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EFFECT OF ELASTASE ON ELONGATION FACTOR 1 FROM WHEAT GERM

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SUMMARY. Elongation factor 1, species A, B and C, were isolated from wheat germ and purified to homogeneity by the following steps: supernatant 100 000 xg, precipitation with ammonium sulphate and column chromatography: Sephadex G-150, DEAE-Sephadex A-50 and hydroxylapatite. On the second column the activity was divided into three peaks: EF1 A, B and C. The pure proteins EF1A, B and C (molecular weight 61 000, 48 000 and 12 500 D, respectively) were treated with elastase. Two products of EF1A digestion, polypeptides b and c, were isolated. The molecular weights of polypeptides b and c were similar to molecular weight of species B and C of EF1. Both digestion products were active in binary complex formation with GDP and in binding of Phe-tRNA to ribosomes. EF1B was converted to polypeptide c or similar and EF1C was rather resistant to elastase treatment.

INTRODUCTION. In many tissues elongation factor 1[§] exists in different molecular forms. The reported MW varies from as low as 30 000 D to over 1 million D (for a review see ref.1). The high molecular weight form of EF1 (over 200 000 D, so called EF1H - heavy) is an aggregate composed of polypeptide(s) with MW in the range of 30 000 - 70 000 D (so called EF1L - light) (2 - 9). Different factors cause conversion from heavy to light form of EF1: GTP (9), elastase (10,11), carboxypeptidase A (12) and enzymatic extract of Artemia salina (13). It is evident that GTP disaggregates (converts) EF1H to EF1L in a different way than do the three above mentioned enzymes (conversion factors) with proteolytic activity. In the case of elastase (10,11), two products of digestion of EF1 from rabbit reticulocytes were observed on polyacrylamide gel (with MW 30 000 and 15 000 D). This limited proteolysis did not affect activity in the binding reaction

§ ABBREVIATIONS: EF1 A, B and C - elongation factor 1 species A, B and C, L - light form, H - heavy form, EF2 - elongation factor 2, PMSF - phenylmethylsulfonylfluoride, BSA - bovine serum albumin, DTT - dithiothretiol, SDS - sodium dodecyl sulphate, MW - molecular weight, D - dalton.

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of EF1. No data were obtained at that time about the biological activity of detected polypeptides. In the present study the effect of elastase on EF1 from wheat germ was observed.

MATERIALS AND METHODS. Elongation factor 1 A, B and C from wheat germ were purified to homogeneity by the steps described in the legend to Fig. 1; details will be published elsewhere. Polyacrylamide gel electrophoresis in the presence of SDS was made according to King and Laemmli (14). The biological activity of EF1 was tested by means of the enzymatic binding of Phe-tRNA to ribosomes in a poly-U system (15). The incubation mixture in 50 µl contained: 50 mM Tris-HCl, pH 7.5, 60 mM KCl, 5 mM $MgCl_2$, 3 mM DTT, 0.1 mM GTP, 10 µg poly-U, about 30 pmoles [^{14}C]Phe-tRNA (530 cpm/pmole), 0.3 A_{260} unit of ribosomes purified as in (3) and different amounts of EF1 as indicated in the legends. After 5 min. incubation in 37°C, 1 ml of cold 10 mM Tris-HCl, pH 8.0, buffer containing 80 mM KCl and 10 mM $MgCl_2$, was added and the mixture was immediately filtered through a Millipore filter 0.45 µm. The filter was washed three times with the buffer, dried and counted in a toluene scintillator. Transfer RNA was purified and the preparative charging reaction was carried out essentially as in (16). The protein concentration was determined by the Lowry method (17). EF1A was incubated with elastase (Boehringer Mannheim) as follows: 5 to 300 µg of factor and 1 to 20 µg of elastase in 50 mM Tris-HCl, pH 7.8, containing 3 mM DTT, for 40 min at 37°C, in total volume of 50 or 300 µl. The products of proteolysis were separated on a Sephadex G-75 column (0.9 x 30 or 60 cm) with flow rate of 12 ml/h. A buffer of 50 mM Tris-HCl, pH 7.5, containing 5 mM $MgCl_2$ and 50 mM KCl, was used in chromatography runs designated for gel electrophoresis. In case of biological activity being tested the above buffer contained 10% glycerol and 3 mM DTT; immediately before chromatography BSA and PMSF in concentration of 1 mg/ml and 1 mM, respectively, were added to the collected fractions (volume of 0.5 ml). EF2 purification and polyphenylalanine synthesis were conducted as in (18). The binary complex was formed according to the procedure described in (5). Other materials were obtained from commercial sources.

