

Methods

Tissue preparation

Pieces of human heart muscle from the right atrium were obtained during surgery of a 34-years old man with a mitral valve disease in accordance with Helsinki Declaration of 1975.

Fixation and preparation of sections

The heart biopsy samples were immediately fixed for 1 h at 4 °C in 0.5 % glutaraldehyde (Fluka, Buchs, Switzerland) and 3 % paraformaldehyde (Merck, New Jersey, USA) in 10 mmol/l phosphate buffer solution (PBS) containing (mmol/l): Na_2HPO_4 (10), NaH_2PO_4 (10), NaCl (85.5), NaN_3 (20), pH 7.4. Fixed specimens were then dehydrated in ethanol, embedded in Lowicryl K4M (Polysciences, Eppelheim, Germany) and polymerized under UV light at –40 °C. Grey/silver sections were mounted on parlodion-coated copper grids (200 mesh). Sections were washed with 2 % BSA (Boehringer, Mannheim, Germany) in 10 mmol/l PBS before incubation with antibodies (see below) and stained with 6% aqueous uranyl acetate (Fluka, Buchs, Switzerland) and lead citrate.

EM immunocytochemistry

Ultrathin sections were labeled with purified polyclonal antiserum obtained by immunization of goats with recombinant human S100A1 protein produced in E. coli cultures (Ilg *et al.* 1996). The serum was diluted 1:300 with 2 % BSA in PBS and applied for one hour. The sections were then washed in PBS, and exposed for 1 h to the rabbit anti-goat secondary antibody coupled with 15-nm gold (Aurion, Wageningen, Netherland) and diluted 1:20 with 2 % BSA in PBS. Stainings with primary antibodies preabsorbed with human recombinant S100A1 protein were used as control.

Prior to application all antibodies were sonicated in water (Sonorex, TK 52H, Berlin, Germany) for 2-3 s. The sections were examined in a Philips EM 400 (Holland) electron microscope at 80 kV, photographed (negatives Kodak SO-163, USA), and printed with total magnification of 17 500× for immunogold counting.

Particle counting

The densities of gold particles on different levels of sarcomere (Z-line, I-band, and A-band), sarcoplasmic reticulum, mitochondria and sarcoplasm of human heart muscle cells were obtained from longitudinal sections.

Three different blocks of tissue were used. From each block 3 different grids with sections were prepared. Sixteen micrographs were taken from each grid and printed at total magnification of 17 500×.

Areas (µm^2) of immunolabeled structural components (Ac) of myofibrils were estimated by the point counting method (Weibel and Bolender 1973) from each micrograph: Ac=Σpx × (d/mag)^2 where p is number of points of two-dimensional test lattice for particular structural components of myofibrils; d is distance of two neighboring points of the test grid (µm), and mag is total magnification of a micrograph. The density of immunolabeling was expressed as the number of gold particles per unit area of the respective structural component. Background labeling was estimated in a similar manner.

Table 1. Immunogold labeling of individual blocks at particular structural components.

| | Gold particles/µm^2 | | |
	Block 1	Block 2	Block 3
Z-line	0.2592 ± 0.0188	0.2883 ± 0.0659	0.6931 ± 0.0802
I-band	0.0318 ± 0.0246	0.1249 ± 0.0364	0.3336 ± 0.0243
A-band	0.0501 ± 0.0087	0.0920 ± 0.0141	0.1454 ± 0.0117
mitochondria	0.0618 ± 0.0134	0.1249 ± 0.0289	0.2946 ± 0.0110
sarcoplasmic reticulum	0.4531 ± 0.2003	0.8973 ± 0.3145	1.1794 ± 0.0418
remaining sarcoplasm	0.1079 ± 0.0577	0.0584 ± 0.0158	0.1642 ± 0.0211

