ACIDIC PROTEINS FROM WHEAT GERM RIBOSOMES

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Received 4 May 1979

1. Introduction

Certain acidic proteins from both prokaryotic and eukaryotic ribosomes are involved in various steps of polypeptide synthesis. In prokaryotes, a number of studies have shown that protein S1 directly participates in translation of synthetic and natural mRNAs at the levels of initiation [1–4] and elongation [5]. Other than S1, acidic proteins from bacterial ribosomes whose function has already been determined are proteins L7 and L12 that are involved in polypeptide chain elongation (reviewed [6]).

The functions of acidic proteins from eukaryotic ribosomes have so far been much less investigated than their counterparts from prokaryotes. It is now believed, however, that at least some eukaryotic acidic proteins are functionally related to their prokaryotic homologs. Thus, functional and immunochemical crossreactivity has been demonstrated between *E. coli* proteins L7 and L12 and two acidic ribosomal proteins from rat liver [7], yeast [8], brine shrimp *Artemia salina* [9,10], Krebs II ascite cells [11] as well as from chicken liver [12]. These findings suggest that despite considerable structural differences between prokaryotic and eukaryotic ribosomes, some of their acidic proteins are highly conservative functionally.

Here we present a preliminary characterization of acidic proteins, from wheat germ ribosomes, which so far have not been described.

2. Methods

Ribosomes and ribosomal subunits from wheat germ were prepared as in [13]. Acidic proteins were extracted from the ribosomes by salt–ethanol treatment as in [14]. After removal of the ribosomal cores the acidic proteins were precipitated with 2.25 vol. acetone at −20°C, dissolved in 50 mM potassium acetate buffer (pH 3.8) containing 5 mM 2-mercaptoethanol and dialyzed overnight against this buffer.

Two-dimensional polyacrylamide gel electrophoresis was performed in 6 M urea according to [15] except that the second dimension gel contained 16% acrylamide. SDS–polyacrylamide gels were prepared as in [16]. The molecular weights of acidic proteins were calculated using cytochrome *c* (12 400), myoglobin (17 800), chymotrypsinogen (25 000), ovalbumin (45 000) and bovine serum albumin (67 000) as the standards.

The binding of [^{3}H]poly(U) to acidic proteins was performed in 20 mM Tris–HCl buffer (pH 7.8) containing 15 mM magnesium acetate and 25 mM KCl. The radioactive complex was detected on Millipore filters (HAWPO 2500) pre-treated with 0.5 N KOH.

Poly(U)-directed polyphenylalanine synthesis was measured in the incubation mixture containing in 50 μl final vol.: 20 mM Hepes–KOH buffer (pH 7.6), 9 mM magnesium acetate, 100 mM potassium acetate, 2 mM ATP, 0.4 mM GTP, 4 mM phosphoenolopyruvate, 1 mM dithiotreitol, 1 μCi [^{14}C]phenylalanine (330 mCi/mmol), 7 μg wheat germ tRNA, 10–50 μg poly(U), 20 μl S-23 fraction from wheat germ and 2–10 μg acidic proteins. The mixture was incubated for 30 min at 30°C. Amino acid incorporation was determined by measuring the radioactivity retained on Whatman 3 MM paper discs after washing with hot and cold trichloroacetic acid and ethanol.

Translation of TMV-RNA was performed in 50 μl

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Elsevier/North-Holland Biomedical Press

final vol. which contained: 20 mM Hepes–KOH buffer (pH 7.6), 3 mM magnesium acetate, 100 mM potassium acetate, 2.5 mM ATP, 0.4 mM GTP, 4 mM phosphoenolopyruvate, 1 mM dithiotreitol, 0.5 mM spermidine, 30 mM each of 20 amino acids, 1 μCi [^{14}C]leucine (290 mCi/mmol), 20 μl wheat germ S-23 fraction, 3–18 μg TMV-RNA and 2–10 μg acidic proteins. Samples were incubated at 30°C for 60 min and the radioactivity was measured as above.

