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ANALYTICAL ULTRACENTRIFUGE SERVICES AND RESEARCH

By: FU-CHUAN CHAO

Prepared for:

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
AMES RESEARCH CENTER
MOFFETT FIELD, CALIFORNIA 94035
Attention: CONTRACTING OFFICER, NAS2-4021

CONTRACT NAS2-4021

SRI Project BU-6289

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Approved:

W. A. SKINNER, *Executive Director*
Life Sciences Division

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SUMMARY

The specific objective of the research performed under this contract (NAS2-4021) was the characterization of biological materials supplied by Ames. Techniques of analytical ultracentrifugation and electron microscopy were used, with the following results.

(1) The fatty acid synthetase from Saccharomyces cerevisiae, strain LK2G12, with a sedimentation coefficient of about 47 S, was purified by the complete removal of ribosomes. The ribosomes of the strain LK2G12 from an aerobic culture were precipitated by lowering the $MgSO_4$ concentration from 0.019 to 0.001 M. The ribosomes of yeast from a standing culture were precipitated by substituting $MgCl_2$ or $CaCl_2$ for $MgSO_4$.

(2) The chemical composition of the ribosomes from strain LK2G12 consisted of protein, RNA, phospholipid, and polysaccharide. It varied with the age and pH of the growth medium. The sedimentation coefficients ranged from 83.7 to 95.8 S, the reduced viscosity from 9.0 to 11.1 ml/g, the specific volumes from 0.70 to 0.74 ml/g, and the molecular weights from 7.0 to 10.9 x 10^6. The total RNA per ribosome remained relatively unchanged.

(3) The ribosomes from commercial baker's yeast, Red Star and Fleischmann, and from Saccharomyces lactis, strain Y14, were characterized. The chemical composition as well as the physicochemical properties of these ribosomes were quite different from each other and also different from those of the strain LK2G12.

(4) The effect of freezing on the ribosomes of S. cerevisiae, strain LK2G12, and of S. lactis, strain Y14, was studied. The ribosomes were altered, with a loss of about 50% into insoluble material which had a protein:RNA ratio ranging from 3.3 to 4.6 as compared with 1.7 to 2.3 for the unfrozen ribosomes. Freezing had no effect on the sedimentation coefficients of the ribosomes. The most drastic changes were in the reduced viscosity, which decreased from 10 to 5 ml/g, and in the molecular weights, which also decreased by about 50%.

(5) The ribosomes from strain LK2G12 were purified by combined procedures of sucrose density gradient and differential centrifugation, and were characterized. The reduced viscosity of the ribosomes thus prepared was 6.0 ml/g and the molecular weight was 6.1 x 10^6.

(6) The acetyl-CoA synthetase from strain LK2G12 had a sedimentation coefficient of about 6.5 S in 0.5 M potassium phosphate at pH 7.5.

(7) The microsomal fraction of strain LK2G12 was found to contain ribosome-like particles.

(8) The phosphoribulokinase appeared as one component with a sedimentation coefficient of 18.4 S for Thiobacillus thioparus and 12.7 S for T. neapolitanus. The carboxydesmutase from T. neapolitanus was partially purified.

(9) The membrane fragments of Halobacterium cutirubrum contained one main component with a sedimentation coefficient of 3.9 S and a minor component with a sedimentation coefficient of about 8.9 to 9.3 S.

(10) The buoyancy density of the DNA from Micrococcus lysodeikticus in CsCl was 1.691 g/ml; that from Proteus mirabilis was 1.693 g/ml. The DNA from an unidentified bacteria showed three bands with buoyancy densities of 1.684, 1.700, and 1.716 g/ml, respectively.

(11) Preliminary characterization of the CH_3-NH_3 polymer was carried out.

(12) Pseudomonas saccharophila in 4-hour N-starved cultures, in the presence of either air or pure oxygen, showed an accumulation of electron-dense structures resembling poly-β-hydroxybutyric acid.