Label over empty plastic of 0.1839 particles/µm^2 has been subtracted. Each block is represented by three sets of data pooled from 16 micrographs obtained from individual grids with sections. Data are expressed as number of gold particles per µm^2; mean values ± S.E.M.
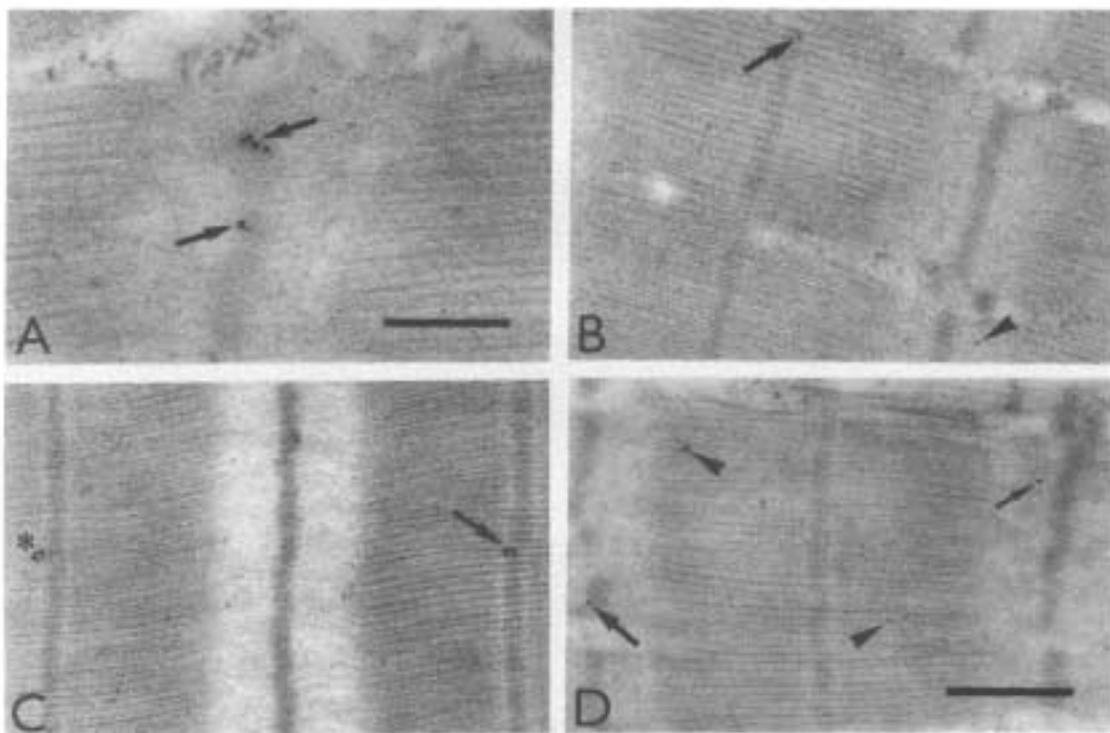

Fig. 1. *Ultrastructural localization of S100A1 protein in human heart muscle at different levels of sarcomere. (A) Presence of gold particles on Z-line (arrows); (B) on I-band (arrowhead) and M-line (arrow); (C) on M-line (arrow) and on H-band (asterisk); (D) on A-band (arrowheads), I-band (thin arrow) and Z-line (thick arrow). Bars: A = 0.3 µm; B-D = 0.5 µm.*
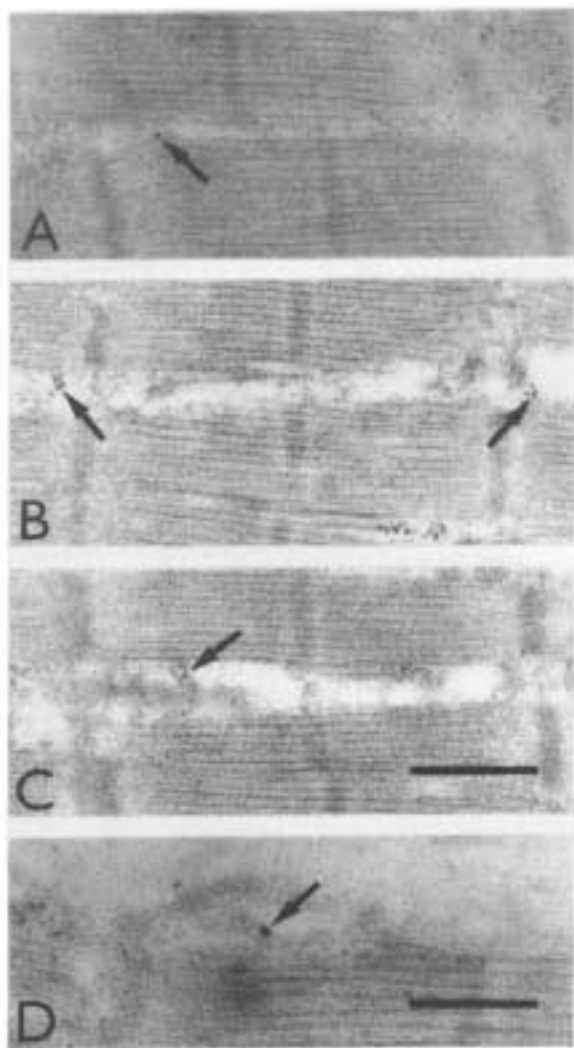
Fig. 2. *The presence of S100A1 protein in elements of the sarcoplasmic reticulum (arrows). Bars: A-C = 0.5 μm; D = 0.3 μm.*

