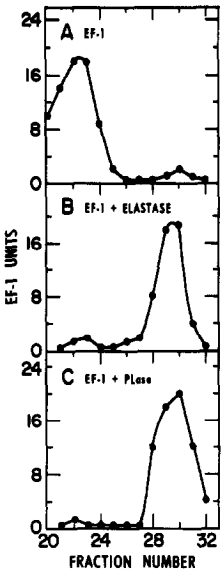

Figure 1: The effect of elastase and phospholipase C on the disaggregation of EF-1. About 150 units of EF-1 (one unit of EF-1 can bind 1 pmole of [^{14}C]-Phe-tRNA to ribosomes per 5 min [see ref. 19]) were incubated for 40 min at 37°C in 40 µl of a solution containing 50 mM Tris-Cl pH 7.4 and 2 mM DTT. Where indicated 2 µg of elastase or phospholipase C (B. cereus, 40% pure) were added. After incubation 60 µl of 0.05 M Tris-HCl pH 7.4 containing 2 mM DTT were added. The mixture (100 µl) was layered on top of 4.2 ml of 5-20% sucrose gradient in a Spinco SW-56 centrifuge tube. The tubes were centrifuged for 2 hours at 50,000 rpm. After centrifugation, 32 fractions (8 drops each), beginning from the bottom, were collected. The EF-1 activity was determined in each fraction. (A) EF-1 alone; (B) EF-1 incubated with elastase; (C) EF-1 incubated with phospholipase C (Plase C).

Thus the disaggregation observed with phospholipase C has been of special concern and indeed the possibility that a contaminant in the phospholipase C preparation is responsible for the observed effect on EF-1 has been considered (11). Disc SDS gel electrophoresis was, therefore, used to determine whether elastase or phospholipase affects the polypeptide gel pattern of EF-1. On SDS gels the reticulocyte EF-1 preparation used in these experiments showed a major band at 56,000 daltons which occassionally indicated the presence of two closely migrating proteins.[1] In addition, minor bands were seen at about 40,000

[1] In the present studies the EF-1 in three separate purifications gave one major band on disc SDS gels with a mol. wt. of about 56,000. Previous results using a similar purification showed two bands of 53,000 and 50,000 daltons (11). The reason for this difference is not known but could be due to variations in the reticulocyte lysates that were used.

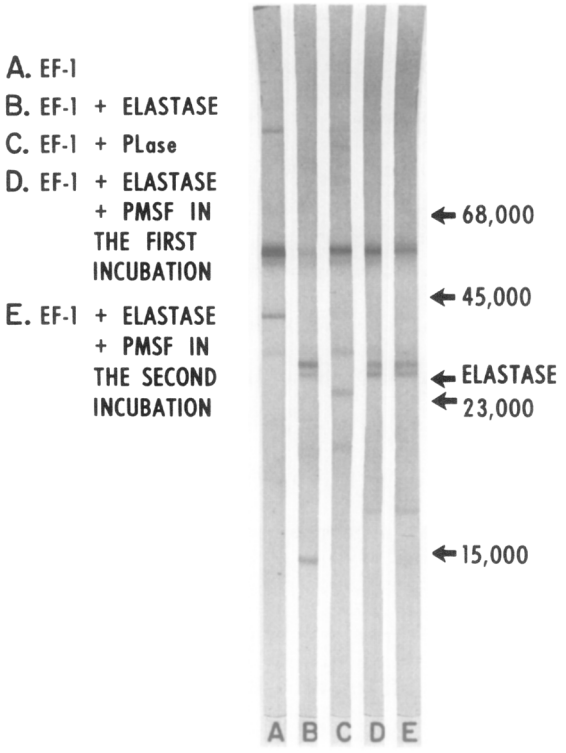

Figure 2: SDS polyacrylamide gel electrophoresis of EF-1 after elastase and phospholipase treatment. The incubation mixture in a total volume of 40 µl contained 50 mM Tris-HCl buffer, pH 7.4, 2 mM DTT and 10-20 µg of EF-1. Where indicated 2 µg of phospholipase C, or 2 µg of elastase and PMSF at a final concentration of 3.1 mM were added. All samples were incubated for 40 min at 37°C; in Sample E, PMSF was added after EF-1 was incubated for 40 min with elastase and the incubations continued for an additional 40 min at 37°C. After the incubations 60 µl of a solution containing: 0.002% bromphenolblue, 17.5% glycerol, 0.1 M Tris-HCl pH 6.8, 1 M β-mercaptoethanol and 33% SDS were added to the reaction mixture. The solution (100 µl) was heated at 100°C for 2 min and electrophoresis done as described elsewhere (20). The arrows show the position of standard proteins: (A) EF-1; (B) EF-1 + elastase; (C) EF-1 + phospholipase C; (D) EF-1 + elastase + PMSF (PMSF added with elastase at the beginning of the incubation); (E) EF-1 + elastase + PMSF (PMSF added after the 40 min incubation of EF-1 plus elastase and the mixture incubated for an additional 40 min).

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and 32,000 daltons (Fig. 2, gel A). After incubating EF-1 with elastase there is a clear change in the gel pattern as seen in Fig. 2, gel B. The major band of about 56,000 daltons and the minor band at 40,000 daltons are no longer seen but two new bands appear at about 30,000 and 15,000 daltons. Presumably, other smaller polypeptides, not detected on the gel, were also formed. A third weak band(s) corresponding to elastase (~28,000 daltons) is also noted.

