Volume 283, number 2, 235–238 FEBS 09766 June 1991

ADONIS 001457939100475G

Cellular mono(ADP-ribosyl) transferase inhibits protein synthesis

Wallace J. Iglewski and Stephen Dewhurst

Department of Microbiology and Immunology, University of Rochester Medical Center, 601 Elmwood Avenue, Box 672, Rochester, NY 14642, USA

Received 20 March 1991

A reticulocyte translation system was depleted of functional EF-2 by treatment with diphtheria toxin (DT) fragment A and NAD. After dialysis to remove NAD, the system was reconstituted using preparations of EF-2 derived from pyBHK cells. Untreated and reconstituted lysates permitted similar rates of translation. As expected, when DT-treated EF-2 was used to reconstitute the system, no translation occurred. Furthermore EF-2, reacting with the endogenous ADP-ribosyl transferase from pyBHK cells, was also unable to restore protein synthesis in the reconstituted system. These studies suggest that eukaryotic cellular ADP-ribosyl transferases may play a role in regulating protien synthesis.

ADP-ribosyl transferase; EF-2; Protein synthesis; Diphtheria toxin; PyBHK cell

1. INTRODUCTION

Diphtheria toxin (DT) and *Pseudomonas* exotoxin kill cells by catalyzing the specific ADP-ribosylation of eukaryotic elongation factor 2 (EF-2) at a modified histidine residue, termed diphthamide [1], thereby functionally inactivating the molecule and inhibiting protein synthesis. Cellular mono (ADP-ribosyl) transferases with similar activity have been isolated from various sources including polyoma virus transformed baby hamster kidney (pyBHK) cells [2,3], turkey erythrocytes [4] and bovine liver [5]. In this report we show that preparations of EF-2 from pyBHK cells, which are modified either by diphtheria toxin or by the endogenous ADP-ribosyl transferase, are no longer functional in an in vitro translation system. These results suggest that cellular ADP-ribosyl transferase may play a role in regulating translation.

2. MATERIALS AND METHODS

2.1. *Preparation and quantitation of EF-2*

EF-2 was isolated from pyBHK cells as described [2,3] and quantitated by the procedure of Gill and Dinius [6]. Briefly, 10 μl of EF-2 was reacted with 0.05 μg of DT fragment A for 20 min at 22°C in 0.1 ml (containing 6.33 μM [U-^{14}C-*adenosine*]-NAD (554 mCi/mmol; NEN), 1 mM dithiothreitol and 25 mM Tris-HCl (pH 8.0)). Trichloroacetic acid (TCA)-precipitable radioactivity was collected on Millipore filters and counted by liquid scintillation. EF-2 content of reticulocyte lysates was determined similarly, except lysates were pretreated with 0.5 M NaCl to free EF-2 from ribosomes, and adsorbed with charcoal (Norit SG) to remove endogenous NAD, and a histamine-containing buffer was used [6].

Correspondence address: W.J. Iglewski, Department of Microbiology and Immunology, University of Rochester Medical Center, 601 Elmwood Avenue, Box 672, Rochester, NY 14642, USA. Fax: (1) (716) 473 9573

2.2. *Modification of EF-2*

Aliquots of EF-2 were modified by addition of either endogenous ADP-ribosyl transferase or 0.05 μg of DT fragment A to 0.1 ml reaction volume (containing 25 mM Tris-HCl (pH 8.0), 1 mM dithiothreitol and 100 μg EF-2 ± NAD or [^{14}C-*adenosine*]-NAD) and were incubated at 22°C, until maximum transfer of radiolabel to a TCA-precipitable form had occurred. Reaction mixtures were then dialyzed extensively against buffer A [7] at 0°C.

2.3. *In vitro translation system*

Rabbit reticulocyte lysates were prepared as described [8]. Aliquots were treated with 37 μg/ml DT fragment A [9] and 50 μg/ml NAD at 20°C for 15 min to inactivate endogenous EF-2. Portions of this inactivated lysate were then dialyzed extensively against de-ionized water at 0°C to remove NAD, and dialyzed lysates were reconstituted by addition of EF-2 (see above). Translation reaction mixture was aliquoted from a stock composed of 800 μl lysate, 10 μl of 10 mg/ml creatine phosphokinase, 50 μl of 0.3 M creatine phosphate in 10 mM Tris-HCl (pH 7.5), 50 μl of 2 M KCl, 100 μl of the 19 essential amino acids at 0.5 mM each, 6 μl of 4 mM hemin, 10 μl of 400 mM MgCl and 6 μl of 200 mM dithiothreitol. EF-2, ADP-ribosylated EF-2 or NAD were added as needed, and reactions were incubated at 30°C for 3 min, chilled to 0°C and then completed by addition of 5 μl of [^{3}H]leucine (56.5 Ci/mmol; NEN), prior to incubation at 30°C. Incorporation of radiolabel into protein was measured by liquid scintillation counting of TCA-precipitable material.

