Eukaryotic elongation factor 2 loses its non-specific affinity for RNA and leaves polyribosomes as a result of ADP-ribosylation

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ADP-ribosylation of rabbit reticulocyte elongation factor 2 (EF-2) catalyzed by the A fragment of diphtheria toxin leads to a loss of its non-specific affinity for RNA. The removal of the ADP-ribose residue from EF-2 in the reverse reaction with nicotinamide restores its affinity for RNA. ADP-ribosylation of EF-2 is accompanied by its dissociation from the complexes with mono- and polyribosomes detected in the rabbit reticulocyte lysate at low ionic strength. The loss of the non-specific affinity of EF-2 for RNA as a result of ADP-ribosylation and, as a consequence, its decompartmentation from polyribosomes is assumed to be a reason for the diphtheria toxin-induced inactivation of the factor in eukaryotic cells.

Elongation factor 2 *ADP-ribosylation* *RNA-binding activity* *Protein synthesis regulation* *Compartmentation-decompartmentation*

1. INTRODUCTION

It is known that almost all the proteins of the eukaryotic translation machinery, including aminoacyl-tRNA-synthetases [1,2], elongation factors [3–6] and most of the initiation factors [4,7–9], possess a sufficiently strong non-specific affinity for different high molecular mass RNAs, i.e., they are RNA-binding proteins. Prokaryotic aminoacyl-tRNA-synthetases [1] and the elongation factors [6] do not possess such an affinity. It was assumed that the evolutionary acquired RNA-binding capability of eukaryotic proteins of the translation machinery serves for their compartmentation (local concentration) on the polyribosomes to provide for their more efficient functioning in the large volume of the eukaryotic cell [10].

Indeed, aminoacyl-tRNA-synthetases [11], elongation factors 1 [12] and 2 [13] were found among the proteins loosely associated with polyribosomes. It has been shown that aminoacyl-tRNA-synthetases [14] and EF-1 [12] can be displaced from polyribosomes by an excess of exogenous RNA, i.e., their association with polyribosomes seems to occur due to their non-specific RNA-binding capability.

Modulation of the affinity for RNA of the translation machinery proteins could be a means of the regulation of protein synthesis in eukaryotic cells [2,15]. Some observations may be relevant to this assumption. First, eukaryotic aminoacyl-tRNA-synthetases for several amino acids exist in two forms – the RNA-binding one and that without affinity for RNA [2]. Second, phosphorylation of RNA-binding proteins can change their affinity for RNA [16].

One of the best known modifications of the translation machinery proteins is ADP-ribosylation of elongation factor 2 by diphtheria toxin. This modification leads to inhibition of protein synthesis [17,18]. The mechanism of such an inhibition is not yet known, inasmuch as all the partial functions of EF-2 studied are retained after its ADP-ribosylation (review [17]).

Here, we show that ADP-ribosylation of EF-2 is

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accompanied by a loss of its non-specific affinity for high molecular mass RNA and its dissociation from the complexes with mono- and polyribosomes.

2. MATERIALS AND METHODS

The elongation factor 2 with a purity of no less than 90% was prepared from rabbit reticulocytes as in [19]. The diphtheria toxin was kindly provided by Drs Vertiev and Ezepchuk (Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow).

The ADP-ribosylation of EF-2 was done using a minor modification of the technique in [20]. 200–500-μl samples were incubated with 1–5 μg diphtheria toxin and 1 pmol nicotinamide [U-^{14}C]adeninedinucleotide (265 μCi/mmol, Amersham) in 20 mM Tris–HCl buffer (pH 8.3) with 10 mM DTT for 10 min at 30°C. [^{14}C]ADP-ribosyl-elongation factor 2 (ADPR-EF-2) was precipitated with 5% trichloroacetic acid and collected on GF/C (Whatman) filters to count radioactivity.

The de-ADP-ribosylation of EF-2 was performed by incubating with 1 μg diphtheria toxin and 20 mM nicotinamide in 50 mM potassium acetate buffer, pH 5.2, with 10 mM DTT for 30 min at 37°C [21].

Affinity chromatography of EF-2 on RNA-Sepharose 4B (with total *Escherichia coli* rRNA) was done as described in [22]. The protein was determined by amido black staining technique on nitrocellulose filters [22,23].

The activity of EF-2 was measured in the poly(U)-dependent system of poly-Phe synthesis [24].

The fraction of mono- and polyribosomes of rabbit reticulocytes was prepared by gel filtration of mitochondria-free extract through a Sephacryl S-300 column (Pharmacia).

