Purification of elongation factor 2 from human placenta and evidence of its fragmentation patterns in various eukaryotic sources

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While preparing human placenta elongation factor 2 (EF-2), whose purification and some molecular properties are reported, we noticed the presence of numerous protein fractions which did not have EF-2 activity, but were ADP-ribosylated by diphtheria toxin in the presence of NAD⁺. All these proteins, like EF-2, were selectively retained by a heparin–Sephadex column, which we used as an affinity-chromatography step. This was also observed when EF-2 was prepared, by this purification step, from other sources, i.e. ox liver and two species of yeasts. In order to assess whether these proteins were a degradation product of EF-2, independent proteins or a mixture of both, they were analysed by subjecting them, after [¹⁴C]ADP-ribosylation, to exhaustive trypsinolysis. Only one radioactive peptide was found, thus suggesting that those proteins originate from EF-2 by some proteolytic process. Our findings indicate that this proteolysis does not occur after cell disruption, but is more or less active in the intact cell, depending on the system considered.

INTRODUCTION

The elongation factor 2 (EF-2) catalyses the last step of the elongation cycle, translocation, in the course of protein biosynthesis. It has been isolated from a variety of eukaryotic cells and consists of a single polypeptide chain ranging in M_r between 90 000 and 110 000 (Bermek, 1978). EF-2 is the only known protein which has the feature of being ADP-ribosylated by diphtheria toxin in the presence of NAD⁺ (Honjo *et al.*, 1968, 1974; Collins *et al.*, 1971). The ADP-ribosylated factor no longer catalyses translocation, although it still retains the ability to bind the ribosome and GTP (Collier, 1967; Chuang & Weissbach, 1972; Bermek, 1972). The specific target of the ADP-ribosylation on EF-2 is the diphthamide, which is the result of a post-translational modification of a histidine residue (Van Ness *et al.*, 1980). The diphthamide has been constantly found in the various eukaryotic cells examined (Pappenheimer, 1977; Brown & Bodley, 1979), thus suggesting an important role for this residue in regulating protein synthesis at the elongation level (Lee & Iglewski, 1984). However, cells containing EF-2 lacking diphthamide have been isolated (Iglewski & Lee, 1983; Herrera *et al.*, 1984); therefore this amino acid does not seem functionally necessary. The existence of an endogenous enzyme which ADP-ribosylates EF-2 has been reported (Sitikov *et al.*, 1984; Iglewski *et al.*, 1984); hence, diphtheria toxin should mimic the action of this possibly regulatory enzyme.

In the present paper we describe the purification and some of the molecular properties of EF-2 from human placenta. We report the finding, in the course of purification, of several proteins, fragments of EF-2, which are substrates of diphtheria toxin in the

ADP-ribosylation reaction. This has also been found in three other eukaryotic sources, i.e. ox liver and two yeasts. The nature of these proteins was investigated.

MATERIALS AND METHODS

Chemicals

ATP, GTP, phosphocreatine, creatine kinase, poly(U), *Escherichia coli* tRNA and dithiothreitol were purchased from Boehringer. [adenosine-U-¹⁴C]NAD⁺ (600 mCi/mmol) was purchased from New England Nuclear. L-[U-¹⁴C]Phenylalanine (513 mCi/mmol) was from Amersham.

Buffer A was 20 mM-Tris/HCl, pH 7.5, 6 mM-mercaptoethanol, 100 μ M-phenylmethanesulphonyl fluoride.

Diphtheria toxin was a product of Istituto 'Sclavo', Siena, Italy, and was given by Dr. I. De Tommaso. The toxin was used as obtained, or fragment A was prepared essentially as described by Kandel *et al.* (1974) by limited tryptic digestion of the toxin, followed by incubation with 0.1 M-dithiothreitol at 37 °C for 90 min.

