Metal Cluster Labeling of Contractile Proteins in Muscle: Its Application to Small-Angle X-ray Scattering/Diffraction Studies.

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With increasing knowledge about the atomic structure of proteins, the focus of protein research has shifted from the function of proteins as a whole from the function of each active site of proteins. It is of general interest to observe the structural changes which occur at active sites when proteins function. Small-angle X-ray scattering/ diffraction technique has advantages over protein crystallography in reporting such structural changes, since experiments can be done under physiological conditions. However, its limited spatial resolution imposes a limit on this technique in reporting local structural changes which may be small compared with the size of the whole protein molecule. To overcome this drawback, we attempted to label specific sites of myosin with metal clusters, which diffract X-ray strongly and thus enhance the signal from the labeled sites. We used actomyosin system from rabbit skeletal muscle as a protein sample, and a metal (gold) cluster as a label. A Nanogold™ label, a culster of 67 gold atoms, was introduced either directly to myosin or via the ATP binding site of rabbit skeletal myosin. In the former, presumably the reactive cysteine residue (SH1) of myosin was labeled. The latter was achieved by synthesizing an ATP analog with a Nanogold covalently attached to the ribose moiety of ATP with an appropriate extension (gold-EDA-ATP). When gold-EDA-ATP was added to the suspension of myosin filaments in the presence of AlF_4, the gold co-precipitated with the filaments, indicating that the stable myosin.gold-EDA-ADP.AlF_4 complex was formed.

The X-ray scattering patterns from the myosin filament and diffraction patterns from skinned muscle fibers were taken using the high-intensity X-ray beams (e = 12 keV) from the undulator beam line (BL45XU small angle scattering station). The detector was a combination of an image intensifier and a cooled CCD camera with 1000 x 1018 pixels. Whether the suspension of myosin filament or muscle fibers, the signal originating from the 14 nm repeat of myosin was enhanced by the addition of gold-EDA-ATP or by the direct labeling of myosin with Nanogold. Although the counting statistics should be improved further for detailed analysis, the present results prove that the metal cluster labeling is a promising technique in structural biology when combined with the small-angle X-ray scattering/diffraction technique.