Arch. Biol. Med. Exper. *12*:367-372, 1979

# The proteins of the cytoplasmic ribosomes of *Euglena gracilis*

## Las proteínas de los ribosomas citoplásmicos de *Euglena gracilis*

C.C. VASQUEZ and G. MORA

(Recibido para publicación el 30 de julio de 1977)

Instituto de Ciencias Biológicas, Departamento de Biología Celular Laboratorio Microbiología e Inmunología, Universidad Católica de Chile, Santiago, Chile

VÁSQUEZ, C.C., MORA, G. The proteins of the cytoplasmic ribosomes of *Euglena gracilis*. (Las proteínas de los ribosomas citoplásmicos de *Euglena gracilis*) Arch. Biol. Med. Exper. *12:* 367-372, 1979.

Two types of cytoplasmic ribosomes of *Euglena gracilis* have been found when the stability *in vitro* of the large subunit at 25°C for 30 min has been considered.

Sucrose density gradient analysis have revealed that the large subunit of ribosomes obtained from cells harvested in the stationary phase of growth was degraded when heated at 25°C. The large subunit of ribosomes obtained from exponentially growin cells was stable in the same experimental conditions.

Studies of the ribosomal RNAs have not explained this differential stability observed *in vitro*. In fact, after SDS-extraction, the largest RNA species always appears degraded in both types of ribosomes. Therefore, an explanation to this phenomenon have been searched for through the analysis of the ribosomal proteins.

One and two dimensional gel electrophoresis techniques have been employed. At least one difference between the two ribosomal protein groups has been observed and this could be associated to the differential stability of the ribosomes.

Approximately 72 protein molecules have been found in Euglena's cytoplasmic ribosome. About 42 proteins belong to the large subunit and 30 to the small one.

Three kinds of ribosomes have been isolated from *Euglena gracilis* cells. They have been found in the cytoplasm, chloroplasts and mitochondria and they have been characterized by their sedimentation coefficients (1).

We have studied the cytoplasmic ribosomes which have a sedimentation coefficients of 87S for the monosome and 65S and 45S for the large and small subunit respectively (2).

Previous experiments carried out in this laboratory have demonstrated that the cytoplasmic ribosomes can be classified into two groups when their stability *in vitro* has been considered.

The subunits of the ribosomes obtained from exponentially growing cells have remained intact after incubation at 25°C for 30 min (6). Therefore, they have been called "stable ribosomes". On the other hand, the large subunit of ribosomes obtained from cells harvested in the stationary step of growth has appeared degraded after the incubation mentioned above.

These ribosomes have been called "unstable". The degradation of the large subunit has been observed by sucrose density gradient analysis.

The presence of a ribonuclease contaminating the preparations of cytoplasmic ribosomes of *Euglena gracilis* has been reported (3, 4, 5). However, this enzyme has only been indirectly demonstrated by the fact that the largest ribosomal RNA molecule has been found degraded after extraction and purification.

According to these reports, it could be that the unstable ribosomes have such contaminating RNase while the stable ribosomes have not. Sucrose gradient analysis of SDS-extracted ribosomal RNAs have revealed that this is not the case since, in both types of ribosomes the largest RNA species have been found degraded into smaller molecules of around 12S and 16S (6).

The study of the rRNAs have not explained the difference in stability between stable and unstable ribosomes, therefore, we have started the analysis of the ribosomal proteins using one and two dimensional gel electrophoresis. These techniques have been succesfully applied to the analysis of ribosomal proteins from eucaryotic and procaryotic cells (7, 8, 9, 10, 11, 12).

In this work we have looked for the presence, absence and/or modification of proteins in the unstable ribosomes compared to the stable one.

## MATERIALS AND METHODS

### *Growth Conditions*

*Euglena gracilis* var. bacillaris, were hetero-trophically grown in the dark in the growth medium described by Myers and Cramer (13) and modified by Buetow and Padilla (14).

For this purpose, a New Brunswick Microferm Formentor was used, containing 12 liters of growth medium which was previously autoclaved for 20 min at 120°C and 15 lb. of pressure. This instrument provided constant aeration and agitation at 25°C. Cell number during growth was measured with a Neubauer counting chamber.

In these conditions, exponential and stationary phases of growth are reached at the $5^{th}$ and $9^{th}$ day with a cell population of $2 \times 10^6$ and $8{,}5 \times 10^6$ cel/ml respectively.

Cell collection was done with the aid of a Sorvall's KSB continous flow system at 8.000 × g.

