THE EFFECT OF INCUBATION WITH PUROMYCIN ON THE DISSOCIATION OF RAT LIVER RIBOSOMES INTO ACTIVE SUBUNITS

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SUMMARY. In sharp contrast to polyribosomes or monomeric ribosomes produced by ribonuclease treatment of polyribosomes, ribosomes which were incubated in the presence of soluble factors, an energy generating system, and puromycin were completely dissociated into subunits when equilibrated with Tris-HCl, pH 7.8 (0.05 M), KCl (0.15 M), $MgSO_4$ (1.0 mM). When separated on a sucrose density gradient, these subunits did not incorporate amino acids into protein when they were tested individually. The recombined subunits were active in phenylalanine incorporation with an absolute requirement for added messenger RNA (poly U). Results obtained from these experiments support the conclusion that a major difference between 70S and 80S ribosomes may be related to the greater degree of stability conferred on the 80S subunit couple by the presence of the peptidyl-transfer RNA.

Liver ribosomes differ from bacterial ribosomes in that the bound Mg^{2+} must be reduced to a much lower level before subunits may be separated (1). Bacterial (70S) ribosomes may be dissociated by lowering the Mg^{2+} concentration to 0.1 mM. This dissociation is reversible and the re-formed 70S particle is active in amino acid incorporation. However, liver ribosomes dissociate only at a Mg^{2+} concentration of 10^{-5} M or less forming subunits of 53S and 28S with the concomitant release of transfer RNA and 5S RNA (1). On restoration of the Mg^{2+} they re-associate mainly to random aggregates, inactive in amino acid incorporation (1).

Petermann et al. (1) have recently shown that treatment of rat

liver ribosomes with 2.2 M urea results in the appearance of 57S and 40S subunits; the 5S RNA is not detached. On restoration of the Mg^{2+} they re-associated to 80S ribosomes which were active in phenylalanine incorporation in the presence of poly U.

This paper shows that when polysome run-off is induced by incubation of polysomes with puromycin, the resulting mono- and dimeric ribosomes, freed of attached messenger RNA and peptidyl-transfer RNA, dissociated into subunits (of approximately 60S and 40S) when the Mg^{2+} concentration was lowered to 1 mM.

METHODS

Conditions for preparation of polysomes, incubation, amino acid incorporation assay, and monitoring of sucrose density gradients were as described previously (2). The pre-incubation was carried out at 37° for 45 minutes in the presence of 7×10^{-4} M puromycin; the ribosome concentration was 1.6 mg/ml. Columns of Sephadex used in these experiments were 2 cm in diameter and 35 cm high. The load on these columns did not exceed 20 mg of ribosomes or 6 ml. Ribonuclease-treated ribosomes were prepared by mixing 25 µg of ribonuclease with 1 ml (10 mg) of polyribosomes for 15 minutes at 4°. Cell sap (1 ml) was added as a ribonuclease inhibitor (3) before the mixture was placed on a column of G-100 Sephadex to separate the ribosomes from ribonuclease and other small molecules.

RESULTS

It was found that the magnesium concentration of ribosomes could be adjusted quickly by passing the ribosome sample through a column of Sephadex G-25 equilibrated with the desired concentration of ions. When rat liver polyribosomes were thus equilibrated with Tris-HCl, pH 7.8 (0.05 M), KCl (0.025 M), $MgSO_4$ (10^{-5} M) subunits were produced as shown in Fig. 1a. These subunits could not

be recombined into active ribosomes by increasing the Mg^{2+} concentration to 5 mM. If the removal of Mg^{2+} was incomplete the preparation contained some subunits in the presence of un-dissociated polyribosomes as shown in Fig. 1b. This dissociation appeared to be directly from polysome to subunits without going through a monomeric intermediate.

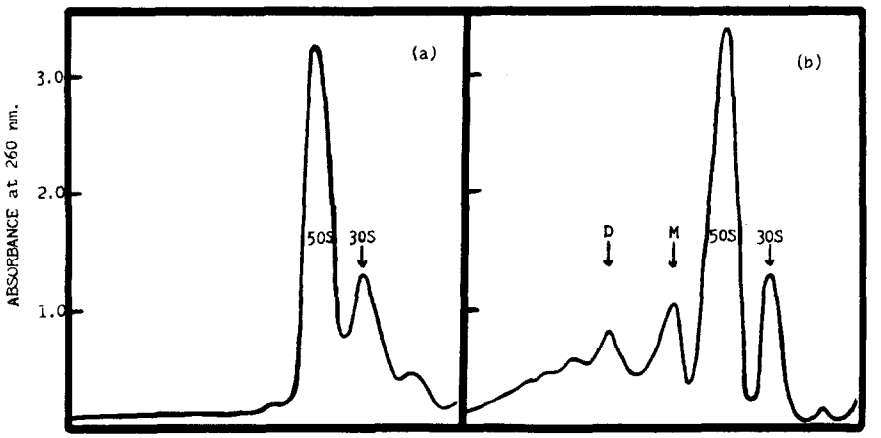

Figure 1 Sucrose Density Gradient Analysis of Ribosomes

(a) after equilibration with Tris-HCl, pH 7.8 (0.05 M), KCl (0.025 M), $MgSO_4$ (10^{-5}M).
(b) after equilibration with Tris-HCl, pH 7.8 (0.05 M), KCl (0.025 M), $MgSO_4$ (5.10^{-5} M).