RESULTS AND DISCUSSION. Three different molecular forms: A, B and C of elongation factor 1 were purified from wheat germ by the steps described in general in the legend to Fig. 1. Purity and MW were determined by polyacrylamide gel electrophoresis at denaturating conditions. All three forms were at least 90% pure with MW: 61 000, 48 000 and 12 500 D for A, B and C, respectively (Fig. 2A, B and C). As one can see in Fig. 1, all three of them were active in the binding of Phe-tRNA to ribosomes, as well as in binary complex formation EF1-GDP, and contained no EF2 as judged by their complete inability to support the synthesis of polyphenylalanine in the absence of EF2.

Species A of EF1 was treated with serine protease elastase. Two main products of digestion were observed on polyacrylamide gel: polypeptides b and c with MW 48 000 and 12 500 D, respectively

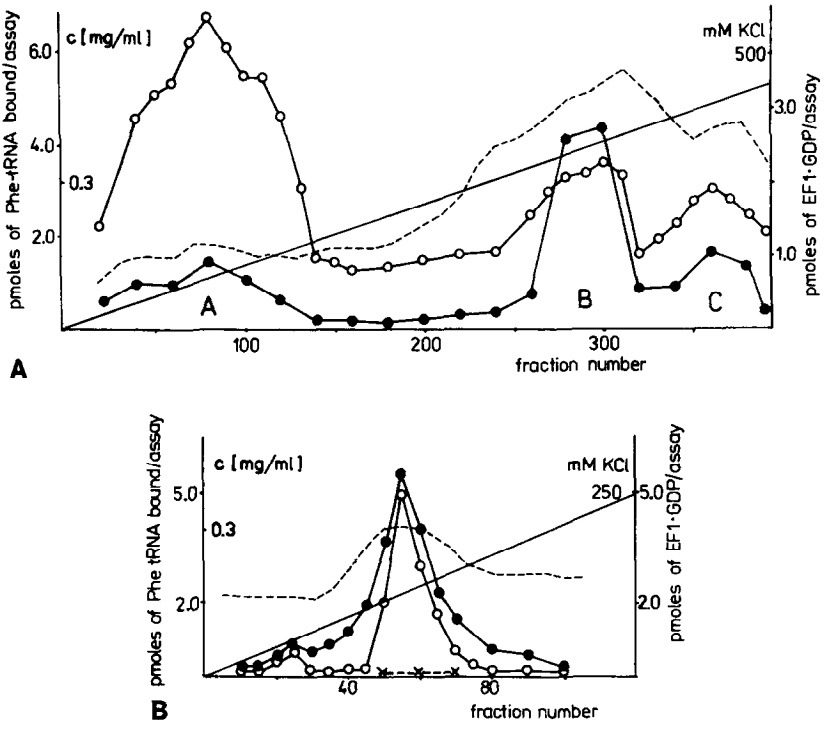

Fig. 1. Purification of EF1 from wheat germ. Supernatant 100 000 xg obtained as in [3] was precipitated in 30% - 60% concentrate of ammonium sulphate. The precipitate was dissolved in buffer 50 mM Tris-HCl, pH 7.2, 10 mM KCl, 10 mM 2-mercaptoethanol, 10% glycerol , applied on a Sephadex G-150 column (9 x 100 cm), equilibrated with the same buffer and eluted at a flow rate of 60 ml/h; fraction volume was 15 ml. Fractions with highest EF1 activity were collected, applied on a DEAE-Sephadex A-50 column (5 x 75 cm) and eluted with a continuous gradient of 10 to 500 mM KCl in a buffer of 10 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$, 5 mM 2-mercaptoethanol and 20% glycerol in total volume of 4000 ml(Fig. 1 A). The fractions marked A, B and C were separately combined and chromatographed on a hydroxylapatite column (2 x 15 cm)using a linear gradient of 10 to 250 mM KCl in 2 x 150 ml of 10 mM potassium phosphate buffer, pH 7.5, containing 1 mM $MgCl_2$, 5 mM 2-mercaptoethanol and 20% glycerol; fraction volume was 2.5 ml (Fig. 1 B shows an example chromatography of EF1A). Key:---, protein concentration;o—o , EF1 activity in the binding reaction;●—● , binary complex formation;×-·-×,polyphenylalanine synthesis. For the binding reaction 10 µl and for binary complex formation 40 µl of every 5th or 10th fraction were taken.

