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Proteins of the chloroplast and cytoplasmic ribosomes of *Euglena*

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The proteins of chloroplast and cytoplasmic ribosomes, isolated from *Euglena gracilis*, have been compared by electrophoresis on SDS-polyacrylamide gels. The proteins of the cytoplasmic ribosomes were more numerous and larger on the average than those of the chloroplast ribosomes.

There were about 14 proteins detected in the small subunit of the chloroplast ribosome, ranging from 11,000 to 43,000 daltons and 16 proteins of 10,000 to 36,000 daltons from the large subunit. The banding patterns of the proteins of the subunits were quite distinct from each other.

The subunits of the cytoplasmic ribosomes were obtained by dissociation of the monomer with EDTA, and in 100 mM and 500 mM KCl and the effects of these conditions of dissociation on the proteins of the subunits compared. Regardless of the means of dissociation, the small and large subunits each gave 20–21 proteins ranging from 10,000 to 49,000 daltons. However, a comparison of scans of the subunits indicated a selective and partial stripping of ribosomal proteins by high salt and by EDTA; i.e. different proteins were sensitive to the two treatments.

Native subunits, presumed to occur free in the cytoplasm were also isolated. In addition to the ribosomal proteins found in small subunits obtained by dissociation, the native small subunit contained substantial amounts of high-molecular-weight proteins.

Small, variable amounts of high-molecular-weight proteins are also associated with chloroplast ribosome subunits, but the quantities depend on the method of purification of the subunits. Because these components are virtually eliminated following two cycles of density gradient centrifugation, we infer that they are adventitious.

These observations reflect on the relative merit among several reported methods of purification of chloroplast and cytoplasmic ribosomes.

The ribosomes of *Euglena* have been previously isolated from the cytoplasm as well as from the chloroplasts and mitochondria and subsequently characterized with regard to some of their physico-chemical properties. Avadhani and Buetow (*1*) have compiled the data from several different sources.

As has been found for nearly all other organisms, the cytoplasmic ribosomes and its subunits were found to have higher sedimentation rates than the corresponding particles obtained from the chloroplasts and mitochondria. The RNA composition was also distinct among these ribosomal populations.

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The ribosomal proteins have been less well characterized. Lyttleton (*8*) analyzed the basic proteins of *Euglena* cytoplasmic ribosomes and those of higher plants. He found 14 to 17 components on polyacrylamide gels. Delihas et al. (*3*) compared the basic proteins of *Euglena* cytoplasmic ribosomes treated with high and low salt and found differences in the amounts of certain protein components separated on polyacrylamide gels. Bickle and Traut (*2*) compared the proteins of bacterial, mammalian and *Euglena* cytoplasmic ribosomes, employing SDS-polyacrylamide gel electrophoresis. They found that *Euglena* cytoplasmic ribosomal proteins resembled those of the eucaryotic rather than the bacterial ribosomes with respect to the number and complexity of the proteins, as well as in the distribution of their molecular weights.

More recently, Freyssinet and Schiff (*4*) estimated that chloroplast ribosomes of *Euglena* contain about 40 proteins. The cytoplasmic ribosomes and their subunits contained many more proteins and had weight-average molecular weights higher than those of the chloroplast ribosome.

Until now, the proteins of *Euglena* chloroplast ribosomal subunits have not been examined. We report here on the proteins of the chloroplast ribosomal subunits as characterized by electrophoresis on SDS-polyacrylamide gels. We have also examined the cytoplasmic ribosomal proteins, the undissociated monomer, the native subunits, and the subunits obtained by dissociation. In addition, we have compared the effects of high salt and of EDTA treatments on the recovery of ribosomal proteins.

Methods

Cultures of *Euglena gracilis* (Klebs) *z* strain were grown heterotrophically in the light or in the dark on a modified Hutner medium (*15*) with glucose as the principal carbon source. The cells were usually harvested from four liters of culture in the exponential phase of growth and washed once with deionized water.

Centrifugation techniques

Centrifugation in density gradients was carried out in swinging bucket rotors (SB-283) or in the B-30A zonal rotor, both from Damon/IEC. For the swinging bucket runs, multiple 11-ml exponential gradients of 0.3–1.4 M sucrose containing the appropriate buffer and salts were prepared according to Noll (*11*).

Equivolumetric gradients (*14*), which are the analogues of isokinetic gradients for zonal rotors, were generated using a Spinco 131 programmable gradient pump.

All gradients were monitored by UV absorption at 260 nm using a continuous-flow photometer.

Ribosomes were pelleted in the fixed-angle A-237 rotor (IEC) at 45,000 to 48,000 rpm for 18 to 20 hr ($\omega^2t=1.4$ to $1.8\ ps^{-1}$). Pelleting through sucrose was achieved by layering 20–22 ml of extract over 7–8 ml of 1.5 M sucrose.

The sedimentation values of the ribosomal particles separated in exponential gradients were estimated from parallel gradients containing *E. coli* ribosomes.

