Short communication

Localization of the Ca^{2+}-binding S100A1 Protein in Slow and Fast Skeletal Muscles of the Rat

B Maco[1], A Brezova[1], B W Schafer[2] B Uhrík[1] and C W Heizmann[2]

1 *Institute of Molecular Physiology and Genetics Slovak Academy of Sciences Vlarska 5 833 34 Bratislava, Slovakia*
2 *Division of Clinical Chemistry and Biochemistry, Department of Pediatrics University of Zurich, Steinwiesstrasse 75 8032 Zurich, Switzerland*

Abstract. The distribution of S100A1 in rat soleus and EDL muscles was studied immunocytochemically at the ultrastructural level using immunogold as marker Antigens were localized mainly in myofibrils at all levels of the sarcomere Immunogold particles along myofibrils were not uniformly distributed The highest density of particles was found at Z-lines An increase in particle density was observed in the middle of half A-bands in EDL and in the middle of half I-bands in the soleus Antigen sites were also present at M-lines and at distinct locations of the sarcoplasmic reticulum

Key words: Ca^{2+}-binding proteins —S100A1 —Myofibrils —Muscle cells — Sarcoplasmic reticulum

Transient increase in intracellular Ca^{2+} is the most common signal transduction mechanism present in a variety of cell types ranging from bacteria to mammalian cells A precise control of Ca^{2+} homeostasis is a prerequisite for many cellular functions (see e g Račay et al 1996, Račay and Lehotský 1996, Berridge 1997) The transmission of the calcium signal is frequently mediated by Ca^{2+}-binding proteins (for a review see Heizmann 1992)

S100 proteins, a subfamily of EF-hand calcium-binding proteins, are assumed to fulfil a number of regulatory roles along the Ca^{2+}-signal-transduction pathway (for a review see Heizmann 1996, Schafer and Heizmann 1996) It is known that skeletal and heart muscle cells highly express S100A1, suggesting an important function within these cell types (Kato and Kimura 1985, for a review see Zimmer et al 1995)

Correspondence to Dr B Uhrík, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Vlarska 5, 833 34 Bratislava, Slovakia Tel (+421 7) 375 266, Fax (+421 7) 373 666, E-mail umfguhrk@savba savba sk

Biochemical determination of target proteins for S100A1 and immunocytochemical detection of S100A1 sites at the ultrastructural level can provide insight into the role played by S100A1 in cells. The higher level of S100A1 in slow-twitch muscle fibres compared with fast-twitch fibres (Haimoto and Kato 1987) helps, in part, to clarify cellular and molecular mechanisms in which S100A1 is involved during the contraction-relaxation cycle or in muscle metabolism.

In the present study two types of muscle cells were used for immunocytochemical localization of S100A1: 1) rat soleus muscle (predominantly slow twitch fibres); 2) rat extensor digitorum longus muscle (EDL) (predominantly fast twitch fibres).

The muscle fibres were fixed with a formaldehyde and glutaraldehyde mixture in phosphate buffer and then either frozen in liquid nitrogen (following pretreatment with sucrose) or dehydrated in acetone and embedded in LR Gold. Purified polyclonal antiserum was obtained by immunization of goats with recombinant human S100A1 produced in *E. coli* cultures (Ilg et al. 1996). The serum was diluted 1:200 in phosphate buffer and applied to ultrathin cryo-sections according to the modified Tokuyasu method. Secondary rabbit antigoat antibodies conjugated with 10 nm gold particles were used to visualize antigen sites. Double contrasting with uranyl acetate showed some cell structures in negative contrast. The sections were embedded in methylcellulose.

Ultrathin sections of specimens embedded in LR Gold were treated either with polyclonal S100A1 antiserum diluted 1:400 or with a monoclonal antibody against bovine S100A1 (Sigma), diluted 1:400 (in this case secondary goat anti-mouse antibodies conjugated with 10 nm gold were used). Background labelling was suppressed with Dulbecco's modified Eagle's medium supplemented with 5% fetal calf serum. For controls the sections were incubated with pre-immune sera.

To determine the distribution of antigen sites gold particles were counted and related to areal densities of structural components of myofibrils. The areal densities of Z-lines and I- and A-bands of myofibrils on longitudinal sections, corresponding to their volume densities, were estimated by the point counting method (Weibel and Bolender 1973). This estimation made it possible to detect differences in distribution of antigen sites along the sarcomeres of the myofibrils. The distribution was considered uniform if the proportion of immunogold particles found in a particular structural component was the same as the areal density of this component. Non-uniform distribution reflects preferential location of antigens at some sites irrespective of their absolute amount. The gold particles were counted in different sections from the same specimen and their numbers were: 1093 (EDL, polyclonal), 217 (EDL, monoclonal), 1100 (soleus, polyclonal), 560 (soleus, monoclonal).