3. Results and discussion

We have already demonstrated that there are 3 acidic proteins in the 40 S wheat germ ribosomal subunit and 7 acidic proteins in the 60 S subunit [13]. These proteins were designated: S7, S13, S26, L2, L4, L6, L8, L11, L16 and L18. The same acidic proteins were obtained from undissociated 80 S ribosomes except protein L18 which was not visible on the electrophoretogram (fig.1A). In this case, however, at least 3 additional proteins are visible which were not present when the separated subunits were investigated. These additional spots may represent proteins tightly bound to intact 80 S particles that are removed during the dissociation of ribosomes into subunits.

Using preparative polyacrylamide gel electrophoresis under denaturing conditions the individual acidic proteins from wheat germ ribosomes were isolated and their molecular weights determined. As is seen from fig.1B, the proteins migrated on the SDS–polyacrylamide gels as single spots, except protein L6 which revealed 2 distinct bands. The apparent molecular weights of the individual acidic proteins are presented in the legend to fig.1. They represent a heterogenous size-group of proteins with molecular weights varying between 10 000 (protein S7) and 68 400 (protein L2).

An important feature of acidic proteins from eukaryotic ribosomes is their susceptibility to phosphorylation. It has already been shown that proteins S13, L2, L8, L11, L16 can be phosphorylated in vitro by yeast protein kinase [13]. Our present experiments indicate that some of the acidic proteins are phosphorylated in vivo, since we have noticed a change in their isoelectric point after digestion with alkaline phosphatase (unpublished observation). These

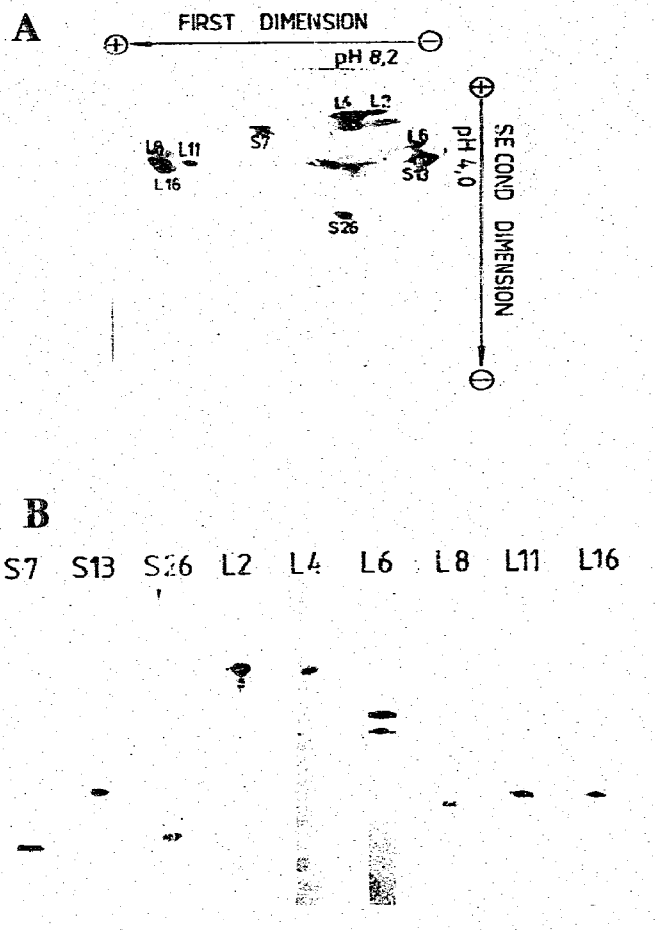

Fig.1. Polyacrylamide gel electrophoresis of acidic proteins from wheat germ 80 S ribosomes. (A) Two-dimensional gel electrophoresis of the total fraction of acidic proteins according to [15]. (B) SDS gel electrophoresis of the isolated acidic proteins. Electrophoresis was carried out in 17.5% polyacrylamide gels according to [16]. Proteins were stained with Coomassie brillant blue R-250. The apparent molecular weights of individual acidic proteins were estimated to be as follows: S7, 10 000; S13, 18 400; S26, 11 400; L2, 68 400; L4, 63 800; L6, 39 400 (34 300); L8, 16 000; L11, 17 200; L16, 17 000.

data are consistent with those published earlier for mammalian acidic ribosomal proteins [17–19].