3. RESULTS

Reticulocyte lysates were determined to contain 90 μg/ml endogenous EF-2, which was reduced to an effective concentration of 2.7 μg/ml after treatment with DT fragment A and NAD. Following dialysis, the cell-free translation system was reconstituted by addition of pyBHK EF-2. Residual EF-2 in the fragment A-treated system accounts for the low level of protein synthesis that is observed in the absence of exogenous

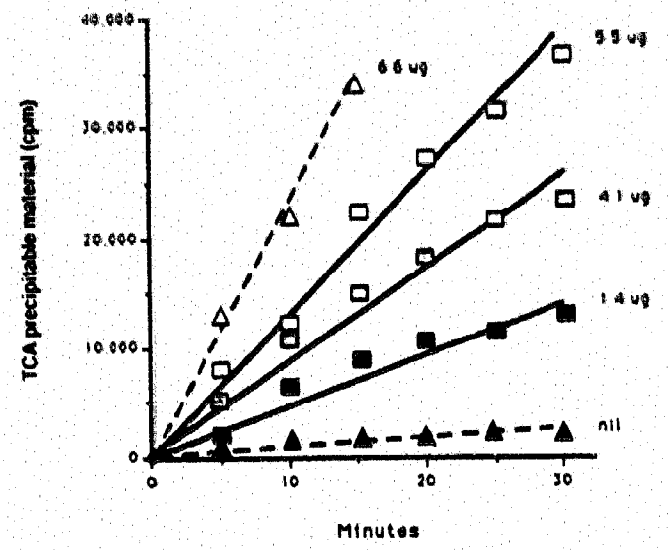


Fig. 1. Reconstitution of the reticulocyte translation system with indicated amounts of exogenous pyBHK-derived EF-2 (lysates were depleted of endogenous EF-2; reaction volume = 0.1 ml; see section 2). Data are averages from at least two experiments.

EF-2, while addition of increasing amounts of pyBHK EF-2 results in progressive augmentation of protein synthesis (Fig. 1). At an exogenous EF-2 concentration of 68 μg/ml the rate of protein synthesis in the reconstituted system is equivalent to that of the untreated reticulocyte lysate (data not shown).

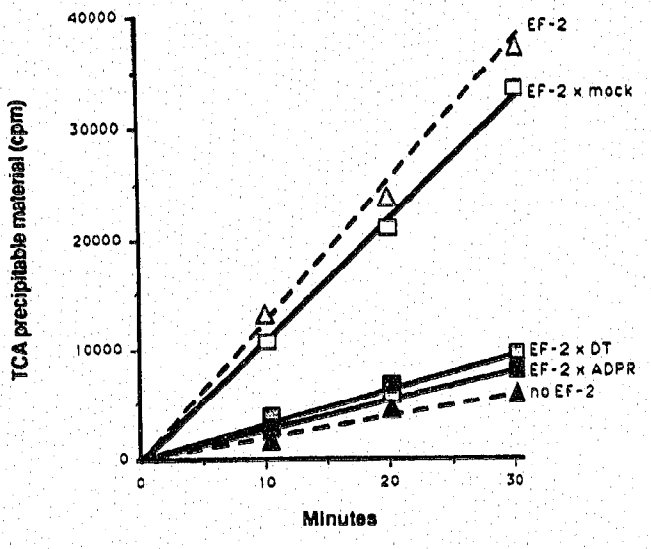


Fig. 2. Reconstitution of the reticulocyte translation system by addition of exogenous EF-2 (55 μg/ml), either untreated (EF-2), mock-treated (EF-2 × mock) or modified using diphtheria toxin (EF-2 × DT) or the pyBHK-derived cellular ADP-ribosyl transferase (EF-2 × ADPR). Data are averages from at least two experiments.

