FRACTIONATION OF YEAST RIBOSOMAL PROTEINS

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Isolation and characterization of individual ribosomal proteins of E. coli has been reported by Traut et al.(1967). This communication is to report on the fractionation and analysis of proteins isolated from the ribosomes of brewer's yeast. It was found that, as in the case of E. coli, the protein moiety of the yeast ribosome is made up of several distinct molecular species. Individual ribosomal protein species have been isolated by preparative gel electrophoresis, characterized by analytical gel electrophoresis and in most cases by amino acid analysis.

Experimental: Ribosomes were isolated from aerobically grown late log phase S. cerevisiae by alumina grinding and extraction with 2 volumes of standard TMSH buffer (50mM TrisCl pH7.4, 10mM $Mg^{++}Ac_2$, 10mM mercaptoethanol) at 2°C. After centrifugation at 20,000 x g for 20 minutes at 2°C the supernatant was centrifuged at 150,000 x g for 1 hour to sediment the ribosomes. Washing of ribosomes: The ribosome pellets were gently rinsed and suspended, by means of vigorous magnetic stirring for 30 minutes at 1°C, in a volume of TMSH sufficient to give an $O.D._{260}$ of 150 - 200 in a 10 mm. light path. An equal volume of cold 10% ammonium sulfate (weight/vol.) containing 30 mM Mg^{++} was added with stirring. After 20 minutes of further stirring the ribosomes were resedimented at 150,000 x g. Preparation of Ribosomal Protein: Ribosomal protein was prepared by a modification of the method of Spitnik-Elson (1965). Washed ribosomes

were suspended in TMSH and, after stirring for 30 mins, one volume of 8M urea - 6M LiCl solution was added. The mixture was allowed to stand in the cold for at least 12 hours before the RNA precipitate was removed by centrifugation at 20,000 x g.

Disk electrophoresis: Electrophoresis was carried out in the pH 4.5 system in 15% polyacrylamide gels containing 8M urea according to the method of Reisfeld et al.(1962) as modified by LeBoy et al.(1964). Identification of individual proteins was achieved by co-electrophoresis with a small aliquot of unfractionated ribosomal protein.

Preparative gel electrophoresis: Preparative electrophoresis of ribosomal proteins was carried out according to Duesberg and Rueckert (1965) using 15% polyacrylamide containing 8M urea at pH 4.5. The height of the small pore gel was 10 cm, that of the 'spacer' and sample gels 1 cm. each. Temperature was maintained at 15°C, voltage between 200 and 300 volts, and amperage at 50 mA. After the tracking dye (crystal violet) had reached the collection chamber electrophoresis was continued for a further 12 hours. Flushing speed was kept constant at 15 ml/hr. by means of a peristaltic pump. The collected fractions were checked for their protein content by analytical disk electrophoresis.

Amino acid analysis: After dialysis into cold 0.1N HCl the protein samples were hydrolyzed for 36 hours in 6N HCl at 110°C in evacuated sealed tubes. After hydrolysis the samples were dried under vacuum. Amino acid analysis was performed with a Beckman Model 120-C Amino Acid Analyzer. No correction was made for the destruction of amino acids during hydrolysis.

Results and Discussion:

Distinguishing between extraneously adsorbed proteins and true structural proteins of the ribosome is a major problem when characterizing "ribosomal proteins". The characteristic electrophoretic pattern of proteins dissociated from ribosomes immediately after isolation in TMSH buffer is shown on the left in Figure 1. Also demonstrated in Figure 1 is the

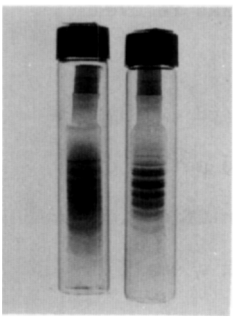
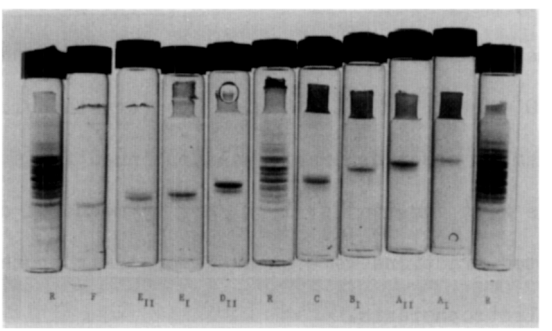


Figure 1 (left). Polyacrylamide gel electrophoresis at pH 4.5 in 8M urea of 'ribosomal proteins' obtained from yeast ribosomes before (left) and after (right) washing in 0.75M ammonium ion-containing buffers. Migration is from top(+) to bottom(-).