Similarly, as with bacterial protein S1 [4], the acidic ribosomal proteins from wheat germ have a capacity to form complex (complexes) with [^{3}H]poly-(U) (fig.2). The complex formation was at 25 mM K^+ and not dependent upon Mg^{2+} at $\leqslant$ 20 mM magnesium acetate (data not shown) which is consistent with earlier data for bacterial protein S1 [4].

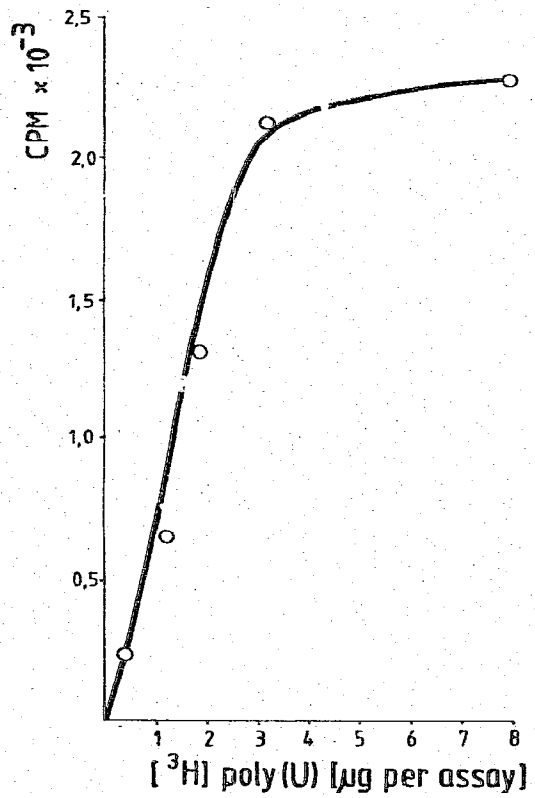

Fig.2. Binding of [^{3}H]poly(U) to acidic proteins from the 80 S wheat germ ribosomes. The reaction was carried out at 37°C for 30 min in the presence of 25 µg acidic proteins. Various amounts of [^{3}H]poly(U) (50 mCi/mmol) were added as indicated. The mixtures were filtered through alkali-treated Millipore filters and counted in a toluene-based scintillator solution.

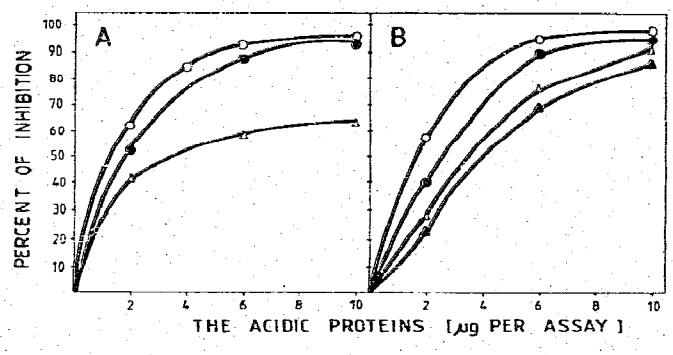

Fig.3. Inhibition of poly(U) and TMV-RNA translation as a function of the amount of acidic proteins added. Assay conditions in an unfractionated wheat germ system are as in section 2. (A) Inhibition of the poly(U)-directed polyphenylalanine synthesis by increasing amounts of acidic ribosomal proteins in the presence of: 10 µg (○——○), 20 µg (●——●) and 50 µg (△——△) poly(U), respectively, per assay. (B) Inhibition of the TMV-RNA translation by the increasing amounts of acidic proteins in the presence of: 3 µg (○——○), 6 µg (●——●) 12 µg (△——△) and 18 µg (▲——▲) TMV-RNA, respectively, per assay.