The absorbance at 260 nm was measured in a Gilford 2400 recording spectrophotometer by pumping the gradients through a flow cell at 1 ml/min. Ribosomes were layered on 29 ml linear gradients, 15-50% sucrose in Tris-HCl, pH 7.8 (0.05 M), KCl (0.025 M), $MgSO_4$ (10^{-4} M) and centrifuged at 24,000 rpm in the SB110 rotor of an International B-50 centrifuge. The positions of monomer and dimer ribosomes are marked by "M" and "D".

When polysomes were incubated at 37° for 45 minutes in the presence of cell sap, an energy generating system and 7×10^{-4} M puromycin, monomer and dimer ribosomes were produced (see Fig. 2c) which were completely devoid of endogenous messenger RNA activity (see Table 1). These ribosomes were completely dissociated into subunits of approximately 60S and 40S when the Mg^{2+} concentration was adjusted to 0.1-1.0 mM. In Fig. 2d, these subunits are shown

Table 1

Incorporation of ^{14}C-Phenylalanine

Source of ribosomes	cpm/mg ribosomes incubated	
	no poly U added	100 ug poly U added
large subunit	0	290
small subunit	0	120
large and small subunit	10	1280
puromycin-treated ribosomes	12	1210

Incubations contained 0.15 mg small subunits or 0.35 mg large subunits or 0.5 mg ribosomes in a total volume of 1 ml. Incubations were at 37° for 30 minutes in the presence of 0.125 µC ^{14}C-phenylalanine as described in reference 2. Puromycin-treated ribosomes were passed through a column of G-25 Sephadex equilibrated with Tris-HCl (0.05 M), KCl (0.025 M), $MgSO_4$ (0.005 M) before being assayed for phenylalanine incorporation.

for comparison, with subunits produced by removal of all Mg^{2+} by treatment with EDTA (2).

Polyribosomes (Fig. 2a) which were equilibrated with Tris-HCl, pH 7.8 (0.05 M), KCl (0.15 M), $MgSO_4$ (1.0 mM) without any preincubation with puromycin did not dissociate into subunits but the proportion of small polysomes and monomers increased (Fig. 2b). This result was characteristic of the behaviour of polysomes in high concentrations of K^+.

In order to demonstrate that dissociation into subunits at 1 mM Mg^{2+} was not a characteristic of all monomer-dimer ribosomes, a comparison was made between monomer-dimer ribosomes prepared by

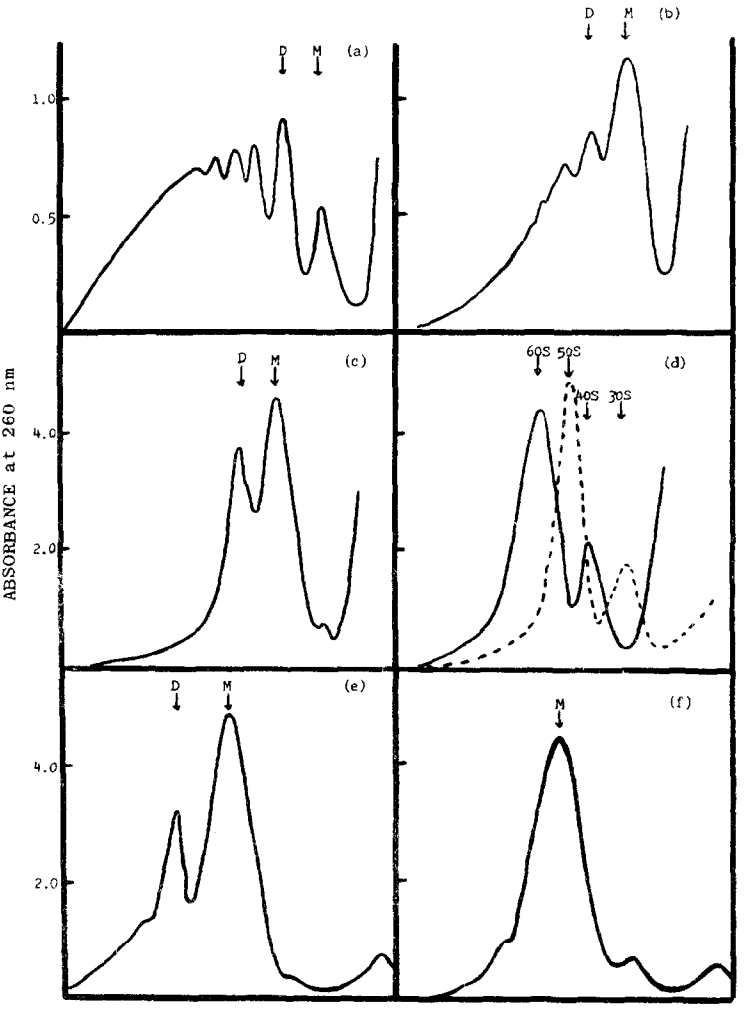

Figure 2 Sucrose Density Gradient Analysis of Rat Liver Polyribosomes after Various Treatments