RESULTS

Purification of Fatty Acid Synthetase from Saccharomyces cerevisiae, Strain LK2G12

The fatty acid synthetase in strain LK2G12 was previously found to be associated with particles having a sedimentation coefficient of about 47 S.[5] The concentration of the 47 S particles was 0.5 to 1 mg/g (wet weight) of yeast as compared with that of ribosomes, which was 40 mg/g of yeast. The problem was to free the 47 S particles from the ribosomes. The use of a salt mixture containing 0.05 M KH_2PO_4, 0.009 M $NaHCO_3$, 0.018 M $MgSO_4$, and 0.001 M $CaCl_2$, at pH 6.0, eliminated a component with sedimentation coefficient of about 70 to 80 S, the presence of which further complicated the purification procedure.

A 48-hour aerobic culture of the strain LK2G12 was studied first. Attempts were made to purify the 47 S particles by a suitable sucrose density gradient centrifugation. Crude yeast homogenate in the above salt mixture was used as the starting material. The residue, centrifuged down at 10,000 rpm (12,100 x g) for 20 minutes in a Servall centrifuge, was discarded. The supernatant fluid (3-18-69) was layered on top of 2.0 ml of 1.5 M sucrose containing the salt mixture and centrifuged at 40,000 rpm (96,500 x g) for 16 hours in a Spinco centrifuge. The pellet with a specific gravity greater than 1.5 M sucrose consisted mainly of ribosomes with a sedimentation coefficient of 96.2 S and a small amount of 52.2 S particles, which were probably identical with the 47 S particles obtained in a different salt mixture. The material collected on top of the sucrose was diluted and again centrifuged at 40,000 rpm for 16 hours. It consisted of a small amount of 23.8 S particles and a large amount of a 9.1 S component.

When the supernatant fluid (4-21-69) obtained by centrifuging at 10,000 rpm was layered on top of 2.0 ml of 1.75 M sucrose and centrifuged for 16 hours, the material in the 1.75 M sucrose contained 46.8 S particles together with a large amount of ribosomes. Centrifuging the 10,000 rpm supernatant fluid on top of 2.0 M sucrose failed to improve the resolution of the 47 S particles.

When the 10,000 rpm supernatant fluid (4-21-69) was layered on top of 2.0 ml of 1.75 M sucrose and centrifuged at 40,000 rpm for 4 hours, three fractions were obtained: (1) a pellet with specific gravity greater than 1.75 M sucrose, which contained ribosomes with an uncorrected sedimentation coefficient of 82.7 S; (2) material in 1.75 M sucrose, which contained the bulk of the ribosomes as well as some components with sedimentation coefficients of 47.4, 28.7, and 47.2 S; and (3) a 5.2 S component with specific gravity less than 1.75 M sucrose.

Attempts were made to precipitate the ribosomes by chemical procedures. The 10,000 rpm supernatant fluid (5-5-69) was centrifuged at 40,000 rpm for 2 hours and the pellet was resuspended in 0.001 M $MgSO_4$ and 0.002 M KH_2PO_4, pH 5.4. The change of $MgSO_4$ concentration from 0.018 M to 0.001 M precipitated a large amount, but not all, of the ribosomes. The precipitate was removed by centrifuging at 10,000 rpm for 15 minutes. The first 40,000 rpm pellet (minus the precipitate formed due to change in $MgSO_4$ concentration) contained 14.5 mg of protein/g of yeast and 0.7 mg of RNA/g of yeast, and consisted of four components with uncorrected sedimentation coefficients of 8.8, 28.7, 49.4, and 91.9 S. Two additional cycles of centrifugation were carried out, using 40,000 rpm and 10,000 rpm for each cycle. Only the salt mixture containing 0.001 M $MgSO_4$ was used. The second 40,000 rpm pellet contained 2.0 mg of protein/g of yeast and 0.12 mg of RNA/g of yeast, and consisted of three components with uncorrected sedimentation coefficients of 9.1, 32.7, and 51.4 S. The third 40,000 rpm pellet contained 0.5 mg of protein/g of yeast and 0.009 mg of RNA/g of yeast, and consisted of one component with uncorrected sedimentation coefficient of 50.6 S.

