CHARACTERIZATION OF ACIDIC 60 S RIBOSOMAL PROTEINS IN *TETRAHYMENA PYRIFORMIS*

Jes SANDERMANN, Anna KRÜGER and Karsten KRISTIANSEN
Department of Molecular Biology, University of Odense, Campusvej 55, DK-5230 Odense M, Denmark

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1. Introduction

The ribosomes of eukaryotes contain acidic proteins that functionally [1–5] and antigenetically [3,6,7] are related to the *Escherichia coli* ribosomal proteins L7/L12. In contrast to their prokaryotic counterparts some of these eukaryotic acidic proteins are subject to phosphorylation in vivo [4,7–15]. Generally, it appears that eukaryotic ribosomes contain two structurally related but distinct acidic proteins each of which may occur in phosphorylated forms [7,11,13,15].

The 60 S ribosomal subunit of *Tetrahymena pyriformis* contains a triplet of acidic protein (L44–46) which according to electrophoretic properties, stainability and molecular weights resembles the putative L7/L12 equivalents of other eukaryotes [16]. However, our previous attempts to demonstrate phosphorylation in vivo of these *T. pyriformis* ribosomal proteins have been uniformly negative [16,17]. Since the L7/L12 equivalents of eukaryotic ribosomes, like their prokaryotic counterparts, seem to be intimately involved in at least elongation factor 1 and 2 dependent reactions [1–4], the possible complete lack of phosphorylation of these proteins in *T. pyriformis* was of interest both from a phylogenetic and a functional point of view. Here we present a more detailed analysis of the complement of acidic ribosomal proteins in *T. pyriformis*. The results indicate that the ribosomal proteins that electrophoretically resemble the putative L7/L12 equivalent of other eukaryotes are not phosphorylated in vivo.

Address correspondence to: Karsten Kristiansen

2. Materials and methods

2.1. *Cell cultures*

Tetrahymena pyriformis, strain GL, was grown axenically in a proteose–peptone/yeast extract medium [18]. Labelling of ribosomal proteins was achieved by adding 2 μCi/ml of a [^{14}C]protein hydrolyzate to the medium.

2.2. *Isolation of ribosomes and extraction of total ribosomal proteins*

Ribosomes were isolated, their proteins extracted with acetic acid and prepared for gel electrophoresis as in [18].

2.3. *Dephosphorylation of ribosomal proteins with alkaline phosphatase*

80 S ribosomes were dissociated into subunits, treated with alkaline phosphatase, and the ribosomal proteins extracted as in [19].

2.4. *Fractionation of acidic ribosomal proteins*

Ribosomes (2500 A_{260} units) were extracted with ethanol/NH_4Cl as in [20]. The extracted ribosomal proteins (P1 proteins) were passed through a column of Sephadex G-25 fine. Elution was with 10% acidic acid and the desalted P1 proteins were lyophilized. The P1 proteins were further fractionated by isoelectric focussing in a LKB 8100 ampholine electrofocussing equipment (110 ml column). The pH gradient was established in 6 M urea (BDH, Aristar) with 1% ampholine ranging from pH 4–6. The focussing was performed at 4°C with the cathode at the top of the column. The initial 600 V was maintained for

18 h followed by 1000 V for 28 h. The column was emptied at 90 ml/h flowrate and the effluent monitored continuously at 254 nm. Fractions (1 ml) were collected and their pH was measured with a glass electrode at 4°C. Appropriate fractions were pooled and the proteins freed of ampholines by passage through a column of Sephadex G-25 fine eluted with 10% acetic acid. The proteins were recovered by lyophilization.

2.5. *Polyacrylamide gel electrophoresis*

Ribosomal proteins were analyzed by two-dimensional polyacrylamide gel electrophoresis according to Kaltschmidt and Wittmann as in [18]. In addition, the acidic ribosomal proteins were analyzed by one-dimensional gel electrophoresis by the procedure in [21]. Autoradiograms were prepared as in [17].

3. Results

3.1. *Treatment of ribosomes with alkaline phosphatase*

The two-dimensional pattern of the *T. pyriformis* ribosomal proteins L44–46 is very similar to those observed for the HeLa cell ribosomal protein L40 [11], the rat liver ribosomal protein P2 [13] and their respective phosphorylated derivatives. In principle, our previous failure to detect phosphorylation of L44–46 in vivo could be due to insufficient incorporation of [^{32}P]phosphate. To test this possibility dissociated ribosomes were treated with alkaline phosphatase under conditions that were found to cause extensive dephosphorylation of phosphorylated S6 [19]. Dephosphorylation of ribosomal proteins is easily detectable by changes in their electrophoretic properties. However, the treatment with alkaline phosphatase had no effect on L44–46 as evaluated by two-dimensional polyacrylamide gel electrophoresis (fig. 1).

