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Comparison of phosphorylation of elongation factor 1 from different species by casein kinase II

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One subunit of EF-1 or EF-1$\beta\gamma$ from *Artemia salina*, wheat germ and rabbit reticulocytes is modified by casein kinase II. The subunit corresponds to the low M_r subunit of EF-1 (26000–36000) which functions along with a higher M_r subunit (46000–48000), to catalyze the exchange of GDP for GTP on EF-1α. The factor from *Artemia* and wheat germ is phosphorylated directly on serine by casein kinase II whereas a modulatory compound is required for phosphorylation of EF-1 from reticulocytes. Polylysine increases the rate of phosphorylation of EF-1 from reticulocytes by 24-fold; both serine and threonine are modified. This suggests that polylysine may be substituting for a physiological regulatory compound which modulates phosphoryation in vivo.

Elongation factor; Casein kinase; Protein kinase; Protein synthesis; Phosphorylation

1. INTRODUCTION

Elongation factor 1 from eukaryotes exists in multiple forms. The heavy form ranges in M_r from 140 000 to 800 000 [1,2]. A significant portion of EF-1 is tightly complexed with valyl-tRNA synthetase [3,4]. EF-1 consists of 3 ($\alpha\,\beta\,\gamma$) or 4 (δ) subunits as shown with *Artemia salina* [5], wheat germ [6] and rabbit reticulocytes [7]. The α-subunit (M_r 50 000) has been isolated alone or in the holoenzyme and mediates the GTP-dependent binding of aminoacyl-tRNA to 80 S ribosomal subunits. The $\beta\gamma$ subunits have been isolated [5,8,9] and shown to catalyze the exchange of GDP bound to EF-1α with exogenous GTP. An additional subunit, δ, is sometimes found with the $\beta\gamma$ complex and with EF-1; the potential role of δ in elongation has yet to be realized.

In *Artemia salina*, one of the subunits, M_r 26 000, has been shown to be phosphorylated at serine 89 [10]; adjacent acidic residues at the carboxyl terminus provide a recognition sequence for casein kinase II [11]. The same subunit is phosphorylated in vitro by a casein kinase II-like protein kinase copurifying with the elongation factor [10]. Recent reports indicate EF-1 is phosphorylated on a M_r 47 000 subunit in maturing *Xenopus* oocytes during meiotic cell division and is modified in vitro by the CDC2 protein kinase [12,13]. Two other subunits, M_r 30 000 and 36 000, also phosphorylated in oocytes, are reported to be modified in vitro by casein kinase II. Previously, EF-1 from reticulocytes was examined as substrate for casein kinase II, but no phosphorylation was observed [14]. The purpose of these studies is to examine the requirements for phosphorylation of EF-1 by casein kinase II from different species, and identify if modulatory compounds are required for phosphorylation to occur.

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2. MATERIALS AND METHODS

2.1. *Materials*

Polylysine (M_r 36 500) was purchased from Sigma. Casein kinase II was purified as described [15]. EF-1 was highly purified from rabbit reticulocytes (Venema, R.C. and Traugh, J.A., manuscript in preparation) and *Artemia salina* [16]. EF-1 $\beta\gamma(\delta)$ was purified from *Artemia* [5] and wheat germ [9]. The factor from *Artemia* was generously provied by Dr Wim Möller (State University of Leiden, The Netherlands), and the factor from wheat germ was a gift of Dr Joanne M. Ravel (University of Texas, Austin).

2.2. *Phosphorylation of EF-1*

Phosphorylation of EF-1 was carried out in 0.07 ml reaction mixtures containing 20 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 50 mM KCl, 0.14 mM [γ-^{32}P]ATP (2000 dpm/pmol), 1 μg of EF-1, 50 units of casein kinase II [15], and 1 μg polylysine, as indicated. Reactions were incubated at 30°C for 30 min and terminated by addition of 0.005 ml of 100 mM ATP, immediately followed by sample buffer containing sodium dodecyl sulfate; the samples were analyzed by electrophoresis in 10% polyacrylamide gels [17]. The gels were stained in Coomassie blue, destained, dried and autoradiographed.

2.3. *Phosphoamino Acid Analysis*

The phosphorylated protein bands were excised, digested with trypsin, and hydrolyzed with 6N HCl for 2 h at 100 – 110°C. Each sample was supplemented with a mixture of phosphoamino acid standards and analyzed by electrophoresis on silica gel plates as described [18]. The labeled amino acids were identified by autoradiography and staining with ninhydrin.