Results

Immunogold electron microscopy of S100A1 distribution in longitudinal sections of normal human heart showed that antigen sites for S100A1 protein were localized mainly in myofibrils at all levels of sarcomere (Fig. 1) and in elements of sarcoplasmic reticulum (SR) (Fig. 2).

The staining with immunogold on other structural components as intercalated discs, sarcolemma and nuclei was sparse. In an effort to suppress nonspecific immunolabeling as much as possible some specific labeling could have been suppressed as well. However, no immunolabeling occurred in controls (Fig. 3).

Immunogold particles were not uniformly distributed but occurred preferentially at particular locations. As summarized in Table 1, the most preferred locations were Z-lines (Figs 1A and 1D) and elements of SR (Fig. 2).

A lower density of S100A1 protein was observed on thin and thick myofilaments (Figs 1B and 1D). Within the A-bands a slightly higher density of immunogold particles was detected on M-lines (Figs 1B and 1C).

To compensate for differences in immunolabeling between individual blocks (Table 1) resulting probably from different time intervals between sectioning and immunolabeling or from differences in blocking conditions, the immunogold densities at particular structural components were normalized to corresponding immunogold densities of mitochondria.

The normalized densities are shown in Figure 4. It is evident that density of immunolabeling on Z-line is about 3 times and in case of SR more than 5 times higher than immunolabeling of remaining structural components. These differences are significant ($p < 0.05$).

Discussion

A precise control of Ca^{2+} homeostasis in cardiac muscle cells is a prerequisite for normal function and its derangement results in various pathological states. Ultrastructural localization studies of S100A1 may contribute to elucidation of subcellular mechanisms of Ca^{2+}-signaling and give us insight into processes resulting in pathological heart.

To our knowledge, the ultrastructural localization and quantitative evaluation of S100A1 distribution in normal human heart has been performed for the first time in the present study. Although S100A1 may be found in different compartments of the heart cell, two areas of the highest occurrence, the Z-line and the elements of the sarcoplasmic reticulum, could be identified. Total amount of S100A1 protein expressed by human heart is 1.84±0.5 μg/mg protein which is about twofold higher than that of human skeletal muscle (Kato and Kimura 1985).

