PROTEIN SYNTHESIS ON RIBOSOMES ISOLATED FROM RAT LIVER MITOCHONDRIA: SENSITIVITY TO ERYTHROMYCIN

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1. Introduction

The mitochondrial ribosome can be clearly differentiated from the cytoplasmic ribosome in its response to inhibitors of protein synthesis [1]. Chloramphenicol and other antibiotics which interfere with protein synthesis on bacterial ribosomes inhibit mitochondrial protein synthesis, while cycloheximide, an inhibitor of protein synthesis on cytoplasmic ribosomes, has no effect on mitochondrial protein synthesis. The ribosome present in liver mitochondria has been reported to sediment in sucrose gradients at 55 S [2–5], although the ribosome of yeast mitochondria sediments at 74 S [6]. Both ribosomes, however, are involved in mitochondrial protein synthesis [6, 7].

A phlyogenetic difference between the mitochondrial ribosome of mammals and yeast has been claimed on the basis of sensitivity to erythromycin [8, 9]. Amino acid incorporation by yeast mitochondria was strongly inhibited by erythromycin, while that of either intact or damaged liver mitochondria was completely resistant [8] suggesting that the ribosome of liver mitochondria was insensitive to erythromycin. In contrast, Kroon and DeVries [10] suggested that the lack of sensitivity to erythromycin was due to a permeability barrier in the mitochondrial membrane, since erythromycin inhibited incorporation in liver mitochondria previously treated with hypotonic sucrose. This conflict can only be satisfactorily resolved by testing the effect of erythromycin on isolated ribosomes. In the present study, we report that low concentrations of erythromycin inhibit protein synthesis on isolated rat liver mitochondrial ribosomes whcih are highly active in catalyzing poly(U)-directed polyphenylalanine synthesis.

2. Methods

Rat liver mitochondria were prepared from 50–60 rats under sterile conditions in 0.34 M sucrose containing 5 mM Tris, pH 7.6, by the procedure of O'Brien and Kalf [2]. The mitochondrial pellet obtained after 7 washes was resuspended at a concentration of 5 mg/ml in a medium (A) containing 0.1 M KCl, 5 mM Tris–HCl, pH 7.6 and 30 mM $MgCl_2$. The mitochondria were lysed by the addition of 0.1 vol of 10% Triton X-100. Deoxyribonuclease, free of ribonuclease, was then added at a final concentration of 5 μg/ml and the solution kept on ice for 5 min. The suspension was then centrifuged for 10 min at 60 000 *g* in the Spinco #30 rotor. The resultant supernatant was layered on 2 ml of medium A containing 24% sucrose and centrifuged for 3 hr at 230 000 *g* in the Spinco #65 rotor. The crude ribosomal pellet was resuspended in 2 ml of medium A containing 6 mM mercaptoethanol and centrifuged at 3000 *g* for 5 min. The supernatant was layered on a 10–30% sucrose gradient containing medium A supplemented with 6 mM mercaptoethanol. After 16 hr at 20 000 *g* in the Spinco #SW 27 rotor, one ml fractions were collected and the absorbance at 260 nm measured for each fraction.

E. coli ribosomes were prepared as described by Nirenberg [11] and yeast mitochondrial ribosomes as described by Grivell et al. [6]. Cell-free protein synthesis was assayed in 0.25 ml of medium containing: 10 mM Tris–HCl, pH 7.8; 50 mM KCl; 12 mM magnesium acetate; 25 μM tyrosine; 50 μM of an amino acid mixture [12]; 30 μM GTP; 1 mM ATP; 6 mM mercaptoethanol; 5 mM P-enol-pyruvate; 25 μg of pyruvate kinase; 1 mM dithiothreitol; 250 μg of poly(U)

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and 50 μM [^{14}C]phenylalanine in the presence of 250 μg of a supernatant fraction from *E. coli* [11]. Mitochondrial ribosomes were added at a concentration of 100 μg of RNA. After 15 min at 30°C, the proteins were precipitated with 5% trichloroacetic acid and prepared for counting [13].

Erythromycin base and nucleotides were obtained from Sigma, [^{14}C]phenylalanine (400 mCi/mmole) from New England Nuclear, and poly(U) from Miles.

3. Results

The sedimentation profile of ribosomes extracted from rat liver mitochondria is compared with that of purified ribosomes from *E. coli* (fig. 1). The mitochondrial ribosomes sediment at 55 S with only a small amount of contaminating material of higher buoyant density and can clearly be distinguished from the bacterial 70 S ribosomes. The concentration of Mg^{2+} ion in the gradient (30 mM) was critical. When the Mg^{2+} ion concentration was lowered to 10 mM, the 55 S ribosomes dissociated to the two subunits of 39 and 28 S. The A_{260}:A_{280} ratio of the purified ribosomes collected from tubes 15–20 of the gradient was 1.51, a value comparable to that reported by O'Brien [3] for ribosomes obtained from rat liver mitochondria.