At the present stage of this investigation it is difficult to estimate the stoichiometry of such a binding when a group of heterogenously sized proteins and poly(U) of unknown chain length were used.

It has been revealed recently that some acidic proteins from eukaryotic ribosomes, and in particular 2 counterparts of bacterial L7/L12 proteins, are involved directly in polypeptide synthesis [9–11]. We therefore decided to investigate the effect of acidic proteins from wheat germ ribosomes on the translation of poly(U) and TMV-RNA in homologous cell-free system. Figure 4A,B show that the acidic proteins strongly inhibit both polyphenylalanine synthesis and translation of TMV-RNA. Moreover, the extent of the inhibition of either poly(U) or TMV-RNA translation was a function of mRNA concentration. As the amount of poly(U) or TMV-RNA was increased, the inhibition by acidic proteins decreased (e.g., for 6 µg acidic proteins from 93% with 10 µg poly(U) to 57% with 50 µg poly(U) per assay; and, respectively, from 95% with 3 µg TMV-RNA to 68% with 18 µg TMV-RNA per assay) (fig.3A,B). Thus, the inhibitory effect of ribosomal acidic proteins was dependent on the mRNA/protein ratio which may suggest that they interact with messenger RNA at a preribosomal level. It should be mentioned that the above experiments were performed at optimal Mg^{2+} concentrations, 9 mM for poly(U) and 3 mM Mg^{2+} for TMV-RNA, in addition to 0.5 mM spermidine in the latter case. The inhibitory effect of acidic proteins was tested also at different Mg^{2+} concentrations, and it was found that the observed inhibition does not depend upon any specific Mg^{2+} requirement (data not shown).

To find out if the observed inhibition could be related to the initiation phase of polypeptide synthesis, we performed an experiment in which the acidic proteins were added prior to incubation and after 20 min of incubation of the TMV-RNA-directed system (fig.4). The acidic proteins added at 0 time considerably decreased the initial rate of polypeptide synthesis, with 2 µg proteins by ~ 50% over 15 min incubation. However, proteins added after 20 min

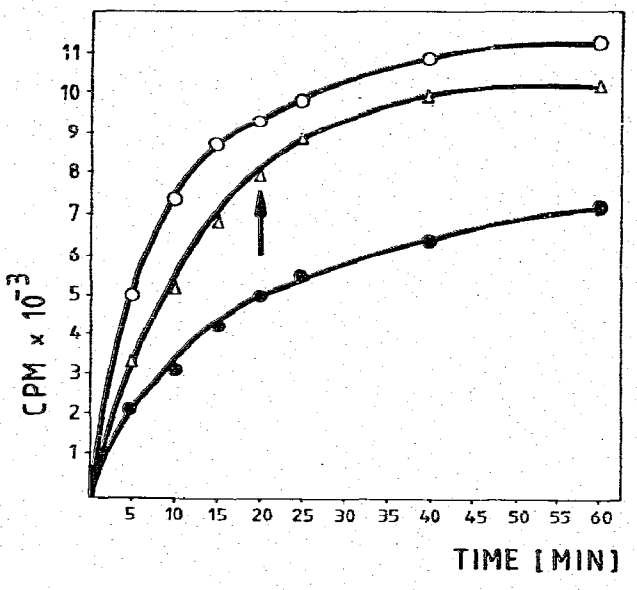

Fig.4. Time kinetics of TMV-RNA translation in the presence of acidic ribosomal proteins. For each curve, an initial incubation mixture of 50 μl containing 6 μg TMV-RNA was employed. The reaction was started by the addition of the wheat germ S23 fraction and 5 μl aliquots were withdrawn at the times indicated. The [^{14}C]leucine incorporation was determined as in section 2. (o——o) Control TMV-RNA translation; (•——•) translation in the presence of acidic proteins (2 μg/assay) added at time 0; (△——△) translation in the presence of acidic proteins (2 μg/assay) added at 20 min. The blank in the absence of TMV-RNA was subtracted from each value.