(Fig. 2 D). Similar, if not the same, MW was observed in the case of forms B and C of EF1. To check the biological activity of the proteolysis products molecular filtration was employed, similarly as in[19 - 21]. Because of the lability of polypeptides b and c special conditions of chromatography were developed (see methods). The influence of different amounts of BSA and PMSF on the level of binding reaction was tested separately and no effect at the used

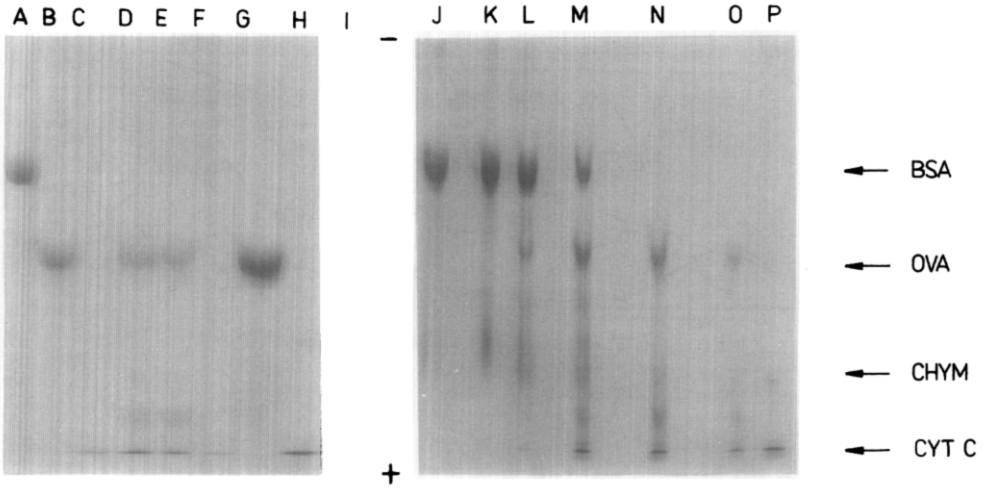

Fig. 2. Polyacrylamide gel electrophoresis at denaturating conditions. A - EF1A (10 µg); B - EF1B (10 µg); C - EF1C (4 µg); D - EF1A (10 µg) incubated with elastase (1 µg); E - EF1B (10 µg) incubated with elastase (1 µg); F - EF1C (4 µg) incubated with elastase (1 µg); G - polypeptide b after molecular filtration (10 µg); H - polypeptide c after molecular filtration (4 µg); I - elastase (5 µg); J to P - EF1A (10 µg) digested with increasing amounts of elastase (0, 0.1, 0.5, 1, 2, 5 and 10 µg, respectively). The arrows show the positions of standards: BSA (65 000 D), ovalbumin (45 000 D), chymotrypsinogen (25 000 D), and cytochrom C (12 400 D).

concentration was observed (data not shown). Factor EF1A digested with protease gave two major peaks (b and c). In the case of each peak the activity in the binding reaction and binary complex test overlapped. The first small peak eluted at void volume of the column is the not completely hydrolysed EF1A. In the same position, in one peak, non-treated factor was eluted (Fig. 3). The ratios of elution volume to void volume for the peaks b and c were 1.3 and 1.8, respectively. The fractions with highest activity of peaks b and c were collected, concentrated and analysed by gel electrophoresis. The position of obtained bands was identical to that described above for non-filtrated polypeptides b and c (Fig. 2 G and H, respectively). According to the purification procedure and polyacrylamide gel electrophoresis all three forms A, B and C and proteolysis products b and c were not cross-contaminated. If any contaminations exist they were not higher than 5% of the total protein amount (see Fig. 2 and 3).

