CHARACTERIZATION OF MITOCHONDRIAL RIBOSOMES FROM SACCHAROMYCES CEREVISIAE

H. SCHMITT

Laboratory of Biological Chemistry, Faculty of Sciences, University of Brussels, Belgium

Received 5 July 1969

1. Introduction

The existence of two distinct protein synthesizing systems in *Saccharomyces cerevisiae* has been demonstrated *in vivo* and *in vitro* by their different sensitivities to particular antibiotics [1,2]. The mitochondrial system is very similar to that occurring in bacteria but differs from the latter by its sensitivity to some phenanthrene alkaloids [3]. The size of the mitochondrial ribosome remains controversial. Rifkin et al. [4] indicated that the *Neurospora* mitochondrial ribosome is of the 80s type while Küntzell and Noll [5] describe a 73s ribosome and its subunits [6]. Many attempts have been made to prove that the 80s mitochondrial ribosome was only a cytoplasmic contaminant [7,8]. By incorporation of radioactive amino acids, it was demonstrated [7] that the monomeric form of rat liver mitochondrial ribosome may be a 55s particle and, more recently [9], that a 77–80s ribosome may occur in mitochondria from *Candida utilis*. It is certain that there is not necessarily a direct relation between the size of the ribosome and its sensitivity to antibiotics. It has been suggested [3] that mitochondria from different species have different protein-synthesizing systems, so that the existence of only two types of ribosomes, 70s and 80s, seems improbable. This paper describes the isolation and distinct dissociation properties of 80s ribosomes from *Saccharomyces cerevisiae* mitochondria.

2. Materials and methods

Diploid strain D261, "grande ρ^+", a prototrophic *Saccharomyces cerevisiae*, was grown until full derepression on the P.G. medium of Imahori and Kudo [10] with sucrose in place of glucose and supplemented with 1 ml $FeCl_3$ 1% per l. The wet weight yield of cells was of 20 g/l of culture or 1.1 g of protein/l. Cells were lysed according to Schatz et al. [11] with the following modifications: the isolation medium was a TMK-sorbitol buffer (Tris-HCl 20 mM pH 7.4, $MgSO_4$ 5 mM, KCl 50 mM, mercaptoethanol 5 mM, sorbitol 500 mM) and the homogenization time was 90 sec at 2000 revs/min. The lysis and all subsequent operations were performed at 0–5°C. The debris were pelleted and the crude supernatant was centrifuged for 20 min at 30,000 × *g*. The supernatant constitutes the crude cytoplasmic fraction from which cytoplasmic ribosomes were sedimented. The 30,000 × *g* pellets (crude mitochondrial fraction) were resuspended and recentrifuged three times in 360 ml of TMK-sorbitol. Three centrifugations were necessary before the mitochondrial fraction had a constant specific cytochrome oxydase activity (per mg of protein). The final, thrice washed mitochondrial pellets were homogenized in 30 ml of TMK-sorbitol, and 10 ml aliquots were layered on 20 ml 20–65% sucrose in TMK gradients. After centrifuging 16 hr at 240,000 rpm in a Spinco SW25.1 rotor, the brown, cytochrome-oxydase-active, mitochondrial zone was collected, diluted with TMK-sorbitol and sedimented. The yield was about 150 mg mitochondrial protein from 5 g total crude protein. The pellet was resuspended in a total volume of 35 ml of TMK medium and 5 ml of 10% sodium deoxycholate (DOC) added. After a clarifying spin, the clear supernatant was layered on 2 ml 65% sucrose-TMK cushions and centrifuged for 3 hr at 105,000 × *g*. The pellet and the sucrose cushion were homogenized with TMK