### *Isolation of ribosomes*

Euglena cytoplasmic ribosomes were obtained according to Freyssinet and Schiff (1). Buffers employed:

Buffer I contains: tris-HCl 100 mM, pH 7,6; KCl 30 mM; magnesium acetate 2 mM.

Buffer II contains: tris-HCl 10 mM, pH 7,6; KCl 30 mM; magnesium acetate 2 mM.

Buffer III contains: tris-HCl 10 mM, pH 7,6; $NH_4Cl$ 100 mM; magnesium acetate 2 mM.

Buffer IV contains: tris-HCl 10 mM, pH 7,6; magnesium acetate, 0,1 mM; mercaptoethanol, 20 mM.

Buffer V contains: tris-HCl 10 mM, pH 7,6; magnesium acetate 0,5 mM.

All the stages in ribosome isolation were performed at 0-4°C.

Ribosome preparations (batches of aprox. 200-300 $DO_{260\text{-}ml}$). were stored at -20°C. or used inmediately.

### *Ribosomal Subunits*

Cytoplasmic ribosomal subunits were prepared dialyzing the ribosomal suspension in buffer II for 4 hrs. at 4°C against buffer IV. 38 $DO_{260}$ of the dialyzed suspension were layered on top of 38 ml of a 5-30% (w/v) linear sucrose gradient prepared in buffer V and centrifuged for 13 hr. at 21.000 rpm in a Beckman SW-27 rotor.

Fractions containing the separated subunits were collected and centrifuges at 105.000 × g for 16 hours. Then, the subunit pellets were resuspended in buffer V at aprox. 100 $DO_{260}$ ml and stored at −20°C.

### *Washed ribosomes*

28 $DO_{260}$ of ribosomal suspension in buffer II were redissolved in buffer A (tris-HCl 10 mM, pH 7,6; KCl 500 mM; magnesium acetate 2 mM) or Buffer B (tris-HCl 10 mM magnesium acetate 2 mM) and centrifuged for 4 hrs. at 105.000 × g. The resulting pellets were resuspended in buffer II and dialyzed overnight the same buffer in order to remove the excess of KCl.

### *Gradient Monitoring*

All gradients were analized by ISCO density gradient fractionator model 183 coupled to an ISCO UA-5 UV absorbance monitor which plots linearly the optical density at 254 nm against the gradient volume.

### *Ribosomal protein extraction*

Ribosomal proteins were extracted according to the method of Hardy *et al.* (15), which employs the 67% acetic acid procedure.

### *Polyacrylamide gel electrophoresis*

One dimensional polyacrylamide gels containing urea 8M (pH 4,5) were performed according to Freyssinet and Schiff (1).

One dimensional polycrylamide slab gels in the presence of SDS 0.1% (pH 8.3) were carried out using the procedure described by Laemmli and Favre (16).

Two dimensional polycrylamide gels were performed according to Kaltschmidt and Wittmann (7,8).

*Protein and rRNA determinations*

Ribosomal protein content was estimated by using the method described by Lowry *et al.* (17), while the rRNA was determined with the orcinol procedure using bovine serum albumin and yeast RNA as standards respectively (18).

*Gel Scanning*

Polyacrylamide-urea cilindrical gels were scanned in a Canalco Europe model K-12 microscanner and the polyacrylamide-SDS slab gels, in a Varian 65 spectrophotometer at 600 nm.

## RESULTS

Ribosomes isolated from cells harvested in the logarithmic growth phase were dissociated and the subunits incubated at 25°C for 30 min after this treatment the subunits have shown in sucrose density gradient analysis an absorbance profile at 260 nm which was considered normal since the ratio between the large and the small subunit was 1.6. However, when the ribosomes were obtained from cells grown until stationary growth phase a degradation of the large subunit was observed after the heating at 25°C for 30 min (Fig. 1). The subunits absorbance ratio determined in this case was less than one. Considering these results the first group of ribosomes was named "stable" and the other group obtained from stationary cells, "unstable".

