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Caldesmon$_{150}$, caldesmon$_{77}$ and skeletal muscle troponin T share a common antigenic determinant

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Caldesmon and troponin are known to control actin-myosin interaction in smooth and striated muscle. We showed that polyclonal antibodies to smooth muscle caldesmon$_{150}$ cross-react with non-muscle caldesmon$_{77}$ and with skeletal muscle troponin T. The epitope recognized by caldesmon antibodies is located in the CB 2 peptide [residues 71–151] of troponin T. It is supposed that the observed cross-reactivity is due to the common structure of the tropomyosin-binding site of caldesmon and troponin T.

Caldesmon; Troponin T; Immunological cross-reactivity; (Chicken gizzard, Rabbit skeletal muscle, Bovine cardiac muscle)

1. INTRODUCTION

Troponin and caldesmon are involved in the thin-filament based regulation of striated and smooth muscle contraction [1–3]. In striated muscles Ca^{2+}-saturated troponin C relieves the inhibition of actomyosin ATPase induced by troponin I and troponin T [1,2]. In smooth muscle as well as in certain non-muscle tissues the interaction of Ca^{2+} with calmodulin (or with some unknown Ca-binding proteins) relieves the inhibition of actomyosin ATPase produced by caldesmon [3–5]. Qualitatively, the mechanisms of troponin-based on caldesmon-based regulation are very similar. A hypothesis has been put forward suggesting that caldesmon can be a precusor of troponin T and/or troponin I [3,6]. However, no direct relationship between caldesmon and troponin components has been established.

The present paper is devoted to the investigation of immunological relationships between caldesmon and troponin components.

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2. MATERIALS AND METHODS

The following proteins and protein complexes were purified by previously described methods: chicken gizzard caldesmon (150 kDa) [7], bovine liver caldesmon (77 kDa) [8], rabbit skeletal and bovine heart troponin complexes and troponin components [9], rat and rabbit skeletal and cardiac myofibrils [10,11]. Chymotrypsinolysis of troponin T was performed according to Tanokura et al. [12] in the absence of urea. The largest (about 45 residues) N-terminal tryptic peptide of troponin T was isolated as described earlier [13]. The method of Jackson et al. [14] was used for the cleavage of troponin T by CNBr. Protein coupling to CNBr-activated Sepharose (Pharmacia) was performed according to manufacturer's instructions.

Aortic smooth muscle cells were isolated from rabbit aorta and grown to confluence according to Colucci et al. [15]. Antibodies to chicken gizzard caldesmon were elicited in rabbits following the protocol of Ngai and Walsh [16]. The antibodies were affinity purified on caldesmon-Sepharose, dialyzed in phosphate buffered saline (PBS), aliquoted and stored at −70°C at a concentration of 1.7 mg/ml. Slab polyacrylamide gel electrophoresis in the presence of SDS was conducted using the method of Laemmli [17]. After electrophoresis the proteins were transferred to nitrocellulose paper in semi-dry blotter operating at 45 V for 1 h at 4°C. In the case of troponin T peptides, immediately after the transfer the nitrocellulose was briefly washed with PBS and soaked in 0.4% glutaraldehyde in PBS for 45 min at room temperature. Nonspecific binding sites were blocked by immersing the nitrocellulose into PBS containing 0.05% Tween-20 for 1 h at room temperature. The nitro-

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cellulose was then incubated with caldesmon antibodies diluted to 6 μg/ml in PBS-Tween-20 for 1 h at room temperature followed by overnight incubation at 4°C in 5-fold diluted solution of the antibodies. After rinsing of the nitrocellulose in PBS-Tween-20 three times for 5 min, the second antibodies conjugated with peroxidase were applied in the same buffer for 1 h at room temperature. Following the incubation the immunoblots were rinsed in PBS-Tween-20 and PBS and developed in a solution of chloronaphthol (0.5 mg/ml) in PBS, to which trace amounts of H_2O_2 were added.