North-Holland Publishing Company – Amsterdam

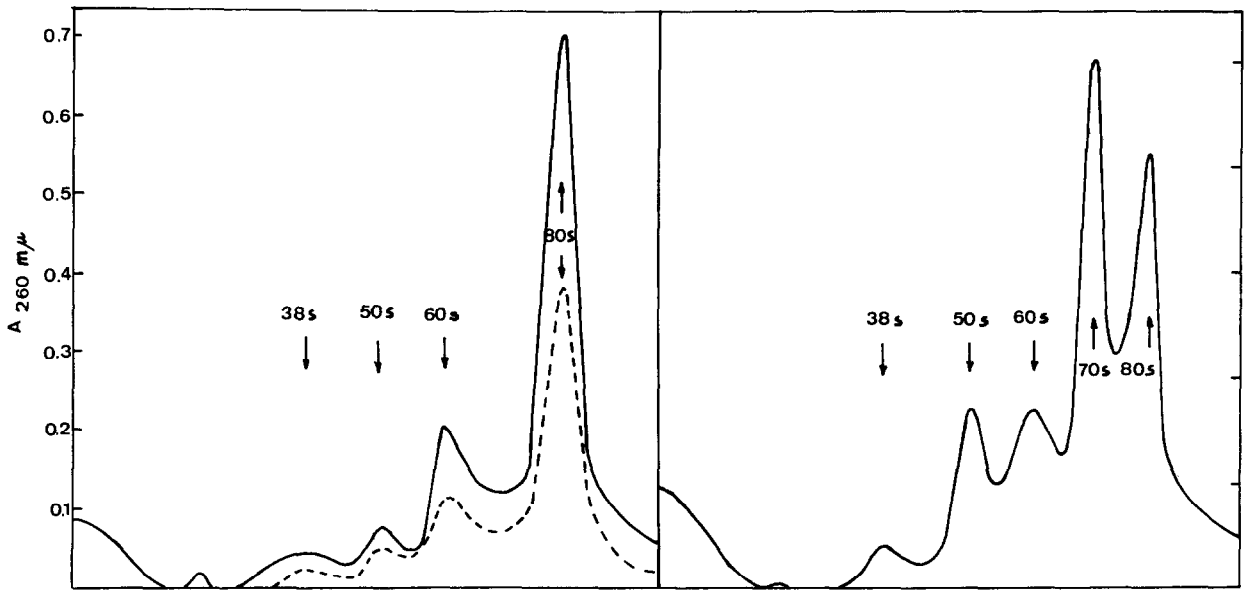

Fig. 1. Left. Sedimentation pattern of mitochondrial ribosomes purified with DOC and triton (- - - -) and of mitochondrial ribosomes purified with DOC and triton mixed with cytoplasmic ribosomes purified with DOC (—). Right. Sedimentation pattern of mitochondrial ribosomes mixed with *E. coli* ribosomes. The gradients contained: Tris-HCl 20 mM pH 7.4, $MgSO_4$ 5 mM, KCl 100 mM. Centrifugation and analysis as described in section 2.

and centrifuged for 20 min at 30,000 × *g*. The supernatant was made 0.5% in triton and the ribosomes pelleted. This purification with triton was essential in order to obtain mitochondrial ribosomes free from contaminating lipoproteins. The yield was about 1.5 mg soluble mitochondrial ribosomes from 5 g total crude protein; about 3 mg of mitochondrial ribosomes remained insoluble after resuspension.

The pellets of cytoplasmic ribosomes were suspended in TMK medium, at about 8 mg protein/ml, and the suspension was made 0.5% in DOC. After 2 hr at 105,000 × *g*, the pellets were resuspended and the suspension was made 0.5% in triton. The ribosomes were sedimented with a yield of about 500 mg ribosomes per 5 g total crude protein.

The ribosomes were analysed by linear sucrose gradients made according to the method of Britten and Roberts [12] between 15 and 30% sucrose in Tris-HCl 20 mM pH 7.4, $MgSO_4$ 5 mM, with variable concentrations of KCl (0–500 mM). The ribosomes were suspended in Tris-HCl 20 mM pH 7.4, $MgSO_4$ 5 mM just before layering. After 90 min at 49,000 rpm, at 4°C, in the Spinco SW50 rotor, the gradients were monitored for absorbance at 260 mμ through a flow cell, in a Beckman-Gilford spectrophotometer. The sedimentation constants were calculated by the method of Martin and Ames [13] with a standard error lower than 3%.

