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THE ROLE OF ETHANOL EXTRACTABLE PROTEINS FROM THE 80S RAT LIVER RIBOSOME

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SUMMARY

80S rat liver ribosomes have been extracted with fifty percent ethanol at varying salt concentrations. The resulting 80S core ribosomes have lost almost all of their protein synthesis activity. The protein synthesis activity could be partially regained when the ethanol extracted proteins were reconstituted with the core ribosomes; however, reconstitution of the ribosome dependent EF-II GTP hydrolysis activity could not be detected. The ethanol extracts were found to contain only a few proteins, one or more of which we believe is necessary for the binding of elongation factor-II.

INTRODUCTION

Extraction of 60S rat liver ribosomal subunits with fifty percent ethanol has been reported to give only a select few proteins (1). These proteins are acidic and phosphorylated (2). Acidic phosphoproteins have been detected in the large ribosomal subunits of other eukaryotes as well (3-5). Ethanol extractable proteins from rat liver 60S subunits have similar physical characteristics to Escherichia coli ribosomal proteins L7/L12. The electrophoretic mobility, molecular weight, phosphorylation, and solubility in fifty percent ethanol are similar for these different proteins which have been isolated from prokaryotic and eukaryotic sources (1). In addition, Wool et al have reported that ethanol extraction of rat liver 60S ribosomal subunits yields proteins which are immunologically cross-reactive to E. coli L7/L12 (5,6). E. coli L7/L12 can interact with yeast ethanol extracted ribosomes to give active hybrids (7). L7/L12 has been shown to be essential for interaction with elongation factor-G, and thus necessary for protein synthesis in E. coli ribosomes (8).

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We have attempted to find the functional analog of E. coli L7/L12 in rat liver ribosomes by using the extraction method of Arpin et al. (1) and the methods described by Hamel et al. (8) for extracting and reconstituting ribosomes.

MATERIALS AND METHODS

[^{3}H]-Phenylalanine and Aquasol were obtained from New England Nuclear. Puromycin hydrochloride, sodium deoxycholate, dithiothreitol, and poly-uridylic acid were purchased from Sigma Chemical Company. E. coli strain B transfer RNA and E. coli paste were obtained from Grand Island Biological. Hydroxyapatite C was from Clarkson Chemical Co., Inc., Williamsport, Pennsylvania. Cellulose phosphate was purchased from Whatman, W. and R. Balston, Maidstone, England. Gamma [^{32}P]-guanosine triphosphate was obtained from Amersham.

Puromycin treated 80S rat liver ribosomes were prepared according to Silve et al. (10). pH 5.2 supernatant containing impure elongation factors I and II was prepared as described by Moldave et al. (11). Elongation factor-II from rat liver was purified according to the procedure of Moldave et al. (12).

Tritiated E. coli phenylalanine transfer RNA was prepared according to the procedure of Moldave et al. (13).

Ribosome dependent EF-II GTPase assays were carried out according to the procedure of Raeburn et al. (14). Controls for background counts due to hydrolysis of GTP by purified EF-II, 80S ribosomes or core ribosomes, and for unabsorbed [^{32}P]-GTP were summed and subtracted from the total counts observed to determine the ribosome dependent GTP hydrolysis.

Rat liver ribosomes in buffer A (0.35 M sucrose, 0.05 M Tris·Cl, 0.05 M KCl, 0.004 $MgCl_2$, 0.001 M DTT) were extracted by adding an appropriate volume of ice-cold ethanol and/or salt solution to the ribosome solution and then allowing the mixture to stand in ice with occasional stirring for 30 minutes. The solution was then centrifuged in a Sorvall SS-34 type rotor for ten minutes at 15,000 RPM, and the pellet was resuspended in buffer A for either further extraction or dialysis against buffer A. The combined supernatants were also dialyzed against buffer A overnight.

Reconstitution was done by adding an appropriate amount of dialyzed extracted protein together with the core ribosome and incubating the two with buffer A at 37° for fifteen minutes before assaying.

