Volume 214, number 2, 331–334 FEB 04618 April 1987

Ca^{2+}/calmodulin-dependent phosphorylation of elongation factor 2

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Received 12 February 1987

Incubation of a ribosome-free extract of rabbit reticulocytes or rat liver with [γ-^{32}P]ATP and Ca^{2+} results in incorporation of ^{32}P predominantly into a single polypeptide of $M_r \sim 100\,000$. This polypeptide is identified as elongation factor 2 (EF-2). Phosphorylation of EF-2 is strictly Ca^{2+}-dependent and can be inhibited by the calmodulin antagonist trifluoperazine. It is suggested that the Ca^{2+}/calmodulin-dependent phosphorylation of EF-2 is involved in regulation of protein biosynthesis.

Protein phosphorylation; Calmodulin; Ca^{2+}; Elongation factor 2; Translation regulation

1. INTRODUCTION

Many proteins of the eukaryotic translational machinery can be phosphorylated in vitro and in vivo. Phosphorylation of initiation factors (see, e.g. [1,2]), ribosomal proteins [3], aminoacyl-tRNA synthetases [3] and elongation factor 1 [4,5] has been reported. Phosphorylation of the initiation factor eIF-2 α-subunit has been studied most extensively and has been shown to play an important role in the regulation of protein biosynthesis (see, e.g. [2,6]).

This work demonstrates that another protein of the eukaryotic translational machinery, elongation factor 2 (EF-2), can be intensively phosphorylated in extracts of animal tissues in a Ca^{2+}/calmodulin-dependent manner. This seems to be the first report on the phosphorylation of EF-2 and also the first indication that calmodulin can be involved in the regulation of translation.

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2. MATERIALS AND METHODS

Ribosome-free extracts from rabbit reticulocytes and rat liver were obtained according to [7,8], respectively.

When required endogenous Ca^{2+} was removed from ribosome-free extracts by gel filtration through a column of Sephadex G-25 coarse (Pharmacia) equilibrated with a buffer containing 50 mM Hepes-KOH (pH 7.6), 10 mM magnesium acetate, 10 mM EGTA and 5 mM dithiothreitol. EF-2 from reticulocytes was purified as in [9] with final purification on a Mono-Q column (Pharmacia). Protein phosphorylation was performed by incubating samples of the extracts with [γ-^{32}P]ATP (Isotope, USSR) for 2 min at 30°C.

Electrophoresis was carried out in a polyacrylamide gradient gel (10–22% acrylamide) in the presence of 0.1% SDS [10]. To obtain a radioautograph of the labeled proteins, gels were dried and exposed overnight at −70°C using an RM-V film (Tasma, USSR). For quantitation of EF-2 phosphorylation, the corresponding bands were excised from the Coomassie blue-stained and dried gel, and ^{32}P radioactivity measured by liquid scintillation spectrometry.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

The concentration of endogenous EF-2 in the rabbit reticulocyte ribosome-free extract was determined by complete ADP-ribosylation of EF-2 with diphtheria toxin and [^{14}C]NAD (260 mCi/mmol, Amersham) [9] and found to be 0.43 μM.

3. RESULTS

Fig.1 demonstrates that incubation of ribosome-free extracts of rabbit reticulocytes with [γ-^{32}P]ATP results in incorporation of ^{32}P into a single polypeptide of about 100 kDa. Addition of purified EF-2 to the incubation mixture drastically enhances the incorporation of ^{32}P into the 100 kDa band. It can be concluded that the 100 kDa band is EF-2. EF-2 itself, without addition of the extract, is not phosphorylated. Thus, a special protein kinase which phosphorylates EF-2 seems to exist. It is calculated that about 50% of EF-2 in the extract can be phosphorylated within 2 min.