A relatively sparse immunogold staining in the present study may result from several reasons: a) strong blocking conditions during specimen preparation; b) the lower S100A1 expression in atrial than in ventricular myocardium (as described during mouse heart development, Kiewitz *et al.* 2000); c) the lower S100A1 expression in the right heart in comparison with the left ventricle (as described for porcine myocardium, Remppis *et al.* 1996). It is not clear if the underlying mitral valve disease could have an effect on the S100A1 expression in the right atrium. There were no clinical signs of right atrium dysfunction.
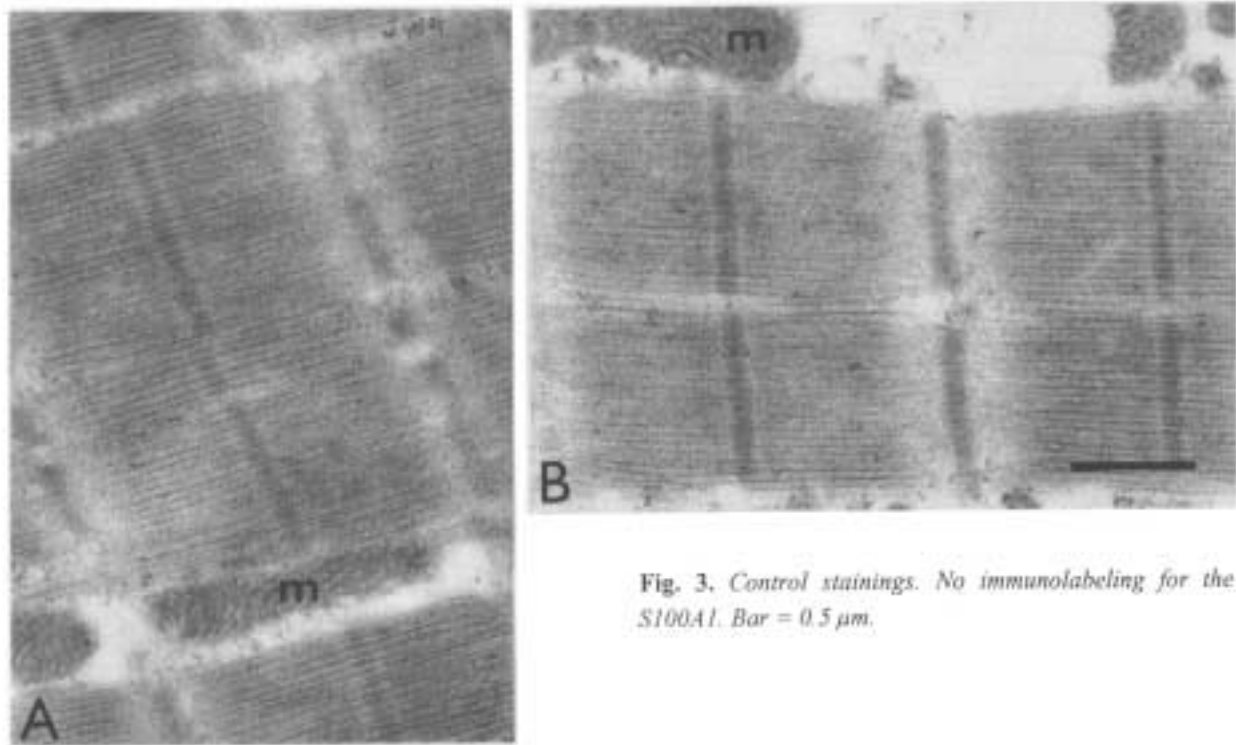
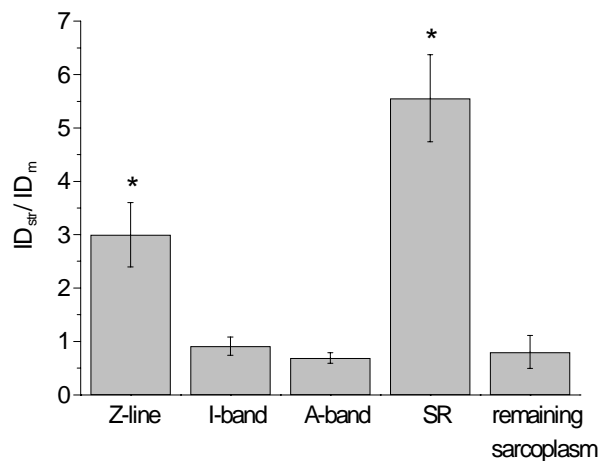Fig. 3. *Control stainings. No immunolabeling for the S100A1. Bar = 0.5 μm.*