The mitochondrial ribosomes prepared in this way were highly active in cell-free protein synthesis measured with poly(U) and phenylalanine. In most experiments, the rate was comparable to that observed with

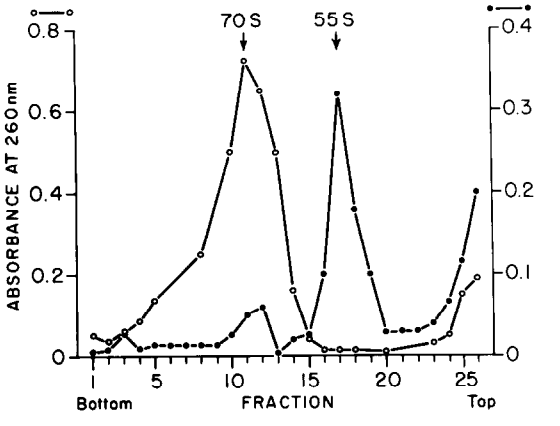

Fig. 1. Sucrose density gradient of ribosomes isolated from rat liver mitochondria or *E. coli* as described in Methods.

Table 1
Activity of mitochondrial ribosomes in poly(U)-directed protein synthesis.

	pmoles/ mg RNA/ 15 min	% Inhibition
Expt. 1.		
Complete (liver)	323	–
–ATP, P-enolpyruvate pyruvate kinase	1	99%
–Supernatant factors	29	91%
–poly(U)	3	99%
+Cycloheximide (1.0 mM)	318	2%
+Chloramphenicol (0.25 mM)	152	56%
+Erythromycin (0.1 mM)	43	87%
Expt. 2.		
Complete (liver)	980	–
+Erythromycin (0.05 mM)	350	64%
(0.1 mM)	60	94%
+Carbomycin (0.05 mM)	320	68%
(0.1 mM)	130	87%
Expt. 3.		
Complete (yeast)	848	–
+Cycloheximide (1.0 mM)	709	17%
+Chloramphenicol (0.25 mM)	490	43%

Ribosomes were incubated as described in Methods. A small zero time of 1 nmole/mg RNA/15 min was substracted from each value.

ribosomes isolated from yeast mitochondria (table 1, expt. 2) and was absolutely dependent on poly(U), supernatant factors from *E. coli* and an energy source. The rate of protein synthesis did vary considerably in different preparations; hence, immediately after preparation, the ribosomes were stored at –70°C and only thawed once before an experiment. Refreezing and thawing the ribosomes resulted in greatly lowered rates of protein synthesis.

Cycloheximide, the inhibitor of cytoplasmic protein synthesis, had no effect on protein synthesis by ribosomes obtained from either yeast or rat liver mitochondria, while chloramphenicol, at a concentration of 0.25 mM, inhibited synthesis approximately 50%. Both erythromycin and carbomycin, at a concentration of 0.1 mM, completely blocked protein synthesis on rat liver mitochondrial ribosomes.

4. Discussion

Previous studies in our laboratory [4, 15] have indicated that erythromycin, as well as carbomycin, causes a significant inhibition of amino acid incorporation by isolated inner membrane-matrix fractions prepared from rat liver mitochondria with digitonin [16]. The amount of erythromycin required for a 50% inhibition of incorporation in this fraction, however, was almost double that needed when isolated yeast mitochondria were used [17]. The differential susceptibility to erythromycin has been explained as due to a change in the ribosome itself [9] or to a permeability barrier to the drug in the liver mitochondrial membrane [10]. In the present study, ribosomes which catalyze the synthesis of polyphenylalanine in a cell-free system at a rate comparable to that obtained with yeast mitochondrial ribosomes, have been isolated from rat liver mitochondria. Protein synthesis catalyzed by these ribosomes was inhibited completely by low concentrations of erythromycin or carbomycin.

Ribosomes isolated from yeast mitochondria are also active in poly(U)-dependent protein synthesis in a cell-free system [6, 13]; however, erythromycin, at concentrations even greater than 0.1 mM inhibit protein synthesis only at a maximum of about 50% [18]. The different responses to erythromycin of the yeast mitochondrial ribosome as compared to the rat liver mitochondrial ribosome is not easily explained; however, it is clear that the mitochondrial ribosome from mammalian liver, despite its different overall size and smaller RNA components, has retained the sensitivity to erythromycin of the mitochondrial ribosomes from primitive eukaryotes.

Summary

Ribosomes with a sedimentation coefficient of 55 S have been isolated from rat liver mitochondria. When combined with supernatant factors from *Escherichia coli*, these ribosomes catalyze poly(U)-dependent polyphenylalanine synthesis at rates up to 1.0 nmole per mg RNA per 15 min. Protein synthesis on these ribosomes was completely inhibited by erythromycin or carbomycin, partially inhibited by chloramphenicol and unaffected by cycloheximide.

Acknowledgements

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