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Isolation Procedures and Partial Characterization of Mitochondrial Ribosomes from Yoshida Ascites Hepatoma A.H. 130 Cells *

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Mitochondrial ribosomes from Yoshida ascites hepatoma A. H. 130 cells have been isolated and their sedimentation coefficient value has been determined.

These ribosomes show a sedimentation coefficient value of about 55 S for the monomeric form and of about 30 S and 40 S for the small and large subunits respectively. These results are in accordance with those obtained from mammalian cells mitochondria of different origins.

The presence of ribosomes has been demonstrated in all mitochondria (1, 4). Mammalians seem to present small mitochondrial ribosomes as first reported by O'BRIEN (12, 13), who described the presence of 55 S mitochondrial ribosome monomers in rat liver mitochondria. Since then, much controversy has been raised about the size of mitochondrial ribosomes (5, 6, 14). At the present time, 55 S ribosomes are considered as the monomeric form of the mitochondrial ribosomes (2, 3) able to carry out all test reactions for ribosomes (7, 8, 11, 15).

In the present paper the isolation and characterization of mitochondrial ribosomes from Yoshida ascites hepatoma A.H.130 cells is described, in order to dispose of isolated ribosomes to carry out studies on protein synthesis in tumour cells mitochondrial ribosomes.

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Materials and Methods

Preparation of mitochondria. Yoshida tumour mitochondria were prepared according to the methods previously described (10). Semisterile conditions have been followed as described by KROON *et al.* (9).

Preparation of mitochondrial ribosomes. Ribosomes were isolated from tumour mitochondria by the following procedures: Mitochondria (10 mg protein/ml) were lysed by dropwise addition of an equal volume of 2 × TTMK medium (2 % Triton X-100, 0.02 M Tris buffer (pH 7.4), 0.02 M Mg acetate, 0.2 M KCl). The lysate was centrifuged at 18,000 × *g* for 20 minutes. The supernatant was again centrifuged at 130,000 × *g* for 3 hours. The pellet obtained was carefully resuspended in a very small quantity of TMK buffer (0.01 M Tris buffer (pH 7.4), 0.01 M Mg acetate, 0.1 M KCl) and centrifuged at 12,500 × *g* for 10 minutes. The clear supernatant was layered on the top of a 10-30 % sucrose in TMK buffer linear gradient and centrifuged in the Spinco S.W. 50 L rotor at 165,000 × *g* for 100 minutes. Fractionation was achieved by means of an ISCO gradient fractionator model D. Absorbance was monitored in a dual beam UA-2 ISCO ultraviolet analyzer.

Preparation of cytoplasmic ribosomes. Cytoplasmic ribosomes were prepared from the postmitochondrial fraction centrifuged twice at 12,500 × *g* for 20 minutes, by the same procedure as described for mitochondrial ribosomes, except that microsomal cytoplasmic fraction protein concentration was about half that of mitochondria during the lysis with 2 × TTMK.

Results and Discussion

The fractionation of the gradients gave for ribosomes the following sedimentation patterns.

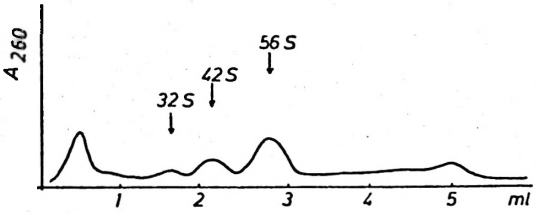

Fig. 1. *Sedimentation profile of mitochondrial ribosomes released by Triton X-100 from the mitochondrial fraction of Yoshida ascites hepatoma A.H. 130 cells.*
The mitochondrial ribosomes were prepared as described in Materials and Methods, and finally resuspended in TMK medium (0.01 M Tris buffer (pH 7.4), 0.01 M Mg acetate, 0.01 M KCl). The resuspended ribosomes were layered on a 10-30 % sucrose gradient in the medium specified above and centrifuged for 100 minutes at 165,000 × *g* in the Spinco S.W. 50 L rotor at 3° C. Gradients were fractionated with an ISCO gradient fractionator model D and absorbance monitored in a dual beam UA-2 ISCO ultraviolet analyzer.

Figure 1 shows the sucrose density gradient profile of mitochondrial ribosomes prepared under the above described methods. Under these conditions mitochondrial ribosomes exhibit a main peak sedimenting at about 55 S and two minor peaks sedimenting at about 30 S and 40 S. Ribosomal particles sedimenting at higher sedimentation coefficients were not present.

The sucrose density gradient profile of the cytoplasmic ribosomes (fig. 2) exhibits two major peaks sedimenting at about 80 S and 110 S, and two minor peaks sedimenting at about 40 S and 60 S. No 55 S peaks were observed in this fraction.

These results show that, under the conditions described above, mitochondrial ribosomes are mainly isolated in the monomeric form with little tendency to dissociate into ribosomal subunits (30 S and 40 S). These values are the same as those accepted for ribosomes from normal rat liver mitochondria. Howeaver, ribosomes

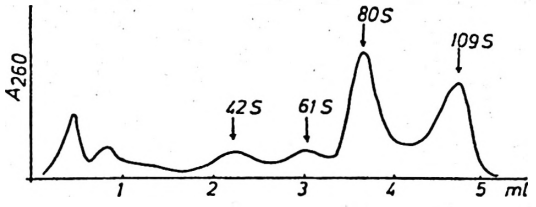

Fig. 2. *Sedimentation profile of cytoplasmic ribosomes released by Triton X-100 from the microsomial fraction of Yoshida ascites hepatoma A.H. 130 cells.*
Experimental conditions as in figure 1.

from normal rat liver mitochondria exhibit a greater tendency to associate into the dimeric form under the same conditions since the 80 S peak is the major one obtained in the sedimentation pattern. In contrast, the sucrose density gradient profile of the ribosomal fraction isolated from Yoshida tumour cytoplasm shows, that under the same conditions, tumour cytoplasmic ribosomes present a lower tendency to disagregate in ribosomal subunits, in relation to normal liver cytoplasmic ribosomes.

We can conclude from these results that tumour mitochondrial ribosomes are very simmilar, if not equal, to the normal rat liver mitochondrial ribosomes. Further characterization of the tumour 55 S particle under different ionic conditions and an investigation of his ability to incorporate aminoacids, are necessary for complete characterization of these tumour mitochondrial ribosomes.

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Resumen

Los ribosomas mitocondriales de células del hepatoma ascítico de Yoshida A.H. 130 han sido aislados y se ha determinado el valor de su coeficiente de sedimentación.

Estos ribosomas presentan un coeficiente de sedimentación de valor próximo a 55 S para el monómero y de 30 S y 40 S para las subunidades menor y mayor, respectivamente. Estos datos son concordantes con los obtenidos de las mitocondrias de células de mamíferos de origen diverso.

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