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Physico-chemical properties of actin cleaved with bacterial protease from *E. coli* A2 strain

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The 36 kDa fragment of actin molecule obtained with the protease from *E. coli* A2 strain [(1988) FEBS Lett. 228, 172] was shown to begin with Val-43 and retain the COOH-terminal amino acid residues of the parent molecule. The *E. coli* protease split actin preserves the NH_2-terminal part of the polypeptide chain as well as the native conformation of actin molecule. However, the *E. coli* protease split actin failed to polymerize in 0.1 M KCl, suggesting that integrity of actin molecule between Gly-42 and Val-43 is crucial for actin polymerization.

Split actin; Bacterial protease; Intrinsic fluorescence

1. INTRODUCTION

Cleavage of actin with different proteases modifies its polymerizability to various degrees. Thus, the cutting off the COOH- and NH_2-terminal portions of actin polypeptide chain with trypsin produced the 33 kDa fragment which does not polymerize and does not interact with intact G-actin under polymerizing conditions [1,2]. On the other hand, the 35 kDa subtilisin and chymotryptic fragments of actin molecule preserving the COOH-terminal amino acid residues and existing in a complex with the NH_2-terminal fragment retain the polymerizability, although the critical concentration for their polymerization is higher than that for intact actin [3,4].

Actin split with the protease from the *E. coli* A2 strain has been shown to produce failure in polymerization in 0.1 M KCl [5,6]. Since the 36 kDa fragment of the *E. coli* protease split actin seems to comprise the COOH-terminal portion of actin polypeptide chain, as do the subtilisin or chymotryptic 35 kDa fragments, it was important to elucidate the structural basis for the functional differences among them. The aim of this work was to establish whether in actin split with the *E. coli* protease both the COOH- and NH_2-terminal portions of the polypeptide chain as well as the native conformation of actin molecule are preserved.

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2. MATERIALS AND METHODS

Rabbit skeletal muscle actin was prepared according to [7] and purified as described in [8].

Actin labeled with *N*-iodoacetyl-*N*-(5-sulfo-1-naphtyl)ethylene diamine (1,5-IAEDANS) was obtained according to the procedure described in [9].

For proteolytic digestion, the actin solution of 1–2 mg/ml was mixed with an equal volume of partially purified protease preparation [10] containing 0.005–0.5 mg of the protein per ml in the buffer used for actin preparation. The digestion was carried out for 1 h at room temperature or overnight at 4°C. When necessary, the digestion was stopped by addition of 5 mM *O*-phenanthroline [10].

The 33 kDa fragment was obtained by cleavage of actin with trypsin at the enzyme to protein mass ratio of 1:25 or 1:50. Digestion was inhibited by addition of soybean trypsin inhibitor at a mass ratio of 1:2.

N-Terminal sequencing of the 36 kDa fragment was performed on an Applied Biosystems model 477A sequencer equipped with on-line model 120 PTH amino acid analyzer, using standard protocols supplied by the manufacturer.

SDS-polyacrylamide gel electrophoresis was carried out according to Laemmli [11] using 12.5% polyacrylamide gel slabs. Gels containing AEDANS-labeled peptides were photographed using an ultraviolet light box ($\lambda = 380$ nm) before staining with Coomassie brilliant blue G250.

Viscosity was measured in a falling ball viscometer [12].

The intrinsic fluorescence of actin was measured in a spectrofluorimeter described in [13]. The fluorescence spectra were characterized by parameter A defined as a ratio of the emission intensities at 320 nm and 365 nm on excitation at 296.8 nm. The value of the parameter A for intact and inactivated actin was earlier determined to be 2.55–2.60 and 1.3, respectively [13].

3. RESULTS AND DISCUSSION

The integrity of the COOH-terminal end of the actin polypeptide chain in the 36 kDa fragment produced by