incubation caused only a slight inhibition of amino acid incorporation — ~10% during the subsequent 15 min. The observed inhibition of amino acid incorporation by acidic proteins added prior to the reaction but not after 20 min of incubation, can be related to the initiation phase of polypeptide synthesis.

The above results indicate that acidic ribosomal proteins added exogenously can modify the efficiency of mRNA translation perhaps at the level of initiation. This suggestion, however, should be confirmed by more detailed studies using the individual acidic proteins. The important question which remains to be answered is the identity of that protein (proteins) which is (are) directly involved in the observed inhibition.

Acknowledgements

This investigation was carried out as project 09.7. of the Polish Academy of Sciences.

References

[1] Van Dieijen, G., Van Der Laken, C. J., Van Knippenberg, P. H. and Van Duin, J. (1975) J. Mol. Biol. 93, 351–366.
[2] Miller, M. J. and Wahba, A. J. (1974) J. Biol. Chem. 249, 3808–3813.
[3] Dahlberg, A. E. and Dahlberg, J. E. (1975) Proc. Natl. Acad. Sci. USA 72, 2940–2944.
[4] Lipecky, R., Kohlschein, J. and Gassen, H. G. (1977) Nucl. Acids Res. 4, 3627–3642.
[5] Linde, R., Quoc Khanh, N., Lipecky, R. and Gassen, H. G. (1979) Eur. J. Biochem. 93, 565–572.
[6] Möller, W. (1974) in: Ribosomes (Nomura, M. et al. eds) pp. 711–731, Cold Spring Harbor Press, NY.
[7] Stöffler, G., Wool, I. G., Lin, A. and Rak, K. H. (1974) Proc. Natl. Acad. Sci. USA 71, 4723–4726.
[8] Richter, D. and Möller, W. (1974) in: Lipmann Symposium: Energy, Regulation and Biosynthesis in Molecular Biology, (Richter, D. ed) pp. 524–533, Walter de Gruyter, Berlin.
[9] Möller, W., Slobin, L. I., Amons, R. and Richter, D. (1975) Proc. Natl. Acad. Sci. USA 72, 4744–4748.
[10] Van Agthoven, A. J., Maassen, J. A. and Möller, W. (1977) Biochem. Biophys. Res. Commun. 77, 989–998.
[11] Leader, D. P. and Coia, A. A. (1978) Biochem. J. 176, 569–572.
[12] Howard, G. A., Smith, R. L. and Gordon, J. (1976) J. Mol. Biol. 106, 623–637.
[13] Sikorski, M. M., Przybył, D., Kudlicki, W., Zając, J., Borkowski, T., Gąsior, E. and Legocki, A. B. (1979) Plant Sci. Lett. in press.
[14] Hamel, E., Koka, M. and Nakamoto, T. (1972) J. Biol. Chem. 247, 805–814.
[15] Kaltschmidt, E. and Wittmann, H. G. (1974) Anal. Biochem. 36, 401–412.
[16] Laemmli, U. K. (1970) Nature 227, 680–682.
[17] Zinker, S. and Warner, J. R. (1976) J. Biol. Chem. 251, 1799–1807.
[18] Leader, D. P. and Coia, A. A. (1977) Biochem. J. 162, 199–200.
[19] Horak, I. and Schiffmann, D. (1977) Europ. J. Biochem. 79, 375–380.