THE IDENTITY OF TROPOCALCIN WITH CALSEQUESTRIN. A SIMPLE METHOD OF ITS PREPARATION

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SUMMARY

Tropocalcin, a protein found by Han and Benson (1,2) in the preparations of troponin, has been found to be identical with calsequestrin, a protein constituent of sarcoplasmic reticulum membrane. A simple method of preparation of calsequestrin from dehydrated fragmented sarcoplasmic reticulum is described.

Han and Benson reported previously (1,2) that one of the constituents of troponin, named by the authors tropocalcin, undergoes calcium dependent conformational changes and suggested that this protein plays an important role in the function of troponin complex, i.e. in conferring calcium sensitivity to the actomyosin system (3). Subsequent studies by Greaser et al. (4) indicated, however, that tropocalcin is present only in those troponin preparations which were obtained according to the method of Yasui et al. (5), the method actually used by Han and Benson for preparation of tropocalcin. Greaser et al. (4) reported moreover that tropocalcin appears not to be related to the function of troponin complex, since the preparations obtained with any other method and devoid of tropocalcin were in the presence of tropomyosin fully active in conferring calcium sensitivity of the Mg^{2+}-stimulated ATP-ase activity of actomyosin. Thus the nature,

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origin and possible role of tropocalcin in muscle has remained obscure.

In the search for a relationship between tropocalcin and any known muscle protein it took our attention that its properties, as the molecular weight close to 47000 and precipitation by few milimolar concentration of calcium are very similar to those of calsequestrin, an acidic protein present in sarcoplasmic reticulum membrane (6). Moreover, similar conformational changes depending on calcium concentration described for tropocalcin by Han and Benson (1,2) were found by Ostwald and MacLennan (7) for calsequestrin.

The first step in the method of preparation of troponin according to Yasui et al. (5) is the dehydration of washed muscle residue with alcohol-ether and subsequent extraction of the troponin-tropomyosin complex from the obtained powder with 1 M KCl (8). Troponin obtained in this way in our laboratory (9) did not contain any protein which migrated in SDS-polyacrylamide gel electrophoresis above troponin-T, the constituent of troponin with the molecular weight about 38 000. The possibility, however, could not be excluded that if the muscle tissue was not homogenized sufficiently enough, the muscle residue used subsequently after dehydration as a source for troponin, could contain unbroken fragments of sarcoplasmic reticulum. If this were the case, calsequestrin should be extracted together with tropomyosin-troponin complex with 1 M KCl from dehydrated muscle residue. To check this possibility we used pure preparation of sarcoplasmic reticulum fragments as a starting material for the procedure of Yasui et al. (5).

Fragmented sarcoplasmic reticulum was prepared from rabbit skeletal muscle as previously described (10,11). It was dehydrated by successive treatment with ethyl alcohol and diethyl ether and finally with ether. After drying the obtained powder was extracted

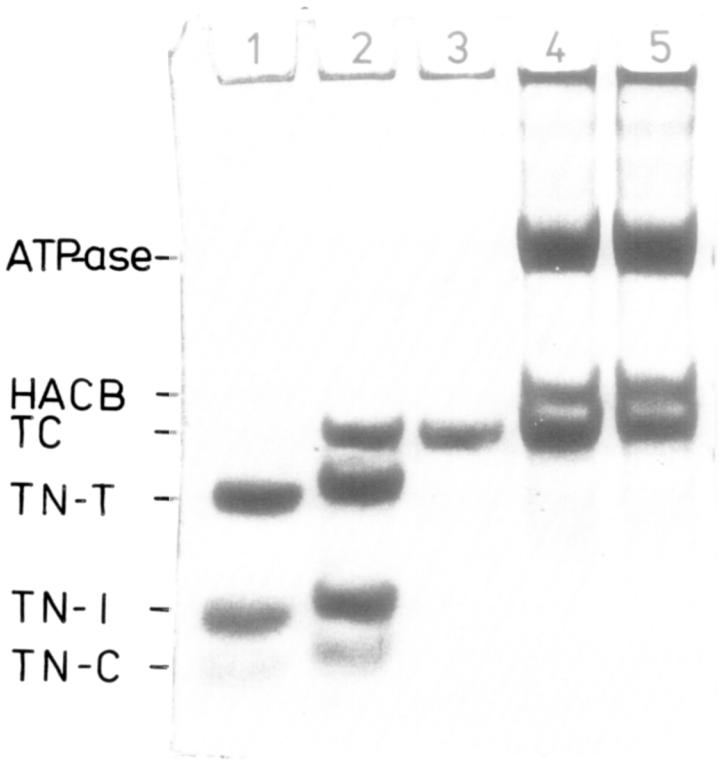

Fig. 1. SDS-polyacrylamide gel electrophoresis of muscle proteins. 1. 20 μg troponin, 2. 20 μg troponin and 20 μg tropocalcin, 3. 20 μg tropocalcin, 4. sarcoplasmic reticulum (50 μg protein) + 20 μg tropocalcin, 5. sarcoplasmic reticulum (50 μg protein), ATPase- 100 000 daltons ATPase, HACB - high affinity calcium binding protein, TC - tropocalcin, TN-T, TN-I and TN-C, individual components of troponin. Electrophoresis was performed according to Weber and Osborn (12) in slabs made from 7.5% acrylamide.

