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Limited proteolysis of actin by a specific bacterial protease

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A 36 kDa fragment of rabbit skeletal muscle actin resistant to further proteolytic breakdown was obtained with a new bacterial protease. This fragment was the only cleavage product obtained from native actin whereas proteolysis of heat-inactivated actin was unlimited. The 36 kDa fragment failed to polymerize and to inhibit DNase I activity. Binding to DNase I protects actin against proteolysis by protease. The results on actin proteolysis by different proteases are compared.

Actin; Actin proteolysis; DNase I; Proteinase

1. INTRODUCTION

It is well known that actin can be cleaved by trypsin or chymotrypsin into a large protease-resistant fragment of 33 kDa which retains the ability to bind ATP but does not bind Ca^{2+} and fails to polymerize or activate the ATPase activity of myosin [1]. This fragment was shown to be a COOH-terminal part of the actin molecule containing residues 69–372 [1,2]. Conversion of native actin to the fragment occurs in two steps and thus the intermediate fragment of greater molecular mass (approx. 36 kDa) may exist during the early stages of proteolysis [1,2]. We have recently found that a stable actin fragment similar to the unstable intermediate product of trypsin hydrolysis can be obtained using an unknown bacterial protease [3]. This protease has been isolated and partly purified. Here we describe the properties of the 36 kDa fragment in more detail. Our results show that this fragment resembles the trypsin fragment of actin but is more stable than the latter. Therefore, this fragment alone or together with the 33 kDa fragment may provide a useful model for the study of actin structure and polymerization.

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2. MATERIALS AND METHODS

Rabbit skeletal muscle actin was prepared using a method slightly modified from that of Spudich and Watt [4] and purified by one cycle of polymerization-depolymerization. To remove actin-binding proteins actin in the filamentous form was treated with trypsin (actin/trypsin, 200:1) for 1 h at 20°C as described [5]. Heat inactivation of actin was achieved by heating the samples to 70°C for 10–15 min [6].

To obtain a crude preparation of the protease, bacteria, identified as the A-2 strain of *E. coli*, were collected by centrifugation, suspended in a solution for the extraction of actin (0.5 mM ATP, 0.2 mM $CaCl_2$, pH 7.5) and disrupted by sonication or several cycles of freeze-thawing. The extract was clarified by centrifugation at 15000 × *g* for 40 min and used as a crude preparation of the protease. A partly purified preparation was obtained after ion-exchange chromatography of the crude extract. Details of this purification will be described elsewhere (in preparation).

For proteolytic digestion actin (c = 1 mg/ml) was mixed with an equal volume of the crude or partly purified protease preparation in buffer A [4] or in the solution for actin extraction containing 0.5–1.0 mg protein per ml. Incubation was allowed to proceed for 1 h at room temperature or at 4°C overnight. Electrophoresis on polyacrylamide gels in SDS was according to [7]. The DNase I inhibition assay was performed as in [8].

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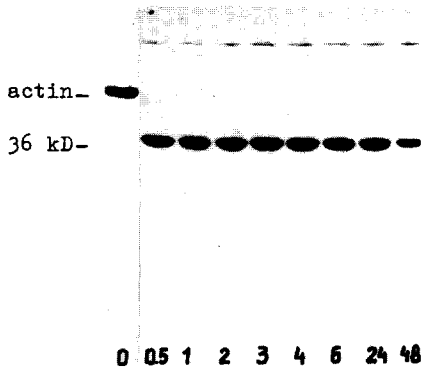

Fig.1. Time course of limited cleavage of G-actin with the bacterial protease at 0, 0.5, 1, 2, 3, 4, 6, 24 and 48 h incubation.

3. RESULTS

The treatment of globular actin with the bacterial extract or partly purified bacterial protease results in the quantitative conversion of actin into one fragment. As shown in fig.1, this fragment did not undergo further cleavage over prolonged incubation periods.

On SDS-polyacrylamide gels the fragment migrates as an intermediate fragment from trypsin [3], subtilisin or thermolysin hydrolysis. Its molecular mass was estimated as 36 kDa [3] which is in reasonable agreement with published data [2]. This fragment is also similar, but not identical, to the main product of proteinase K hydrolysis.

In contrast to hydrolysis of the native form, cleavage of heat-inactivated actin is unlimited. Three fragments of approx. 40, 39 and 36 kDa are obtained as the products during the early stages of the reaction (not shown).

