Short Communication

Immunofluorescent Localization
of Z-Nin in the Z-Disk

Atsushi SUZUKI and Yoshinobu NONAMI

Department of Animal Science, Faculty of
Agriculture, University of Niigata,
Niigata 950-21, Japan

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On the basis of the success or failure of reconstitution of Z-disks, we proposed that the proteins required for the reconstitution of Z-disks, in other words, the principal constituents of the Z-disk, were a high molecular weight protein having a subunit molecular weight of 300,000~400,000 dalton, α -actinin and tropomyosin.^{1,2)} From sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoretic analysis, amino acid analysis of the isolated proteins and electron micrographs of the Z-disks reconstituted, it was clear that the protein of high molecular weight was a new protein different from myofibrillar proteins reported up to now and was indispensable for the reconstitution of Z-disks. To indicate this, we have named the protein Z-nin.²⁾ This paper describes indirect immunofluorescent locali-

zation of Z-nin in the Z-disks of myofibrils.

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.³⁾ 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 was stopped by adding sufficient EDTA to chelate all Ca^{2+} . The reaction mixture was centrifuged at $30,000 \times g$ for 30 min, and the supernatant was concentrated with Collodion-Bags (Sartorius membranfilter GmbH) and fractionated by gel permeation chromatography on a 2.4×90 cm Sepharose 6B column under the conditions described in a previous paper.¹⁾ The proteins in Fraction A eluted first from the Sepharose 6B column were collected, concentrated with Collodion-Bags and applied to a 1.2×80 cm Bio-gel A 50 m column under the conditions described in a previous paper.²⁾ As shown in the previous paper,²⁾ the proteins in Fraction A-C 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 was dialyzed against the same solution and con-

FIG. 1. Indirect Immunofluorescent Staining of a Rabbit Skeletal Myofibril.

A rabbit myofibril was treated with 16-fold-diluted antiserum against Z-nin and then treated with 16-fold-diluted fluorescein-conjugated anti-chicken IgG (Miles-Yeda Ltd.). Upper, phase contrast image; lower, fluorescent image. Scale mark represents 10 μm .

centrated 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 by the procedures described by Weber and Osborn.⁴⁾ The stained bands of Z-nin as shown in a previous paper²⁾ were excised with a razor blade from the SDS-polyacrylamide gels. The gel pieces were homogenized in a solution of 10 mM sodium phosphate, pH 7.2, and 0.15 M NaCl, and about 0.2 mg of Z-nin in Freund's incomplete adjuvant was injected into a chicken four times at intervals of two weeks. Two weeks after the final injection, the resultant antiserum was collected.

In immunoprecipitation tests, the obtained antiserum reacted with Z-nin, but not with α -actinin, the established constituent of Z-disk^{5~7)} or titin (connectin), one of the high molecular weight proteins in myofibrils.^{8,9)} An indirect immunofluorescent experiment was conducted in accordance with the procedures described by Ohashi *et al.*¹⁰⁾ As shown in Fig. 1, the Z-disks of rabbit *psaos* muscle were exclusively fluorescent, when treated with antiserum against the Z-nin. This is immunological evidence that the Z-nin is located in the Z-disk, in other words, it is a constituent of the

Z-disk.

A further immunochemical study of Z-nin is now under way in our laboratory.

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