The precipitate formed by change of $MgSO_4$ concentration from 0.018 to 0.001 M contained 20.0 mg of protein/g of yeast and 7.1 mg of RNA/g of yeast. The precipitate was resuspended in the salt mixture containing 0.018 M $MgSO_4$ and become partly dissolved. The soluble material amounted to 8.4 mg of protein/g of yeast and 3.8 mg of RNA/g of yeast. There were four components with uncorrected sedimentation coefficients of 8.1, 29.0, 85.2, and 129 S. The insoluble material consisted of 12.0 mg of protein/g of yeast and 2.3 mg of RNA/g of yeast.

The effect of using 0.10 M $MgSO_4$ was studied (10-2-69). The 10,000 rpm supernatant fluid in 0.10 M $MgSO_4$ + 0.002 M KH_2PO_4-K_2HPO_4 (1.9:0.1), pH 4.9, was centrifuged at 40,000 rpm for 4 hours and the pellet was resuspended in 0.002 M KH_2PO_4-K_2HPO_4 (1:4), pH 7.35. Insoluble material was removed from the suspension by centrifuging at 10,000 rpm for 15 minutes. The first 40,000 rpm pellet contained 13.3 mg of protein/g of yeast (wet weight) and 7.9 mg of RNA/g of yeast, and consisted of four components with uncorrected sedimentation coefficients of 10.0, 31.2, 55.0, and 83.3 S. Two additional cycles of centrifugation were carried out, using 40,000 rpm and 10,000 rpm for each cycle. The second 40,000 rpm pellet was resuspended in 0.40 M $MgSO_4$ + 0.002 M KH_2PO_4-K_2HPO_4 (1.9:0.1). The precipitate formed overnight at 4°C was removed by centrifuging at 10,000 rpm. This precipitate contained 2.4 mg of RNA/g of yeast and no protein. No analyses were made on the second 40,000 rpm pellet minus the RNA precipitated in 0.40 M $MgSO_4$. The third 40,000 rpm pellet was resuspended in 0.002 M KH_2PO_4-K_2HPO_4 (1:4). It contained 0.50 mg of protein/g of yeast and 0.011 mg of RNA/g of yeast. There was one component with an uncorrected sedimentation coefficient of 34.9 S.

The effect of 0.1% Triton X-100 on the 47 S particles was studied (10-16-69). The yeast was ruptured in 0.020 M $CaCl_2$ + 0.002 M KH_2PO_4 K_2HPO_4 (4:1), pH 6.0. A large precipitate, formed when the first

40,000 rpm pellet was resuspended in 0.020 M $CaCl_2$, was removed by centrifuging at 10,000 rpm. What remained in solution contained 3.5 mg of protein/g yeast and 0.078 mg of RNA/g of yeast. It consisted of three components with uncorrected sedimentation coefficients of 9.2, 30.4, and 49.8 S. The 47 S particles after the first cycle of purification were treated with 0.1% Triton X-100 and centrifuged immediately at 40,000 rpm. The second 40,000 rpm pellet was resuspended in the 0.020 M $CaCl_2$ without Triton X-100. It contained 1.1 mg of protein/g of yeast, and 0.019 mg of RNA/g of yeast, and consisted of three components with uncorrected sedimentation coefficients of 12.1, 31.3, and 51.8 S. From these results it is concluded that 0.1% Triton X-100 caused partial dissociation of the 47 S particles.