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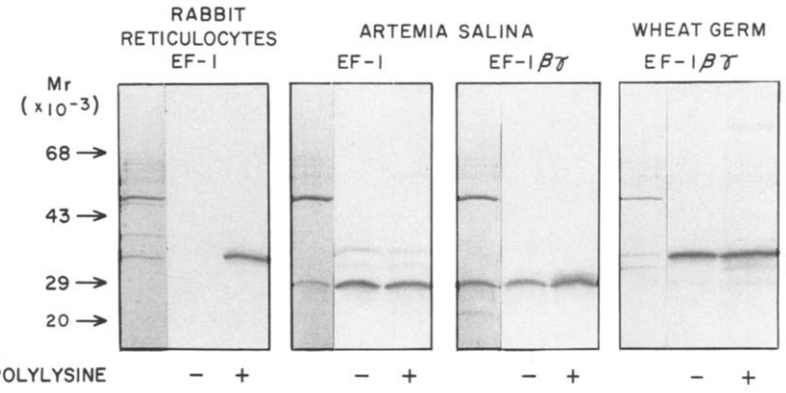

Fig. 1. Requirements for phosphorylation of EF-1 by casein kinase II. The first lane in each panel is the protein pattern of purified EF-1 from the species indicated, as visualized with Coomassie blue. The following two lanes are the corresponding autoradiograms of EF-1 phosphorylated by casein kinase II in the absence or presence of polylysine, as described in section 2.

3. RESULTS

Phosphorylation of highly purified EF-1 from rabbit reticulocytes and *Artemia salina* and EF-1 $\beta\gamma$ from *Artemia* and wheat germ was examined with casein kinase II purified from reticulocytes (Fig. 1). With EF-1 from *Artemia* and wheat germ, a single subunit was phosphorylated. No phosphorylation of EF-1 from reticulocytes was observed in the absence of effector compounds. The subunit from *Artemia* was phosphorylated both in the EF-1 complex or as EF-1$\beta\gamma$. EF-1 from reticulocytes was a substrate for casein kinase II when phosphorylation was carried out in the presence of the effector compound, polylysine. Polylysine has been shown to have similar stimulatory effects when calmodulin is used as substrate [19,20]. The subunit of EF-1 phosphorylated by casein kinase II has a M_r of 33 000 in reticulocytes, 36 000 in wheat germ and 26 000 in *Artemia*.

Phosphorylation of reticulocyte EF-1 was stimulated 24-fold by polylysine (Table I). With *Artemia*, phosphorylation of EF-1$\beta\gamma$ was stimulated 1.8-fold by polylysine and phosphorylation of EF-1 was slightly inhibited. With wheat germ, little or no effect was observed with polylysine. Thus, mammalian EF-1 was the only factor to require polylysine for phosphorylation by casein kinase II in vitro, and phosphorylation of EF-1 from other eukaryotes was affected only to a small extent, if at all, by the modulatory compound.

Phosphoamino acid analysis of EF-1 from *Artemia* and wheat germ showed only phosphoserine in the absence of polylysine. A small amount of threonine was observed in the factor from wheat germ with polylysine, but was less than 10% of the total phosphate incorporated. With reticulocyte EF-1, which can be phosphorylated by casein kinase II only in the presence of polylysine, approximately equal amounts of phosphoserine and phosphothreonine were observed.

Table I

Phosphorylation of EF-1 by casein kinase II in the presence and absence of polylysine

EF-1	^{32}P Incorporated into EF-1		
	− Polylysine (cpm)	+ Polylysine (cpm)	Stimulation (-fold)
EF-1 rabbit Reticulocytes	490	11648	23.8
EF-1 *Artemia salina*	5047	4765	0.9
EF-1$\beta\gamma$ *Artemia salina*	3591	6595	1.8
EF-1$\beta\gamma$ Wheat germ	15510	18590	1.2

Following phosphorylation of EF-1 by casein kinase II, the phosphorylated subunit was excised from the gel and the radiolabel was quantified by scintillation counting.

