Volume 299, number 2, 175–178 FEBS 10809 March 1992

Coexistence of two calsequestrin isoforms in rabbit slow-twitch skeletal muscle fibers

Donatella Biral, Pompeo Volpe, Ernesto Damiani and Alfredo Margreth

Centro di Studio per la Biologia e la Fisiopatologia Muscolare del CNR, Istituto di Patologia Generale dell'Università di Padova, via Trieste 75, 35121 Padova, Italy

Received 16 December 1991

The cardiac and skeletal muscle isoforms of calsequestrin (CS), the low affinity, high capacity Ca^{2+} binding protein localized in the lumen of sarcoplasmic reticulum, are the products of two different genes (Fliegel, L., Leberer, E., Green, N.M. and MacLennan, D.H. (1982) FEBS Lett. 242, 297–300), and can be both purified from slow-twitch skeletal muscle of the rabbit (Damiani, E., Volpe, P. and Margreth, A. (1990) J. Muscle Res. Cell Motil. 11, 522–530). Here we show that both CS isoforms coexist in slow-twitch muscle fibers as indicated by indirect immunofluorescent staining of cryosections with affinity-purified antibodies specific for each CS isoform.

Skeletal muscle; Sarcoplasmic reticulum; Calsequestrin; Ca^{2+}

1. INTRODUCTION

Calsequestrin (CS) is the main Ca^{2+} storage protein [1] localized within the lumen of sarcoplasmic reticulum (SR). Adult rabbit slow-twitch skeletal muscle, unlike fast-twitch muscle, expresses two isoforms of CS, the cardiac and the fast-twitch skeletal muscle forms [2,3], which are the products of two different genes [4,5]. The present immunofluorescent localization study shows that the coexistence of two CS isoforms is a common feature of slow-twitch fibers, distinguishing these fibers from the overall fast fiber population.

The promiscuity of slow-twitch muscle fibers with respect to CS isoforms contrasts with the segregation in the same fibers, during postnatal development of skeletal muscles, of a type-specific complement of myosin heavy and light chains [6], troponins [7] and, with regard to SR proteins, a cardiac isoform of the Ca^{2+}-ATPase [8,9]. However, a common skeletal muscle isoform of the SR Ca^{2+}-release channel, distinct from the cardiac isoform [10], appears to be invariantly expressed in both fast-twitch and slow-twitch muscle fibers [11,12]. Consequently, the SR membrane system of slow-twitch muscle fibers, unlike that of fast-twitch fibers, can be regarded as a mosaic of proteins encoded by either cardiac or fast-twitch muscle genes or both.

2. EXPERIMENTAL

2.1. *Anti-(CS) polyclonal antibodies*

Anti-(canine cardiac CS) ascites was raised in SLJ mice as previously described [3]. The IgG fraction contained antibodies which cross-reacted with the fast-twitch muscle CS isoform (cf. Fig. 3 in [3]). Antibodies specific for cardiac CS were affinity-purified as described by Bisson and Schiavo [13] on electroblotted cardiac CS.

Antibodies against rabbit fast-twitch muscle CS were obtained in adult hens, and were purified by affinity chromatography, as previously described [3].

2.2. *Isolation of terminal cisternae (TC) from slow-twitch skeletal muscle of the rabbit*

TC were isolated essentially as described by Saito et al. [14] and as detailed by Damiani et al. [3].

2.3. *Gel electrophoresis and Western blotting*

SDS-slab gel electrophoresis was carried out on 10% polyacrylamide gels as described [15]. Electrophoretic transfer to nitrocellulose, Ponceau red staining and indirect immunoenzymating staining were carried out as described [16].

2.4. *Immunofluorescence*

Rabbit gastrocnemius, a mixed predominantly fast-twitch muscle, and soleus, a pure slow-twitch muscle, on account of its isomyosin composition [17], were removed, frozen by immersion in liquid nitrogen, and stored at −80°C until used. Both longitudinal and transverse sections (10 μm thick) were cut out on a freezing microtome and mounted on glass slides.

Indirect immunofluorescence staining was carried out as previously described [18] using 50 μg/ml and 100 μg/ml of anti-(cardiac CS) and anti-(skeletal CS) antibodies, respectively. Cryosections were examined in a Zeiss Axioplan fluorescent microscope.