The effect of substituting $CaCl_2$ for $MgSO_4$ on the 47 S particles from strain LK2G12 grown under aerobic conditions was studied. A 15.0-g aliquot of the yeast (8-11-69) was used. Results of the fractionation and the determination of fatty acid synthetase activity in each fraction are summarized in Table I. Specific activity was defined as the amount of malonyl CoA (mμM) converted/mg of protein in the fraction. Total activity was the product of specific activity and total protein (mg) in the fraction. After centrifuging the 10,000 rpm supernatant fluid at 40,000 rpm for 2 hours, the total synthetase activity was about equally divided between the pellet and the 40,000 rpm supernatant fluid. The first 40,000 rpm pellet that was soluble in 0.020 M $CaCl_2$ contained the 47 S particles and the bulk of the synthetase activity. The first 40,000 rpm pellet that was insoluble in 0.020 M $CaCl_2$ contained the bulk of the ribosomes and was low in synthetase activity. Much of the enzyme activity in the first 40,000 rpm pellet was recovered in the second 40,000 rpm pellet. There was an overall 10-fold increase of specific activity after the two cycles of centrifugation. The 50% loss of synthetase activity by the first 40,000 rpm centrifugation in the supernatant fluid was probably due to the high viscosity of the soluble protein in the 10,000 rpm supernatant fluid.

Attempts were made to improve the yield of the 47 S particles from strain LK2G12, which was grown in an aerobic culture for 48 hours. The effect of using 0.020 M $MgSO_4$ + 0.002 M KH_2PO_4-K_2HPO_4 (4:1), pH 6.0, as the disruption medium was studied (12-3-69). The residue, contrifuged down at 10,000 rpm for 30 minutes, was discarded. The supernatant fluid was centrifuged at 40,000 rpm. The pellet obtained after 10 minutes of centrifugation was discarded and that after four hours was resuspended in 0.020 M $CaCl_2$ + 0.002 M KH_2PO_4-K_2HPO_4 (4:1), pH 6.0. Insoluble material was removed from the suspension by centrifuging at 10,000 rpm for 15 minutes. The 40,000 rpm pellet contained 4.0 mg of protein/g of wet yeast and 0.10 mg of RNA/g of yeast, and consisted of four components with uncorrected sedimentation coefficients of 9.0, 30.0, 38.3, and 50.0 S, as well as aggregated material, which spread out rapidly. An aliquot of the second 40,000 rpm pellet (4 hours) was resuspended in 0.001 M $MgSO_4$ + 0.002 M KH_2PO_4-K_2HPO_4 (4:1), pH 6.0. It contained 0.80 mg of protein/g of yeast and 0.02 mg of RNA/g of yeast, and

Table I

PURIFICATION OF THE 47 S PARTICLES FROM STRAIN LK2G12

Fractions	Chemical Composition (mg/g of yeast)		Fatty Acid Synthetase Activity*		Sedimentation Coefficient	
	Protein	RNA	Sp. Act.	Total Act.	S	Run #
1st 40,000 pellet	21.3	8.1	6.3	134		
0.02 M $CaCl_2$ ppt (ribosomes)	15.8	8.9	2.6	41		
0.02 M $CaCl_2$ soluble	5.0	0.18	33	165	7.8, 29.2, 49.9	591
1st 40,000 rpm supernatant	46.0	1.4	3.7	170		
2nd 40,000 rpm pellet	2.0	0.02	56	112	36.7, 52.9	592

* Specific activity of fatty acid synthetase is defined as the mμM of malonyl CoA converted/mg of protein. Total activity is the product of specific activity and total protein in the fraction.

consisted of four components with uncorrected sedimentation coefficients of 9.0, 30.2, 37.6, and 49.2 S, in addition to aggregated material. Another aliquot of the second 40,000 rpm pellet (4 hours) was resuspended in 0.020 M $MgSO_4$ + 0.002 M KH_2PO_4-K_2HPO_4 (4:1), pH 6.0. It contained 1.1 mg of protein/g of yeast and 0.02 mg of RNA/g of yeast; there were four components with uncorrected sedimentation coefficients of 11.2, 29.5, 36.1, and 29.4 S, in addition to aggregated material.