with 30 ml of 1 M KCl per one g powder for 2 hours at 4°. After subsequent centrifugation at 20 000 x g the obtained solution was dialysed against 1 mM $NaHCO_3$. A small precipitate formed during dialysis was removed by centrifugation at 100 000 x g for 30 min. The obtained extract showed on SDS-polyacrylamide gel electrophoresis one protein band whose molecular weight was about 45 000 (Fig. 1). This protein showed typical properties attributed by Han and Benson (1,2) to tropocalcin, as the reversible changes of fluorescence intensity depending on calcium concentration and precipitation by few milimolar calcium chloride. The protein ob-

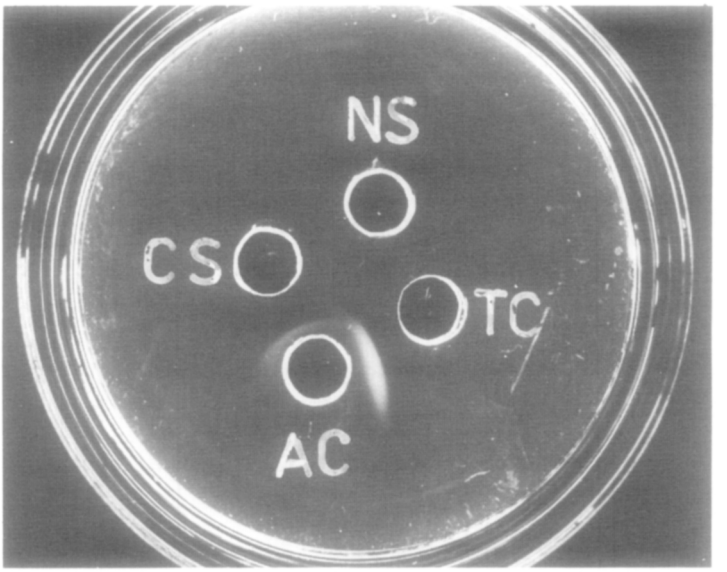

Fig. 2. Ouchterlony double diffusion test of tropocalcin. CS - calsequestrin, isolated from rabbit sarcoplasmic reticulum according to MacLennan (14), TC - tropocalcin, NS - normal sheep serum, AC - sheep anti-rabbit calsequestrin serum.

tained in this way co-migrated in SDS-gel electrophoresis with calsequestrin prepared according to MacLennan and Wong (6), and could be easily separated from actin (molecular weight 42 000). Ouchterlony double diffusion test showed a single precipitin line between sheep antirabbit calsequestrin serum and either calsequestrin isolated from rabbit sarcoplasmic reticulum (13) or tropocalcin. No precipitin line was obtained between normal sheep serum and tropocalcin. This specific immunological test clearly shows the identity of tropocalcin with calsequestrin (Fig. 2).

The extraction of dehydrated sarcoplasmic reticulum vesicles with high salt solutions provides a simple method of preparation of calsequestrin. The only method available so far, developed by MacLennan (14), is much more elaborate and involves several steps of purification including column chromatography on DEAE-cellulose hydroxylapatite and Sephadex G-200. Calsequestrin obtained according

to the method described here is more than 90% pure. Even in the overloaded gels only traces of other protein bands are visible. The yield is up to 80 mg of calsequestrin per 1 g of sarcoplasmic reticulum protein. The dehydration is a necessary prerequisite, sin the extraction of fresh sarcoplasmic reticulum vesicles with high K solutions, the procedure often used for the removal of myofibrillar contaminations (15), does not lead to the release of calsequestrin from sarcoplasmic reticulum membrane.

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REFERENCES

1. Han, M.H. and Benson, E.S. (1970) Biophys. J. 10, 245.
2. Han, M.H. and Benson, E.S. (1971) J. Gen. Physiol. 57, 247.
3. Ebashi, S. (1974) in Essays in Biochemistry (Campbel, P.N. and Dickens, F., eds.), Vol. 10, pp. 1-36, Academic Press, London and New York.
4. Greaser, M.L., Gergely, J., Han, M.H. and Benson, E.S. (1972) Biochem. Biophys. Res. Commun. 48, 358-361.
5. Yasui, B.C., Fuchs, F. and Briggs, F.N. (1968) J. Biol. Chem. 243, 735-742.
6. MacLennan, D.H. and Wong, P.T.S. (1971) Proc. Natl. Acad. Sci. U.S. 68, 1231-1235.
7. Ostwald, T.J. and MacLennan, D.H. (1974) J. Biol. Chem. 249, 5867-5871.
8. Bailey, K. (1948) Biochem. J. 43, 271-279.
9. Drabikowski, W., Dąbrowska, R. and Baryłko, B. (1973) Acta Biochim. Polon. 20, 181-199.
10. Drabikowski, W., Sarzała, M.G., Wroniszewska, A., Łągwińska, E. and Drzewiecka, B. (1972) Biochim. Biophys. Acta 274, 158-170.
11. Sarzała, M.G., Zubrzycka, E. and Drabikowski, W. (1974) in Calci Binding Proteins (Drabikowski, W., Strzelecka-Gołaszewska, H. an Carafoli, E. eds.), pp. 315-346, Elsevier Scientific Publishing Company, Amsterdam, PWN - Polish Scientific Publishers, Warszawa
12. Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406-4412.
13. Zubrzycka, E. and MacLennan. D.H., J. Biol. Chem., in press.
14. MacLennan, D.H. (1974) in Methods in Enzymology (Fleischer, S. and Packer, L., eds.), Vol. 32, pp. 291-302, Academic Press, New York, San Francisko and London.
15. Martonosi, A. (1968) J. Biol. Chem., 243, 71-81.