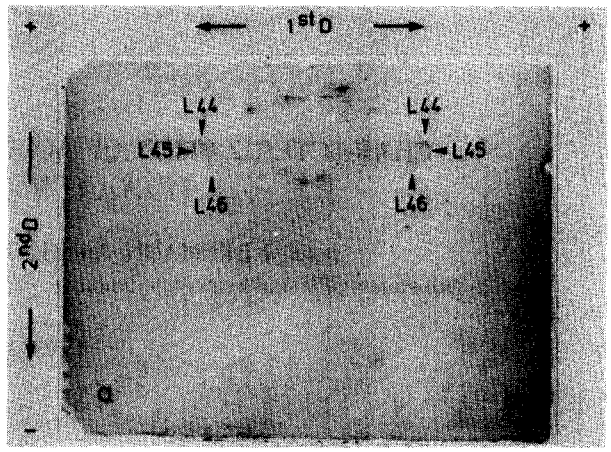

Fig. 1. Two-dimensional polyacrylamide gel electrophoresis of *T. pyriformis* acidic ribosomal proteins, untreated (a) and treated (b) with alkaline phosphatase. Dissociated 80 S ribosomes were treated with 0.5 mg/ml alkaline phosphatase for 2 h at 28°C. Samples (400 μg) of the extracted ribosomal proteins were used for electrophoresis according to Kaltschmidt and Wittmann. The anodic sides of the first dimensional gels were arranged symmetrically for electrophoresis in the second dimension. For nomenclature of ribosomal proteins see [16].

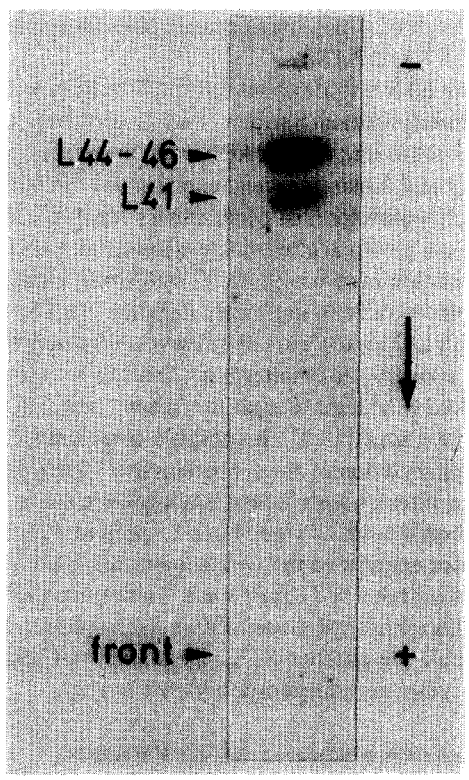

Fig. 2. One-dimensional polyacrylamide gel electrophoresis of ^{14}C-labelled ribosomal proteins. Ribosomal protein (200 μg) was subjected to electrophoresis in an 8% acrylamide slab gel at pH 6.0 according to [21]. The gel was dried and autoradiographed as in [17].

3.2. *One-dimensional polyacrylamide gel electrophoresis of acidic ribosomal proteins*

Like most eukaryotic acidic ribosomal proteins the acidic ribosomal proteins of *T. pyriformis* stain very poorly. During our previous analyses of *T. pyriformis* ribosomal proteins we might, therefore, have overlooked or lost the putative L7/L12 equivalents. To overcome these problems, ^{14}C-labelled ribosomal proteins were analyzed by one-dimensional polyacrylamide gel electrophoresis by the procedure in [21]. Neither the Coomassie blue stained gels (results not shown) nor the autoradiograms (fig. 2) indicated the presence of acidic ribosomal proteins in addition to those already catalogued [16].