Figure 3 (below). Analytical re-electrophoresis of fractions obtained by preparative gel electrophoresis. Gels 1, 6 and 11 are reference unfractionated ribosomal protein patterns from different preparations and demonstrate the variability of the 'slower bands' and 'fast bands' from preparation to preparation. Although some components (identified below each gel) may not exactly align with the reference their identity was unequivocally established by co-electrophoresis in the presence of trace amounts (5 - 10 µg.) of unfractionated ribosomal protein as background.



considerable reduction in complexity and "background stain" which results from a single washing of the ribosomes in 0.75M NH_4^+ prior to the isolation of ribosomal proteins. The major protein bands remaining after this washing procedure we have designated (in order of increasing mobility) A_I, A_{II}, B_I, B_{II}, B_{III}, $C_{(I \& II?)}$, D_I, D_{II}, E_I, E_{II} and F. We have observed bands migrating slower than A_I and faster than F but these bands are variable from preparation to preparation (see reference gels 1, 6 and 11 in Figure 3).

Although the unsupplemented endogenous incorporation of amino acids by ammonium-washed ribosomes is markedly reduced (presumably due to the removal of adsorbed activating enzymes, transfer factors etc.) evidence

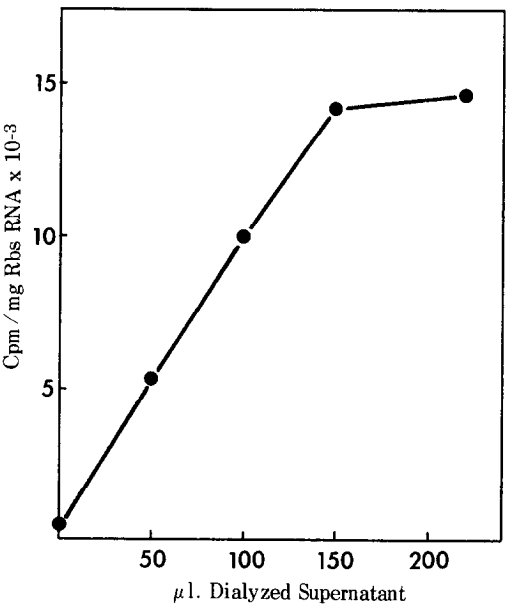


Figure 2. Amino acid incorporation by ammonium sulfate-washed (0.75M NH_4^+) ribosomes. 0.4 mg. ribosomes were preincubated 5 mins. at 25°C in a medium of 9mM TrisCl pH 7.4, 4mM Mg^{++}, 281 µg/ml poly U and varying amounts of dialyzed supernatant (7 mg/ml protein and 1 mg/ml sRNA) in a final volume of 0.24 ml. The tubes were then chilled on ice and 0.09 ml. of a mixture containing 1.4 µmoles creatine phosphate, 12.6 µg creatine kinase, 0.28 µmoles ATP, 0.025 µmoles GTP, 15 µmoles KCl, 0.12 µmoles spermine and 1.0 µg C-14 L-phenylalanine containing 2.97×10^5 cpm was added to each. After further incubation for 20 mins. at 25°C the tubes were rapidly chilled and 0.1 ml. aliquots were removed, plated onto filter paper disks, precipitated with TCA and washed with hot TCA, ethanol and ether according to a modification of the procedure of Mans and Novelli (1961) before scintillation counting to determine incorporation into protein.

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that this procedure does not disrupt ribosome function is seen in Fig. 2. The ribosomes isolated after this wash procedure are still perfectly competent to carry poly U-directed polyphenylalanine synthesis when supplemented with appropriate soluble factors. It is nevertheless obvious that even after the removal of the adsorbed material the ribosomal protein pattern is similar in complexity to that observed in E. coli.

