Short Communication

A 34,000 Dalton Protein Located in the Z-Disk

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On the basis of the success or failure of reconstitution of the Z-disks, we proposed that the proteins required for this, in other words, the principal constituents of the Z-disks, were one high molecular weight protein of 300,000-400,000 dalton, 100,000 and 34,000 dalton proteins. From sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoretic analysis and amino acid analysis of the proteins isolated from the gels, we presumed that the proteins mentioned above were Z-nin, α-actinin and tropomyosin, respectively. Z-nin is a new high molecular weight protein named by us and immunofluorescent localization of Z-nin in the Z-disk has appeared in our latest paper. This paper describes the indirect immunofluorescent localization of the protein having a subunit molecular weight of 34,000 dalton and presumed identical with tropomyosin.

Partially purified CAF (Ca\(^{2+}\)-activated factor) and myofibrils were prepared from rabbit skeletal muscle immediately after death by the procedures described in a previous paper. The myofibrils were incubated with the partially purified CAF (myofibrils:CAF=20:1, by weight) at 25°C for 3 hr in 100 mM KCl, 25 mM Tris-HCl, pH 7.0, 1 mM Ca\(^{2+}\), 5 mM β-mercaptoethanol (β-MCE) and 1 mM NaN\(_3\), and the reaction terminated by adding sufficient EDTA to chelate all Ca\(^{2+}\). The reaction mixture was centrifuged at 30,000 \(\times g\) for 30 min, the supernatant concentrated with Collodion-Bags (Sartorius membranfilter GmbH) and then fractionated by gel permeation chromatography on a 2.4 x 90 cm Sepharose 6B column under the conditions described in a previous paper. The proteins in Fraction A, which were eluted first from the Sepharose 6B column, were collected, concentrated with Collodion-Bags and applied to a 1.2 x 80 cm Bio-gel A 50 m column under the conditions described in a previous paper. As shown in the previous paper, the proteins in Fraction A-C, which were eluted last from the Bio-gel A 50 m column, were collected and lyophilized with sucrose. The lyophilized materials in Fraction A-C were dissolved in a solution of 4 mM Tris-HCl, pH 7.6 and 2 mM β-MCE, and the solution then dialyzed against the same solution and concentrated with Collodion-Bags. The concentrated solution was denatured in 1.0% SDS, 1% β-MCE and 0.01 M sodium phosphate, pH 7.0 by warming at 37°C for 2 hr, and then electrophoresed in 5% polyacrylamide gels in the presence of 0.1% SDS using the procedures described by Weber and Osborn. The stained bands of the protein with a molecular weight near 34,000 dalton were excised with a razor blade from the SDS-polyacrylamide gels as shown in a previous paper. The gel pieces were homogenized in a solution of 10 mM sodium phosphate, pH 7.2 and 0.15 M NaCl, and about 0.4 mg of 34,000 dalton protein in Freund’s incomplete adjuvant was injected into a chicken four times at interval of two weeks. Ten days after the final injection, the resultant antiserum was collected.

In immunoprecipitation tests, the antiserum obtained reacted with the antigen, but not with tropomyosin (presumed identical with the antigen), α-actinin, actin, myosin or titin (connectin). However, the antiserum obtained without dilution showed a weak precipitation line reacting with the Z-nin. An indirect immunofluorescent experiment was conducted in accordance with the procedures described by Ohashi et al. As shown in Fig. 1, the Z-disks of rabbit psoas muscle were exclusively fluorescent when treated with antiserum against the 34,000 dalton protein. This is immunological
evidence that the 34,000 dalton protein is located in the Z-disks, in other words, it is a constituent of the Z-disk.

From the results obtained in this paper, it was clear that the 34,000 dalton protein was not identical with tropomyosin. The release of the protein named pseudo-tropomyosin from myofibrils (probably from the Z-disks) on Ca²⁺-treatment with the same subunit molecular weight and amino acid composition as tropomyosin was reported by Nakamura and Takahashi.⁷) Immunoprecipitation tests suggested the possibility that the 34,000 dalton protein was identical with the protein named pseudo-tropomyosin, but the immunochimical localization of the protein named pseudo-tropomyosin in the Z-disk has not been apparent so far. Therefore, it is not certain whether or not the 34,000 dalton protein located in the Z-disk is identical with the protein named pseudo-tropomyosin.

A further immunochimical study to elucidate the relationship between the structure of Z-disk and the Z-disk constituents, including Z-nin and 34,000 dalton protein, is now in progress in our laboratory.

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REFERENCES