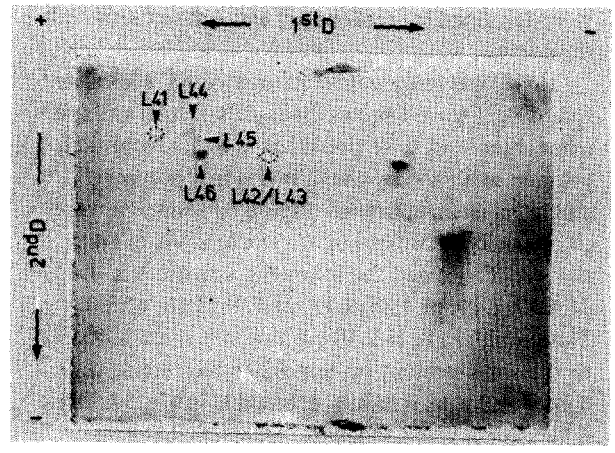

Fig. 3. Two-dimensional polyacrylamide gel electrophoresis of P1 proteins extracted from *T. pyriformis* ribosomes. A P1 protein fraction extracted from 40 A_{260} units of ribosomes was analyzed.

3.3. *Preparation and fractionation of P1 proteins*

To obtain greater quantities of the acidic ribosomal proteins for their characterization a P1 protein fraction was prepared. In addition to the acidic ribosomal proteins L41–46 this fraction also contained a number of basic ribosomal proteins (fig. 3). Similar results

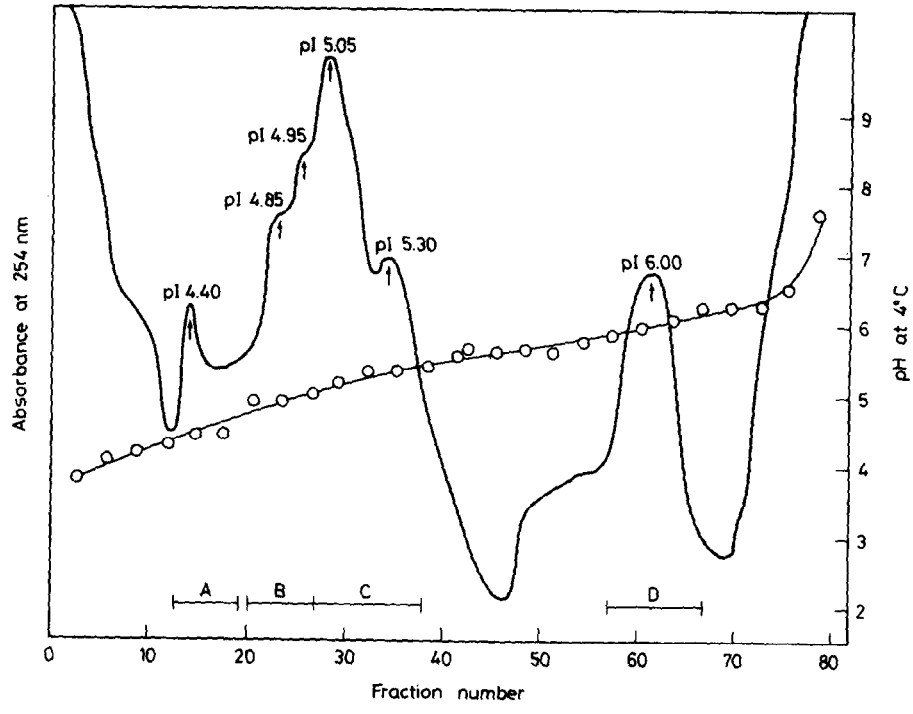

Fig. 4. Isoelectric focussing of P1 proteins extracted from *T. pyriformis* ribosomes. P1 proteins extracted from 2500 A_{260} units of ribosomes were focussed in a pH 4–6 gradient of ampholine in a 110 ml LKB 8100 electrofocussing equipment. The column was emptied with continuous recording of the A_{254}. Fractions (1 ml) were collected and pooled as indicated. (——), A_{254}; (o–o), pH at 4°C.

were described for rat liver [5] and *Artemia salina* [15] ribosomes.

The P1 proteins were fractionated by isoelectric focussing. Figure 4 shows the results of such an experiment. Fractions were pooled as indicated and their proteins analyzed by two-dimensional polyacrylamide gel electrophoresis. Figure 5a shows the results of the analysis of fraction C. The analysis of fractions A–C allowed the following assignments: L41, pI 4.40; L44, pI 4.85; L45, pI 4.95; L46, pI 5.05. The shoulder at pH 5.30 corresponds most probably to the small subunit protein S29 which stains very lightly and thus is hardly detectable in fig. 5a. Two-dimensional polyacrylamide gel electrophoresis analysis showed fraction D to be an aggregate of L41 and L45 (fig. 5b). We have in total performed 4 isoelectric focussing experiments and in all observed a distinct peak around pH 6. Thus it appears that aggregation of L41 and L45 occurs regularly in the P1 protein fraction.