Absorbances at 260 nm were recorded as described for Fig. 1. All gradients were 29 ml linear gradients, 15-50% sucrose. Gradients (a), (c), and (e) contained Tris-HCl, pH 7.8 (0.05 M), KCl (0.025 M), $MgSO_4$ (5.0 mM); (b), (d), and (f) contained Tris-HCl, pH 7.8 (0.05 M), KCl (0.15 M), $MgSO_4$ (1.0 mM). The gradients were centrifuged in the SB 110 rotor of an International B-50 centrifuge at 24,000 rpm. The positions of monomer and dimer ribosomes are marked by "M" and "D".

(a) Polyribosomes were layered on the gradient and centrifuged for 3 hr.

(b) Polyribosomes were equilibrated with Tris-HCl, pH 7.8 (0.05 M), KCl (0.15 M), $MgSO_4$ (1.0 mM), layered on the gradient and centrifuged for 3 hr.

(c) Polyribosomes were incubated with puromycin, layered on the gradient and centrifuged for 4 hr.

(d) Polyribosomes were incubated with puromycin, adjusted to Tris-HCl, pH 7.8 (0.05 M), KCl (0.15 M), $MgSO_4$ (1.0 mM), layered on the gradient and centrifuged for 6.5 hr (———). Polyribosomes were treated with EDTA (0.6 µmole/mg ribosomes) (2), layered on a gradient containing Tris-HCl, pH 7.8 (0.05 M), KCl (0.025 M), $MgSO_4$ (0.1 mM), and centrifuged for 6.5 hr (-----).

(e) Polyribosomes were treated with ribonuclease, chromatographed on a column of Sephadex G-100, layered on a gradient and centrifuged for 5 hr.

(f) Polysomes were treated with ribonuclease, chromatographed on a column of Sephadex G-100, adjusted to Tris-HCl, pH 7.8 (0.05 M), KCl (0.15 M), $MgSO_4$ (1.0 mM), layered on a gradient and centrifuged for 5 hr.

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puromycin treatment (Fig. 2c) and those prepared by ribonuclease treatment of polyribosomes (Fig. 2e). Ribosomes from which peptidyl-transfer RNA had been removed by incubation with puromycin were dissociated to subunits in 1 mM Mg^{2+} (Fig. 2d). Ribonuclease treatment, however, produced monomer-dimer ribosomes with peptidyl-transfer RNA intact and these ribosomes did not dissociate in 1 mM Mg^{2+} (Fig. 2f).

Subunits produced at 0.1-1.0 mM Mg^{2+} from pre-incubated ribosomes were found to be very sensitive to the ratio K^+/Mg^{2+}. At low K^+/Mg^{2+} the small subunit tended to dimerize and co-sediment with the large subunit. At high potassium concentrations the small subunit showed a tendency to break down from 40S to 30S.

In order to test the activity of the ribosomal subunits prepared from puromycin-treated ribosomes, the peak fractions from the sucrose density gradients were pooled and the subunits concentrated by centrifuging them into pellets. When the subunits were resuspended and tested for phenylalanine incorporation (according to the methods described earlier, 2) they were each found to be relatively inactive alone but large and small subunits together were active in the presence of added poly U (see Table 1). This activity was equal to that of a control preparation of ribosomes which

had received a puromycin treatment only. Each type of subunit, when placed on a second sucrose density gradient, sedimented as a single peak with the same sedimentation rate as it had originally.

DISCUSSION

The dissociation of 80S ribosomes into subunits which may be re-constituted to active ribosomes has recently been accomplished by a variety of methods in various laboratories (1,4,5). The method used in this investigation was to mimick the process of polysome runnoff which occurs in the cell. The dissociation into subunits of puromycin-treated ribosomes from rat liver appears very similar to the behaviour of E. coli ribosomes under similar conditions (6). This implies that 70S and 80S ribosomes may behave similarly with respect to dissociation once the peptidyl transfer RNA has been released. Thus we are presently seeking to establish whether or not a dissociation factor similar to that found in E. coli (7) is operative in rat liver.

The dimerization tendency of small subunits produced by exposing puromycin-treated ribosomes to 1 mM Mg^{2+} has also been noted with "runoff" 70S ribosomes at 2 mM Mg^{2+} (6), suggesting that this phenomenon is not a peculiar characteristic of 40S subunits.

The results of this study show that 80S ribosomes are similar to 70S ribosomes in that they are stabilized by the presence of peptidyl-transfer RNA; however, a major difference between the two types of ribosomes appears to be the extent to which this stabilization occurs. With peptidyl-transfer RNA in place, 70S ribosomes are dissociated by removal of Mg^{2+} to a concentration of 0.1 mM, whereas 80S ribosomes require the removal of so much Mg^{2+} (10^{-5} M) that the subunits themselves become unstable and break down, apparently irreversibly, to smaller particles.

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