RESULTS

When 80S rat liver ribosomes were extracted two to three times with fifty percent ethanol at zero degrees under varying salt concentrations, the resulting core ribosomes showed significant reduction in protein synthesis activity. When the same 80S core ribosomes were reconstituted with an equivalent amount of the dialyzed extracted protein solution, the activity of the reconstituted ribosome was as much as six times the activity of the core ribosome, and thirty percent of the original activity (Figure 1). The dialyzed extracted protein showed no activity itself under any extraction condition and did not stimulate the activity of the unextracted ribosomes in any way. A variety of extraction conditions were tried,

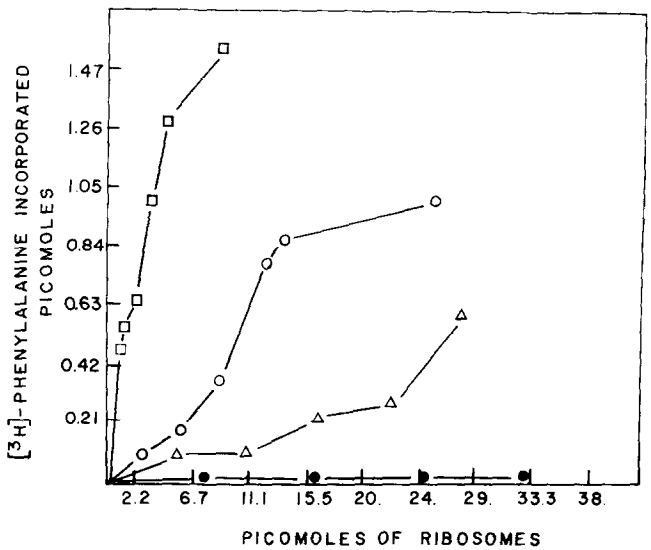

Figure 1: Measurement of protein synthesis activity of extracted, unextracted, and reconstituted ribosomes. All measurements were made with the same amounts of poly-uridylic acid, GTP, EF-I, EF-II, and salt concentrations. Unextracted ribosomes (□), ribosomes extracted with fifty percent ethanol two times (△), the same ribosomes reconstituted with their extracts (○), and the extracts alone (■).

TABLE I. PROTEIN SYNTHESIS ACTIVITY OF EXTRACTED AND RECONSTITUTED RIBOSOMES

EXTRACTION METHOD	80S CORE	80S CORE + EXTRACTED PROTEINS	EXTRACTED PROTEINS
50 % Ethanol, 1X, 0° C	39.8	45.0	0
50 % Ethanol, 2X, 0° C	5.4	28.7	0
50 % Ethanol, 3X, 0° C	4.6	8.9	0
50 % Ethanol, 2X, 30° C	2.1	4.4	0
33 % Ethanol, 3X, 0° C	42.4	43.5	0
75 % Ethanol, 2X, 0° C	25.7	24.4	0
50 % Ethanol/0.5 M KCl, 1X	1.1	7.3	0
50 % Ethanol/0.5 M KCl, 2X	2.4	8.1	0
50 % Ethanol/1 M NH_4Cl, 1X	0.44	0.55	0

Measurements in this table were made by varying the ribosome concentration holding concentrations of all other components constant, as shown in Figure 1. The activities were calculated from the average slope of the ribosome titration curve determined for at least eight different values between 0 and 20.0 picomoles of ribosomes. The values given are then the percentage of the control or untreated ribosome activity.

including: varying the temperature, the salt concentration, and the percentage of ethanol (Table 1). Repeated extraction with fifty percent ethanol at zero degrees yielded the best results since this method appears to cause the least damage to the ribosomes themselves. This procedure removes only a few proteins from the 80S ribosome.

One major protein band was determined to have a molecular weight of 12,700 daltons by SDS polyacrylamide gel electrophoresis. Two other SDS gel bands had molecular weights of 14,800 and 11,500 daltons. These results are consistent with that obtained by Arpin et al.(1) who have analyzed these proteins by two-dimensional gel electrophoresis. We have not as yet examined our ribosomal extracts on 2-D gels to determine if the one dimensional SDS gel bands that we are observing are single proteins (Figure 2).