For labelling the ribosomes, the cells were grown until full derepression in a medium containing per l: yeast extract 10 g, glucose 20 g, KH_2PO_4 1 g, $FeCl_3$ 1% 1 ml; supplemented with 3 mC of $^{35}SO_4$ per l. The labelled ribosomes were sedimented as described above and washed in TMK. An amount of labelled ribosomes was added to the crude extract and the mitochondrial and cytoplasmic ribosomes were purified with DOC and triton as mentioned above. The specific radioactivity of the total cytoplasmic ribosomes (x cpm/$A_{260m\mu}$) and of the mitochondrial ribosomes (y cpm/$A_{260m\mu}$) were measured in toluene-triton (tt 21, Patterson and Greene [14]) in a Packard liquid scintillation counter. The percentage contamination of the mitochondrial ribosomes by cytoplasmic ones was equal to $y/x \times 100$.

3. Results and discussion

An indication of cytoplasmic contamination of the successive mitochondrial fractions comes from the disappearance of the radioactivity of added "tracer" cytoplasmic ribosomes to the crude extract. After three washings of the mitochondrial pellet, as precisely described in section 2, the contamination of mitochondrial ribosomes by cytoplasmic ribosomes did not exceed 8%. After isopycnic centrifugation, which eliminates cytoplasmic ribosomes as a pellet, the calculated contamination of the mitochondrial ribosomes was less than 3%. These results are in contrast with the difficulties encountered with liver microsomes [7,8] and in agreement with the results obtained with mitochondria of *Neurospora* purified by isopycnic centrifugation [4].

The mitochondrial ribosomes were calibrated against cytoplasmic ribosomes considered as 80s (we calculated a value of 79s using *E. coli* 70s as a standard). Fig. 1 shows that no separation could be obtained between cytoplasmic and mitochondrial monomers while the latter was clearly separated from *E. coli* ribosomes under the same conditions. As the gradient contained 100 mM KCl, the mitochondrial ribosomes are already partially dissociated. Without KCl, only the 80s peak was observed. Thus the mitochondrial monomer is of the 80 ± 1 S type. However there is a