ADP-ribosylation reaction

The incubation mixture (100 μ l) contained: 50 mM-Tris/HCl, pH 7.8, 1 mM-EDTA, 5 mM-dithiothreitol, 1 μ g of diphtheria toxin fragment A/ml, 20 000 c.p.m. of [¹⁴C]NAD and 200 μ g of bovine serum albumin. The proteins were precipitated by adding 2 ml of 5% (w/v) trichloroacetic acid and then collected by filtration on glass-fibre filters. The filters were rinsed twice with 2 ml of 5% trichloroacetic acid, dried and counted for radioactivity.

Abbreviations used: EF-2, elongation factor 2; PAGE, polyacrylamide-gel electrophoresis.

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Polyphenylalanine synthesis assay

The poly(U)-directed polyphenylalanine synthesis assay was performed as described by Merrick (1979). The components of the assay mixture (ribosomes, aminoacyl-tRNA synthetases etc.) were prepared by the same procedure.

Gel electrophoresis

SDS/polyacrylamide-gel electrophoresis was performed as described by Laemmli (1970). The M_r markers used were: phosphorylase *b* (M_r 94 000), albumin (M_r 67 000), ovalbumin (M_r 43 000), carbonic anhydrase (M_r 30 000).

Protein preparation

Preparation of extract. Fresh placenta (300–500 g) from full-term parturition was processed within 30 min of delivery or stored at -80°C after dissection from the fetal membrane. After washing with ice-cold buffer A containing 150 mM-NaCl, the placenta was homogenized in a Waring blender for 2 min at 4°C in the same buffer containing a proteinase-inhibitor cocktail (10 μM -soyabean trypsin inhibitor, 10 μM -pepstatin A, 10 μM -leupeptin, 1 mM- NaN_3). The homogenate was centrifuged for 50 min at 20 000 *g*. The same proteinase-inhibitor cocktail was included in the homogenization media also when preparing EF-2 from the other sources used in this work.

Protein precipitation. The post-mitochondrial fraction was centrifuged at 105 000 *g* for 2 h. The supernatant was adjusted to pH 5 by dropwise addition of 1 M-acetic acid and centrifuged for 10 min at 30 000 *g*. The pH 5 precipitate was dissolved in a small volume of buffer A, the pH was adjusted to 7.5 by addition of 0.1 M-KOH and the resulting suspension was clarified by centrifugation.

Alternatively, solid $(\text{NH}_4)_2\text{SO}_4$ to give 70% saturation was added to the post-mitochondrial-supernatant fraction. The suspension was stirred at 4°C for 30 min and then centrifuged for 30 min at 20 000 *g*. The pellet was resuspended in the minimum volume of buffer A and dialysed against two changes of the same buffer. After dialysis, the solution was centrifuged for 40 min at 30 000 *g* and loaded on to a Sepharose CL-6B column (70 cm $\times$ 5 cm) equilibrated in buffer A. The fractions containing EF-2 were pooled.

DEAE-cellulose column. The solution obtained by acid precipitation or by the $(\text{NH}_4)_2\text{SO}_4$ procedure was loaded on a DEAE-cellulose column (25 cm $\times$ 2.5 cm) equilibrated in buffer A. The column was washed with buffer A until the A_{280} of the eluate was below 0.2, and then eluted with a 500 ml linear gradient of 0–150 mM-KCl in the same buffer. From this column a single peak of activity was obtained at about 100 mM-KCl.

Heparin-Sepharose column. The active EF-2 fractions from DEAE-cellulose were pooled, dialysed against buffer A and loaded on a heparin-Sepharose column (15 cm $\times$ 1 cm) equilibrated in the same buffer. The column was washed with buffer A and eluted with a 500 ml linear gradient of 0–200 mM-KCl in buffer A.

Bio-Gel P-200 column. The fractions from the heparin-Sepharose column, which were active in

supporting poly(U)-directed polyphenylalanine synthesis, were pooled, concentrated by ultrafiltration (with a Millipore CX-30 immersible unit) and loaded on a column (150 cm $\times$ 2 cm) of Bio-Gel P-200, equilibrated in buffer A containing 150 mM-KCl and 10 mM- MgCl_2 . The eluted fractions were analysed by SDS/PAGE; those containing EF-2 without contamination were pooled.

