Short Communication

Immunoelectron Microscopic
Localization of Rabbit Z-Nin
and 34,000 Dalton
Protein in the Z-DiskAstushi SUZUKI, Makoto SAITO*
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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, *i.e.*, the principal constituents of the Z-disk, were Z-nin (a high molecular weight protein of 300,000~400,000 dalton named by us), α -actinin and 34,000 dalton protein.¹⁾ The indirect immunofluorescent localizations of Z-nin and 34,000 dalton protein have already been published.^{2,3)} This

paper describes the immunoelectron microscopic localization of Z-nin and 34,000 dalton protein in the Z-disk to elucidate the relationship between the structure of the Z-disk and the Z-disk constituents.

Z-nin and 34,000 dalton protein were prepared from rabbit skeletal muscle according to the procedures described in a previous report.¹⁾ The solution of Z-nin or 34,000 dalton protein in 10 mM sodium phosphate of pH 7.2 and 0.15 M NaCl was mixed with Freund's incomplete adjuvant, and about 0.2 mg of this Z-nin or 34,000 dalton protein mixture was injected into an adult male chicken four times at intervals of two weeks. Ten days after the final injection, the resultant antiserum was collected. The γ -globulin fraction was prepared from the antiserum by the method of Nicol and Deutch.⁴⁾ The γ -globulin was dialyzed against 10 mM sodium phosphate of pH 7.2 and 0.15 M NaCl, and then stored at -20°C . Antibody labelling was carried out as described by Craig and Offer.⁵⁾ Small bundles of glycerinated fibers prepared from rabbit *psaos* muscle were washed in 0.1 M KCl, 1 mM MgCl_2 and 5 mM sodium phosphate of pH 6.8 to remove the glycerol and then incubated

FIG. 1. Electron Micrograph of Unlabelled (Control) Fiber Bundles.

Glycerinated rabbit *psaos* muscle was teased into thin bundles (0.05×5 mm) and incubated overnight at 0°C in 0.1 M KCl, 1 mM MgCl_2 and 5 mM sodium phosphate, pH 6.8. This and all other samples for electron microscopy were fixed with glutaraldehyde- OsO_4 , embedded in Epon, and stained with uranyl acetate-lead citrate. Each scale mark on the electron micrographs indicates $0.5 \mu\text{m}$. ($\times 40,000$)

FIG. 2. Electron Micrograph of Fiber Bundles Labelled with Antibody against Z-Nin.

Antibody labelling was carried out by incubating the antibody against Z-nin (1 mg/ml) with 3 pieces of fiber bundles (0.05×5 mm) under the same conditions as specified for Fig. 1. ($\times 40,000$)

FIG. 3. Electron Micrograph of Fiber Bundles Labelled with Antibody against 34,000 Dalton Protein.

Antibody labelling was carried out by incubating the antibody against 34,000 dalton protein (1 mg/ml) with 3 pieces of fiber bundles (0.05×5 mm) under the same conditions as specified for Fig. 1. ($\times 40,000$)

overnight at 0°C in the same buffer containing about 1 mg of γ -globulin. After washing in this buffer to remove any unbound antibody, the fiber bundles were fixed and embedded according to the procedures described by Ohtsuki.⁶⁾ Thin sections were stained with saturated uranyl acetate and poststained with lead citrate. The specimens were examined with a Hitachi H-300 electron microscope operated at 75 kV.

The interaction between the antibody to Z-

nin or 34,000 dalton protein and the Z-disk regions observed in light microscopy^{2,3)} was confirmed at the ultrastructure levels. As shown in Figs. 1 ~ 3, the center of the I-band corresponding to the Z-disk was heavily stained when treated with γ -globulin against the Z-nin or the 34,000 dalton protein. The increases in the density and the width of the Z-disks incubated with the antibody in comparison with those of the control Z-disks are

probably due to the deposit of the reaction products between the antibody and the antigen. In this experiment, an ordered pattern analogous to that obtained with the antibody to troponin⁷⁾ or C-protein⁸⁾ was not observed.

From the results obtained in this paper, it is clear that the Z-nin and the 34,000 dalton protein are completely located in the Z-disk.

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