3. RESULTS AND DISCUSSION

The specificity of affinity purified antibodies for each CS isoform was ascertained by Western blotting. Fig. 1 shows TC from slow-twitch muscle after probing with either anti-(cardiac CS) antibodies (lane a) or anti-(skeletal CS) antibodies (lane b). The apparent molecular weight of immunostained bands was approximately 64

Correspondence address: D. Biral, Centro di Studio per la Biologia e la Fisiopatologia Muscolare del CNR, Istituto di Patologia Generale dell'Università di Padova, via Trieste 75, 35121 Padova, Italy.

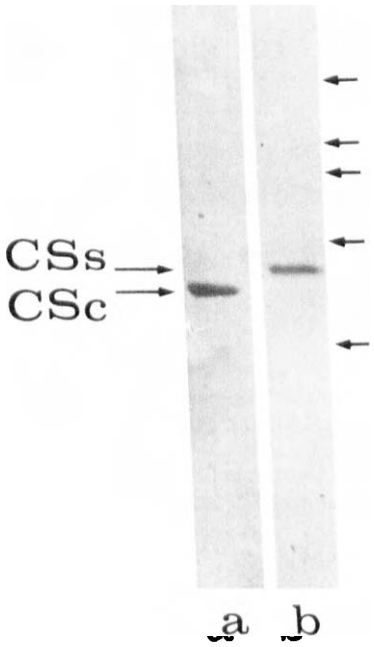

Fig. 1. Specificity of affinity purified anti-(CS) polyclonal antibodies. Slow-twitch skeletal muscle TC (10 μg of protein) were separated on 10% polyacrylamide gels, electroblotted and probed with either anti-(cardiac CS) antibodies (lane a) or anti-(skeletal CS) antibodies (lane b) at a concentration of 5 μg/ml. Arrows indicate the position of molecular mass standards: myosin heavy chain, 200,000; β-galactosidase, 116,250; phosphorylase *b*, 97,400; bovine serum albumin, 66,200; ovalbumin, 44,700.

and 54 kDa for skeletal CS (CS_s) and cardiac CS (CS_c), respectively (cf. [3]).

Immunofluorescence staining of transverse cryosections of gastrocnemius, a mixed predominantly fast-twitch muscle [17] showed that only a minority of fibers were reactive with anti-(cardiac CS) antibodies (Fig. 2A, B). On the other hand, immunofluorescent staining of serial sections with anti-(skeletal CS) antibodies (cf. panels B and C) did not distinguish between fast- and slow-twitch fibers that were all found to be similarly reactive (Fig. 2C). With both antibodies the fluorescence pattern was punctated, string-like or a combination of the two, as expected for the intermyofibrillar disposition of the SR. In longitudinal sections after immunofluorescent staining, both anti-(cardiac CS) antibodies (Fig. 2D) and anti-(skeletal CS) antibodies (Fig. 2E) labelled CS at the A-I band region, i.e. at the same region where the junctional TC is localized (cf. [19]).

Serial transverse sections of the soleus, a relatively pure slow-twitch muscle in the rabbit [17], showed that all fibers were reactive with antibodies specific to either cardiac or fast-skeletal muscle CS (Fig. 3A, B).

The present results indicate that the cardiac and fast-skeletal CS isoforms coexist in slow-twitch muscle fibers and that there are also no apparent differences in distribution of the two isoforms between successive sarcomeres, as well as between parallel sarcomeres within the same fiber. That does not, however, exclude that the complement of CS isoforms of individual TC may vary