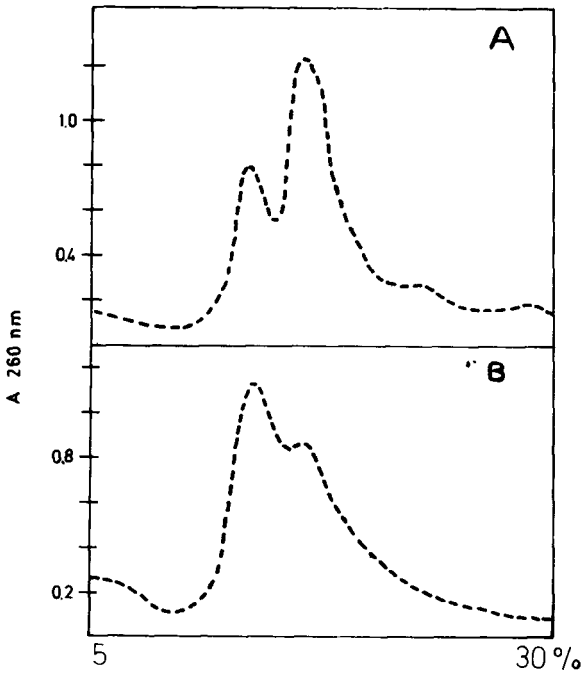


*Fig. 1.* Sedimentation profiles of cytoplasmic ribosomes. Before centrifugation, the ribosomes were dialyzed against buffer IV at 4°C for 150 min and incubated at 25°C for 30 min.
A. Stable ribosomes
B. Unstable ribosomes.

To explain the difference of the stability *in vitro* of the large subunits, the ribosomal proteins of both types of ribosomes were analyzed by gel electrophoresis.

The proteins were extracted from the monosome and from the purified subunits according to Hardy *et al.* (15). Then, one and two dimensional polycrylamide gel electrophoresis were performed with the lyophilized protein extract.

One dimensional polycrylamide gels containing urea 8 M at pH 4.5 were carried out according to Freyssinet and Schiff (1). The observation of these gels did not revealed any difference between stable and unstable ribosome when the proteins were extracted from the monosome or the small subunit. But an additional band in the unstable ribosome as compared to the stable one was found when the proteins of the large subunits were electrophoresed (Fig. 2).

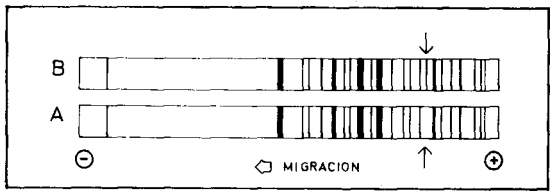


*Fig. 2.* Polyacrylamide gel electrophoresis patterns employing Freyssinat and Schiff technique (1).
A. Stable large subunit proteins
B. Unstable large subunit proteins.

The fact that this additional band was not observed with the monosome proteins could be due to the great number of bands present in the gels.

Similar results were obtained using the one dimensional gel technique described by Laemmli and Favre (16), which is carried out in the presence of 0.1% sodium dodecyl sulfate at pH 8.3. In these conditions the large subunit

of the unstable ribosomes revealed again a band not present in the large subunit of the stable ribosomes (Fig. 3). In figure 4 the densitometric tracing of these gels are shown.

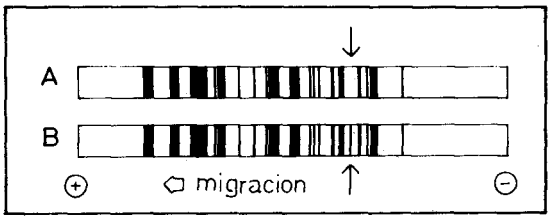


*Fig. 3.* Polyacrylamide-SDS electrophoresis patterns employing Laemmli and Favre technique (16).
A. Stable large subunit proteins
B. Unstable large subunit proteins.

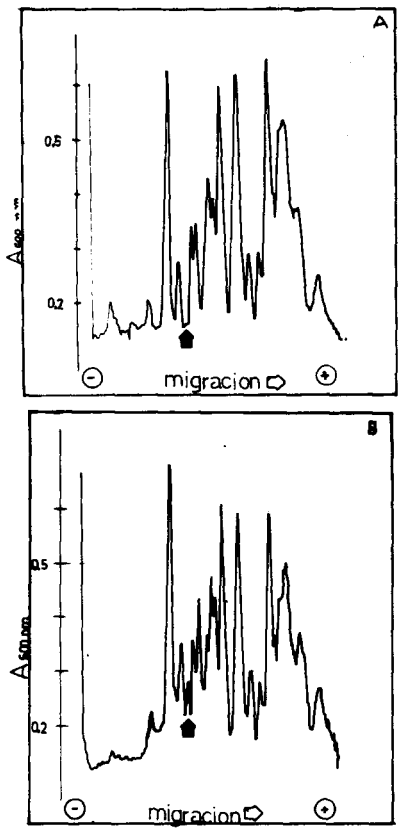


*Fig. 4.* Densitometric tracing of polyacrylamide-SDS gels. gels.
A. Stable large subunit
B. Unstable large subunit.

