Endogenous ADP-ribosylation of elongation factor 2 in polyribosome fraction of rabbit reticulocytes

A.S. Sitikov, E.K. Davydova and L.P. Ovchinnikov

Institute of Protein Research, Academy of Sciences of the USSR, 142292 Pushchino, Moscow Region, USSR

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Several polypeptides of about 120, 96, 85, 60 and 38 kDa are shown to be radiolabeled during incubation of the mono- and polyribosome fraction of rabbit reticulocytes with [^{32}P]NAD. Among them is a polypeptide coinciding with elongation factor 2 (EF-2) in its electrophoretic mobility in SDS-polyacrylamide gel. The addition of pure EF-2 to the polyribosome fraction results in an increase of the radioactive label in this polypeptide band. From this it is concluded that both endogenous and added EF-2 is ADP-ribosylated by an enzyme associated with polyribosomes. A possibility of regulation of protein synthesis through endogenous ADP-ribosylation in vivo is considered.

ADP-ribosylation *Elongation factor 2* *Polyribosome* *Rabbit reticulocyte* *Protein biosynthesis regulation*

1. INTRODUCTION

Post-translational modifications of proteins play an important role in the regulation of many cellular processes. It has been accepted that regulation of such processes as DNA replication [1] and repair [2], cell differentiation [3] and others, involves poly- and mono (ADP-ribosyl)ation of proteins. Authors in [4] found an endogenous ADP-ribosyltransferase modifying the components of the adenylate cyclase system, and thus inducing stimulation of cAMP synthesis [4]. ADP-ribosyltransferase activities have been shown to be associated with ribosomes and polyribosomes [5] and mRNPs [6].

This paper reports that an ADP-ribosyltransferase modifying EF-2 is present among the proteins associated with rabbit reticulocyte polyribosomes.

2. MATERIALS AND METHODS

Reticulocytes were prepared from blood of rabbits injected subcutaneously with a phenylhydrazine solution as in [7]. The cells were lysed with an equal volume of 5 mM $MgCl_2$ and the lysate was clarified from cellular debris and mitochondria by centrifugation at 12000 rpm for 15 min in JA-14 rotor of the J-21B (Beckman) centrifuge.

The polyribosome fraction was obtained by gel filtration of the mitochondria-free extract through a Sephacryl S-300 column equilibrated with a buffer containing 10 mM Tris–HCl (pH 7.6), 1 mM $MgCl_2$. The preparation contained about 90% ribosomes and less than 0.1% hemoglobin of the initial extract.

Rabbit reticulocyte EF-2 was isolated as in [8].

ADP-ribosylation was performed in 40 μl of 50 mM K-phosphate buffer (pH 7.0) containing 0.5 mM $MgCl_2$, 2 μCi [^{32}P]NAD (Amersham, spec. act. 5 Ci/mmol), 1.0 A_{260} unit (about 60 μg protein) of the polyribosome fraction or 20 μg EF-2, or both. The reaction mixture was incubated for 15 min at 37°C.

Gradient polyacrylamide gel (10–22% acrylamide) electrophoresis of the samples was carried out in the presence of SDS [9]. The gels were dried and exposed for 5 days at −70°C using RM-V film (Tasma, USSR).

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3. RESULTS

The radioautograph of the polyribosome fraction incubated with [^{32}P]NAD reveals several polypeptides with molecular masses of about 38, 60, 85, 96 and 120 kDa which incorporated the radioactive label (fig.1A). The band of the 96-kDa polypeptide coincides with that of EF-2.

An addition of a purified EF-2 to the incubation mixture with [^{32}P]NAD and the polyribosome fraction results in the increase of the label incorporation into the 96-kDa polypeptide band, which becomes the main radiolabeled component (fig.1B). At the same time, the control incubation of the EF-2 with [^{32}P]NAD does not lead to a noticeable incorporation of the label into the protein (fig.1C). Hence, it is the polyribosome fraction that contains an enzymic activity responsible for the ADP-ribosylation of EF-2.