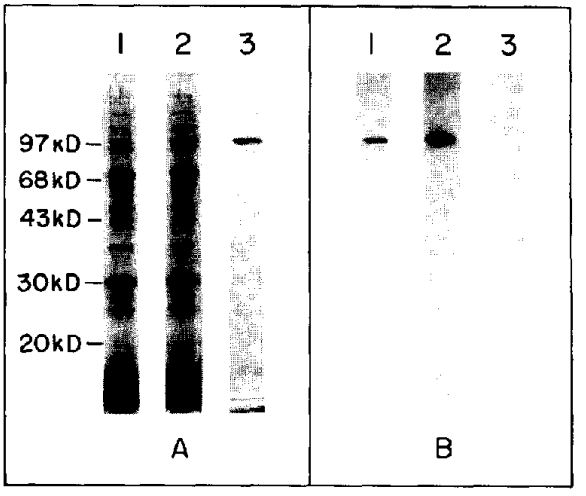

Fig.1. Identification of the 100 kDa band in a ribosome-free extract of rabbit reticulocytes as EF-2. (A) Coomassie blue-stained gel after electrophoresis of proteins of the ribosome-free extract without (1) and with (2) 3 μg EF-2; (3) 3 μg pure EF-2. (B) Radioautograph of the same gel. The reaction was carried out in the following buffer: 50 mM Hepes (pH 7.6), 10 mM magnesium acetate, 5 mM dithiothreitol, 50 μM [γ-^{32}P]ATP (600 cpm/pmol) and 4 μl of the extract in a final volume of 40 μl. Samples were incubated for 2 min at 30°C. The reaction was stopped by the addition of 10 μl of SDS-containing buffer for electrophoresis and 20 μl were applied on the track.

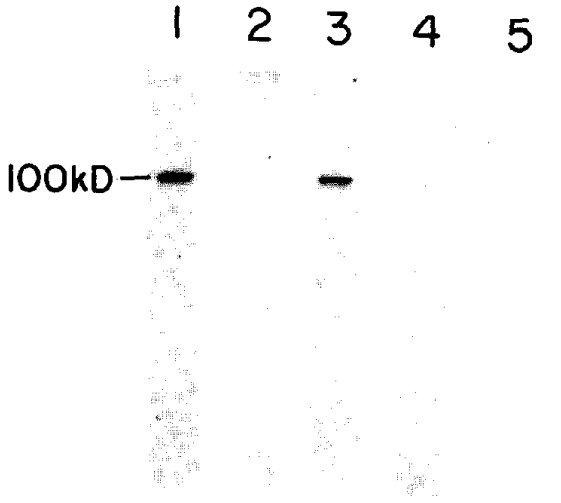

Fig.2. Dependence of EF-2 phosphorylation on the presence of Ca^{2+} and trifluoperazine. The experiment was done as described in the legend to fig.1. (1) Control (same as in fig.1B, track 1); (2) extract was gel filtered through Sephadex G-25 before incubation; (3) 150 μM Ca^{2+} was added to the gel-filtrated extract; (4,5) incubation was carried out under standard conditions (as for track 1), but 50 μM (4) or 100 μM (5) trifluoperazine was added to the reaction mixture.

The phosphorylation of EF-2 is Ca^{2+}-dependent. It cannot be detected if the extract has been gel filtered through Sephadex G-25 before incubation (see fig.2). Phosphorylation of EF-2 in the gel-filtered extract becomes visible after addition of 150 μM $CaCl_2$. The phosphorylation seems to be calmodulin-dependent since it is inhibited by the calmodulin antagonist, trifluoperazine (see fig.2).

A new Ca^{2+}/calmodulin-dependent protein kinase has recently been identified in different rat tissues [8,11]. The major substrate of this kinase, termed Ca^{2+}/calmodulin-dependent protein kinase III, is an abundant cellular protein of about 100 kDa. However, judging from different criteria, e.g. molecular mass, isoelectric point and cellular abundancy, it is similar to EF-2 [12]. This fact prompted a direct comparison of these proteins. According to Palfrey's data [8], Ca^{2+}/calmodulin-dependent phosphorylation of the 100 kDa protein is most clearly seen in the extract from rat liver. The conditions for phosphorylation of the 100 kDa protein were