3. RESULTS

When the EF-2 preparation is applied to the RNA–Sepharose column both the protein material and EF-2 activity are completely adsorbed on the column at low ionic strength and eluted with 1 M KCl (fig.1A). This result correlates with the earlier observations on the affinity of EF-2 for RNA [4].

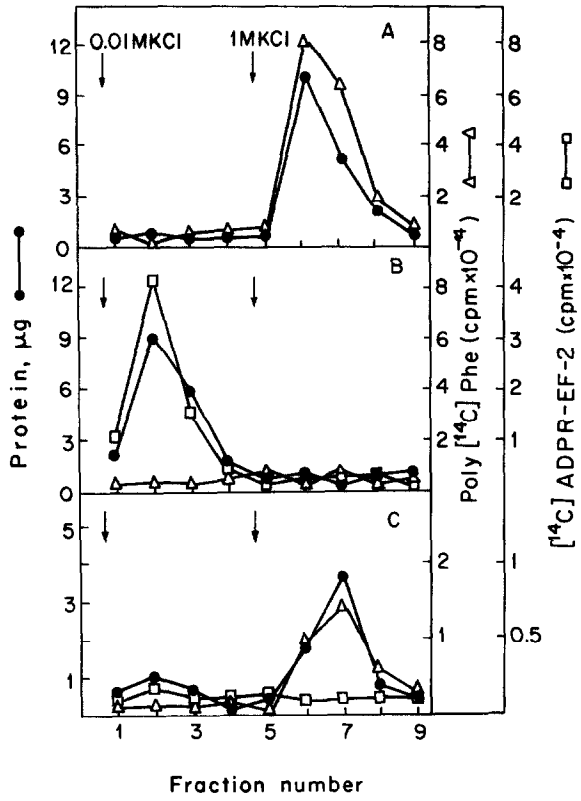

Fig.1. Chromatography of EF-2 (A), [^{14}C]ADP-ribosylated EF-2 (B) and EF-2 after de-ADP-ribosylation (C) on the RNA–Sepharose column. The preparation of EF-2 in the buffer containing 10 mM Tris–HCl (pH 7.6), 10 mM KCl, 0.2 mM EDTA, 7 mM β-mercaptoethanol and 10% glycerol was applied onto the RNA–Sepharose column, equilibrated with the same buffer. The protein adsorbed was eluted with 1 M KCl buffer solution.

After ADP-ribosylation EF-2 loses its non-specific affinity for RNA and is not adsorbed on RNA–Sepharose (fig.1B).

Incubation of [^{14}C]ADPR-EF-2 with nicotinamide in conditions of de-ADP-ribosylation restores activity of the factor and its capability to be adsorbed on RNA–Sepharose (fig.1C). The small amount of the protein that has passed through the column without being adsorbed, in this case may represent diphtheria toxin or [^{14}C]ADPR-EF-2 which has not undergone demodification.

It can be concluded that ADP-ribosylation of elongation factor 2 results in a loss of its transla-

tion activity and a simultaneous loss of its non-specific affinity for RNA, whereas demodification restores both functions.

In subsequent experiments we studied the effect of ADP-ribosylation on the association of endogenous EF-2 with mono- and polyribosomes in rabbit reticulocyte extracts. Mono- and polyribosomes were separated from free proteins by gel filtration of the mitochondria-free extract through Sephacryl S-300. It turned out that about 60% of EF-2 passes through Sephacryl together with mono- and polyribosomes if Sephacryl is equilibrated with a buffer of low ionic strength (preparation no.1). If Sephacryl is equilibrated with a buffer containing 150 mM KCl, only 10–15% of EF-2 is detected in the fraction of mono- and polyribosomes (preparation no.2). Centrifugation of the preparations of mono- and polyribosomes in the sucrose gradient prepared in a buffer without KCl has demonstrated that about a half of EF-2 of preparation no.1 sediments in the zone of mono- and polyribosomes (fig.2A). All EF-2 of preparation no.2 is found to be associated with monoribosomes (fig.2B).

The addition of an excess of exogenous RNA (*E. coli* 16 S rRNA) to the preparation of mono- and polyribosomes before centrifugation shifts all EF-2 from polyribosomes to the post-polyribosomal zone. At the same time, the factor remains associated with monoribosomes (fig.2C,D).

From the above results it can be concluded that a significant portion of EF-2 in the rabbit reticulocyte extract is associated with mono- and polyribosomes. Complexes of EF-2 with polyribosomes are labile and reversible, but they can be stabilized by lowering the ionic strength of the solution. Complexes of EF-2 with monoribosomes are sufficiently more stable. The loose association of EF-2 with polyribosomes may be explained by the interaction of its RNA-binding center with the RNA of polyribosomes.