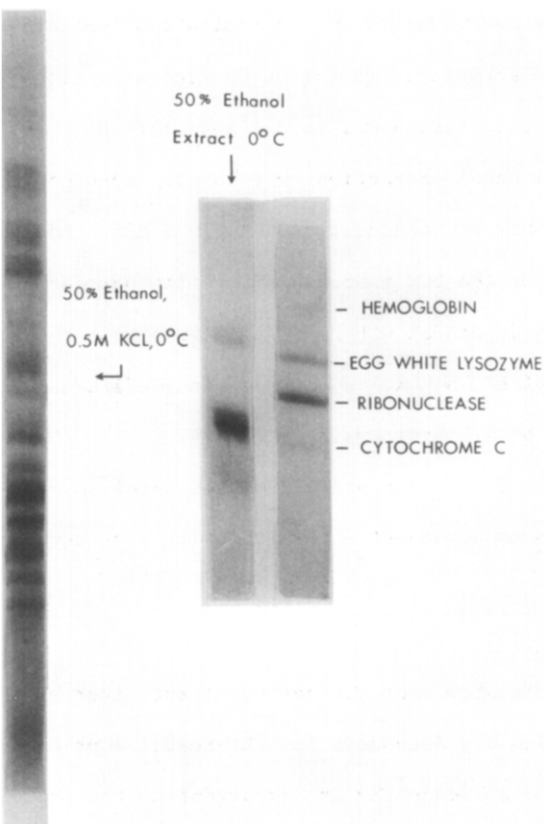

Figure 2: SDS polyacrylamide electrophoresis gels of ethanol extracts from rat liver 80S ribosomes.

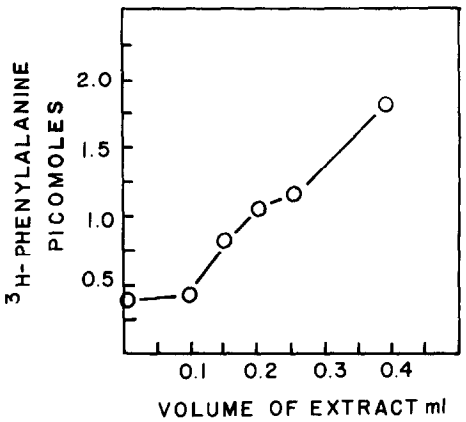

Figure 3: Concentration dependence of dialyzed ethanol extract in the reactivation of protein synthesis of ethanol extracted 80S ribosomes.

Figure 3 shows that the reconstituted protein synthesis activity is dependent on the amount of the protein extract added back to the ribosome. In that particular experiment, up to two equivalents of extracted protein were used in order to maximize activity. In the other reconstitutions however, only one equivalent of extracted protein was used (Table 1).

The effect of ethanol extraction on ribosome dependent elongation factor-II GTP hydrolysis was more pronounced than on poly U directed protein synthesis assays. Extraction of the 80S ribosome with fifty percent ethanol at zero degrees reduces the ribosome dependent GTP hydrolysis to almost the background of EF-II alone. Extraction with 0.5 M KCl and fifty percent ethanol completely removes ribosome dependence of GTP hydrolysis (Figure 4).

With the sensitivity of our present GTPase assay we were unable to detect an increase in ribosome dependent GTPase activity upon reconstitution of the extracted ribosome.

DISCUSSION

These experiments show that extraction of rat liver ribosomes with ethanol removes proteins which are necessary for the reaction of elongation factor-II with the ribosome and subsequently are important to the protein elongation cycle. The major protein component in these extracts has a molecular weight of 12,700 daltons.