Ultracentrifugal measurements

(a) **Determination of M_r .** Samples of EF-2 at different concentrations were dialysed overnight against 100 mM-KCl/10 mM-Tris/HCl, pH 7.4, bubbled with N_2 all though the dialysis period. The M_r was determined by equilibrium sedimentation in a Beckman L8-70 ultracentrifuge, equipped with a Prep-UV scanner. Double-sector cells with charcoal-filled Epon centrepieces and quartz windows were centrifuged in an AN-D aluminium rotor. The measurements were made at two different rotor speeds (6000 and 8000 rev./min) on three cells containing EF-2 at different concentrations, whose initial A_{280} values were 0.15, 0.33 and 0.52. Sedimentation equilibrium was checked by comparing the sedimentation profiles at 6 h intervals. Before the calculations, the profiles were corrected for the baseline absorbance by accelerating the rotor to 42 000 rev./min for 6 h, and then decelerating it to initial speed, just before the scan.

(b) **Determination of sedimentation coefficient.** The same samples used for the equilibrium measurements were analysed for the determination of the sedimentation rate at a rotor speed of 40 000 rev./min. The temperature was 20°C . The data were analysed by the moving-boundary method, plotting the logarithm of the distances (*r*) of the sedimentation-profile inflection point from the rotation axis as a function of time (*t*). The sedimentation coefficient was then calculated from the line slope according to the equation:

$$s = \frac{1}{\omega^2} \frac{d \ln r}{dt}$$

where ω is the rotor angular velocity.

RESULTS

EF-2 characterization

The homogeneity of the EF-2 obtained from the Bio-Gel P-200 column was demonstrated by the presence of a single protein band on PAGE, under both native and

Fig. 1. SDS/PAGE of purified EF-2

A 10%-acrylamide gel was used.

Fig. 2. Determination of M_r of EF-2 by sedimentation equilibrium

The $\ln$ of the A_{280} in the cell at the distance (r) from the rotation axis was plotted versus the square of that distance. The data were obtained at 6000 rev./min (▲) and 8000 rev./min (●). The solid lines were derived from least-squares analysis on the data points. Details are reported in the Materials and methods section.

denaturing conditions (Fig. 1). In addition, the presence of only one symmetrical boundary in the sedimentation-velocity experiments, as well as the linearity of the plot of $\ln A_{280}$ versus r^2 from sedimentation-equilibrium analysis, confirmed that the protein was essentially homogeneous. The $s_{20,w}$, determined under the experimental conditions reported in the Materials and methods section, was 5.65 S. No concentration-dependence of the sedimentation coefficient in the three different samples was detected. Fig. 2 reports the equilibrium sedimentation profile of the more concentrated sample at 6000 and 8000 rev./min, plotted as $\ln A_{280}$ versus r^2 . A least-squares analysis on the data groups, obtained both at 6000 and at 8000 rev./min, gave a regression coefficient for the

straight line higher than 0.99. An M_r of 94000 ± 3000 was calculated from the slopes, which are equal to $M(1 - \bar{v}\rho)\omega^2/2RT$, where ω is the rotor angular velocity, R the gas constant, T the absolute temperature, ρ the solvent density, M the protein M_r , and $\bar{v}$ its partial specific volume respectively. The $\bar{v}$ of EF-2 was assumed to be 0.73 ml/g.

The M_r of 94000 for EF-2 obtained by sedimentation equilibrium has furthermore been confirmed by SDS/PAGE and gel-filtration techniques. In particular, in SDS/PAGE, EF-2 shows the same mobility as phosphorylase b (M_r 94000), used as a marker.