In accordance with the analysis by one-dimensional polyacrylamide gel electrophoresis, isoelectric focussing of P1 proteins did not lead to the detection of additional acidic ribosomal proteins.

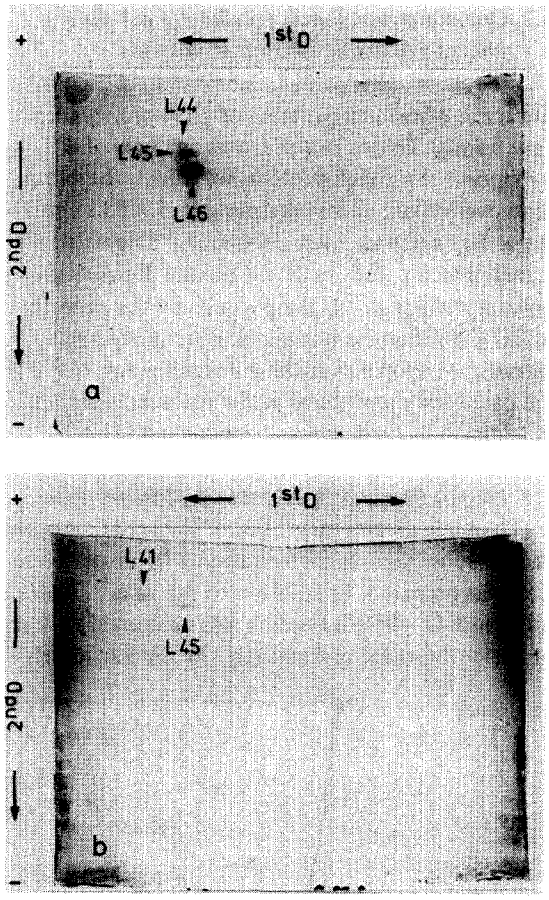

Fig. 5. Two-dimensional polyacrylamide gel electrophoresis of ribosomal proteins fractionated by isoelectric focussing. (a) Proteins from fraction C, fig.4. About 50 μg was used for the analysis. (b) Proteins from fraction D, fig. 4. About 25 μg was used for the analysis.

4. Discussion

In common with other eukaryotes (and *E. coli*) the ribosomes of *T. pyriformis* contain a complement of acidic proteins that are detached by NH_4Cl/ethanol treatment (fig. 3). These proteins are necessary for the activity of *T. pyriformis* ribosomes in a poly(U)-dependent cell-free system and, furthermore, they are capable of restoring the polyphenylalanine synthesizing capacity of *E. coli* ribosomal core particles lacking L7/L12 (K. K., unpublished). Thus, *T. pyriformis* ribosomes contain proteins homologous to L7/L12.

Our results show that the 60 S subunit of *T. pyriformis* ribosomes contains 4 proteins (L41, L44–46) possessing isoelectric points comparable to those of *E. coli* L7/L12. Estimated from dodecyl sulphate gels L41 has mol. wt 26 300, whereas L44–46 have mol. wt 15 000–15 500 (results to be presented elsewhere). The phosphorylated putative L7/L12 equivalents in other eukaryotes have molecular weights similar to L44–46 [2,7–11,14]. In contrast, the rat liver ribosomal proteins L40 and L41 that share antigenic determinants with L7/L12 [6], but apparently are not phosphorylated in vivo, have mol. wt 25 500 [22] which is conspicuously close to our estimate of 26 300 for L41.

The isoelectric points of L44 (pI 4.85), L45 (pI 4.95) and L46 (pI 5.05) are higher than those reported for the presumably analogous ribosomal proteins eL12 (pI ~ 4.5) of *A. salina* [2] and L_γ (pI ~ 4.40–4.45) of mouse ascites cells [12]. Indeed, L44–46 are slightly less acidic than *E. coli* L7/L12 (pI 4.70, 4.85 [23]). The complement of acidic ribosomal proteins from different strains of *Tetrahymena* was analyzed by one-dimensional

polyacrylamide gel electrophoresis [24]. A protein, designated Lδ, appears to be common to all the strains so far analyzed (D. H. Hayes, personal communication). Lδ most probably corresponds to our triplet proteins L44–46, which also in our one-dimensional analysis fail to resolve into three bands (fig. 2). In agreement with our determination of isoelectric points, Lδ appears slightly less acidic than *E. coli* L7/L12 [24]. Also in accordance with our results a more acidic ribosomal protein, designated L_ζ, was detected [24]. This protein corresponds to our L41. The isoelectric point of L41 (pI 4.40) is similar to those of eL12 and L_γ, but as noted above, its molecular weight is ~2-times greater than that of eL12 and L_γ.