Preparation of chloroplasts

The harvested cells were washed once with a breaking mixture containing

0.15 M sucrose, 0.15 M sorbitol, 1% (w/v) Ficoll (Pharmacia), 5 mM HEPES buffer, pH 7.6 and 2 μg/ml polyvinyl sulfate (*20*). The cells were then resuspended in the same breaking mixture (ca. 15 g wet weight to 50 ml total suspension) and passed through a French pressure cell at about 1500 p.s.i. The resulting brei was diluted with an equal volume of breaking medium and clarified by centrifugation for 2 min at 1000 rpm (IEC rotor No. 870). The supernatant was centrifuged at 3000 rpm for 10 min to pellet the chloroplasts. The chloroplast fraction was washed once with the breaking medium and then sedimented as before. The post-chloroplast supernatant obtained from the first pelleting was set aside for the isolation of cytoplasmic ribosomes.

Isolation of chloroplast ribosomes

The washed chloroplast pellet was resuspended and homogenized in a mixture containing 10 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 500 mM KCl, 5 mM β-mercaptoethanol and 1% of the detergent LDAO (lauryl dimethylamine oxide, obtained as "Ammonyx LO," Onyx Chem. Co., Jersey City, N.J.). After centrifugation at 20,000 rpm for 20 min (IEC rotor No. A-321), a clear dark-green extract containing the ribosomes was obtained. Ribosomes were then isolated and purified by two stages of centrifugation in gradients of sucrose. In the first stage, the clarified extract was introduced into a B-30A zonal rotor containing an equivolumetric gradient of 0–35% (w/w) sucrose, also containing TMK (10 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 100 mM KCl), 5 mM β-mercaptoethanol and 1% of the detergent LDAO throughout. Centrifugation was carried out at 4°C at 28,000 rpm overnight ($\omega^2t=0.5$ ps^{-1}). Under these conditions, the chloroplast ribosomal subunits (30S and 50S) are separated in the gradient while virtually all of the green material remained in the sample zone. The fractions corresponding to the peaks of the two subunits were pooled separately, diluted with TMK buffer and recovered by pelleting. The surface of the pellets was rinsed carefully with ice-cold deionized water and the pellets were resuspended in TMK buffer containing 5 mM β-mercaptoethanol. At this point, the A_{260}/A_{280} ratio of the material corresponding to the small (30S) and large (50S) subunits was 1.2 and 1.6, respectively.

The ribosomes were further purified by a second stage of centrifugation in sucrose gradients containing the buffer-salts mixture described above, except that the detergent was omitted. Five to 10 A_{260}-ml of material was layered on each gradient and centrifuged for 18 hr at 20,500 rpm ($\omega^2t=0.3$ ps^{-1}) in an SB-283 rotor. The subunits were reocvered from the gradients and pelleted as before. The A_{260}/A_{280} of the pellets after resuspension was 1.5–1.9 for the 30S and 2.0–2.3 for the 50S subunits.

In an alternative procedure, the ribosomes from the chloroplast extract were pelleted through a cushion of 1.5 M sucrose containing the buffer-salts mixture, as a substitute for the first stage of density gradient centrifugation. The pellets, which were still green, were rinsed with ice-cold deionized water, resuspended in TMK buffer containing 5 mM β-mercaptoethanol, and centrifuged at 20,000 rpm for 20 min to remove most of the contaminating green material. The ribosomal subunits were then isolated by centrifugation in exponential gradients of sucrose as before.

Isolation of cytoplasmic ribosomes

Cytoplasmic ribosomes were prepared from whole cells of dark-grown *Euglena* or from the post-chloroplast supernatant from green cells. In the former case, the cells from 4 liters of culture were harvested, washed once with deionized water, and once with TMK buffer. The washed cells were then resuspended in TMK buffer containing 14 mM β-mercaptoethanol and 2 μg/ml polyvinyl sulfate, passed through the French pressure cell at 5000 p.s.i., and the resulting brei made 2% with respect to Triton X-100 (Rohm and Haas, Philadelphia, Pa.). The mixture was allowed to stir gently in the cold for 30 min, clarified by two successive centrifugations at 17,000 rpm for 20 min (IEC rotor No. 870), layered over a cushion of 40% (w/w) sucrose containing the TMK buffer-salts mixture described above and centrifuged overnight. The pellets were rinsed with ice-cold deionized water, resuspended in TMK buffer and centrifuged at 17,000 rpm for 20 min. The clarified suspension (15–20 ml, containing 35 to 65 A_{260}-ml) was subjected to zonal centrifugation as described for chloroplast ribosomes, except that centrifugation was at 23,000 rpm for about 18 hr ($\omega^2t=0.32$ ps^{-1}). Under these conditions, one obtains a large, well-defined peak of the cytoplasmic monomeric ribosomes, previously described as 87S or 89S particles (*10*, *16*), and smaller peaks, which correspond to the cytoplasmic subunits. The 89S ribosomes were recovered from the gradient by pelleting.