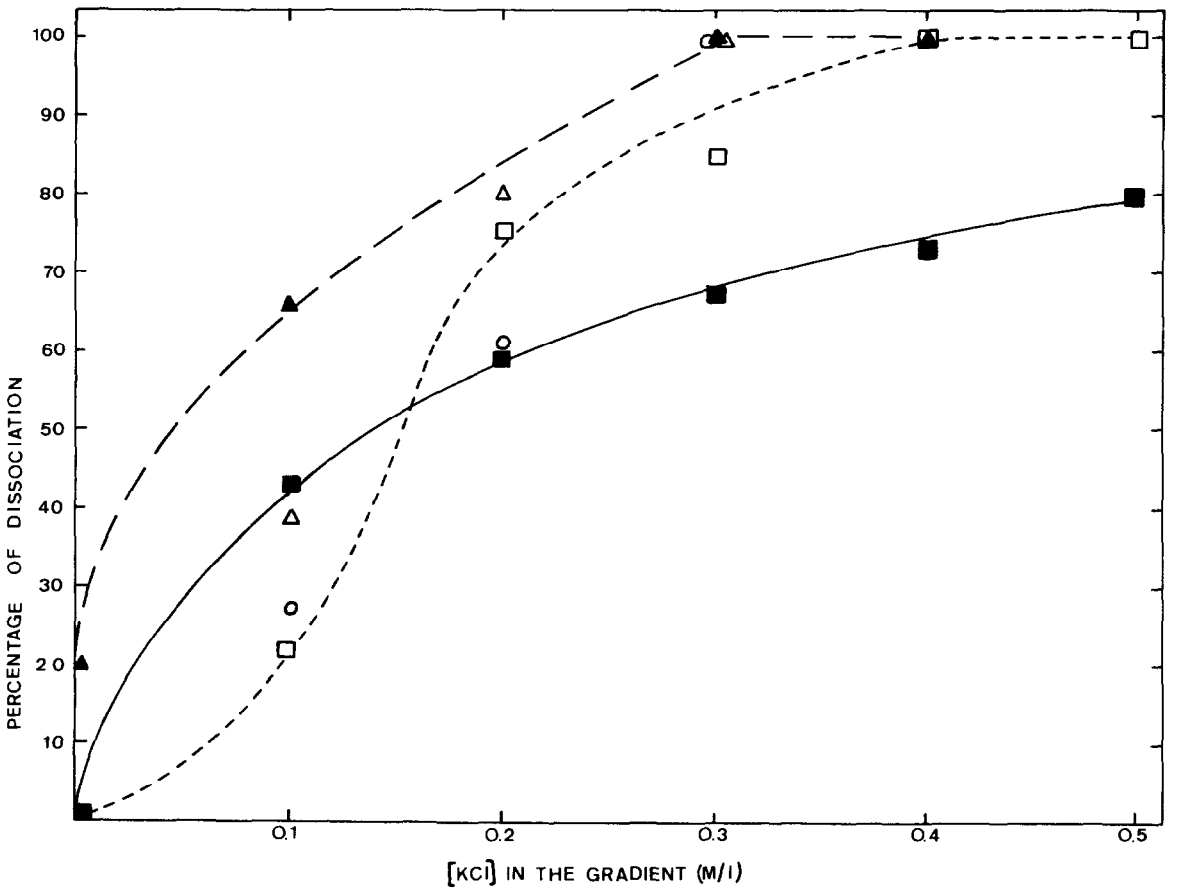

Fig. 2. Dissociation curves of cytoplasmic ribosomes washed in TMK (□- - -□), purified with 0.5% DOC (○) or 0.5% triton (△) or both (▲— —▲) and of mitochondrial ribosomes purified with DOC and triton (■—■). Experimental conditions are described in section 2.

distinction between the cytoplasmic and the mitochondrial ribosomes in their dissociation properties, when treated with detergents under the same conditions. Dissociation can be obtained in eucaryotic ribosomes by an increase in monovalent ion concentration (KCl) which presumably acts as a competitor of Mg^{++} without being able to play the same stabilizing role.

We preferred this way of dissociation (experimental conditions are given in section 2) to the use of EDTA which though more gentle is less reproducible, and also to low Mg^{++} concentrations which sometimes induces unfolding of the 80s monomer in the absence of dissociation. Fig. 2 shows the dissociation of cytoplasmic, with and without treatment by detergents, and of mitochondrial ribosomes. The treatment of cytoplasmic ribosomes with detergent facilitates dissociation.

A purification with DOC followed by a treatment with triton yields cytoplasmic ribosomes which are nearly fully dissociated at 0.2 M KCl, as shown in fig. 3; while with the mitochondrial ribosomes dissociation does not exceed 59%. The cytoplasmic ribosomes are visualized as a small 80s monomer peak, and 57s and 38s subunit peaks (67s peak is probably an unfolded form of 80s). For the mitochondrial ribosomes we distinguish the 80s monomer and three peaks of 60s, 50s and 38s respectively. The cytoplasmic subunits, calibrated as 60s and 38s, unfold stepwise at higher KCl concentrations into 50s and 28s entities. The 60s subunit unfolds before the small subunit. Thus at 0.2 M KCl the cytoplasmic 60s is already partially unfolded. At 0.3 M KCl it is completely unfolded to 50s (fig. 3). Mitochondrial ribosomes, under these conditions, and even at 0.5 M KCl, still show the 80s and 60s peaks. We can conclude that the light mitochondrial subunit has a sedimentation coefficient of 38s and the heavy one 60s, which unfolds to 50s. A complete dissociation of the mitochondrial 80s monomer is obtained by suspending the ribosomes in Tris-HCl 20 mM pH 7.4 and sedimenting them through a gradient containing Tris-HCl 20 mM pH 7.4 and KCl 100 mM. Under these conditions the 60s subunit is completely unfolded to 50s.

It is certain that these differences in dissociation properties do not necessarily represent the situation *in vivo*, but they are sufficient to distinguish the mitochondrial ribosomes from cytoplasmic ones.

