Abstract:

The chemical reagent, the PBM (phenylenebismaleimide), can cross-link Lysine-191 and Cysteine-374 between two adjacent actin subunits along the genetic helix of the actin filament. Different columns for gel filtration chromatography were used to purify the cross-linked actin dimers, trimers and tetramers. In order to optimize the purification of actin oligomers, Fast Protein Liquid Chromatography (GE Pharmacia FPLC System®) has been used. The purified actin oligomers showed the ability to interact with actin binding proteins, such as gelsolin, different gelsolin segments, formins, DnaseI and Arp2/3, by native gel electrophoresis and DnaseI inhibition assay. The functionality of these purified actin oligomers was confirmed by their ability to polymerize into filaments and to nucleate actin polymerization. ADP-ribosylation of these actin oligomers on Arg-177 by the iota toxin of Clostridium botulinum and the binding of gelsolin segment 1-3 to the barbed end of these actin-oligomers inhibit the polymerization of these actin oligomers, which was verified by electron microscopy. The ATPase assay has been used to test the interaction between these non-polymerizing actin oligomers and myosin heads. The results of ATPase assay showed that the purified actin trimer and tetramer stimulated the ATPase activity of myosin heads much better than the actin dimer. The binding of non-polymerizing actin oligomers to the myosin heads is verified by size exclusion chromatography and chemical cross-linking by EDC and glutaraldehyde. Moreover, the EDC cross-linking experiment followed by Western blotting demonstrated that the cross-linked actomyosin complex is formed by actin oligomer, gelsolin segment1-3, and myosin head subfragment1. The results indicate that highly purified actin- tetramer and -trimer, in complex with gelsolin segment 1-3, should be the optimal myosin head binding scaffold.