Subunits of the cytoplasmic ribosomes were prepared from the purified 89S monomer by dissociation with 20 mM EDTA (ethylenediamine tetraacetate), essentially according to the method of Rawson and Stutz (*16*), or in varying concentrations of KCl up to 500 mM, containing 1 mM or 10 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.5, and 7 mM β-mercaptoethanol. The subunits were subsequently separated in gradients of sucrose.

Electrophoresis of ribosomal proteins

Polyacrylamide gels containing 0.1% SDS (sodium dodecylsulfate) were prepared and run according to a modification of the procedure of Laemmli (*7*). The separation gel contained 12.5% acrylamide and 0.33% N,N′-methylene bisacrylamide and the stacking gel contained 3% acrylamide. The gels were set in glass tubes, 17 cm long with an internal diameter of 0.5 cm. The electrode buffer consisted of Tris-glycine at pH 8.4, containing 0.1% SDS.

Ribosome samples were mixed with sample buffer consisting of 62.5 mM Tris-HCl, pH 6.8, 5% β-mercaptoethanol, 3% SDS, and 10% glycerol, and heated at 70–80°C for 10 min. Samples representing 50 to 100 μg of ribosomes (assuming $E_{260}^{1\%}=15$) were mixed with tracking dye (2 μl of 0.1% bromphenol blue) and applied to the tops of the stacking gels. The following proteins of known molecular weights were also run as markers: bovine serum albumin, 68,000; ovalbumin, 45,000; carbonic anhydrase, 29,000; β-lactoglobulin, 18,400; RNase A, 13,600; and cytochrome *c*, 12,400.

Electrophoresis was carried out with a current of 1 mamp/gel until the samples had just entered the lower gel. Electrophoresis was continued at 2.5 mamp/gel until the tracking dye was about 1 cm from the bottom of the tubes. Total electrophoresis time was 5 1/2 to 6 hr. The gels were processed for staining (E. F.

Eikenberry, unpublished) and, after destaining, photographed and subsequently scanned at 563 nm in the Gilford spectrophotometer with a scanner attachment at a speed of 2 cm/min and a chart speed of 2 in./min.

Analysis of RNA components by gel electrophoresis

Electrophoretic separations of the ribosomal RNA components were carried out essentially as described before (*10*), based on the method of Peacock and Dingman (*13*).

Results

When a clarified extract of chloroplasts was centrifuged in a zonal rotor as described under **Methods,** a typical sedimentation profile was obtained as represented in Fig. 1a. The zones designated II and III correspond to the small 30S and large 50S subunits, respectively, of the chloroplast ribosome, as verified by the banding patterns of their RNA's on agarose-polyacrylamide gels. The zone designated I which formed a shoulder off the small subunit peak II contained no detectable RNA and appeared therefore not to contain ribosomes. We have routinely found only small amounts of 70S ribosomes in our chloroplast extracts under a variety of conditions tested, in contrast to the reports of others (*1*, *17*).

When the material in zones II and III was subjected to a second centrifugation in sucrose gradients, the sedimenting components were almost exclusively the small and large particles, respectively (Fig. 1b and 1c). Again the respective

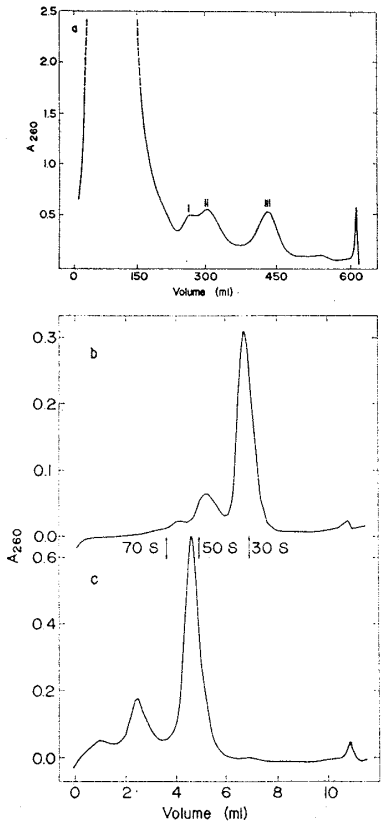

Fig. 1. *Sedimentation profiles of chloroplast ribosomes.* a) Separation of subunits from chloroplast extracts centrifuged through sucrose gradients in the B-30A zonal rotor. b) 30S ribosomes (peak II) and c) 50S ribosomes (peak III) from 1a, recentrifuged through exponential gradients of sucrose as described in the text.

RNA banding patterns were characteristic of 30S and 50S ribosomes of *Euglena* chloroplasts as reported previously (*10*). The small zones of faster sedimenting material, which were resolved from each of the isolated subunits, yielded RNA which was identical to the main subunit peak; so that the faster sedimenting particles may represent the respective subunit in a different conformation.

Purified 30S and 50S subunits were alternatively obtained from chloroplast extracts when the ribosomes were first pelleted through 1.5 M sucrose and subsequently fractionated on gradients of sucrose as above.