The knowledge of the $M(1 - \bar{v}\rho)$ term from the sedimentation-equilibrium data, along with the sedimentation coefficient, allowed us to calculate the Stokes radius (R_s) for EF-2, from the equation:

$$R_s = \frac{M(1 - \bar{v}\rho)}{6\pi\eta Ns}$$

where N is Avogadro's number and η the solvent viscosity. The value we found for R_s was 3.8 nm, which corresponds well to the value of 3.75 nm reported for EF-2 from rabbit reticulocytes and calculated by gel-filtration experiments (Merrick *et al.*, 1975).

Pattern of diphtheria-toxin ADP-ribosylatable protein

Table 1 shows the correlation between the purification steps of human placenta EF-2 and the activity, measured both as diphtheria-toxin [^{14}C]ADP-ribosylation and as polyphenylalanine synthesis. The activity and the recovery are essentially the same when the pH 5 precipitation procedure is replaced by the $(NH_4)_2SO_4$ and Sepharose steps. Table 1 shows that the percentage recovery falls sharply after the heparin-Sepharose step, as far as the ADP-ribose-accepting activity is concerned. On the other hand, the recovery measured as polyphenylalanine-synthesis activity is much less affected by this step. This can be easily understood by looking at Fig. 3, which shows the elution pattern of that column: two peaks of activity, measured as diphtheria-toxin-catalysed ADP-ribosylation, are eluted, but only the fractions constituting the second peak are able to support protein synthesis.

Table 1. Correlation between purification steps and activities during purification of EF-2 from human placenta

Purification step	ADP-ribose incorporation		Polyphenylalanine synthesis	
	Specific activity (pmol of ADP-ribose bound/mg of protein)	Recovery (%)	Specific activity (pmol of phenylalanine polymerized/min per mg of protein)	Recovery (%)
Homogenate	1	100	—	100
pH 5 precipitation	9	75	5	75
DEAE-cellulose	50	65	28	65
Heparin-Sepharose	7500	32	5900	54
Bio-Gel P-200	9500	15	7000	26
$(NH_4)_2SO_4$ *	3	96	—	—
Sepharose CL-6B*	8	74	—	—

* Alternatively, $(NH_4)_2SO_4$ precipitation, followed by Sepharose CL-6B chromatography, was employed as starting steps, instead of pH 5 precipitation.

Fig. 3. Elution pattern of human placental EF-2 from heparin-Sepharose

The presence of EF-2 in the fractions (5 ml) was assayed either by diphtheria-toxin reaction (●) or by its ability to support poly(U)-directed polyphenylalanine synthesis (▲). The assay conditions are reported in the Materials and methods section.

Fig. 4. SDS/PAGE and fluorography of the ADP-ribosylatable fractions in the human placenta EF-2 preparation after the heparin-Sepharose column

Lane 1: mixture of the two pooled peaks from the heparin-Sepharose column incubated in the presence of [^{14}C]NAD $^{+}$. Lane 2: first peak from heparin-Sepharose column incubated in the presence of [^{14}C]NAD $^{+}$ and diphtheria toxin. Lane 3: second peak from heparin-Sepharose column incubated in the presence of [^{14}C]NAD $^{+}$ and diphtheria toxin. The incubation conditions are reported in the Materials and methods section.

The result in Table 1, together with that in Fig. 3, appears to be in contrast with the belief that the only target for the ADP-ribosylation catalysed by diphtheria toxin is EF-2 (Bermek, 1978). In addition, further investigations on the two heparin-Sepharose peaks have revealed an unexpected complexity in the pattern of the proteins sensitive to ADP-ribosylation. Each peak was pooled, ADP-ribosylated by diphtheria toxin, by using [adenine- ^{14}C]NAD $^{+}$, and subjected to SDS/PAGE. Fluorography of the gel, reported in Fig. 4, shows the presence of a remarkable number of radioactive bands in both the pools. In the first pool (lane 2) most of the radioactivity is associated with bands of M_r in the range 30000–43000, whereas in the second pool (lane 3) the most radioactive band shows an M_r of 94000, which is that found for the native protein. The ADP-ribosylation of each band is dependent on the diphtheria-toxin reaction, since in lane 1, where the toxin is omitted from the incubation mixture, no radioactive band is visible.

