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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 [1C] 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

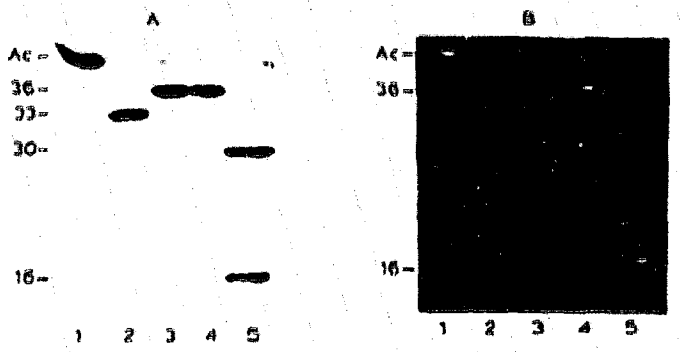

Fig. 1. Limited proteolysis of 1,5-AEDANS-labeled actin (1) with trypsin (2), bacterial protease (3,4) and V8 *S. aureus* protease (5). The same gel was stained with Coomassie blue (A) and irradiated with UV light (B).

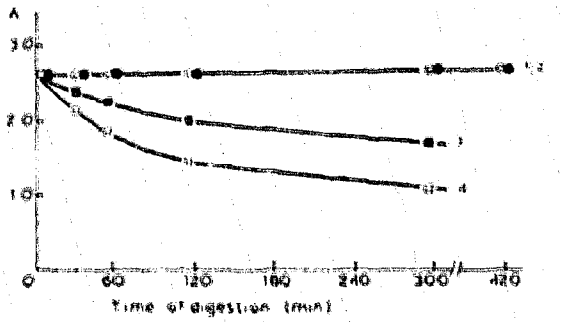

Fig. 3. Parameter *A* of the fluorescence spectrum of actin cleaved with the *E. coli* protease (1,2) and trypsin (3,4). G-actin was digested as shown in Fig. 2. Closed symbols denote measurements performed immediately after cleavage; open symbols denote measurements performed next day after cleavage.

the *E. coli* protease was checked using actin specifically labeled at Cys-374 with a fluorescent dye 1,5-IAEDANS [2]. In order to verify the specificity of labeling under our experimental conditions, the fluorescently labeled actin was also cleaved by trypsin and by protease V8 from *Staphylococcus aureus*, known to split actin into a COOH-terminal 16 kDa fragment and an NH_2-terminal 26 kDa one [2]. The results are shown in Fig. 1. It is evident that the *E. coli* protease 36 kDa fragment as well as the COOH-terminal fragment produced by *S. aureus* V8 protease retained the fluorescent label, whereas the NH_2-terminal product of V8 proteolysis and the 33 kDa fragment produced by trypsin treatment lost it.

The N-terminal sequence of the *E. coli* protease 36 kDa fragment was determined to be Val-Met-Val-Gly-Met. According to the known sequence of actin [14], this peptide corresponds to the cleavage site between Gly-42 and Val-43.

The 36 kDa fragment of actin obtained by digestion with the *E. coli* protease is resistant to further proteolytic breakdown [6]. Fig. 2 shows that a small fragment of about 9 kDa was also produced upon treatment of actin by this protease. This latter fragment, too, was fairly stable during further incubation with the protease. The data presented elsewhere (Strzelecka-Golaszewska, Moraczewska, Khaitlina, manuscript in preparation) showed that in the presence of divalent cations the 9 kDa fragment remained associated with the 36 kDa fragment.

It is known that the spectrum of the intrinsic fluorescence of actin changes during polymerization, inactivation or denaturation of the protein [15,16]. This allows to use the UV-fluorescence spectrum as a measure of the native structure of actin [13,16]. In contrast to the effect induced by trypsin treatment the values of the parameter *A* of the fluorescence spectra of samples taken from actin solution at different stages of the *E. coli* protease hydrolysis were equal to that for intact actin (Fig. 3), indicating that the fluorescence spectrum of actin did not change upon proteolysis with