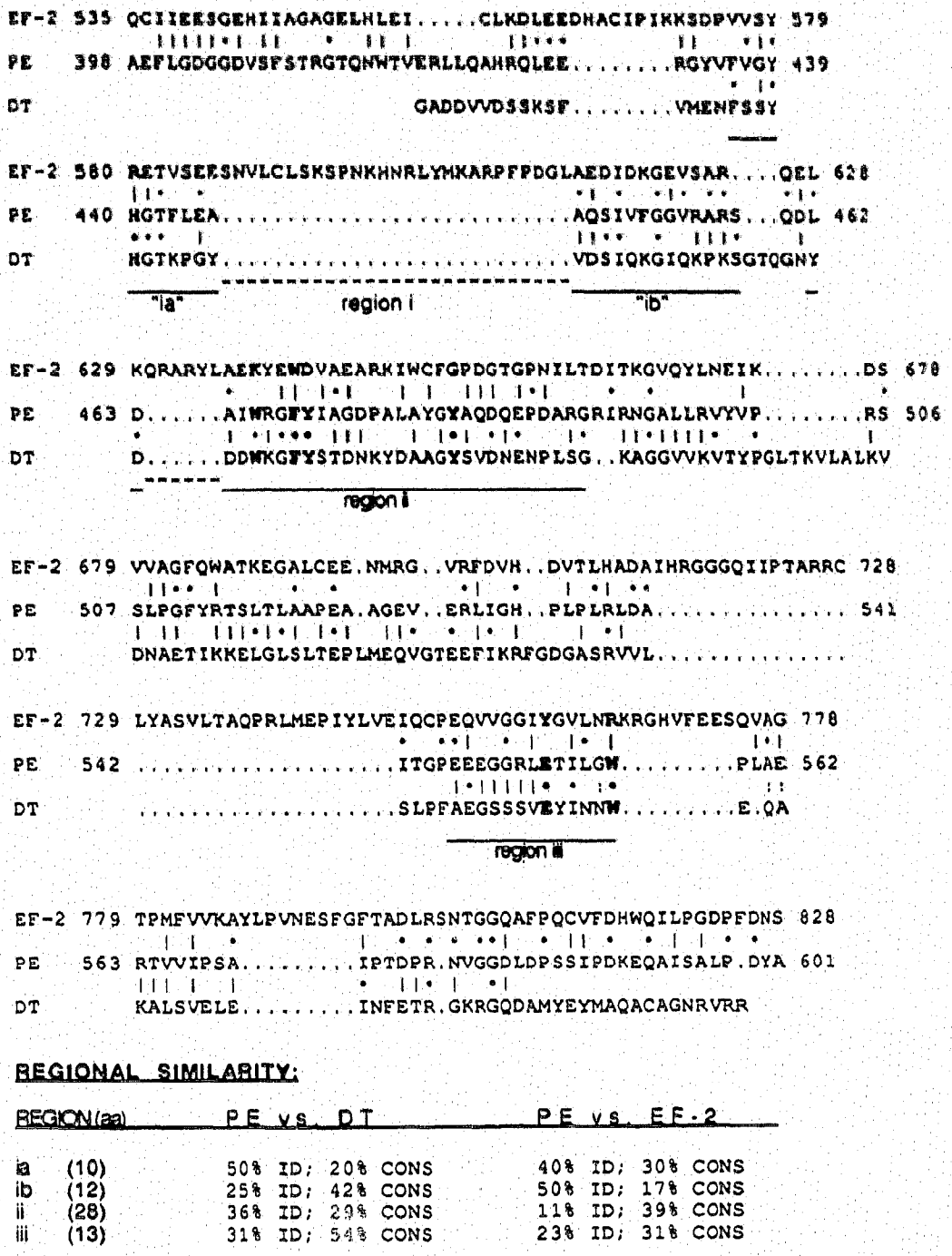
PyBHK EF-2 was modified in vitro by the endogenous ADP ribosyl transferase or by DT fragment A in the presence of [^{14}C-*adenosine*]-NAD. This enabled us to determine the reaction kinetics for the two enzymes and thereby to ascertain the incubation time required for complete modification of EF-2. The ability of ADP-ribosylated EF-2 to reconstitute the fragment A-treated translation system was then examined. As expected, ADP-ribosylation of EF-2 by DT fragment A abolished its ability to reconstitute the cell-free protein synthesis system. In addition, EF-2 modified by the endogenous ADP-ribosyl transferase was also inactive in the in vitro system, whereas mock-treated EF-2 (reacting with transferase in the absence of NAD) retained its functionality (Fig. 2).

4. DISCUSSION

We have previously identified an endogenous ADP-ribosyl transferase in mammalian cells which is able to modify the same diphthamide residue of EF-2 that is affected by bacterial proteins such as *Pseudomonas* exotoxin and diphtheria toxin [2,3]. We now provide evidence that the cellular enzyme not only ADP-ribosylates EF-2, but that it also leads to functional inactivation of the molecule, thereby inhibiting protein synthesis.