3. RESULTS

Polyclonal antibodies to chicken gizzard caldesmon recognized only caldesmon (150 kDa) in the immunoblots of the total protein transfer of rabbit aorta smooth muscle (fig.1). It is well known that non-muscle cells contain another isoform of caldesmon with a molecular mass of 77 kDa [4,5,8]. This isoform is expressed in proliferating smooth muscle cells [18], and is stained by caldesmon antibodies in the total protein transfer of subcultured rabbit aorta smooth muscle cells (fig.1). Thus, the antibodies obtained specifically recognized two isoforms of caldesmon among all proteins of the smooth muscle.

In our search for immunological relationships between caldesmon and the regulatory proteins of striated muscle we studied the interaction of caldesmon antibodies with proteins of cardiac and skeletal myofibrils. Immunoblot analysis of myofibrils from mixed rabbit skeletal muscles revealed a number of closely separated proteins bands of 38 kDa. Similar results were obtained with rat skeletal muscle myofibrils, whereas none of the proteins was stained in the case of cardiac myofibrils (fig.2). The apparent molecular mass of the protein recognized by caldesmon antibodies in skeletal muscle myofibrils is similar to that of troponin T, while the multiplicity of stained bands can be due to the presence of troponin T isoforms [19,20]. Indeed, in separate experiments we found that isolated skeletal troponin T is effectively stained by caldesmon antibodies, whereas cardiac troponin T is not (fig.3). To obtain a troponin T-specific pool of caldesmon antibodies, the latter were purified on a column with immobilized skeletal troponin T. Thus purified antibodies retained their ability to recognize two isoforms of caldesmon as well as skeletal troponin T.

In order to identify the epitope recognized by caldesmon antibodies in troponin T, we cleaved

Fig.1. Control of caldesmon antibodies specificity. A SDS-gel electrophoretic pattern of total proteins of rabbit aorta media (1), subcultured rabbit aortic smooth muscle cells (3) and corresponding blots (2,4) stained with caldesmon antibodies. The arrowheads indicate the positions of caldesmon$_{150}$ and caldesmon$_{77}$; F, filamin; M, myosin heavy chains.

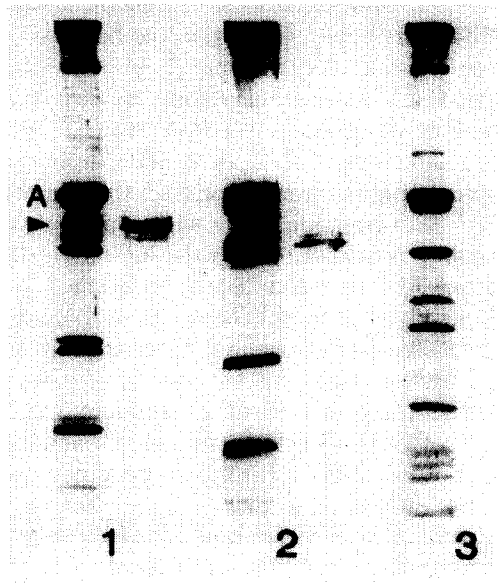

Fig.2. Staining of myofibrillar proteins with caldesmon antibodies in immunoblotting. Each lane represents a SDS-electropherogram (left) and a corresponding immunoblot (right) Lanes: 1, rabbit skeletal muscle myofibrils; 2, rat skeletal muscle myofibrils; 3, rabbit cardiac myofibrils. The arrowhead indicates the position of troponin T (38 kDa), A, actin.