The main results of immunolabelling using polyclonal S100A1 antiserum are summarized as follows: a) Antigens were localized mainly in myofibrils at all levels of sarcomere, in both muscle types studied. b) Immunogold particles along myofibrils were not uniformly distributed but occurred preferentially at particular locations.

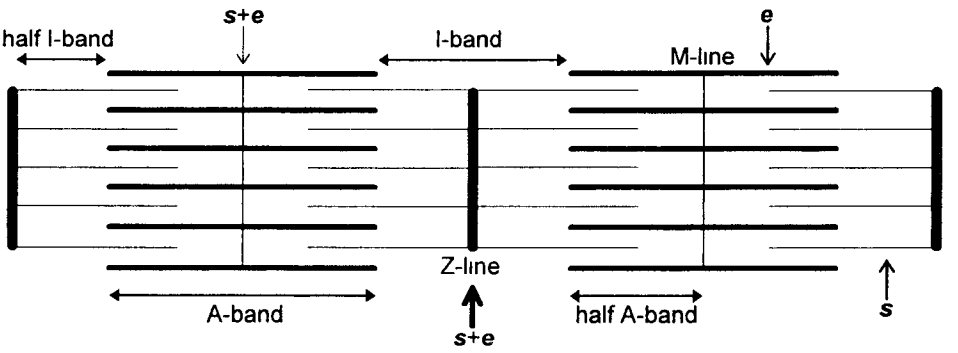

Figure 1. Schematic representation of sarcomere bands and lines Arrows indicate locations of preferential S100A1 immunoreactivities (s – soleus, e – EDL)

c) In Z-lines the particles were found 2 (soleus) to 3 times (EDL) more frequently than expected from respective areal densities d) The staining of I-bands with immunogold in soleus was 32% higher, and in EDL nearly the same than expected from the areal densities The density of particles in soleus I-bands was highest in the middle of half I-bands e) The occurrence of antigens in A-bands of soleus was 40% less whereas in EDL it was nearly the same than expected from areal densities However, in EDL about 30% more particles were present in the central parts of half A-bands (in the middle between M line and A/I-border) than expected from the uniform distribution f) S100A1 was also found to be localized in terminal cisternae of sarcoplasmic reticulum

The incubation of tissue sections with the monoclonal S100A1 antibody resulted in an identical but much less intense staining pattern, especially at the Z-lines

Using the same blocking protocol, the density of immunogold was much higher in soleus than in EDL, in accordance with the results of biochemical studies There it has been shown that S100A1 levels in slow-twitch skeletal muscles are much higher than in fast-twitch muscles (Haimoto and Kato 1987, Zimmer et al 1995) The presence of antigen sites along the sarcomere suggests an association of S100A1 with contractile filaments and/or with some other components associated with myofibrils In our study the sarcomere lengths differed slightly in different regions of the sections and we had the impression that at shorter sarcomeres (more extensive overlap of actin and myosin filaments in A-bands) the number of immunogold particles in the middle of half A-bands was increasing This observation points to an association of S100A1 with F-actin but it should be corroborated with a study on fibres in different contractile states In addition to a possible affinity to actin, the

higher density of antigen sites in the middle of half I-bands in soleus may reflect an interaction of S100A1 with some glycogenolytic and glycolytic enzymes described by others (Zimmer et al 1995, Landar et al 1996), as it is known that these types of enzymes bind preferentially to F-actin (Arnold and Pette 1968, Pette 1975)

The relatively high concentration of immunogold particles seen at Z-lines may result from a combination of S100A1 binding to F-actin and to CapZ, the actin capping protein (Ivanenkov et al 1996) In both muscle types investigated in the present study, antigen sites were also detected at M-lines This location may be interesting as a site of titin kinase (for a review see Labeit et al 1997) and the possibility that this enzyme is regulated by S100A1 in a similar way as twitchin kinase is regulated in invertebrates (Heierhorst et al 1996)

The location of S100A1 antigen sites in elements of sarcoplasmic reticulum observed in our study corresponds to similar findings of others (Haimoto and Kato 1987) and may be related to S100A1-stimulation of Ca^{2+}-induced Ca^{2+} release from isolated sarcoplasmic reticulum vesicles (Fanò et al 1989), to increased caffeine-induced Ca^{2+} release by S100A1 in permeabilized skeletal muscle fibres (Weber et al 1997) and to interaction of S100A1 with the ryanodine receptor (Treves et al 1997)

Acknowledgements. This work was supported by the Slovak grant agency for science (grant No 95/5305/515)

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Final version accepted December 20, 1997