There is strong biochemical evidence to suggest that the EF-2 molecule itself contains an ADP-ribosyl transferase activity, which can be activated during purification of the protein in vitro. We believe that this is probably due to conformational changes in the protein, since we expect ADP-ribosyl transferase activity to be under stringent control in vivo. Interestingly, there is significant similarity between the enzymic domains of the bacterial toxins [10,11] and mammalian EF-2 (Fig. 3). We are currently investigating the possibility that carboxy terminal subfragments of EF-2 may contain an active enzymic site. The data presented suggest that auto-ADP-ribosylation of EF-2 may represent an important control mechanism in eukaryotic cells, perhaps regulating peptide chain elongation (e.g. during the translocation of proteins across the endoplasmic reticulum [12]).

Finally, the potential toxicity of an endogenous ADP-ribosyl transferase could be exploited in anti-tumor or anti-viral drugs. *Pseudomonas* exotoxin (PE), for example, has been fused to the cellular ligand CD4, to produce a hybrid PE-CD4 protein which possesses potent cytotoxicity for HIV-infected cells [13]. However, PE-CD4 is highly immunogenic and antibodies against the protein are likely to interfere with its efficacy in vivo [14]. In contrast, a hybrid toxin based on the cellular enzyme would be expected to be far less immunogenic and thus considerably more effective.



REGIONAL SIMILARITY:

REGION(aa)		PE vs. DT	PE vs. EF-2
ia	(10)	50% ID; 20% CONS	40% ID; 30% CONS
ib	(12)	25% ID; 42% CONS	50% ID; 17% CONS
ii	(28)	36% ID; 29% CONS	11% ID; 39% CONS
iii	(13)	31% ID; 54% CONS	23% ID; 31% CONS

Fig. 3. Amino-acid similarity between the enzymic domains of *Pseudomonas* exotoxin (PE) and diphtheria toxin (DT), and the carboxyl terminal half of human EF-2, showing identical (·, ID) and conservatively substituted residues (|, CONS). Experimentally identified active site residues in PE and DT are indicated in bold [10,11]; regions (i)–(iii) were identified by Collier [10]. Sequence analysis was performed with GCG software [15], using the algorithm of Needleman and Wunsch [16].

Acknowledgement: We thank P. Muller for excellent technical assistance.

REFERENCES

[1] Iglewski, B.H., Liu, P.V. and Kabat, D. (1977) Infect. Immun. 15, 138–144.
[2] Lee, H. and Iglewski, W.J. (1984) Proc. Natl. Acad. Sci. USA 81, 2703–2707.
[3] Fendrick, J.L. and Iglewski, W.J. (1989) Proc. Natl. Acad. Sci. USA 86, 554–557.
[4] Moss, J. and Vaughn, M. (1978) Proc. Natl. Acad. Sci. USA 75, 3621–3624.
[5] Iglewski, W.J., Lee, H. and Muller, P. (1984) FEBS Lett. 173, 113–118.

[6] Gill, D.M. and Dinius, L.L. (1973) J. Biol. Chem. 248, 654–658.
[7] Sokol, P.A., Iglewski, B.H., Hager, T.A., Sadoff, J.C., Cross, A.S., McManus, A., Farber, B.F. and Iglewski, W.J. (1981) Infect. Immun. 34, 147–153.
[8] Pelham, H.R.B. and Jackson, R.J. (1976) Eur. J. Biochem. 134, 237–240.
[9] Pappenheimer Jr., A.M., Uchida, T. and Avery-Harper, A. (1972) Immunochemistry 9, 891–906.
[10] Carroll, S.F. and Collier, R.J. (1988) Mol. Microbiol. 2, 293–296.
[11] Domenighini, M., Montecucco, C., Ripka, W.C. and Rappuoli, R. (1991) Mol. Microbiol. 5, 23–31.
[12] Iglewski, W.J. and Fendrick, J.L. (1990) In: ADP-Ribosylating Toxins and G Proteins: Insights into Signal Transduction (Moss, J. and Vaughn, M. eds) pp. 511–524, ASM, Washington, DC.
[13] Chaudhary, V.K., Mizukami, T., Fuerst, T.R., Fitzgerald, D.J., Moss, B., Pastan, I. and Berger, E.A. (1988) Nature 335, 369–372.
[14] Palca, J. (1989) Science 246, 1559–1560.
[15] Devereux, J., Haeberli, P. and Smithies, O. (1984) Nucleic Acids Res. 12, 387–395.
[16] Needleman, S.B. and Wunsch, C.D. (1970) J. Mol. Biol. 48, 443–453.