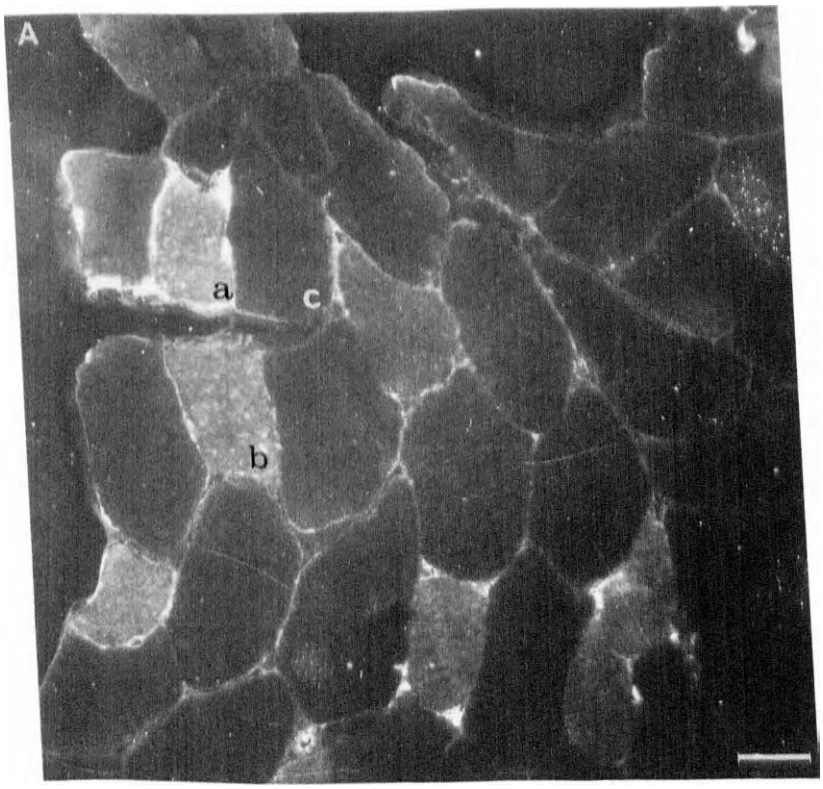

Fig. 2. *(for legend see next page)*

along the length of muscle fibers, given the presence of multiple nuclei. The present immunofluorescent data also cannot distinguish between the coexistence of two distinct CS isoforms and the presence of CS molecular hybrids.

It is known that the cardiac and the fast-twitch isoforms of CS are the products of two different genes [2,4,5]: in adult fast-twitch muscle fibers the cardiac gene for CS (as well as all other cardiac genes coding for SR proteins) appears to be totally repressed. The control of gene expression is more elaborate in slow-twitch fibers: in some cases, there is coding for alternative isoforms, i.e. only the cardiac type of Ca^{2+}-ATPase and only the fast-twitch type of Ca^{2+} release channel; in other cases there is coding for both isoforms, i.e. CS (present study) and tropomyosin [20].

The reason why the junctional SR of mammalian skeletal muscle fibers, independent of their twitch characteristics, expresses an identical Ca^{2+} release channel isoform [11,12], encoded by a distinct gene from the cardiac Ca^{2+} release channel gene [10], whereas the cardiac CS gene is not coordinately down-regulated at the same membrane area, is of considerable interest. Preliminary observations that denervation of rabbit slow-twitch muscle is associated with up-regulation of the fast-skeletal as well as the cardiac isoform of CS (A. Martini, E. Damiani and A. Margreth, unpublished), seem to argue against an epigenetic influence from the specific motor innervation to the muscle on the pattern of expression of CS genes.

From the functional point of view, the two CS isoforms display different structural and Ca^{2+} binding properties in vitro [3,21]. The significance of the present results remain conjectural inasmuch as it is not known whether each CS isoform affects differentially SR intralumenal Ca^{2+} buffering in situ.

