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# Purification and characterization of the proteinase ECP 32 from Escherichia coli A2 strain

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## Abstract

The proteinase previously described as an unidentified component of E. coli A2 extracts which hydrolyses actin at a new cleavage site (Khaitlina et al. (1991) FEBS Lett. 279, 49) was isolated and further characterized. A chromatographic method of proteinase purification was developed by which a purity of more than 80% was attained. The enzyme was identified as a single, 32 kDa polypeptide (ECP 32) by SDS-PAGE and non-denaturing electrophoresis as well as by ion-exchange chromatography and gel filtration. The N-terminal sequence of ECP 32 was determined to be: AKTSSAGVVIRDIFL. The activity of ECP 32 is inhibited by o-phenanthroline, EDTA, EGTA and zincone. The EDTA-inactivated enzyme can be reactivated by cobalt, nickel and zinc ions. Based on these properties ECP 32 was classified as a metalloproteinase (EC 3.4.24). Limited proteolysis of skeletal muscle actin between Gly-42 and Val-43 was observed at enzyme substrate mass ratios of 1:25 to 1:3000. Two more sites between Ala-29 and Val-30, and between Ser-33 and Ile-34 were cleaved by ECP 32 in heat- or EDTA-inactivated actin. Besides actin, only histones and DNA-binding protein HU were found to be substrates of the proteinase, confirming its high substrate specificity. Its molecular mass, N-terminal sequence and enzymatic properties distinguish ECP 32 from any known metalloproteinases of E. coli, and we therefore conclude that it is a new enzyme.

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## Keywords

Characterization, E. coli, Gel filtration (size exclusion), Ion-exchange chromatography, Metalloproteinase, Proteinae ECP 32, Purification