Elongation factor 1 A was digested with increasing amounts of elastase (Fig. 2 J - P). At low level of elastase concentration three bands were visible: not completely hydrolysed EF1A, and

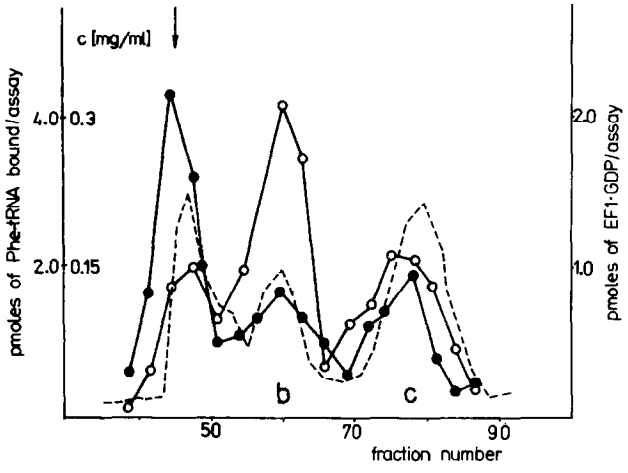

Fig. 3. Molecular filtration of EF1A after treatment with elastase. The mixture of 300 μg of EF1A and 20 μg of elastase after standard incubation was applied on a Sephadex G-75 column (see methods). Key: ---, protein concentration; o—o, binding of Phe-tRNA to ribosomes; ●—●, binary complex formation (EF1-GDP). The arrow shows the position of elution of non-treated with elastase EF1A at void volume of the column. Every third fraction was tested in binding reaction (30 μl per assay) and in binary complex formation (40 μl per test).

polypeptides b and c. Only polypeptides b and c were detected at an average amount of protease (Fig. 2 N and O). Finally, at relatively high concentration of elastase (Fig. 2 P) polypeptide c, with tendency to disappear was observed. The gels were overloaded to detect the minor amounts of digestion products.

Species B and C of EF1 were also treated with elastase and analysed by polyacrylamide gel at identical conditions as those for EF1A. In the case of form B one band in a similar position as for EF1C and polypeptide c was detected. Factor EF1C was rather resistant (in comparison with EF1A and B) to elastase. We found the band on the gel at a similar position as in the case of non - treated EF1C, but the intensity of the band was significantly lower.

Summarising the data, we can say that elastase converts the form A of EF1 to smaller polypeptides: b and c; EF1B is converted to c, while EF1C is less sensitive than EF1A and B to digestion with elastase. Both products of proteolysis were active in two tests typical for binding factor: binary complex formation and binding reaction. The sum of the masses of both polypeptides b and c is close to the molecular weight of EF1A. The activities of all three forms were variable in different isolations, dependent on the conditions of procedure. The products of proteolysis had low but

significant activity (see Fig. 1 and 3). On the average the specific activity of B and C were 30 - 40% and b, c were about 10% of EF1A.

We can suppose that the smallest fragment obtained after elastase treatment (polypeptide c, probably identical to EF1C) could be the structural and functional unit of EF1.

Observations on mild digestion of bacterial factor EFTu with trypsin have been reported (19 - 21). The authors of those studies (20,21) have obtained 4 polypeptides, but complete destruction of AA-tRNA binding to ribosomes after treatment with trypsin has been observed. However, the existence of a fully active fragment with MW of 37 000 D after digestion at similar conditions has also been noticed. Other authors(1,5,8,22,23,) reported the existence of three forms (A,B and C or α, β, γ and a, b and c) in different materials. These forms might be related to our A, B, C and b, c peptides. However their MW are different. Especially nobody, as far as we know, found the light form with MW about 15 000 D.

It is possible that binding factors are under some kind of regulation of proteolytic enzymes. However, it is not known at this time whether change in molecular weight represents a true in vivo situation or is only the results of protease activity. Evidently, further studies are needed to clarify the properties of the eukaryotic binding factor.

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