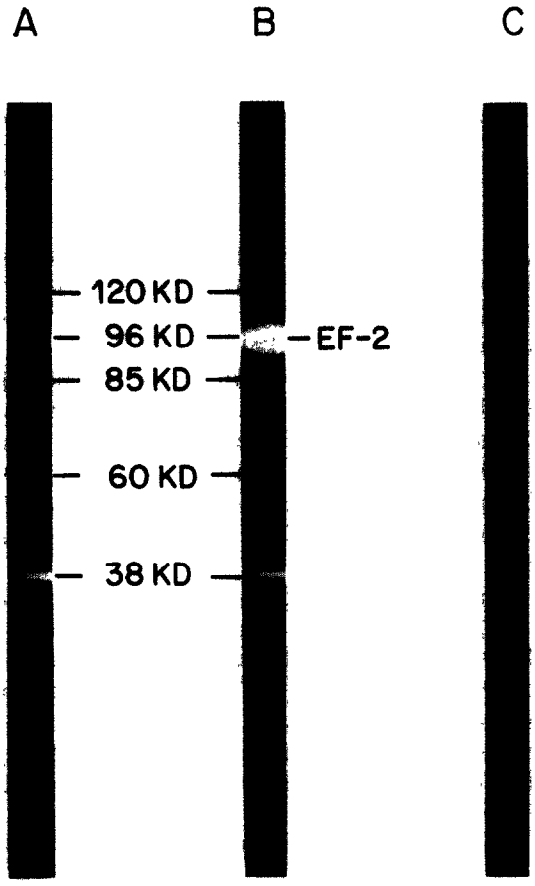

Fig.1. Radioautograph showing ADP-ribosylation of proteins in the polyribosome fraction (A), in EF-2 preparation (C) and in their mixture (B). The samples were prepared and analysed as described in section 2. KD, kDa.

4. DISCUSSION

It is known that EF-2 is a specific target of diphtheria toxin [10]. The A-fragment of diphtheria toxin ADP ribosylates EF-2, using NAD as a donor of ADP-ribose. The unique ADP-ribose attachment site in EF-2 is diphthamide [11]. ADP-ribosylation of EF-2 leads to its functional inactivation.

It has been shown [12] that diphthamide is a result of post-translational modification of histidine. Cultures of hamster ovary cells were obtained which were resistant to diphtheria toxin. Among them were clones without mutations in the EF-2 structural gene but with mutations in the system of modifying enzymes. In the cells of these clones EF-2 contained non-modified histidine instead of diphthamide and this explained their resistance to diphtheria toxin. Nonetheless, the cells functioned normally.

It seems that the presence of diphthamide is not strictly required for the translational activity of EF-2. It is more likely that diphthamide is a site of regulatory modifications of EF-2.

Here we have shown that ADP-ribosyltransferase activity modifying EF-2 is present in rabbit reticulocytes among the proteins associated with polyribosomes. It may be that, as in the case with diphtheria toxin, dipthamide of EF-2 is the acceptor of ADP-ribose. Such a modification of EF-2 could have an important regulatory function, in particular in its partial decompartmentation from polyribosomes [13].

While preparing our paper, authors in [14] published a paper where they showed ADP-ribosylation of EF-2 at the diphthamide residue by an endogenous ADP-ribosyltransferase present in the EF-2 preparations from polyoma virus-transformed baby hamster kidney cells. Our results differ from those in [14]: (i) We did not succeed in revealing noticeable ADP-ribosyltransferase activity in rabbit reticulocyte EF-2 preparations (~95% purity); (ii) ADP-ribosyltransferase activity in rabbit reticulocyte mono- and polyribosome fractions is several orders of magnitude lower than the cor-

responding activity in EF-2 preparations from transformed hamster cells.

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