As shown in Fig. 2, gel C, phospholipase C, unlike elastase, did not alter the polypeptide pattern of EF-1 to a great extent. The only significant change always observed was the disappearance of the minor 40,000 dalton species (minor bands at 32,000, 23,000 and 20,000 daltons seen on the gel were present in the phospholipase preparation used for these studies). These results suggest that a protease with elastase-like activity is not present in the phospholipase C preparation. As expected, phenylmethanesulfonylfluoride (PMSF), an inhibitor of proteases with serine at the active site, significantly inhibited the change in the gel pattern of EF-1 caused by elastase (Fig. 2, gel D) when it was added at the beginning of the incubation with elastase. However, an unexplained result was noted in a control experiment in which EF-1 was incubated with elastase for 40 min (as in gel B), PMSF then added, and the incubation allowed to proceed for an additional 40 min. A typical gel analysis after this procedure (Fig. 2 gel E) shows that the effect of elastase is partially reversed with the band at ~15,000 daltons disappearing, some diminution of the band at 30,000 daltons and the reappearance to a small extent of the major EF-1 band at 56,000 daltons. This result suggested that a small amount of rejoining of the polypeptide chains was occurring under these incubation conditions. In order to explore this observation further, sucrose gradient analysis was used to see whether the results obtained by gel analysis could be confirmed by another technique. One would expect that if rejoining occurred, the 56,000 dalton polypeptide chain might aggregate to form $EF\text{-}1_H$. This conversion could be determined by sucrose gradient analysis. The gradient profile of EF-1 alone (Fig. 3A) and EF-1 plus elastase (Fig. 3B) are similar to those in Fig. 1. PMSF, added initially to the incubation, prevented the conversion of $EF\text{-}1_H$ to $EF\text{-}1_L$ (Fig. 3C). However, when PMSF was added after EF-1 was incubated with elastase for 40 min, and the incubation continued for an additional 40 min, (as in Fig. 2, gel E) a significant amount of $EF\text{-}1_H$ (fractions 24-27) reappeared. These results indicated that not only was a 56,000 dalton polypeptide formed under these conditions but that significant aggregation to $EF\text{-}1_H$ occurred.

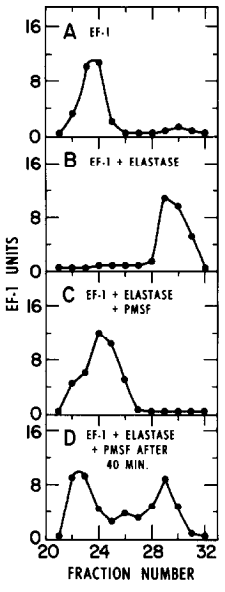

Figure 3: Sucrose gradient analysis of EF-1 after incubation with elastase and PMSF. About 100 units of EF-1 were used. (A) EF-1 alone incubated for 40 min at 37°C; (B) EF-1 incubated with elastase (2 µg) for 40 min at 37°C; (C) EF-1 incubated with elastase (2 µg) and 3.1 mM PMSF for 40 min at 37°C; (D) EF-1 incubated with elastase (2 µg) for 40 min at 37°C; after this incubation PMSF was added (3.1 mM final concentration) and the incubation continued for an additional 40 min at 37°C. The EF-1 activity was analyzed on sucrose gradients. For further details see text and Figure 1.

The present results clearly show a difference between the effect of phospholipase C and elastase on EF-1. Although both cause disaggregation of the enzyme on sucrose gradient analysis, elastase results in a specific cleavage of the 56,000 dalton polypeptide to 30,000 and 15, 000 dalton species on SDS gels, whereas phospholipase C does not. These data indicate that a protease contamination is not the active factor in the phospholipase C preparations. This was also suggested by the observation that PMSF prevented the disaggregation of EF-1 by elastase but not by phospholipase C (11).

It should be noted that some EF-1 preparations have yielded minor polypeptide bands on SDS gels that might be indicative of limited proteolysis (8,11). It will be of special interest to purify EF-1 from either 30 day old nematodes (16) or from A. salina after hatching (17), both of which are

tissues where the enzyme is in the light form. If SDS gel analysis shows that these enzyme preparations are composed of polypeptides of 30,000 and 15,000 daltons, with little or no 50,000 dalton species, it will be indicative that a protease is responsible for the disaggregation observed *in vivo*.

The ability of elastase to cleave EF-1 into 2 major polypeptides without loss of activity suggests that the 2 fragments are able to interact non-covalently and maintain enzymatic activity. This situation may be similar to the well known studies on ribonuclease S and other nucleases (21). In addition, the observation has been made that the polypeptide products, formed after elastase cleavage of EF-1, appear to rejoin in the presence of PMSF (see Fig. 2, gel E) to form a complex not dissociated by SDS. A precedent for this reaction comes from the studies of Laskowski (22) on the effect of trypsin on soybean trypsin inhibitor. He has demonstrated that the modified inhibitor (cleaved by trypsin) is in equilibrium with the native inhibitor. The effect of elastase on EF-1, described in the present experiments, may be similar to the results seen with trypsin and trypsin inhibitor. Further studies are needed to clarify this phenomenon.

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