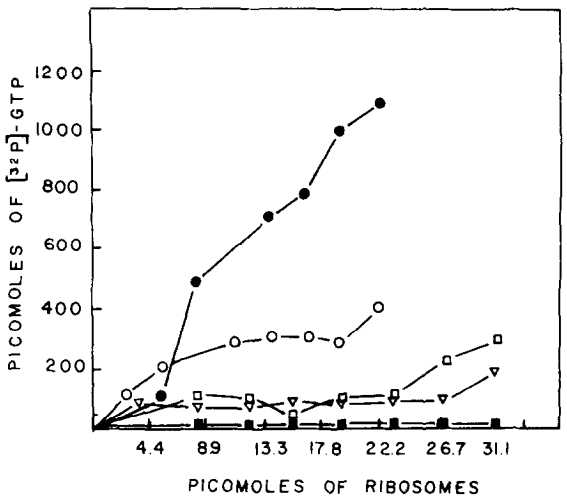

Figure 4: GTPase activities of extracted, unextracted, and reconstituted 80S ribosomes with and without EF-II. Unextracted 80S ribosomes alone (○), extracted 80S ribosomes assayed with EF-II present (▽), extracted 80S ribosomes alone (■), extracted 80S ribosomes reconstituted with their extracts, then assayed with EF-II present (□). All assays were carried out with identical concentrations of EF-II, GTP, and salts.

E. coli ribosomal protein L7/L12 extracted from 70S ribosomes with fifty percent ethanol and 1 M NH_4Cl has been shown to be essential for the binding of EF-G, the prokaryotic analog of rat liver EF-II (8,9). L7/L12 has a molecular weight of 12,300 daltons. The possibility exists that the functional analog of E. coli L7/L12 in rat liver ribosomes is removed by ethanol extraction without high salt concentrations.

As shown by the poly U directed protein synthesis, the loss of these ethanol soluble proteins does not completely stop the elongation cycle. This residual protein synthesis activity in the core ribosomes could be due to an incomplete removal of the ethanol soluble proteins, however this conclusion is not consistent with the fact that further extractions of the ribosome yield core particles with similar residual activity.

Extraction with 0.5 M potassium chloride and fifty percent ethanol, which removes a far greater number of proteins from the ribosome (Figure 2) yields core ribosomes with almost zero residual activity in the protein synthesis (Table 1). The dialyzed 0.5 M potassium chloride/fifty percent ethanol extracts stimulate the residual activity of their core ribosomes six fold.

The reconstitution of GTPase activity for ribosomes extracted with either fifty percent ethanol or 0.5 M KCl/fifty percent ethanol has not yet been observed. Our inability to see the reconstitution is probably a result of a lack of sensitivity in our present GTPase assay due to high background GTP hydrolysis.

REFERENCES

1. Arpin, M., Madjar, J., Reboud, J. P., (1978) Biochimica et Biophysica Acta 519, 537.
2. Sanchez-Madrid, F., Reyes, R., Conde, P., and Ballesta, J., (1979) Eur. J. Biochem. 98, 409.
3. Horak, I. and Schiffman, D., (1977) Eur. J. Biochem. 79, 375.
4. Zinker, S. and Warner, J. R., (1976) J. Biol. Chem. 251, 1799.
5. Stoffler, G., Wool, I. G., Lin, A., and Rak, K. H., (1974) Proc. Nat. Acad. Sci. 71, 4723.
6. Wool, I. G., (1979) Ann. Rev. Biochem. 48, 719.
7. Richter, D. and Moller, W., (1976), in Lipmann Symposium, ed. D. Richter, p. 524.
8. Hamel, E., Koch, M., and Nakamoto, T., (1972) J. Biol. Chem. 247, 805.
9. Acharya, A. S., Moore, P. B., and Richards, F. M., (1979) Biochemistry 12, 3108.
10. Silve, M. and Moldave, K., (1969) Biochem. et Biophys. Acta 195, 123.
11. Moldave, K. and Skogerson, L., (1967) in Meth. Enzymology, (L. Grossman and K. Moldave ed.), vol. XII, Part A, 478.
12. Moldave, K., Galasinki, W., and Rao, P., (1971), in Meth. Enzymology, (L. Grossman and K. Moldave ed.), vol. XX, Part C, 337.
13. Moldave, K., (1963), in Meth. Enzymology, (L. Grossman and K. Moldave ed.) vol. VII, 758.
14. Raeburn, S., Collins, J. F., Moon, H. M., and Maxwell, E. S., (1971) J. Biol. Chem. 246, 1041.