By scanning densitometry of the fluorogram of Fig. 4, we calculated the relative abundance of each labelled protein. The component of M_r 94000 showed a relative abundance of about 50%, that of M_r 40000 was roughly 30%, and the remaining 20% was represented by the other bands.

In order to establish whether these proteins are fragments produced from EF-2 during the purification or whether they existed before cell rupture, the two pools from the heparin-Sepharose step were mixed, subjected

Fig. 5. SDS/PAGE fluorography of ADP-ribosylated EF-2 fraction from human placenta under different labelling conditions

The pool of the two peaks from the heparin-Sepharose column was subjected to ADP-ribosylation with [^{14}C]NAD $^{+}$ and analysed according to the following scheme. For lane 1, the mixture was [^{14}C]ADP-ribosylated and immediately subjected to SDS/PAGE analysis. For lane 2, the mixture was [^{14}C]ADP-ribosylated, left for 20 h at 37 °C and then analysed by SDS/PAGE. For lane 3, the mixture was left for 20 h at 37 °C and [^{14}C]ADP-ribosylated just before the SDS/PAGE analysis.

Fig. 6. SDS/PAGE fluorography of the material ADP-ribosylated by diphtheria toxin in the human placenta post-mitochondrial fraction

Lane 1: the post-mitochondrial fraction was [^{14}C]ADP-ribosylated and immediately subjected to SDS/PAGE analysis. Lane 2: the post-mitochondrial fraction was left for 5 h at 37 °C and then subjected to [^{14}C]ADP-ribosylation. Interference by endogenous NAD^+ was avoided as described in the Results section.

Fig. 7. SDS/PAGE fluorography of the material, ADP-ribosylated by diphtheria toxin, from different eukaryotic sources

The pool of the two peaks from the heparin–Sephacose column of each preparation was subjected to ADP-ribosylation with [^{14}C]NAD $^+$ and analysed on a 10%-acrylamide gel. Lanes: *a*, ox liver; *b*, brewer's yeast; *c*, baker's yeast; *d*, human placenta.

Fig. 8. SDS/PAGE fluorography of the material, ADP-ribosylated by diphtheria toxin, in brewer's yeast

The pool of the two peaks from the heparin–Sephacose column of protein preparations from cells at different growth stages was subjected to ADP-ribosylation with [^{14}C]NAD $^+$ and analysed on a 10%-acrylamide gel. Lanes: *a*, preparation from early-exponential-phase cells; *b*, preparation from mid-exponential phase cells; *c*, preparation from resting cells.

to [^{14}C]ADP-ribosylation by the toxin and analysed by SDS/PAGE. The ADP-ribosylation was performed by the scheme reported in Fig. 5 legend. As shown in Fig. 5, fluorography showed no difference between the pattern of the original components (lane 1) and that of the mixture after the incubation at 37 °C.

On the other hand these patterns are very close to those obtained in another set of experiments, where the post-mitochondrial fraction was [^{14}C]ADP-ribosylated soon after preparation and after 5 h of incubation at 37 °C (Fig. 6). Interference by endogenous NAD^+ in this last experiment was avoided by adding DEAE-cellulose resin to the post-mitochondrial fraction, washing the resin with buffer A and then releasing the proteins with buffer A plus 0.3 M-KCl.