Fig. 4. *Normalization of immuno-gold densities at particular structural components (ID_{str}) to corresponding immunogold densities of mitochondria (ID_m). Data are means ± S.E.M.; *$p<0.05$ with respect to the I-band, A-band and remaining sarcoplasm.*

The presence of antigen sites for S100A1 along the sarcomere (Figures 1A-D) suggests an association of S100A1 with contractile filaments and/or with some other components associated with myofibrils. While an interaction of S100A1 with skeletal muscle myosin heavy chain has been detected by gel overlay technique (Zimmer 1991), an interaction with cardiac myosin heavy chain has not been observed (Donato *et al.* 1989). These differences could be due to the different myosin isoforms in cardiac and skeletal muscle fibers. Recently Mandinova *et al.* (1998) have shown a direct interaction of S100A1 protein with rabbit skeletal F-actin in Ca²⁺-dependent manner in vitro and with actin stress fibers of human smooth muscle cells.

Location of antigen sites at M-lines (Figs 1B and 1C) may be interesting since M-lines are a site of titin kinase (Labeit *et al.* 1997) which is equivalent to twitchin kinase in invertebrates. Twitchin kinase is regulated by S100A1 and it is assumed that cardiac titin may be regulated in a similar way (Heierhorst *et al.* 1996, 1997). The high concentration of immunogold particles seen at the Z-line (Figs 1A and 1D) may result from a combination of interaction of S100A1 binding to F-actin and CapZ, the actin capping protein (Ivanenkov *et al.* 1996). S100A1 protein interacts with CapZ protein at the same binding site as phosphatidylinositol 4-monophosphate (PIP), a regulator of CapZ activity. It is assumed that S100A1 protein modulates the inhibitory

effect of PIP on activity of CapZ protein and can participate in the regulation of length, orientation and/or anchoring of actin filaments to the Z-line (Caldwell et al. 1989, Xu et al. 1999).

The location of S100A1 antigens at the sarcoplasmic reticulum observed in our study (Fig. 2) corresponds to similar findings from mouse (Haimoto and Kato 1988) and porcine heart (Donato et al. 1989) or rat skeletal muscle (Haimoto and Kato 1987, 1999, Maco et al. 1997, 2000a). It may be related to S100A1 stimulation of Ca^{2+}-induced Ca^{2+} release from isolated SR vesicles (Fanò et al. 1989b, Marsili et al. 1992), to increased caffeine-induced Ca^{2+} release by S100A1 in permeabilized fast and slow twitch skeletal muscle fibers (Weber et al. 1997) and to interaction of S100A1 with the ryanodine receptor (Treves et al. 1997). The localization of S100A1 at the level of SR could also be related to its stimulatory effect on the basal activity of adenylate cyclase (Fanò et al. 1989a) associated with the sarcolemma, T-tubules and junctional SR where this enzymatic complex has been identified histochemically (Slezak and Geller 1984; for review see Schulze and Buchwalow 1998).

The growing list of S100A1 target proteins also includes some enzymes of energy metabolism such as glycogen phosphorylase a and phosphoglucomutase (for review see Zimmer et al. 1995, Landar et al. 1996) in slow-twitch skeletal muscle. Although many glycogenolytic and glycolytic enzymes are preferentially bound to F-actin (Arnold and Pette 1968) both above-mentioned enzymes are associated with the membranes of SR (Entam et al. 1976, Lee et al. 1992). Recently, other glycolytic enzymes were also found to be localized on cytoplasmic surface of rabbit skeletal and heart SR vesicles and it was suggested that the ATP generated by SR-associated glycolytic enzymes may be coupled to SR active calcium transport (Xu and Becker 1998).