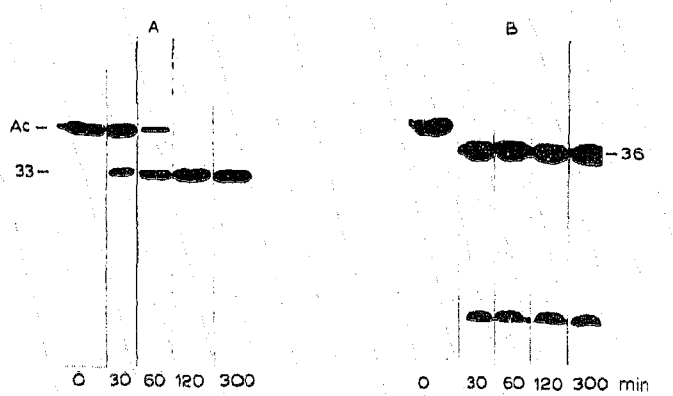

Fig. 2. Time course of actin digestion with trypsin (A) and the *E. coli* protease (B). AC, actin; '36,33' the 36 kDa and the 33 kDa fragments of actin.

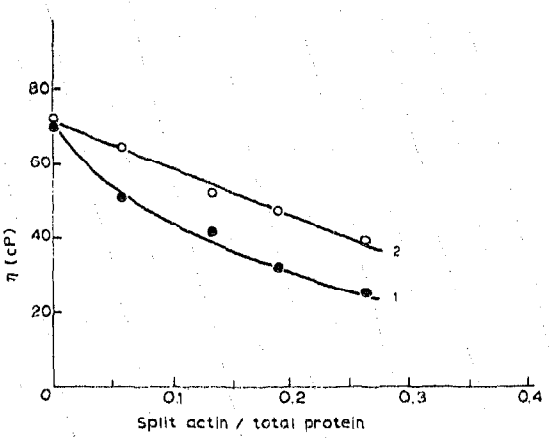

Fig. 4. Influence of the *E. coli* protease-cleaved actin on the viscosity of intact actin solutions. (1) Intact actin (1 mg/ml) in 0.5 mM ATP, 0.2 mM $CaCl_2$, 2 mM Tris-HCl, pH 7.5 was polymerized with 0.1 M KCl in the presence of the *E. coli* protease-cleaved actin (0.1–0.7 mg/ml). (2) Intact actin polymerized in the presence of *O*-phenanthroline in the concentrations equal to those in the mixtures of intact and fragmented actin.

the *E. coli* protease. No changes in the parameter *A* occurred also upon storage of the *E. coli* protease fragmented actin.

Thus, the fluorescence measurements indicate that the cleavage of actin with the *E. coli* protease does not lead to changes in the conformation of the molecule. In contrast, proteolysis of actin with trypsin is accompanied by transformation of the native structure into the structure of inactivated actin.

To elucidate whether the *E. coli* protease-split actin can be copolymerized with the intact one, various amounts of cleaved actin were added to the solution of intact actin up to the final concentration varying from 0.1 mg/ml to 0.7 mg/ml. Polymerization was induced by 0.1 M KCl and samples were left standing overnight at room temperature. Since the protease introduced with the cleaved actin was expected to split the intact actin, *O*-phenanthroline was used in these experiments to inhibit the protease [10].

In agreement with the results obtained earlier [6], the 36 kDa fragment cosedimented with actin although the major portion of it was found in the supernatant fraction (data not shown). The viscosity of the solutions of intact actin polymerized in the presence of the split actin decreased (Fig. 4), suggesting some interaction of the cleaved actin with the intact one.

Thus, the results of this work show that the proteolysis of actin with the *E. coli* protease does not induce the large changes of actin structure. One could therefore expect that actin split by the *E. coli* protease would polymerize and copolymerize with intact actin. However, viscosity measurements, as well as data earlier obtained in sedimentation experiments failed to detect polymerization of this preparation in 0.1 M KCl. The presence of split actin decreased the viscosity of intact actin polymerized with KCl. However, this effect was not large when corrected for the effect of *O*-phenanthroline introduced with the split actin.

It is known that actin split by subtilisin between Met-47 and Gly-48 or by chymotrypsin between Met-44 and Val-45 can be polymerized in 0.1 M KCl [3,4]. Thus, small differences in the site of cleavage appear to significantly affect the functional properties of split actins. These observations may be useful in mapping the functional sites on actin molecule.

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