We also tested certain variations in the standard method of extraction and subsequently compared the composition of ribosomal proteins. In one, chloroplast preparations were extracted with the buffer-salts mixture containing sodium deoxycholate instead of LDAO and then centrifuged on density gradients of sucrose. Although the yield of ribosomes was about equal with the two detergents, as judged from the sedimentation profiles obtained, the use of LDAO in the extraction mixture and in the sucrose gradients resulted in cleaner separations, with the green material, presumably in membrane fragments, confined to the sample zone. We also found that increasing the concentration of KCl in the extraction mixture from 100 to 500 mM increased the yield of the chloroplast ribosomal subunits.

Extraction with Triton X-100 gave good yields of chloroplast ribosomal subunits. However, this detergent also extracted significant amounts of high molecular weight proteins, which persistently co-sedimented with the small subunit even after pelleting through 1.5 M concentrated sucrose and centrifugation in density gradients.

Purification of cytoplasmic ribosomes and their dissociation into subunits

Cytoplasmic ribosomes were extracted from whole cells, as described in **Methods,** and purified by pelleting through concentrated sucrose and zonal centrifugation into density gradients of sucrose. A typical sedimentation profile is illustrated in Fig. 2. The large, rapidly sedimenting zone (III) consisted of cytoplasmic 89S ribosomes, as shown by RNA analyses as well as by its sedimentation rate. There were also two slower, partially resolved zones (I and II) of the small and large subunits, respectively. We refer to these as native cytoplasmic subunits,

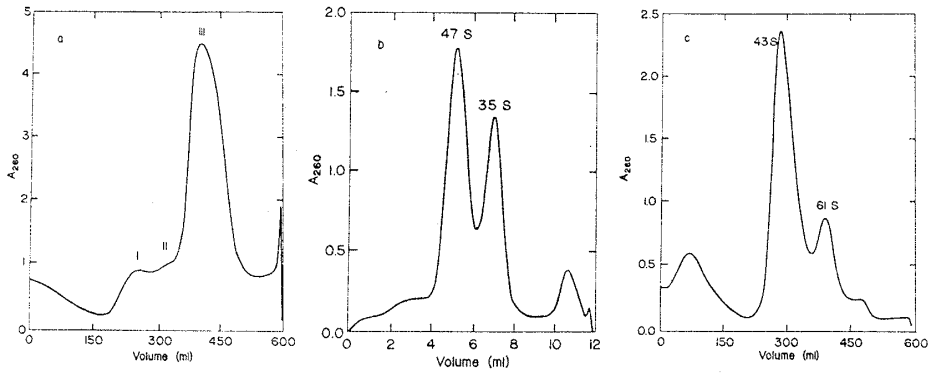

Fig. 2. *Sedimentation profiles of cytoplasmic ribosomes.* a) Separation of 89S monomers and native subunits in the B-30A zonal rotor. b) Separation of cytoplasmic subunits obtained by dissociation in EDTA. c) Separation of cytoplasmic subunits obtained by dissociation in 500 mM KCl.

since the ribosomes were not previously exposed to dissociating conditions. Curiously the amount of the small subunit always exceeded that of the large subunit. When the centrifugation was prolonged, the native subunits were much better resolved.

Cytoplasmic ribosomal subunits were prepared from the 89S monomer by exposure to EDTA or high concentrations of KCl and separated by centrifugation through exponential gradients of sucrose. Dissociation with EDTA following the method of Rawson and Stutz (*16*) resulted in almost complete dissociation of the monomer into the two subunits, in the expected ratio of approximately 2 : 1 for the large to the small subunit, respectively (Fig. 2b). However, the subunits had low sedimentation values of 35S for the small and 47S for the large subunit.

When the monomer was dissociated in mixtures containing 500 mM KCl and 10 mM $MgCl_2$ and separated in gradients containing the same concentrations of salts, two subunits were obtained with sedimentation coefficients, 43S and 61S, approximating the expected values, but with only small amounts of the large subunit (Fig. 2c). However, when the ribosomes were treated with high salt and then centrifuged through gradients containing lower KCl concentrations (100 instead