Another hypothesis how to explain the presence of S100A1 antigen sites at the level of SR is provided by gel-overlay experiments performed by Zimmer (1991) who found an interaction of S100A1 with different SR proteins. Two of these proteins with the molecular weights of 30 kDa and 100 kDa could be tentatively identified as phospholamban and Ca^{2+}-ATPase (SERCA2), respectively. Thus S100A1 may not only be involved in the Ca^{2+} release from SR but could also play a role in the calcium uptake into SR. In such a case S100A1 would be an interesting candidate to explain the impaired Ca^{2+}-homeostasis in dilated cardiomyopathy (Minamisawa et al. 1999).

Acknowledgements

References

ARNOLD H, PETTE D: Binding of glycolytic enzymes to structural proteins of the muscle. *Eur J Biochem* **6**: 163-171, 1968.

BERRIDGE MJ: Elementary and global aspects of calcium signaling. *J Physiol Lond* **499**: 291-306, 1997.

CALDWELL JE, HEISS SG, MERMALL V, COOPER JA: Effect of CapZ, an actin capping protein of muscle, on the polymerization of actin. *Biochemistry* **28**: 8506-8514, 1989.

CRIVICI A, IKURA M: Molecular and structural basis of target recognition by calmodulin. *Annu Rev Biophys Biomol Struct* **24**: 85-116, 1995.

DONATO R: Functional roles of S100 proteins, calcium-binding proteins of the EF-hand type. *Biochim Biophys Acta* **1450**: 191-231, 1999.

DONATO R, GIAMBANCO I, AISA MC, DI GERONIMO G, CECCARELLI P, RAMBOTTI MG, SPRECA A: Cardiac S-100a$_0$ protein: purification by a simple procedure and related immunocytochemical and immunochemical studies. *Cell Calcium* **10**: 81-92, 1989.

EHLERMANN P, REMPPIS A, GUDDAT O, WEIMANN J, SCHNABEL PA, MOTSCH J, HEIZMANN CW, KATUS HA: Right ventricular upregulation of the Ca^{2+} binding protein S100A1 in chronic pulmonary hypertension. *Biochim Biophys Acta* **1500**: 249-255, 2000.

ENGELKAMP D, SCHÄFER BW, ERNE P, HEIZMANN CW: S100α, CAPL, and CACY: Molecular cloning and expression analysis of three calcium-binding proteins from human heart. *Biochemistry* **31**: 10258-10264, 1992.

ENGELKAMP D, SCHÄFER BW, MATTEI MG, ERNE P, HEIZMANN CW: Six S100 genes are clustered on human chromosome 1q21: identification of two genes coding for the two previously unreported calcium-binding proteins S100D and S100E. *Proc Natl Acad Sci USA* **90**: 6547-6551, 1993.

ENTAM ML, KANIKE K, GOLDSTEIN MA, NELSON TE, BORNET EP, FUTCH TW, SCHWARTZ A: Association of glycogenolysis with cardiac sarcoplasmic reticulum. *J Biol Chem* **251**: 3140-3146, 1976.

FANÒ G, ANGELELLA P, MARRIGIO D, AISA MC, GIAMBANCO I, DONATO R: S100a$_0$ protein stimulates the basal (Mg^{2+}-activated) adenylate cyclase activity associated with skeletal muscle membranes. *FEBS Lett* **248**: 9-12, 1989a.

FANÒ G, MARSILI V, ANGELELLA P, AISA MC, GIAMBANCO I, DONATO R: S100a$_0$ protein stimulates Ca^{2+}-induced Ca^{2+} release from isolated sarcoplasmic reticulum vesicles. *FEBS Lett* **255**: 381-384, 1989b.

HAIMOTO H, KATO K: S100a$_0$ ($\alpha\alpha$) protein, a calcium-binding protein, is localized in the slow-twitch muscle fiber. *J Neurochem* **48**: 917-923, 1987.

HAIMOTO H, KATO K: S100a$_0$ ($\alpha\alpha$) protein in cardiac muscle. Isolation from human cardiac muscle and ultrastructural localization. *Eur J Biochem* **171**: 409-415, 1988.

HEIERHORST J, KOBE B, FEIL SC, PARKER MW, BENIAN GM, WEISS KR, KEMP BE: Ca^{2+}/S100 regulation of giant protein kinases. *Nature* **380**: 636-639, 1996.