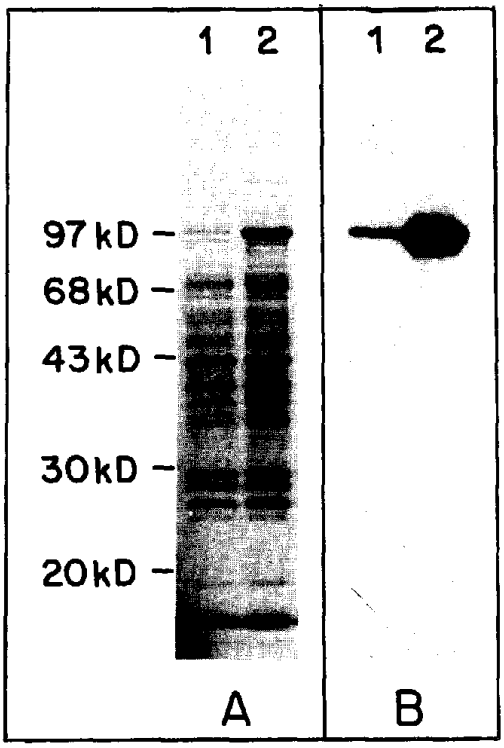

Fig.3. Comparison of EF-2 with the 100 kDa substrate of Ca^{2+}/calmodulin-dependent protein kinase III. (A) Coomassie blue-stained gel after electrophoresis of proteins of the ribosome-free extract of rat liver without (1) or with (2) 5 μg EF-2. (B) Radioautograph of the same gel. The reaction was carried out in the following buffer: 50 mM Hepes (pH 7.6), 10 mM magnesium acetate, 5 mM dithiothreitol, 1 mM EGTA, 1.5 mM $CaCl_2$, 50 μM [γ-^{32}P]ATP (800 cpm/pmol) and 20 μg of the extract. The final volume was 50 μl. Incubation conditions and subsequent manipulations were the same as those described in the legend to fig.1.

reproduced in detail. As seen in fig.3, the 100 kDa protein not only co-migrates with EF-2 upon electrophoresis, but its phosphorylation is also enhanced after the addition of purified EF-2. Thus, EF-2 is found to be the major substrate of the recently described Ca^{2+}/calmodulin-dependent protein kinase III.

4. DISCUSSION

This work demonstrates that EF-2 can be phosphorylated in vitro. However, it is most interesting that the phosphorylation of EF-2 is specific and EF-2 is the only visible ^{32}P-labeled protein in extracts of such different animal tissues as rabbit reticulocytes and rat liver. Another interesting characteristic of this phosphorylation is that a large portion (about 50%) of the EF-2 molecules can be rapidly phosphorylated.

Since this phosphorylation is predominant in different animal tissues, it was noted in many studies on protein phosphorylation (for references see [11]).

Recently Nairn et al. [11] have described a new Ca^{2+}/calmodulin-dependent protein kinase which they termed Ca^{2+}/calmodulin-dependent protein kinase III. The major substrate of this kinase in different tissues was an abundant cellular protein of about 100 kDa. The present results clearly demonstrate that the 100 kDa protein phosphorylated by Ca^{2+}/calmodulin-dependent protein kinase III is identical to EF-2.

Thus, there is a special Ca^{2+}/calmodulin-dependent protein kinase which phosphorylates predominantly EF-2. This kinase is present in all tissues studied [11] and may be involved in some crucial regulatory process.

Since phosphorylation of EF-2 takes place only in the presence of a sufficient concentration of Ca^{2+}, it may be a part of a regulatory mechanism connecting the rate of protein biosynthesis with the Ca^{2+} level in the cell. This is not unlikely as the elevation of the Ca^{2+} concentration was reported to activate protein synthesis in culture cells and in cell-free systems [13].

ACKNOWLEDGEMENTS

I am grateful to Professor A.S. Spirin for encouragement and discussion and to E.A. Shestakova and P.G. Natapov for help in performing the experiments.

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