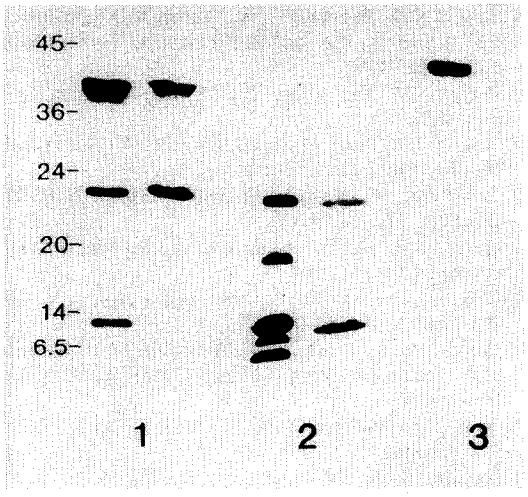

Fig.3. Cross-reactivity of troponin T and its fragments with caldesmon antibodies. Each pair of lanes represents a SDS-electropherogram in 15% gel (left) and a corresponding protein transfer stained with caldesmon antibodies (right). Lanes: 1, rabbit skeletal troponin T and two of its chymotryptic peptides; 2, CNBr peptides of rabbit skeletal troponin T; 3, bovine cardiac troponin T. On the left, an apparent molecular mass scale (in kDa).

this protein by chymotrypsin [12]. Among the two peptides thus obtained, only the larger one (so-called Tn T_1 fragment, residues – 1–158) was stained by caldesmon antibodies (fig.3). Thus, the epitope is located in the N-terminal part of troponin T. At the same time, the largest N-terminal tryptic peptide (about 45 residues) was not recognized by caldesmon antibodies in dot-blot experiments. This indicates that the epitope is located somewhere in between residues 45–158 of troponin T. To verify this assumption we cleaved troponin T by CNBr [14] and separated the peptides by SDS gel electrophoresis. In this case caldesmon antibodies stained only two bands of 22 and 10 kDa. These bands correspond to peptides CB 1 (residues 1–151) and CB 2 (residues 71–151) of rabbit skeletal troponin T. The data presented indicate that the epitope recognized by caldesmon antibodies is located in the N-terminal part (residues 71–151) of skeletal troponin T. Since this determinant was expressed both in glutaraldehyde-treated and untreated protein, it seems likely that this determinant is not a spatial one which is assembled in the course of troponin T refolding on nitrocellulose.

4. DISCUSSION

In 1984 Lim et al. [21] observed that monoclonal antibodies to troponin T cross-react with all muscle types. This finding led to a suggestion that troponin T and caldesmon are related proteins and therefore monoclonal antibodies to troponin T recognized caldesmon [3]. Later on, it was found that mono- and polyclonal troponin T antibodies cross-react with glyceraldehyde phosphate dehydrogenase present in the smooth muscle [22]. Moreover, a new protein of 34–36 kDa termed calponin was recently isolated from smooth muscle [23]. Antibodies to this protein recognized the epitope located in the C-terminal part of skeletal troponin T. Thus, at present there are at least two smooth muscle proteins immunologically related to troponin T. At the same time, there is no direct evidence of a relationship between caldesmon and troponin T.

Caldesmon and troponin T share many common features. Both proteins tightly interact with tropomyosin [1,2,14,24] and actin [3,4,7,25]. Under appropriate conditions both caldesmon and troponin T inhibit actomyosin ATPase [3,26]. This effect is enhanced by tropomyosin and is partly abolished by Ca-binding proteins (troponin C and calmodulin) [3,26]. Thus, the hypothesis that caldesmon and troponin T are related proteins seems to be very plausible.

The data of the present paper provide the first direct evidence that caldesmon and troponin T share a common antigenic determinant. The epitope is located in the CB 2 peptide (residues 71–151) of skeletal troponin T. This peptide is involved in the interaction of troponin T with tropomyosin [1,2,22,25,27]. Since this peptide contains the antigenic determinant common to caldesmon and troponin T, one can suppose that the corresponding region of caldesmon is also involved in the interaction with tropomyosin. If this assumption is correct the question arises as to why this epitope is absent in cardiac troponin T which is also able to interact with tropomyosin. A comparison of the primary structure of cardiac and skeletal muscle troponin T in the vicinity of tropomyosin-binding site reveals a strong homology [27]. Nevertheless, the sequence between residues 133 and 143 of rabbit skeletal muscle troponin T differs from the corresponding se-

quence of bovine cardiac troponin T (residues 160–170) by eight conservative substitutions. Since this stretch of amino acids contains the largest number of replacements, one may suppose that caldesmon antibodies recognized the epitope restricted by residues 133–143 of skeletal muscle troponin T. Our future investigations will be aimed at the verification of this hypothesis and at the evaluation of the physiological significance of the common determinant found in caldesmon and troponin T.