HEIERHORST J, MANN RJ, KEMP BE: Interaction of the recombinant S100A1 protein with twitchin kinase, and comparison with other Ca^{2+}-binding proteins. *Eur J Biochem* **249**: 127-133, 1997.

HEIZMANN CW, COX JA: New perspectives on S100 proteins: a multi-functional Ca^{2+}-, Zn^{2+}- and Cu^{2+}-binding protein family. *Biometals* **11**: 383-397, 1998.

HEIZMANN CW, HUNZIKER W: Intracellular calcium-binding proteins: more sites than insights. *Trends Biochem Sci* **16**: 98-103, 1991.

HEIZMANN CW, SCHÄFER BW: S100 protein family. In: *Encyclopaedia of Molecular Medicine*. TE CREIGHTON (ed), John Wiley, New York, 2001 (in press).

ILG EC, SCHÄFER BW, HEIZMANN CW: Expression pattern of S100 calcium-binding proteins in human tumors. *Int J Cancer* **68**: 325-332, 1996.

IVANENKOV VV, DIMLICH RV, JAMIESON GA: Interaction of S100a$_0$ protein with the actin capping protein, CapZ: characterization of a putative S100a$_0$ binding site in CapZα-subunit. *Biochem Biophys Res Commun* **221**: 46-50, 1996.

KATO K, KIMURA S: S100a$_0$ ($\alpha\alpha$) protein is mainly located in the heart and striated muscles. *Biochim Biophys Acta* **842**: 146-150, 1985.

KAWASAKI H, NAKAYAMA S, KRETSINGER RH: Classification and evolution of EF-hand proteins. *Biometals* **11**: 277-295, 1998.

KIEWITZ R, LYONS GE, SCHÄFER BW, HEIZMANN CW: Transcriptional regulation of S100A1 and expression during mouse heart development. *Biochim Biophys Acta* **1498**: 207-219, 2000

LABEIT S, KOLMERER B, LINKE WA: The giant protein titin. Emerging roles in physiology and pathophysiology. *Circ Res* **80**: 290-294, 1997.

LANDAR A, CADDELL G, CHESSHER J, ZIMMER DB: Identification of an S100A1/S100B target protein: phosphoglucomutase. *Cell Calcium* **20**: 279-285, 1996.

LEE YS, MARKS AR, GUERECKAS N, LACRO R, NADAL-GINARD B, KIM DH: Purification, characterization, and molecular cloning of a 60-kDa phosphoprotein in rabbit skeletal sarcoplasmic reticulum which is an isoform of phosphoglucomutase. *J Biol Chem* **267**: 21080-21088, 1992.

MACO B, BREZOVÁ A, SCHÄFER BW, UHRÍK B, HEIZMANN CW: Localization of the Ca^{2+}-binding S100A1 protein in slow and fast skeletal muscles of the rat. *Gen Physiol Biophys* **16**: 373-377, 1997.

MACO B, UHRÍK B, HEIZMANN CW: Changes in distribution of the Ca^{2+}-binding S100A1 protein at different sarcomere lengths of slow and fast rat skeletal muscles. *Physiol Res* **48:** 12P, 1999.

MACO B, UHRÍK B, HEIZMANN CW: Distribution of the Ca^{2+}-binding S100A1 protein at different sarcomere lengths of slow and fast rat skeletal muscles. *Gen Physiol Biophys* **19**: 237-244, 2000a.

MACO B, MANDINOVA A, DÜRRENBERGER M, SCHÄFER BW, UHRÍK B, HEIZMANN CW: Ultrastructural distribution of S100A1 protein in human heart. *Physiol Res* **49:** P8, 2000b.

MANDINOVA A, ATAR D, SCHÄFER BW, SPIESS M, AEBI U, HEIZMANN CW: Distinct subcellular localization of calcium binding S100 proteins in human smooth muscle cells and their relocation in response to rises in intracellular calcium. *J Cell Sci* **111**: 2043-2054, 1998.

