Purification and characterization of actin gelation protein of *Amoeba proteus*

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SUMMARY

In the cytoplasm of the free living amoeba, *Amoeba proteus*, there are gel and sol layers, and gel–sol transformation is a dynamic and an interesting phenomenon. To help understand the mechanism of gel–sol transformation, we purified an actin gelation protein from *Amoeba proteus* and characterized it. After being suspended in extraction buffer, cells were ruptured using a Teflon homogenizer. The homogenate was centrifuged, and EGTA was added to the supernatant. The mixture was centrifuged and the precipitate was washed with a washing buffer, and suspended in a solution of higher Ca^{2+} concentration. After centrifugation, the supernatant was collected. Hereafter the supernatant thus prepared is called crude extract. Actin gelation activity of the crude extract was measured by the falling ball assay. To purify the actin gelation protein, the crude extract was successively separated by an anion exchange column, a hydroxyapatite column and a gel filtration column. Finally we obtained a 150-kDa protein that showed actin gelation and F-actin binding activity in a Ca^{2+}-independent manner. However, the crude extract showed these activities only at low Ca^{2+} concentrations. It is speculated that the crude extract contained an actin severing factor(s) and/or an actin depolymerization factor(s). Alternatively, the crude extract could contain a factor(s) that inhibits actin gelation activity of the 150-kDa protein at high concentrations of Ca^{2+}.