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REFERENCES

[1] El-Saleh, S.C., Warber, K.D. and Potter, J.D. (1986) J. Muscle Res. Cell Motil. 7, 387–404.
[2] Leavis, P.C. and Gergely, J. (1984) Cr. Rev. Biochem. 16, 235–305.
[3] Marston, S.B. and Smith, C.W.J. (1985) J. Muscle Res. Cell Motil. 6, 669–708.
[4] Sobue, K., Kanda, K., Tanaka, T. and Ueki, N. (1988) J. Cell Biochem. 37, 317–325.
[5] Ball, E.H. and Kovala, T. (1988) Biochemistry 27, 6093–6098.
[6] Fuji, T., Imai, M., Rosenfeld, G.C. and Bryan, J. (1987) J. Biol. Chem. 262, 2757–2763.
[7] Lynch, W. and Bretscher, A. (1986) Methods Enzymol. 134, 37–42.
[8] Litchfield, D.W. and Ball, E.H. (1987) J. Biol. Chem. 262, 8056–8060.
[9] Gusev, N.B., Barskaya, N.V., Verin, A.D., Duzhenkova, I.V., Khuchua, Z.A. and Zheltova, A.O. (1983) Biochem. J. 213, 123–129.
[10] Perry, S.V. and Grey, T.C. (1956) Biochem. J. 64, 184–192.
[11] Solaro, R.J., Pang, D.C. and Briggs, F.N. (1971) Biochim. Biophys. Acta 245, 259–262.
[12] Tanokura, M., Tawada, Y. and Ohtsuki, I. (1982) J. Biochem. 91, 1257–1265.
[13] Gusev, N.B., Dobrovolskii, A.B. and Severin, S.E. (1980) Biochem. J. 189, 219–226.
[14] Jackson, P., Amphlett, G.W. and Perry, S.V. (1975) Biochem. J. 151, 85–97.
[15] Colucci, W.S., Brock, T.A., Atkinson, W.J., Alexander, R.W. and Gimbrone, M.A. (1985) J. Cardiovasc. Pharmacol. 7, Suppl. 6, 79–86.
[16] Ngai, P.K. and Walsh, M.P. (1985) Biochem. Biophys. Res. Commun. 127, 533–539.
[17] Laemmli, U.K. (1970) Nature 227, 680–685.
[18] Glukhova, M.A., Kabakov, A.E., Ornatsky, O.I., Vasilevskaya, T.D., Koteliansky, V.E. and Smirnov, V.N. (1987) FEBS Lett. 218, 292–294.
[19] Briggs, M.M., Klevit, R.E. and Schachat, F.H. (1984) J. Biol. Chem. 259, 10369–10375.
[20] Briggs, M.M., Lin, J.J.-C. and Schachat, F.H. (1987) J. Muscle Res. Cell Motil. 8, 1–12.
[21] Lim, S.S., Tu, Z.H. and Lemanski, L.F. (1984) J. Muscle Res. Cell Motil. 5, 515–526.
[22] Sanders, C., Stewart, D.I.H. and Smillie, L.B. (1987) J. Muscle Res. Cell Motil. 8, 118–124.
[23] Takahashi, K., Hiwada, K. and Kokubu, T. (1988) Hypertension 11, 620–626.
[24] Horiuchi, K.Y. and Chacko, S. (1988) Biochemistry 27, 8388–8393.
[25] Heeley, D.H. and Smillie, L.B. (1988) Biochemistry 27, 8227–8232.
[26] Chong, P.C.S., Asselbergs, P.J. and Hodges, R.S. (1983) FEBS Lett. 153, 372–376.
[27] Leszyk, J., Dumaswala, R., Potter, J.D., Gusev, N.B., Verin, A.D., Tobacman, L.S. and Collins, J.H. (1987) Biochemistry 26, 7035–7042.