Our attempts to fractionate yeast ribosomal proteins into individual components by a variety of ion-exchange methods have been unsuccessful. However preparative polyacrylamide gel electrophoresis in 8M urea has

Table 1. Amino acid composition of individual ribosomal proteins from yeast. Data expressed as moles per 100 moles of all amino acids.

Fraction	F	E_{II}	E_I	D_{II}	D_I	C	B_I	A_{II}	A_I	RP
Lys	13.7	12.5	12.5	12.2	10.9	11.5	10.9	10.8	11.1	10.5
His	2.2	2.0	2.1	2.0	1.6	2.2	1.9	2.2	2.0	2.1
Arg	9.6	8.1	8.0	7.6	7.8	8.8	8.5	8.3	7.1	7.5
Asp	7.4	8.1	7.9	7.5	8.5	7.6	8.5	8.8	9.1	8.9
Thr	5.3	4.8	5.1	4.5	4.7	5.2	4.9	5.0	5.3	5.4
Ser	5.6	6.6	7.6	7.5	5.7	5.3	5.3	5.2	5.2	5.4
Glu	9.7	9.7	10.3	10.6	10.2	9.7	10.5	10.0	10.3	10.3
Pro	3.9	4.2	3.0	4.1	4.0	4.2	4.3	4.2	4.3	4.1
Gly	7.5	7.9	8.6	9.2	7.7	7.3	7.4	8.1	7.3	7.2
Ala	10.7	10.1	9.6	8.9	10.0	9.6	9.3	9.0	9.5	9.7
Val	7.7	8.0	8.0	8.4	9.5	8.6	8.6	8.5	8.6	8.2
Met	0.3	0.0	0.2	0.5	0.3	0.6	0.7	0.7	0.5	0.7
Ileu	4.6	5.1	4.4	4.1	5.0	5.3	5.4	5.6	5.5	5.5
Leu	7.5	7.9	7.5	7.3	8.1	8.0	7.8	7.8	8.3	8.2
Tyr	1.8	2.0	2.2	2.3	2.5	2.5	2.4	2.3	2.2	2.7
Phe	3.0	2.9	2.9	3.3	3.6	3.5	3.3	3.3	3.4	3.5
Basic	25.5	22.6	22.6	21.8	20.3	22.5	21.3	21.3	20.2	20.1
Acidic	17.1	17.8	18.2	18.1	18.7	17.3	19.0	18.8	19.4	19.2
B/A	1.49	1.27	1.24	1.20	1.09	1.30	1.12	1.13	1.04	1.05

RP = total ribosomal protein containing the acidic proteins not fractionated by this electrophoretic procedure.
Cys was found (as cysteic acid) in some fractions in amounts no greater than 0.3 moles percent.

yielded promising results as shown in Figure 3. Again as in the case of E. coli, the protein complexity appears to be due to a genuine heterogeneity rather than to aggregation phenomena between a small number of protein species. This is suggested by the observation that upon equilibration in 8M urea and re-electrophoresis, the band patterns of individual protein fractions remain unaltered. Further evidence is provided by the amino acid composition of several different protein fractions as shown in Table 1 - however the similarities between the composition of certain fractions do not rule out aggregation patterns. This problem will only be solved

by characterizing the peptide patterns from each yeast ribosomal protein band, and this is currently under investigation in this laboratory.

Discussion: The results of preparative gel electrophoresis of yeast ribosomal protein point to the fact that this is made up of a genuinely heterogeneous mixture of protein species which, furthermore, appear to separate in 15% polyacrylamide on the basis of their respective net charge rather than on the basis of major size differences. The present work has been carried out with whole 80S ribosomes and makes the isolation of absolutely pure protein species from such a complex mixture extremely difficult. Attempts at prior coarse fractionation by ion exchange chromatography has not been helpful in our hands but prefractionation of 60S and 40S ribosomal subunits at the preparative level appears to be a promising method of reducing the complexity of the sample subjected to preparative gel electrophoresis. Results of such experiments will be presented at a later date. A more detailed characterization of individual ribosomal proteins is indispensable to any investigation of how their structure and function are correlated and necessitates the isolation of all the major bands appearing in analytical gel electrophoresis patterns. Such work is in progress in this laboratory.

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