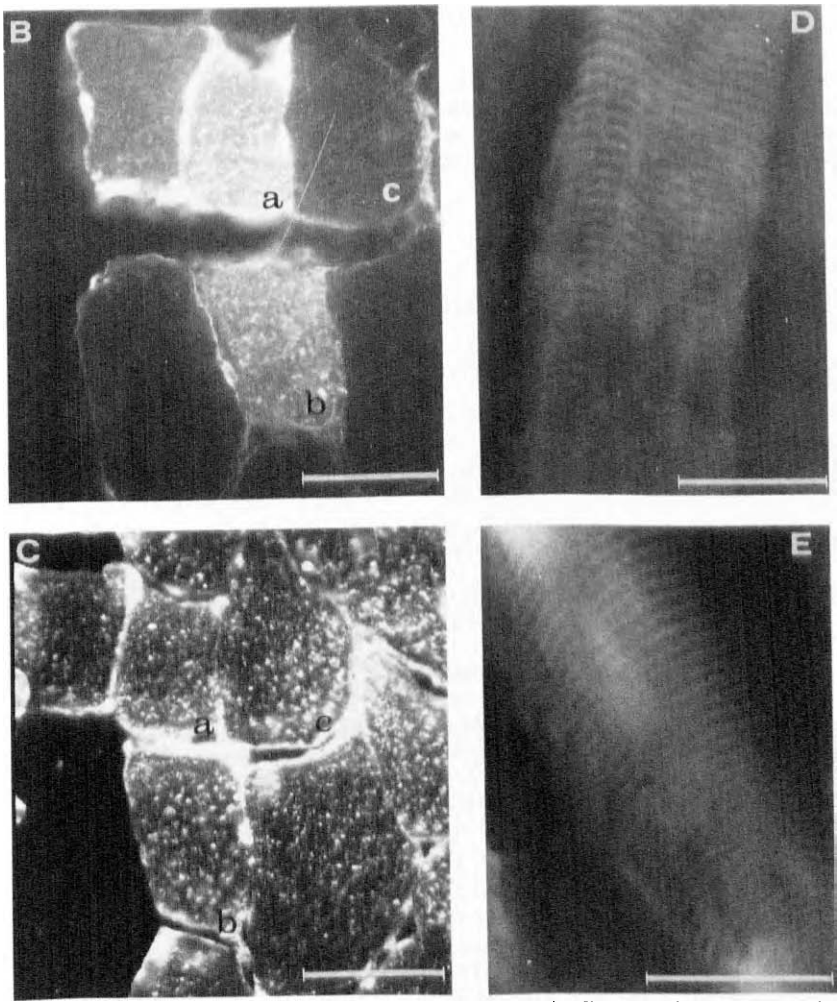

Fig. 2. Immunofluorescence of rabbit gastrocnemius cryosections with anti-(CS) antibodies. (A) Transverse section immunostained with anti-(cardiac CS) antibodies. (B) Higher magnification of upper left corner of A. (C) Serial section immunostained with anti-(skeletal CS) antibodies. (D) Longitudinal section immunostained with anti-(cardiac CS) antibodies. Fibers flanking the one shown were negative. (E) Longitudinal section immunostained with anti-(skeletal CS) antibodies. Bar = 20 μm in A–C, and 5 μm in D and E.