To obtain a better resolution two dimensional polyacrylamide gel technique described by Kaltschmidt and Wittman (7,8) was also employed. Again, no difference in the electrophoretograms of stable and unstable ribosomes was found when the proteins of the monosome and small subunit were electrophoresed. On the other hand, the large subunit proteins of the unstable ribosome show one more spot in the electrophoretograms (as pointed out by and arrow in figure 5) than the stable large subunit proteins.

The visual inspection of the electrophoretograms also revealed that a group of acidic proteins present in the protein extract of the monosome is absent in the protein extracts obtained from the purified subunits. Evidenly, these proteins which have an isolectric point below pH 8.6 in the first dimension were lost during the dissociation of the ribosome. However, washing of the ribosomes (as monosomes) with high molar KCl solutions failed to remove these proteins. In other words these proteins fall off from the ribosome by dissociation but not by washing of them.

The washing of the ribosomes caused in the large subunit an increase of the lability to the heating at 25°C. This phenomenon is observed once the ribosomes are dissociated into subunits (Fig. 6).

The 87S particle sedimentation properties were also altered by the washing of the ribosomes with KCl solutions. Degradation of the 87S particle in sucrose density gradient was observed and a new particle of a smaller sedimentation coefficient appeared in the gradient, which probably has less protein and/or RNA content.

Finally, the protein spots in the electrophoretograms of the isolated and purified ribosomal subunits were counted. The group of acidic proteins found at the top of the gels were not considered.

42 spots in two dimensional gels were revealed when the proteins of the large subunit were run. With the proteins of the small subunit 30 spots were found. These counts made a total of 72 proteins in the cytoplasmic ribosome of Euglena gracilis.

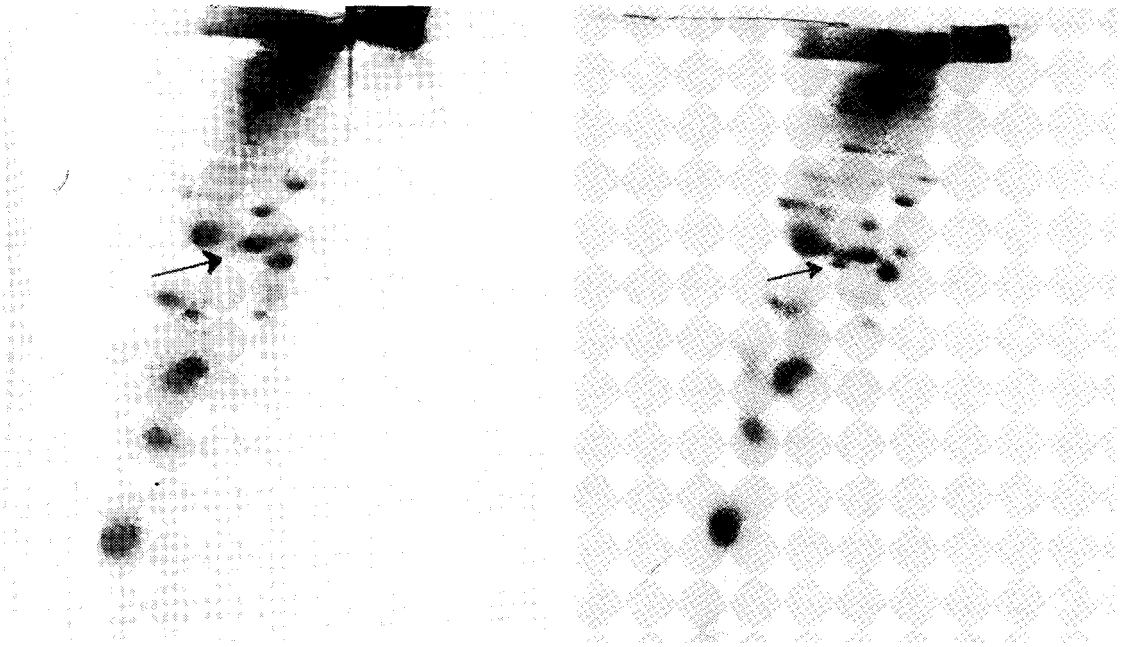


*Fig. 5* Two dimensional polyacrylamide gel electrophoresis.
A. Large stable subunit proteins
B. Large unstable subunit proteins.