The absence of detectable [^{32}P]phosphate incorporation into L44–46 [16,17] combined with the findings presented in this report that treatment with alkaline phosphatase does not alter the electrophoretic mobilities of L44–46 (fig. 1) makes it highly unlikely that these proteins are phosphorylated in vivo in *T. pyriformis*. Thus, it is not obvious how L41 and L44–46 are related to *E. coli* L7/L12 and their equivalents in other eukaryotes. It is intriguing that L41 forms an aggregate with L45 (fig. 5b). In solution the *A. salina* protein eL12/eL12-P occurs mainly as a dimer [15] like the *E. coli* proteins L7/L12 [23]. Similarly, aggregation of the putative L7/L12 equivalents in other eukaryotes has also been observed [12]. We are considering the possibility that a precursor–product relationship exists between L41 and L44–46. L41 being a protein that evolutionary arised by an intragenic duplication and thus contains two regions showing extensive sequence homology.

Acknowledgements

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References

[1] 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.
[2] Möller, W., Slobin, L. I., Amons, R. and Richter, D. (1975) Proc. Natl. Acad. Sci. USA 72, 4744–4748.
[3] Howard, G. A., Smith, R. L. and Gordon, J. (1976) J. Mol. Biol. 106, 623–637.
[4] Van Agthoven, A. J., Maassen, J. A. and Möller, W. (1977) Biochem. Biophys. Res. Commun. 77, 989–998.
[5] Reyes, R., Vazquez, D. and Ballesta, J. P. G. (1977) Eur. J. Biochem. 73, 25–31.
[6] Stöffler, G., Wool, I. G., Lin, A. and Rak, K.-H. (1974) Proc. Natl. Acad. Sci. USA 71, 4723–4726.
[7] Leader, D. P. and Coia, A. A. (1978) Biochem. J. 176, 569–572.
[8] Zinker, S. and Warner, J. R. (1976) J. Biol. Chem. 251, 1799–1807.
[9] Leader, D. P. and Coia, A. A. (1977) Biochem. J. 162, 199–200.
[10] Issinger, O.-G. (1977) Biochim. Biophys. Acta 477, 185–189.
[11] Horak, I. and Schiffmann, D. (1977) Eur. J. Biochem. 79, 375–380.
[12] Leader, D. P. and Coia, A. A. (1978) Biochim. Biophys. Acta 519, 213–223.
[13] Tsurugi, K., Collatz, E., Todokoro, K., Ulbrich, N., Lightfoot, H. N. and Wool, I. G. (1978) J. Biol. Chem. 253, 946–955.
[14] Arpin, M., Madjar, J.-J. and Reboud, J.-P. (1978) Biochim. Biophys. Acta 519, 537–541.
[15] Van Agthoven, A., Kriek, J., Amons, R. and Möller, W. (1978) Eur. J. Biochem. 91, 553–565.
[16] Kristiansen, K. and Krüger, A. (1978) Biochim. Biophys. Acta 521, 435–451.
[17] Kristiansen, K., Plesner, P. and Krüger, A. (1978) Eur. J. Biochem. 83, 395–403.
[18] Kristiansen, K., Hartmann, B., Plesner, P. and Krüger, A. (1978) Eur. J. Biochem. 83, 389–394.
[19] Kristiansen, K. and Krüger, A. (1979) Exp. Cell Res. 118, 159–169.
[20] Hamel, E., Koka, M. and Nakamoto, T. (1972) J. Biol. Chem. 247, 805–814.
[21] Li, K. and Subramanian, A. R. (1975) Anal. Biochem. 64, 121–129.
[22] Lin, A. and Wool, I. G. (1974) Mol. Gen. Genet. 134, 1–6.
[23] Möller, W., Groene, A., Terhorst, C. and Amons, R. (1972) Eur. J. Biochem. 25, 5–12.
[24] Cuny, M., Milet, M. and Hayes, D. H. (1979) FEBS Lett. 101, 77–84.