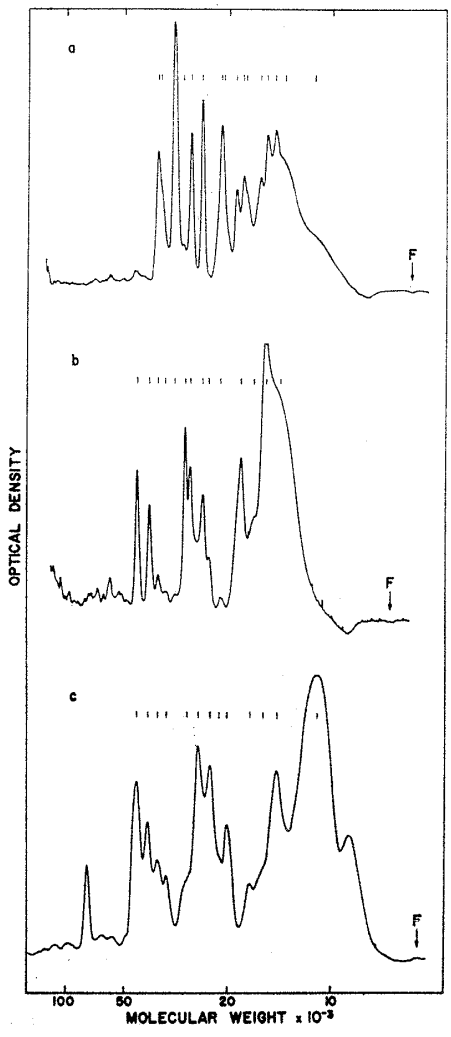

Fig. 3. *Spectrophotometric scans of chloroplast ribosomal proteins analyzed on SDS-polyacrylamide gels.* a) 50S ribosomal proteins and b) 30S ribosomal proteins obtained from ribosomes purified by 2 cycles of centrifugation in density gradients; c) 30S ribosomal proteins from ribosomes which had been purified by pelleting through 1.5 M sucrose, followed by fractionation in density gradients. Molecular weights were estimated from the mobilities of marker proteins as described in text. F indicates the position of the tracking dye. Fiducial marks indicate discernible polypeptide components. All gels were stained with Coomassie brilliant blue.

of 500 mM) most of the ribosomes reassociated and sedimented as 89S particles, which indicates that the particles are not sensitive to mere exposure to high concentrations of KCl.

Numerous other combinations of KCl and $MgCl_2$ concentrations were tested for their ability to induce dissociation of the cytoplasmic ribosomes. The mixture of 500 mM KCl and 10 mM $MgCl_2$ gave a high percentage of dissociation with minimal reduction of the sedimentation coefficients of the subunits, presumably caused by unfolding. For example, when the cytoplasmic ribosomes were treated with 100 mM KCl and 1 mM $MgCl_2$ and run in gradients containing the same salt concentrations, incomplete dissociation into subunits resulted, with poor recovery of the large subunit. This is in contrast to the observations of Delihas et al. (*3*) who reported complete dissociation of *Euglena* cytoplasmic ribosomes under similar conditions.

SDS-polyacrylamide gel electrophoresis of ribosomal proteins

In order to characterize and compare the protein components of the ribosomal subunits, purified samples were subjected to electrophoresis on SDS-polyacrylamide gels. The spectrophotometric scans of the stained gels obtained with *Euglena* chloroplast ribosomes are shown in Fig. 3, and in the photographs of the gels in Fig. 6. The difference in the banding patterns of the proteins between the two subunits is apparent. On the whole, the large subunit shows a more even distribution of proteins ranging in molecular weight from about 36,000 to 10,000 whereas the proteins of the small subunit range in size from 43,000 to 11,000 molecular weight. Furthermore, several of the proteins in the small subunit are present in relatively

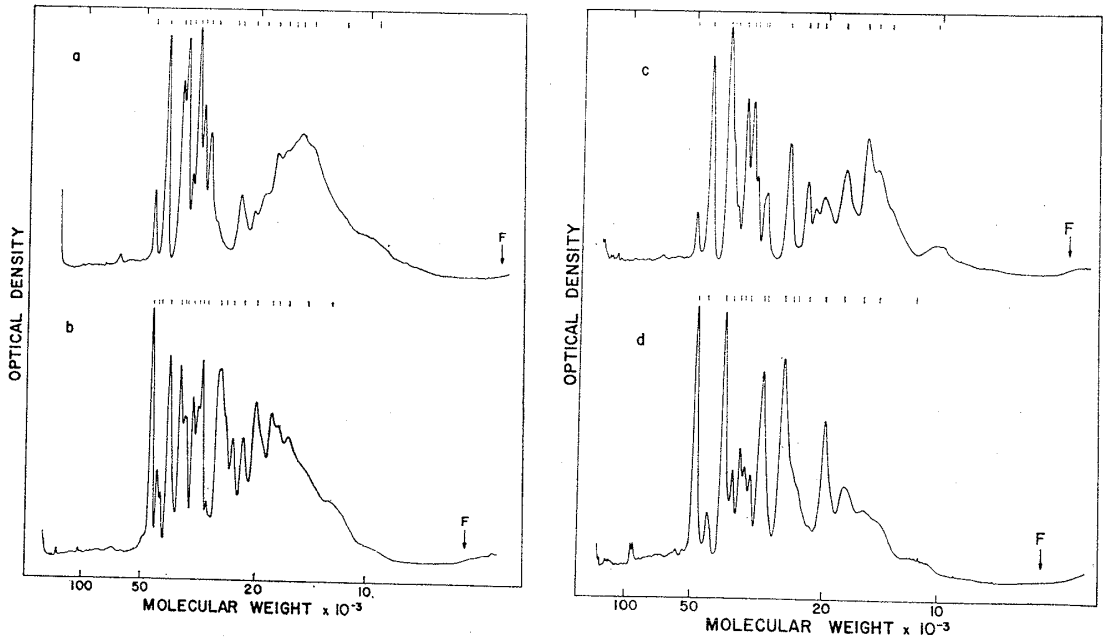

Fig. 4. *Spectrophotometric scans of cytoplasmic ribosomal proteins analyzed on SDS-polyacrylamide gels.* a) EDTA-derived small subunit; b) EDTA-derived large subunit; c) 500 mM KCl-derived small subunit and d) 500 mM KCl-derived large subunit.