MARSILI V, MANCINELLI L, MENCHETTI G, FULLE S, BALDONI F, FANÒ G: S100ab increases Ca^{2+} release in purified sarcoplasmic reticulum vesicles of frog skeletal muscle. *J Muscle Res Cell Motil* **13**: 511-515, 1992.

MINAMISAWA S, HOSHIJIMA M, CHU G, WARD CA, FRANK K, GU Y, MARTONE ME, WANG Y, ROSS J Jr, KRANIAS EG, GILES WR, CHIEN KR: Chronic phospholamban-sarcoplasmic reticulum calcium ATPase interaction is the critical calcium cycling defect in dilated cardiomyopathy. *Cell* **99**: 313-322, 1999.

MOORE BW: A soluble protein characteristic of the nervous system. *Biochem Biophys Res Commun* **6**: 739-744, 1965

REMPPIS A, GRETEN T, SCHÄFER BW, HUNZIKER P, ERNE P, KATUS HA, HEIZMANN CW: Altered expression of the Ca^{2+}-binding protein S100A1 in human cardiomyopathy. *Biochim Biophys Acta* **1313**: 253-257, 1996.

RIDINGER K, ILG EC, NIGGLI FK, HEIZMANN CW, SCHÄFER BW: Clustered organization of S100 genes in human and mouse. *Biochim Biophys Acta* **1448**: 254-263, 1998.

SCHÄFER BW, HEIZMANN CW: The S100 family of EF-hand calcium-binding proteins: functions and pathology. *Trends Biochem Sci* **21**: 134-140, 1996.

SCHULZE W, BUCHWALOW IB: Adenylyl cyclase in the heart: an enzymocytochemical and immunocytochemical approach. *Microsc Res Technique* **40**: 473-478, 1998.

SLEZAK J, GELLER SA: Cytochemical studies of myocardial adenylate cyclase after its activation and inhibition. *J Histochem Cytochem* **32**: 105-113, 1984.

TREVES S, SCUTARI E, ROBERT M, GROH S, OTTOLIA M, PRESTIPINO G, RONJAT M, ZORZATO F: Interaction of S100A1 with the Ca^{2+} release channel (ryanodine receptor) of skeletal muscle. *Biochemistry* **36**: 11496-11503, 1997.

USUI A, KATO K, SASA H, MINAGUCHI K, ABE T, MURASE M, TANAKA M, TAKEUCHI E: S-100a$_o$ protein in serum during acute myocardial infarction. *Clin Chem* **36**: 639-641, 1990.

WEBER C, REMPPIS A, HEIZMANN CW, KATUS HA, FINK RHA: Increased caffeine-induced Ca^{2+} release by the calcium-binding protein S100A1 in permeabilized mammalian skeletal muscle fibers. *J Muscle Res Cell Motil* **18**: 192, 1997.

WEIBEL ER, BOLENDER RP: Stereological techniques for electron microscopic morphometry. In: *Principles and Techniques of Electron Microscopy, Vol. 3.* MA HAYAT (ed), Van Nostrand Reinhold Co., New York, 1973, pp 237-296.

XU J, CASELLA JF, POLLARD TD: Effect of capping protein, CapZ, on the length of actin filaments and mechanical properties of actin filament networks. *Cell Motil Cytoskeleton* **42**: 73-81, 1999.

XU KY, BECKER LC: Ultrastructural localization of glycolytic enzymes on sarcoplasmic reticulum vesicles. *J Histochem Cytochem* **46**: 419-427, 1998.

ZIMMER DB: Examination of the calcium-modulated protein S100α and its target proteins in adult and developing skeletal muscle. *Cell Motil Cytoskeleton* **20**: 325-337, 1991.

ZIMMER DB, CORNWALL EH, LANDAR A, SONG W: The S100 protein family: history, function, and expression. *Brain Res Bull* **37**: 417-429, 1995.

Reprint requests

B. Uhrík, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Vlárska 5, 833 34 Bratislava, Slovak Republic. Fax: +421-2-5477-3666, E-mail: umfguhrk@savba.savba.sk