4. DISCUSSION

The phosphorylation of a single subunit (M_r 26 000) of EF-1 and EF-1$\beta\gamma$ from *Artemia salina* by casein

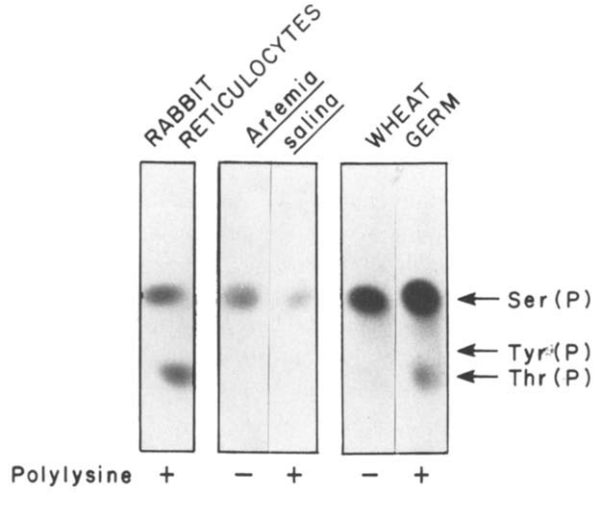

Fig. 2. Phosphoamino acid analysis of EF-1 phosphorylated by casein kinase II in the presence or absence of polylysine. The phosphorylated subunit of EF-1 was excised from the polyacrylamide gel and subjected to phosphoamino acid analysis as described in section 2.

kinase II confirms the results obtained by Janssen and Möller [10] using EF-1$\beta\gamma$ containing traces of a casein kinase II-like enzyme. This subunit is modified on serine 89 [10], in a sequence which agrees with the consensus sequence for casein kinase II [11]. Casein kinase II also modifies a single subunit in wheat germ (M_r 36 000). One subunit of EF-1 (M_r 33 000) from reticulocytes is also phosphorylated by casein kinase II, but only in the presence of a modulatory compound, such as polylysine. A similar requirement was observed with EF-1 $\beta\gamma$ from reticulocytes and liver (data not shown). Polylysine has little or no effect on phosphorylation of EF-1 from the other species. With *Artemia* and wheat germ, the primary phosphorylated amino acid is serine. With reticulocytes, both serine and threonine are observed in approximately equal amounts.

The observation that a single subunit from each species is modified by casein kinase II leads us to suggest that the M_r 26 000 subunit of *Artemia* EF-1 (denoted as β in reference [10]) is equivalent to the M_r 33 000 subunit from reticulocytes (denoted as δ in reference [7]) and the wheat germ polypeptide M_r 36 000 [9]. This subunit would correspond to the low M_r subunit of EF-1 (Table II) which, along with the higher M_r subunit, catalyzes the exchange of GDP bound to EF-1α for GTP. At this time, sequence data have been determined only for the *Artemia* factor subunit [21]. Phosphorylation at serine 89 in *Artemia* has been reported by Janssen et al. [10] to decrease the EF-1$\beta\gamma$-dependent guanine nucleotide exchange rate with α.

It is interesting that an effector molecule is required for phosphorylation by casein kinase II only with the purified factor from mammals. Although polylysine is not a physiological activator, other effector compounds could carry out this role in vivo. Other studies (Palen, E., Venema, R.C. and Traugh, J.A., unpublished), have shown that the low M_r subunit of EF-1 is phosphorylated in reticulocytes in vivo at the same site(s) modified in vitro by casein kinase II, as shown by two-dimensional phosphopeptide mapping. The requirement for modulation of substrates prior to phosphorylation has been observed recently with another component of protein synthesis, the cap binding protein, which can be isolated either alone (eIF-4E) or as the p25 subunit of the cap binding protein complex, eIF-4F. The cap binding protein is phosphorylated at a significant rate by protein kinase C only when present in the eIF-4F complex [22]. The polylysine requirement for phosphorylation of the low M_r subunit of EF-1 from reticulocytes suggests that complexation of EF-1 with a modulatory compound is required for phosphorylation in vivo.

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Table II
Comparison of phosphorylated subunits of EF-1 from three different species

Rabbit reticulocytes	Wheat germ ($M_r \times 10^{-3}$)	*Artemia salina*
52 (α)	52 (α)	50 (α)
48 (β)	47	46 (γ)
38 (γ)		
33 (δ)	36, 34	26 (β)

Subunits phosphorylated by casein kinase II are underlined. Values were taken from [7] for reticulocytes, from [9] for wheat germ and [5] for *Artemia salina*. Letters in parentheses denote the nomenclature used in the original paper.

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