ADP-ribosylatable proteins from other sources

In order to investigate whether the pattern of ADP-ribosylatable proteins is a peculiarity of the full-term placenta, EF-2 was purified from ox liver,

baker's yeast and brewers' yeast. The purification procedures were similar to those used with placenta, except in the initial step there was a precipitation with 70% satd. $(\text{NH}_4)_2\text{SO}_4$ instead of at pH 5. In the purification steps before the heparin-Sepharose column we did not observe loss of appreciable amounts of ADP-ribosylatable material. In fact, after the $(\text{NH}_4)_2\text{SO}_4$ step, in each case, we recovered about 95% of the initial activity, and all this material, after desalting and loading on the DEAE-cellulose column, was eluted as a single peak, whose pooled fractions, after dialysis, were loaded on the heparin-Sepharose column. In each case, the heparin-Sepharose step gave the same elution pattern as obtained in the purification from placenta, with two peaks of ADP-ribose-accepting activity, and in each case only the fractions constituting the peak eluted at higher ionic strength were able to support polyphenylalanine synthesis. Fig. 7 reports the fluorography of the first and the second peaks from the heparin-Sepharose column, pooled and ADP-ribosylated, for ox liver (Fig. 7a), brewer's yeast (Fig. 7b), baker's yeast (Fig. 7c) and human placenta (Fig. 7d).

The fragmentation patterns were different, among the various species, in terms of both degree of degradation and the sizes of the fragments produced. Moreover, we examined the pattern of the ADP-ribosylatable proteins

in brewer's yeast at various growth stages. As shown in Fig. 8, the pattern is not constant, but depends strongly on the growth phase. We found, by scanning densitometry, that the component of M_r 94000, which accounts for more than 95% in the early exponential phase, dramatically decreases with time and becomes less than 5% in the resting cells.

At this point the question arises as to the nature of these ADP-ribosylatable proteins. Are they EF-2 fragments, other distinct proteins, or a mixture of both? To answer this question, the pool of the two peaks from heparin-Sepharose obtained in the purification procedure from baker's yeast (because of its higher yield of ADP-ribosylatable material) was subjected to ADP-ribosylation with $[^{14}\text{C}]\text{NAD}^+$ and then to exhaustive trypsinolysis for 5 h at 37 °C in 50 mM- NH_4HCO_3 with a trypsin/protein ratio of 1:50 (w/w). The incubation mixture was loaded on a Sephadex G-25 column. Two radioactive peaks were obtained (Fig. 9a), the first containing the $[^{14}\text{C}]\text{ADP}$ -ribosylated peptide and the second $[^{14}\text{C}]\text{NAD}^+$ that had not reacted; the first radioactive peak was pooled and subjected to fast protein liquid chromatography on a Mono Q column. Only one radioactive peak was eluted (Fig. 9b); its constitutive fractions were pooled, freeze-dried, dissolved in 10 mM-ammonium formate, pH 4, and chromato-

Fig. 9. Purification steps of the $[^{14}\text{C}]\text{ADP}$ -ribosylated tryptic peptide from baker's yeast

Chromatographic steps employed during the purification of EF-2 tryptic peptide labelled by $[^{14}\text{C}]\text{ADP}$ -ribosylation. (a) Sephadex G-25 column elution pattern of ADP-ribosylated and trypsin-digested EF-2 fragments. The column was equilibrated in 50 mM- NH_4HCO_3 . (b) Fast protein liquid chromatography on a Mono-Q column (5 mm $\times$ 50 mm) of the radioactive pool from step A. The eluents a and b were 50 mM- and 500 mM- NH_4HCO_3 respectively. The stippled area represents the radioactive peak. (c) H.p.l.c. on an ODS column (4.6 mm $\times$ 150 mm) of the radioactive pool from step B. The eluents a and b were 10 mM-ammonium formate, pH 4.0, and methanol respectively. The stippled area represents the radioactive peak, which was used for amino acid composition and N-terminal analysis.

Table 2. Amino acid composition of the ADP-ribosylated peptide from baker's yeast

Amino acid	Content (residues/molecule)*
Asp	3.2 (3)
Thr	0.8 (1)
Ala	1.9 (2)
Val	2.3 (2)
Ile	2.1 (2)
Leu	2.1 (2)
His	0.9 (1)
Arg	1.0 (1)
Diphthamine†	0.7 (1)

* Calculated assuming one arginine residue/molecule.