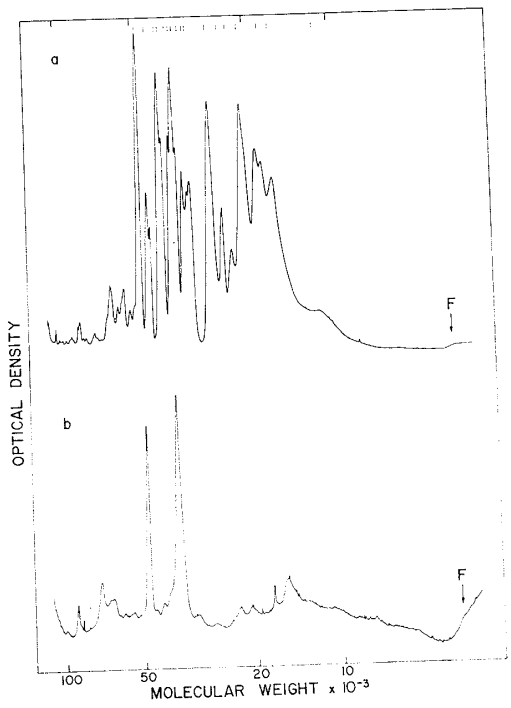

Fig. 5. *Spectrophotometric scans of cytoplasmic ribosomal proteins.* a) Undissociated 89S monomer and b) proteins solubilized by EDTA.

small amounts, with the smallest proteins predominating and forming large heterogeneous peaks. The smaller amounts of proteins in certain zones may be real or may be due to the more labile nature of these proteins, and hence their preferential loss during purification. In any event there are 16 discernible protein components in the large subunit and at least 14 components in the small subunit, or a total of about 30 proteins in the chloroplast ribosome. This is a minimal estimate, since there may be overlaps between proteins of similar molecular weights. Freyssinet and Schiff (*4*) distinguished only 12 protein zones on similar SDS gels of the chloroplast ribosomes, but on the basis of the calculated amounts of protein in each zone, they inferred the presence of about 40 proteins.

When our chloroplast ribosomes were prepared by the alternative procedure of pelleting the extracts through concentrated sucrose solution, followed by centrifugation in density gradients of sucrose, variable numbers and amounts of additional polypeptides appear in the upper portion of the gel where only minor peaks normally appear (Fig. 3c).

Compared to the chloroplast ribosomes, the proteins of the cytoplasmic ribosomes are more complex and numerous. The spectrophotometric scans in Fig. 4 and the photographs of the gels in Fig. 6 show the proteins of the large and the small subunits derived by dissociation with EDTA (Fig. 4a, b) and with 500 mM KCl (Fig. 4c, d). The scan for the 89S monomer is also shown in Fig. 5a. Regardless of the means of dissociation, the banding patterns of the proteins of the small and large subunits are quite distinct, especially in the region of the proteins of highest molecular weights, although the patterns of distribution of the proteins are more similar between these subunits than for those of the chloroplast ribosome. There are about 20 detectable protein components in the large and small subunits, ranging in molecular weight from approximately 49,000 to 10,000. It is interesting