Fig.2E,F shows the sedimentation distribution of EF-2 in mono- and polyribosome preparations after its ADP-ribosylation. It is seen that ADP-ribosylation of EF-2 in such preparations leads to a dissociation of the factor from complexes both with poly- and monoribosomes.

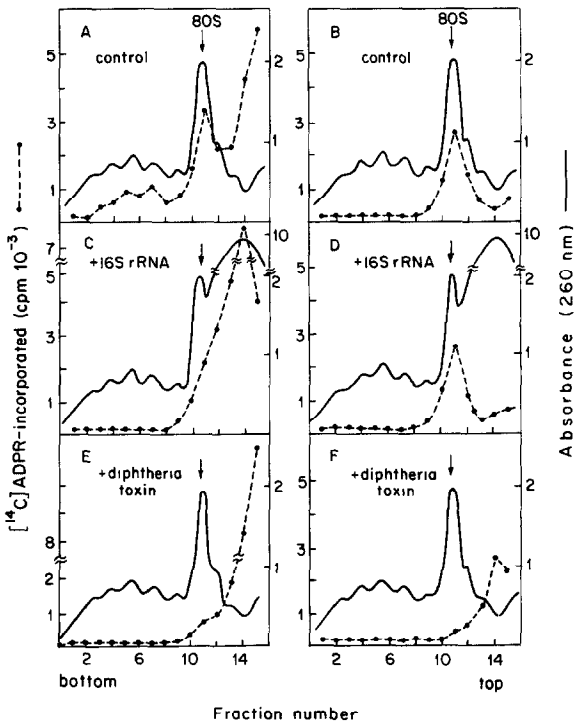

Fig.2. Sedimentation distribution of EF-2 upon sucrose gradient centrifugation of the fraction of mono- and polyribosomes. The preparation of mono- and polyribosomes was isolated by gel filtration through Sephacryl S-300 equilibrated with a buffer without KCl (10 mM Tris–HCl, (pH 7.6), 1 mM $MgCl_2$) (A,C,E), or with the same buffer containing 150 mM KCl (B,D,F). A,B, the mono/polyribosome fraction as such was layered on sucrose gradient; C,D, 20 A_{260} units of *E. coli* 16 S RNA was added to the mono/polyribosome fraction before centrifugation; A–D, the EF-2 distribution was traced by the reaction of ADP-ribosylation with [^{14}C]NAD after centrifugation; E,F, the mono/polyribosome fraction was [^{14}C]ADP-ribosylated before centrifugation and then layered on sucrose gradient. Centrifugation was performed in 15–33.5% sucrose gradient in the buffer without KCl, SW-41 rotor, 40000 rpm, 45 min (Spinco L5-50).

4. DISCUSSION

Up to the present it is unclear how ADP-ribosylation of EF-2 disengages protein synthesis. A comparison of intact and ADP-ribosylated EF-2 in partial reactions, occurring in the process of translocation, has shown that the ADP-ribosylated factor binds with GTP [25,26], forms an

EF-2·GTP·ribosome complex [26,27] and possesses GTPase activity in such a complex [28,29]. Finally, ADP-ribosylated EF-2 can, in principle, perform translocation when it is added to ribosomes in stoichiometric amounts [30].

On the other hand, as far back as 1971, authors in [3] mentioned that ADP-ribosylation of EF-2 leads to a loss of its affinity for RNA. However, no experimental data validating this conclusion were given and no subsequent publications devoted to the inactivation mechanism of EF-2 as a result of ADP-ribosylation discussed this fact.

Our results have shown that ADP-ribosylation of EF-2 indeed leads to a loss of its non-specific affinity for RNA, and to its release from complexes with poly- and monoribosomes. The non-specific RNA-binding capability seems to be the only function of EF-2 which is known by now to be definitely lost in response to ADP-ribosylation.

From the results obtained the RNA-binding capability of EF-2 can be assumed to serve for its interaction with the exposed regions of polyribosomal RNA (mRNA and/or rRNA) and the formation of loose complexes, thus leading to a local concentration of the factor near the sites of translation, i.e., to its partial compartmentation on polyribosomes. ADP-ribosylation of EF-2, inducing the loss of its non-specific affinity for RNA, leads to its dilution throughout the volume of the cytoplasm, i.e., to its decompartmentation. The drastic decrease of the effective concentration of the factor near polyribosomes may result in an abrupt drop of the protein synthesis rate. Anyhow, the proposed mechanism of protein synthesis inhibition, as a result of ADP-ribosylation of EF-2, does not contradict the available experimental facts.

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