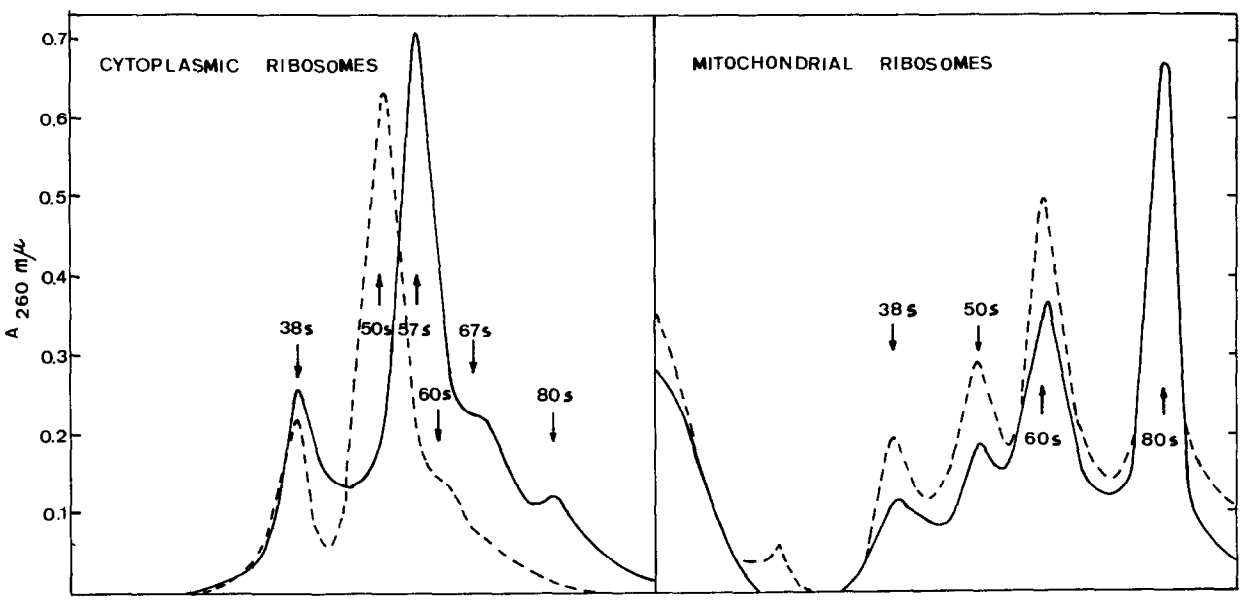

Fig. 3. Sedimentation pattern of mitochondrial and cytoplasmic ribosomes, both purified with DOC and triton, in a gradient containing: Tris-HCl 20 mM pH 7.4, $MgSO_4$ 5 mM, KCl 200 mM (—) and in a gradient containing: Tris-HCl 20 mM pH 7.4; $MgSO_4$ 5 mM, KCl 300 mM (- - - -).

Acknowledgements

We wish to thank Prof. H.Chantrenne, Dr. A.Sels and Prof. P.van Gansen for very helpful discussions. H.S. holds a predoctoral fellowship from the Institut pour l'encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture.

References

[1] G.D.Clark-Walker and A.W.Linnane, Biochem. Biophys. Res. Commun. 25 (1966) 8.
[2] A.J.Lamb, G.D.Clark-Walker and A.W.Linnane, Biochem. Biophys. Acta 161 (1968) 415.
[3] J.M.Haslam, P.H.Davey, A.W.Linnane and M.R.Atkinson, Biochem. Biophys. Res. Commun. 33 (1968) 368.
[4] M.R.Rifkin, D.D.Wood and D.J.L.Luck, Proc. Natl. Acad. Sci. US 58 (1967) 1025.
[5] H.Küntzell and H.Noll, Nature 215 (1967) 1340.
[6] H.Küntzell, J. Mol. Biol. 40 (1969) 315.
[7] T.W.O'Brien and G.F.Kalf, J. Biol. Chem. 242 (1967) 2172.
[8] J.G.Georgatsos and N.Papasarantopoulou, Arch. Biochem. Biophys. 126 (1968) 771.
[9] P.V.Vignais, J.Huet and J.André, FEBS Letters 3 (1969) 177.
[10] K.Imahori and G.Kudo, Biochim. Biophys. Acta 78 (1963) 525.
[11] G.Schatz, H.F.Penefsky and E.Racher, J. Biol. Chem. 242 (1967) 2553.
[12] R.J.Britten and R.B.Roberts, Science 131 (1960) 32.
[13] R.G.Martin and B.N.Ames, J. Biol. Chem. 236 (1961) 1373.
[14] M.S.Patterson and R.C.Greene, Anal. Chem. 37 (1965) 854.