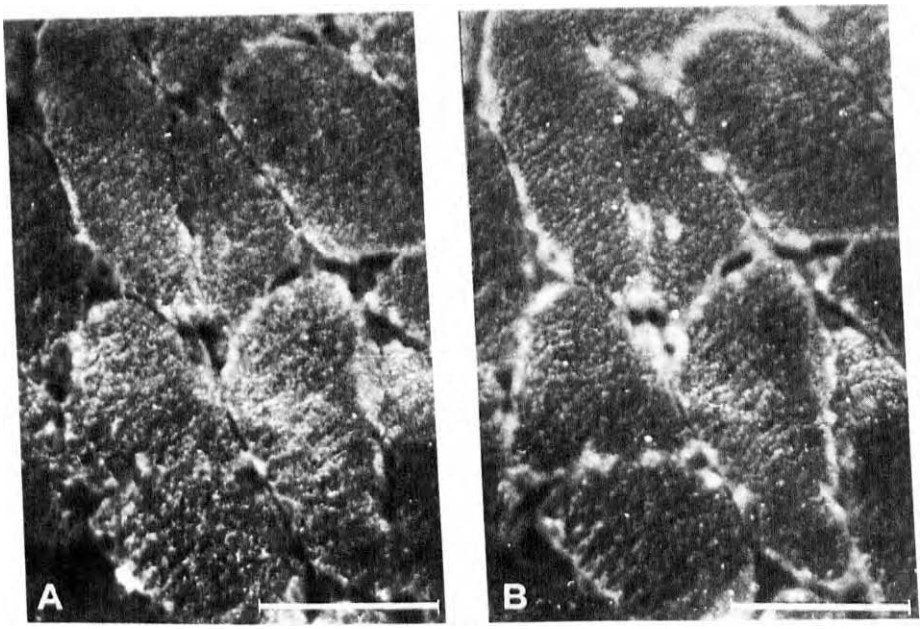

Fig. 3. Immunofluorescence of rabbit soleus serial cryosections with anti-(CS) antibodies. (A) Transverse section immunostained with anti-(cardiac CS) antibodies and rhodamine-labelled anti-mouse IgG. (B) Serial section immunostained with anti-(skeletal CS) antibodies and fluorescein-labelled anti-chicken IgG. Bar = 20 μm.

Acknowledgements: The financial support of Telethon-Italy to the project 'Ca^{2+} release channels of the sarcoplasmic reticulum in relation to fiber types and the specific motor innervation to the muscle' is gratefully acknowledged.

REFERENCES

[1] MacLennan, D.H. and Wong, P.T.S. (1971) Proc. Natl. Acad. Sci. USA 68, 1231–1235.
[2] Fliegel, L., Leberer, E., Green, N.M. and MacLennan, D.H. (1989) FEBS Lett. 242, 297–300.
[3] Damiani, E., Volpe, P. and Margreth, A. (1990) J. Muscle Res. Cell Motil. 1, 522–530.
[4] Fliegel, L., Ohnishi, M., Carpenter, M.R., Khanna, V.K., Reithmeier, R.A.F. and MacLennan, D.H. (1987) Proc. Natl. Acad. Sci. USA 84, 1167–1171.
[5] Scott, B.T., Simmerman, H.K.B., Collins, J.H., Nadal-Ginard, B. and Jones, L.R. (1988) J. Biol. Chem. 263, 8858–8864.
[6] Caplan, A.I., Fiszman, M.Y. and Eppenberger, H.M. (1983) Science 211, 921–927.
[7] Dhoot, G.H. and Perry, S.V. (1979) Nature 228, 714–718.
[8] Damiani, E., Betto, R., Salvatori, S., Volpe, P., Salviati, G. and Margreth, A. (1981) Biochem. J. 197, 245–248.
[9] MacLennan, D.H., Brandl, C.J., Korczak, B. and Green, N.M. (1985) Nature 316, 696–700.
[10] Otsu, K., Willard, H.F., Khanna, V.K., Zorzato, F., Green, N.M. and MacLennan, D.H. (1990) J. Biol. Chem. 265, 13472–13483.
[11] Takeshima, H., Nishimura, S., Matsumoto, T., Ishida, H., Kangawa, K., Minamino, N., Matsuo, H., Ueda, M., Hanaoka, M., Hirose, T. and Numa, S. (1989) Nature 339, 439–445.
[12] Zorzato, F., Fujii, J., Otsu, K., Phillips, M., Green, N.M., Lai, A.F., Meissner, G. and MacLennan, D.H. (1990) J. Biol. Chem. 265, 2244–2256.
[13] Bisson, R. and Schiavo, G. (1986) J. Biol. Chem. 261, 4373–4376.
[14] Saito, A., Seiler, S., Chu, A. and Fleischer, S. (1984) J. Cell Biol. 99, 875–885.
[15] Laemmli, U.K. (1970) Nature 227, 680–685.
[16] Volpe, P., Alderson-Lang, B.H., Madeddu, L., Damiani, E., Collins, J.H. and Margreth, A. (1990) Neuron 5, 713–721.
[17] Biral, D., Damiani, E., Volpe, P., Salviati, G. and Margreth, A. (1982) Biochem. J. 203, 529–540.
[18] Biral, D., Damiani, E. Margreth, A., Scarpini, E. and Scarlato, G. (1985) Neurology 35, 1360–1363.
[19] Jorgensen, A.O., Kalnins, V. and MacLennan, D.H. (1979) J. Cell Biol. 80, 372–384.
[20] Salviati, G., Betto, R. and Danieli-Betto, D. (1982) Biochem. J. 207, 261–272.
[21] Mitchell, R.D., Simmerman, H.B.K. and Jones, L.R. (1988) J. Biol. Chem. 263, 1376–1381.