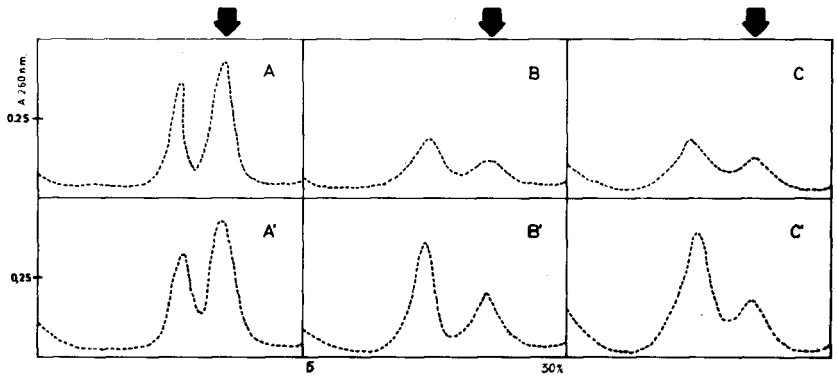


*Fig. 6.* Sedimentation profiles of stable and unstable ribosomes.
A. Stable ribosomes; unwashed and dissociated
B. Stable ribosomes; washed and dissociated
C. Stable ribosomes; washed, dissociated and incubated
A' Unstable ribosomes; unwashed and dissociated
B' Unstable ribosomes; washed and dissociated
C' Unstable ribosomes; washed, dissociated and incubated.

## DISCUSSION

We have apparently found two types of cytoplasmic ribosomes in Euglena gracilis and the stability *in vitro* of the large subunit is what has marked difference between these two groups. Now, the fact that these two groups of ribosomes are obtained from cells in two different physiological states makes this observation very interesting. It is possible, that somehow, the cell controls the activity of the ribosomes modifying its structure, and it is the unstability

of the large subunit of the ribosomes from stationary cells the target of this hypothetical modification.

We have observed in the one dimensional gel electrophoresis techniques employed a band of protein that is only present among the proteins of the large unstable subunit. Therefore a new proteins or a modified old one is being observed in the gels. This is confirmed in the electrophoretograms of the two dimensional gel technique. In this gels, a new spot is found in the large unstable subunit which is absent in the large stable subunit. However, the total number of spots is the same for both type of large subunits.

We are not certain whether one of the proteins has been modified and has moved to another position in the gels or it is in fact a new protein which has been associated to the ribosome of the stationary cells.

Since after extraction the largest ribosomal RNA has been found specifically cleaved in both types of ribosomes, perhaps, the integrity of this molecule is preserved by the protein content of the ribosome and modification of this protein content might determine the collapse *in vitro* of the ribosome structure.

We think that our observation might be a sign that a regulation of this kind might be controlling the activity of the ribosome. Supporting this idea is the fact that our "stationary ribosomes" are always 60-80% less active than the "exponential ribosomes" in an *in vitro* protein synthetizing system (not published).

## BIBLIOGRAPHY


1. FREYSSINET, G., SCHIFF, J.A. Plant Physiol. *53:*543 (1974.).
2. DELIHAS, N., JUPP, A., LYMAN, H. Biochim. Biophys. Acta *262:*344 (1972).
3. AVADHANI, N.G., BUETOW, D.E. Biochem. Journal *128:*353 (1972).
4. HEIZMANN, P. Biochim. Biophys. Acta *224:*144 (1970).
5. RAWSON, J.R., STUTZ, E., CROUSE, E.J. Biochim. Biophys. Acta *246:*507 (1971).
6. MORA, G., RIVAS, A. Arch. Biol. Med. Expert. *10:*61 (1976).
7. KALTSCHMIDT, E., WITTMANN, H.G. Proc. Nat. Acad. Sci. *67:* 1276 (1970).
8. KALTSCHMIDT, E., WITTMANN, H.G. Analyt. Biochem. *36:*401 (1970).
9. DELAUNAY, J., SCHAPIRA, G. Biochim. Biophys. Acta *259:*243 (1972).
10. HANNA, N., GODIN, C. Biochim. Biophys. Acta *374:* 342 (1974).
11. HUYNH-VAN-TAN, DELAUNAY, J. SCHAPIRA, G. FEBS. Letters *17:*163 (1971).
12. SHERTON, C., WOOL, I.G. J. Biol. Chem. *247:*4460 (1972).
13. MYERS, J., CRAMER, M. Arch. Microbiol. *17:*384 (1952).
14. BUETOW, D.E., PADILLA, G.M. J. Protozool. *10:*121 (1963).
15. HARDY, S., KURLAND, C., VOYNOW, P., MORA G. Biochemistry *8:*2897 (1969).
16. LAEMMLI, U.K., FAVRE, M. J. Mol. Biol. *80:*575 (1973).
17. LOWRY, O.H., ROSEBROUGH, N.J., FARR, A., RANDALL, R. J. Biol. Chem *193:*265 (1951).
18. CERIOTTI, G. J. Biol. Chem. *214:*59 (1955).