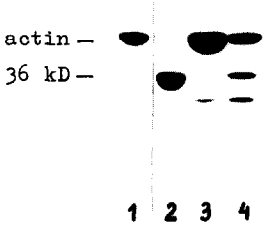

Fig.2. Co-polymerization of G-actin and the 36 kDa fragment. (1) Actin, (2) 36 kDa fragment, (3) actin pellet, (4) pellet of the fragment-actin mixture (1:4, w/w), (5) 'pellet' of the 36 kDa fragment.

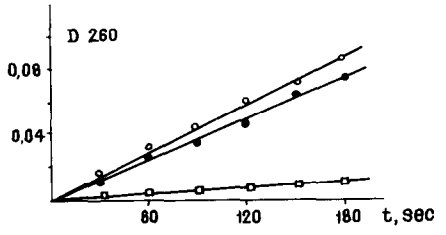

Fig.3. Interaction of actin and the 36 kDa fragment with DNase I assayed by inhibition of enzymatic activity. (○) DNase I standard (2.5 μg DNase I/100 μg DNA), (□) DNase I + 1 μg G-actin, (●) DNase I + 2 μg 36 kDa fragment.

It was shown previously that the 36 kDa fragment of actin fails to polymerize [3]. To determine whether it can be incorporated in actin polymers globular actin was polymerized in the presence of the fragment. Whereas actin in 0.1 M KCl was quantitatively sedimented at 100000 × g for 2 h, the 36 kDa fragment was not pelleted under these conditions (fig.2). However, a rather high proportion of the 36 kDa fragment was found in the pellet of a fragment-actin mixture, suggesting that the 36 kDa fragment is incorporated into actin filaments.

The other functional property of actin is its ability to interact with DNase I, thus inhibiting the enzyme activity [8]. Similar to the trypsin fragment [9] the 36 kDa fragment of actin does not inhibit DNase I activity (fig.3).

In addition, binding to DNase I protects actin against proteolysis by protease (fig.4).

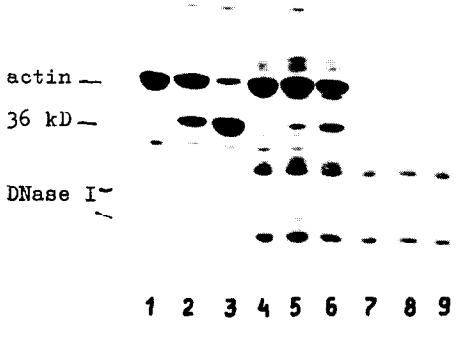

Fig.4. Proteolysis of G-actin–DNase I complex by the bacterial protease. G-Actin (2 mg/ml) was mixed with an equal volume of DNase I (3 mg/ml) and 1 vol. of crude protease preparation was added. (1–3) G-Actin, (4–6) G-actin–DNase I complex, (7–9) DNase I (at 0, 1 and 24 h of incubation with protease).

4. DISCUSSION

Comparing the results on the proteolysis of actin by a new bacterial protease with those of known proteases [1,2], three important points merit attention. (i) The 36 kDa fragment is the only cleavage product obtained with this protease whereas several fragments usually occur as a result of treatment with other proteases [2]. Even the stable trypsin or chymotrypsin fragment can be obtained only under appropriate experimental conditions. (ii) The 36 kDa fragment is resistant to further proteolytic breakdown whereas trypsin-derived and other actin fragments are degraded extensively to give cleavage products of lower molecular mass. (iii) Proteolysis of actin by the bacterial protease does not require any inhibition. Taken together these data show that the bacterial protease attacks only one site on the native actin molecule. The similarity between the 36 kDa species and an intermediate trypsin fragment allows one to conclude that it is the same bond between Arg-62 and Gly-63 at the N-terminal end of the molecule which undergoes attack. Since it has been shown that DNase I interacts with the N-terminal part of the actin molecule [9,10] the lack of inhibition of DNase I activity by the 36 kDa fragment and the protective effect of DNase I against actin proteolysis with the bacterial protease appear to support this proposal. However, further experiments must be performed to localize the 36 kDa fragment in the primary amino acid sequence of actin.

Heat inactivation of actin leads to the appearance of at least three additional fragments. Heat inactivation is known to be accompanied by conformational changes in the actin molecule [6,11]. Thus, the 'restrictase' properties of the bacterial protease are due not only to the properties of the protease itself but also to the conformation of native actin.

In summary, a stable 36 kDa actin fragment was obtained with a new bacterial protease. This fragment has lost the ability to polymerize and to inhibit the DNase I activity but does retain (according to preliminary experiments) the conformation of native actin. This species may therefore prove to be a useful model in studies of the structure and polymerization of actin.

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