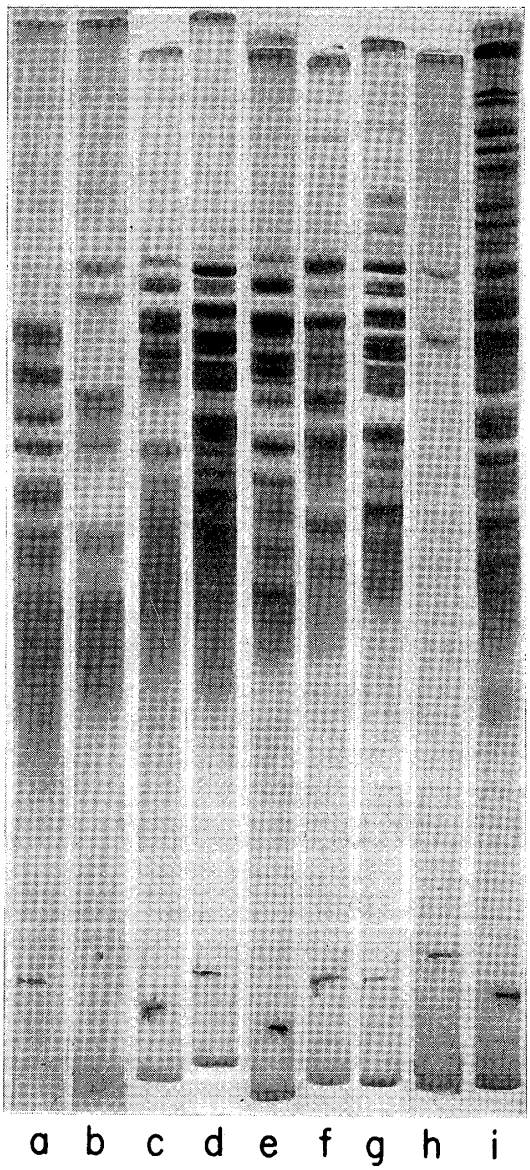

Fig. 6. *Comparison of ribosomal proteins of* Euglena. Photographs of stained gels. Chloroplast ribosomes: a) 50S and b) 30S. Cytoplasmic ribosomal subunits: c) EDTA-derived, small; d) EDTA-derived, large; e) KCl-derived, small and f) KCl-derived, large subunits. g) 89S cytoplasmic monomer. h) proteins solubilized by EDTA. i) native small cytoplasmic subunit.

to note the similarities in the banding patterns of the proteins between the 89S ribosome and the large subunits. The scans of the cytoplasmic ribosomal subunits obtained from green cells were quite similar to those of the dark-grown cells, except for some variations in the amounts of certain components.

In contrast, when the proteins of the native small cytoplasmic subunit described above were similarly analyzed on SDS gels, we found substantial amounts of high molecular weight proteins (57,000 to 100,000), in addition to the proteins previously observed in small subunits obtained by dissociation with EDTA or high salt. The cytoplasmic 89S ribosomes which were recovered from the very same gradients contained only small amounts of a few proteins exceeding 50,000 daltons (cf. Fig. 6i).

Effects of dissociation by EDTA or high salt on the ribosomal proteins

On the whole, there are no qualitative differences between the banding

patterns of the proteins of the subunits derived by EDTA and those dissociated in high salt. There are, however, marked differences in the relative amounts of certain protein components. For instance, scans of the large subunit obtained by dissociation with high salt show significantly smaller amounts of several proteins when compared to the corresponding regions of the scan of the EDTA-derived large subunit (cf. Fig. 4b and d). When the ribosomes were dissociated at lower salt concentrations (1 mM $MgCl_2$, 100 mM KCl), the pattern of the proteins of the large subunit was very similar to that of the large subunit derived by EDTA. It thus appears that the high salt treatment results in the partial stripping of some of the ribosomal proteins of the large subunit. Although this stripping also occurs to a certain extent in the case of the small subunit (Fig. 4a and c), there are protein components which are present in comparatively *increased* amounts in the high salt-derived and intermediate salt-derived subunits compared to those dissociated in EDTA. The amount of the largest protein was highest in intermediate salt indicating that this particular component may be susceptible to both high salt and EDTA.

If the EDTA treatment does result in the stripping of ribosomal proteins, one would expect to find these proteins in the sample zone when the subunits are fractionated by density gradient centrifugation following dissociation. Indeed, when the nonsedimenting material was analyzed on SDS gels, we found protein components having molecular weights within the range of ribosomal proteins, including some components of molecular weights higher than 50,000 which were previously observed in the scan of the 89S monomer (Fig. 5a). However, as can be seen in Fig. 5b, two large proteins with estimated molecular weights of 48,000 and 37,000 predominated, indicating that these particular proteins were selectively solubilized by EDTA.

Discussion

The proteins of the chloroplast ribosomes of *Euglena* show approximately 30 detectable protein bands on SDS-polyacrylamide gels compared to more than 40 in the case of the cytoplasmic ribosomes. This trend is similar to the observations of others with higher plants (*5*, *12*, *18*, *19*). In addition, the chloroplast ribosomal proteins are distributed over molecular weight ranges which are lower than those of the cytoplasmic ribosomes, as has been observed in comparing procaryotic and eucaryotic ribosomes (*2*).

Freyssinet and Schiff (*4*) also found that the number- and weight-average molecular weights of the cytoplasmic ribosomal proteins were higher than those of the chloroplast in *Euglena*. However, these authors report the presence of ribosomal components of higher molecular weights than we have found, for the chloroplast as well as for the cytoplasmic ribosomes. We also find a few proteins of molecular weights higher than 43,000, notably one of 55,000–56,000, in crude preparations of the small subunit, when the ribosomes are purified by pelleting through a cushion of concentrated sucrose solution or when the chloroplasts had been extracted with Triton X-100. In contrast, chloroplast ribosomes obtained by two cycles of density gradient centrifugation yield little, if any, of the high-molecular-weight components. Since these high-molecular-weight proteins

(>43,000) are present in variable amounts in the purified subunits and since their presence depends on the method of isolation, we think that they are probably adventitious. Nonetheless, the association of these proteins with the small but not the large subunit may indicate a selective affinity. We did not look at the proteins of the 70S ribosomes since the yield of this monomer was always very low under a variety of conditions of extraction.