† The peak emerging before histidine was assumed to be diphthine, to which the same colour yield as histidine was attributed.

graphed by h.p.l.c. on a C_{18} reverse-phase column. In this case too, only one radioactive peak was detected (Fig. 9c). The pool of the radioactive peak was used for *N*-terminal amino acid analysis as described by Gray (1967) and for determination of amino acid composition. Valine was the only amino acid found in the *N*-terminal analysis. The amino acid composition is reported in Table 2.

DISCUSSION

The purification of EF-2 from human placenta, described in this paper and summarized in Table 1, can start by precipitation of the post-mitochondrial supernatant either with $(NH_4)_2SO_4$ or by acidification at pH 5. The two procedures gave the same protein recovery: therefore the pH 5 precipitation was preferred by us, because it was quicker.

During the EF-2 purification we took advantage of its ability to bind to RNA, by using chromatography on heparin-Sepharose column. The column proved to be highly selective, because, after this chromatography, the EF-2 specific activity increased about 150-fold. However, the elution pattern of the column was very surprising; in fact, it showed only one peak of activity, measured as ability to support polyphenylalanine synthesis, but two activity peaks (of the same magnitude) when the ADP-ribose incorporation was measured. This finding explains why, in this step of purification (see Table 1), the percentage of recovery of ADP-accepting activity decreases by 50%, whereas the translocation activity remains roughly constant.

SDS/PAGE of the two peaks revealed that both of them contained several proteins sensitive to ADP-ribosylation. It was difficult to explain with confidence the origin of these bands, which were also found in a similar pattern in three other eukaryotic sources, i.e. ox liver, baker's yeast and brewer's yeast.

So far, no protein, except EF-2, has been reported to be a substrate of the diphtheria toxin. On the other hand, the large number of bands, the finding that none of them correspond to an M_r higher than 94000, and the observation that the protein of M_r 94000 possesses translocation activity, let us suppose that all these

polypeptides which are sensitive to ADP-ribosylation originate from native EF-2 by some proteolytic cleavage. It is known that some EF-2 fragments, produced by tryptic cleavage, are still substrates of diphtheria toxin (Van Ness *et al.*, 1980). We obtained evidence of the common origin from EF-2 of all those ADP-ribosylatable proteins by subjecting a pool of them to [^{14}C]ADP-ribosylation and then to trypsinolysis. In fact, only one radioactive peptide was produced, as demonstrated by various chromatographic procedures and by the finding of an unique *N*-terminal amino acid. The peptide shows the same composition (15 residues) and the same *N*-terminal amino acid (valine) as the peptide containing diphthamide produced by tryptic cleavage of EF-2 from baker's yeast (Brown & Bodley, 1979).

The production of the fragment seems to be due neither to a trivial microbial contamination nor to proteinases acting after cell disruption. In fact, the same pattern in the fragment composition was also obtained by incubation for 20 h at 37 °C of the pools from the heparin-Sepharose column or by leaving the post-mitochondrial fraction for 5 h at 37 °C. From these results it can be concluded that, most probably, the EF-2 fragments are already present in the cell.

The fragmentation pattern of EF-2 is quite different, depending on the specific source examined. The ox liver EF-2 shows a higher relative abundance of the native protein relative to the fragments than does the placenta. The larger proportion of fragments in the term placenta could be explained by considering that it is no longer a functioning organ, in which EF-2 breakdown could not be counterbalanced by its biosynthesis. On the other hand, the EF-2 breakdown by itself could represent a cellular regulation mechanism of protein synthesis at the level of the elongation cycle. The data obtained for yeast support this consideration. In this species, the fragmentation pattern appears to depend on the growing phase: in the early-exponential phase, the native protein accounts for more than 95% of the ADP-ribosylatable material, but its relative abundance, with respect to the fragments, decreases in the mid-exponential phase and becomes negligible in the resting cells.

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