In the case of the proteins of the cytoplasmic ribosomes, we find components in the molecular weight range of 10,000 to 49,000, similar to the ranges found by Bickle and Traut (*2*) with earlier preparations from this laboratory. Freyssinet and Schiff (*4*) reported a molecular weight range of 11,200 to 98,000 for the cytoplasmic ribosomes of a strain of *Euglena* similar to that used in the present study. Judging from their scans, the proteins of molecular weights exceeding 54,000–57,000 were present in comparatively minor and variable amounts. In our scans of the 89S monomer we find only minor protein bands corresponding to molecular weights higher than 52,000. In the subunits the amounts of these proteins were variable and not always detectable. We therefore question whether they are true structural components of the ribosome.

High molecular weight proteins have previously been found associated with the small subunit of cytoplasmic ribosomes of other organisms. Hirsch et al. (*6*) reported that the native small subunit found free in the cytoplasm of Ehrlich ascites tumor cells are combined with substantial amounts of exchangeable, nonribosomal proteins of 50,000 to 200,000 molecular weight. The proteins of the derived subunit (obtained by dissociation of the ribosomes in 0.5 M KCl), on the other hand, were found to have molecular weights less than 50,000. These large, nonribosomal proteins were found to have a high and quite specific affinity for the 40S subunit since, after their release with high salt, they recombined quantitatively with the small subunit when the salt concentration was lowered, even in the presence of a large excess of cytoplasmic proteins.

We have also observed the presence of substantial amounts of high molecular weight proteins associated with the native small cytoplasmic subunit of *Euglena*, whereas these same proteins were not detected in the undissociated monomer nor in any of the derived subunits. It is thus possible that in *Euglena* as in mammals, the native small cytoplasmic subunit is normally associated with large, nonribosomal proteins which may contain initiation factors. However, we have not yet determined if the association of the large proteins with the native small subunit in *Euglena* is truly specific nor have we analyzed the proteins of the native large subunit. Our preliminary experiments indicate that, unlike the case with mammalian ribosomes, the large proteins remain associated with the ribosomes even after treatment of the native subunit with 500 mM KCl.

Our present studies have shown that treatment of *Euglena* cytoplasmic ribosomes with EDTA or high concentrations of KCl results in the partial and selective stripping of certain ribosomal proteins. Delihas et al. (*3*) similarly observed a decrease in the amounts of certain proteins of *Euglena* cytoplasmic ribosomes after treatment with high salt, as compared to ribosomes which had been washed with lower salt concentrations. Our own results indicate on the one hand that subunits dissociated in high salt can reassociate, but that high salt adversely affects the proteins of both subunits, especially the large subunit. The results of McConkey

(*9*) concerning the protein content of mammalian ribosomal subunits may be relevant. The latter author found that the subunits obtained by dissociation with high salt contained less total protein than the corresponding ribosomes obtained by dissociation with EDTA. This was especially true of the large subunit, where the protein content was 45% for dissociation with EDTA compared to 33% for the high salt-derived particle. Furthermore, the high salt-derived subunits, although containing less protein, were found to be highly active in terms of their ability to polymerize phenylalanine in the presence of poly(U).

We have also shown that some ribosomal proteins are lost after EDTA treatment, since protein components having mobilities on the gels similar to those of the ribosomes were recovered in the nonsedimentable material after density gradient centrifugation of the dissociated subunits. The presence of substantial amounts of two stripped protein components (molecular weights approx. 48,000 and 37,000) was particularly striking. Although our data do not permit us to say which subunit these proteins are derived from, in view of the lower sedimentation rates that we obtained and as observed by others for the subunits dissociated with EDTA, it is quite probable that the loss of these proteins from both subunits leads to the unfolding of the particles.

The yield of the large and small subunits prepared by dissociation in EDTA was in the expected ratio of 2 to 1. In contrast, the cytoplasmic subunits obtained by dissociation in 500 mM KCl were present in disproportionate amounts, the yield of the small subunit being much larger than that of the large subunit (Fig. 2b, c). In this case, however, the sedimentation rates of the subunits were close to the values reported for particles from *Euglena* obtained by dissociation in low magnesium and low salt (*1*) and unlike the low sedimentation values reported by Delihas et al. (*3*) who also used 500 mM KCl but lower magnesium concentration.

Finally, regardless of the means of dissociation of the cytoplasmic ribosomes, the banding patterns of the proteins of the large and small subunits are characteristic and distinguishable between the subunits, especially in the range of 30,000 to 50,000. At the same time, the mobilities of the protein constituents of the two cytoplasmic subunits are distributed over a similar range, whereas this distribution is markedly different between the subunits of the chloroplast ribosomes. This comparison of mobilities is similar to the findings of Gualerzi and Cammarano (*5*) for the ribosomal proteins of spinach. In addition, these authors noted a striking homology between the banding patterns of the proteins of the large subunit and the monomer of spinach cytoplasmic ribosomes. We observe a similar homology in the case of *Euglena* cytoplasmic monomer and the large subunit derived by EDTA or intermediate salt concentrations. We expect that the proteins of the small subunit are largely obscured in the monomer due to the preponderance of proteins with similar mobilities from the large subunit.

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