The effect of using 0.001 M $MgSO_4$ + 0.002 M KH_2PO_4-K_2HPO_4 (4:1, pH 6.0, was studied (12-10-69). The first 40,000 rpm pellet (2 hours) contained 23.6 mg of protein/g of yeast and 8.1 mg of RNA/g of yeast, and consisted of seven components with uncorrected sedimentation coefficients of 8.5, 30.9, 51.6, 72.3, 88.5, 132.7, and 179.7 S. The second 40,000 rpm pellet (2 hours) contained 18.8 mg of protein/g of yeast and 7.4 mg of RNA/g of yeast, and had four components with uncorrected sedimentation coefficients of 31.8, 54.0, 91.6, and 140.8 S. The portion of the second 40,000 rpm pellet (2 hours) that was soluble in 0.020 M $CaCl_2$ + 0.002 M KH_2PO_4-K_2HPO_4 (4:1), pH 6.0, contained 0.50 mg of protein/g of yeast and 0.07 mg of RNA/g of yeast, and consisted of three components with uncorrected sedimentation coefficients of 7.6, 30.0, and 50.7 S, in addition to aggregated material.

The effect of using a solution containing 0.050 M KH_2PO_4, 0.009 M $NaHCO_3$, 0.018 M $MgSO_4$, and 0.001 M $CaCl_2$, pH 6.0, was studied (12-17-69). The 40,000 rpm supernatant fluid (10 minutes) contained 60.0 mg of protein/g of yeast and 9.0 mg of RNA/g of yeast, and had four components with uncorrected sedimentation coefficients of 5.3, 21.8, 35.2 and 59.4 S. The 59.4 S component represented the ribosomes. The 40,000 rpm pellet (2 hours) contained 22.2 mg of protein/g of yeast and 5.8 mg of RNA/g of yeast, and consisted of five components with uncorrected sedimentation coefficients of 7.9, 29.2, 49.1, 83.6, and 120.6 S, in addition to aggregated material. An aliquot of the 40,000 rpm supernatant fluid (10 minutes) was treated with 0.4 M $MgSO_4$ overnight. There were three components with uncorrected sedimentation coefficients of 4.8, 24.1, and 39.0 S. An aliquot of the 40,000 rpm supernatant fluid (10 minutes) was treated with an equal volume of cold ethanol. The precipitate was centrifuged down at 10,000 rpm for 30 minutes and then resuspended in 0.002 M KH_2PO_4-K_2HPO_4 (4:1), pH 6.0. The solution contained 11.8 mg of protein/g of yeast and 0.26 mg of RNA/g of yeast; there were two components with uncorrected sedimentation coefficients of 6.4 and 19.0 S. An aliquot of the 40,000 rpm supernatant fluid (2 hours) was layered on top of 1.5 M sucrose and centrifuged at 40,000 rpm for 16 hours. A pellet with specific gravity greater than 1.5 M sucrose was resuspended in the same starting salt solution. It contained 0.60 mg of protein/g of yeast and 0.13 mg of RNA/g of yeast, and consisted of three components with uncorrected sedimentation coefficients of 22.5, 50.2, and 84.3 S.

A 48-hour standing culture of strain LK2G12 followed by 2.5-hour aeration was studied next. The effect of changing the $MgSO_4$ concentration was again examined. The 10,000 rpm supernatant fluid (6-18-69) was centrifuged at 40,000 rpm for 2 hours and the pellet was resuspended in 0.001 M $MgSO_4$ and 0.002 M KH_2PO_4-K_2HPO_4 (4:1), pH 6.0. A small precipitate was removedfrom the suspension by centrifuging at 10,000 rpm

for 15 minutes. The first 40,000 rpm pellet contained 15.5 mg of protein/g of yeast and 9.6 mg of RNA/g of yeast. It consisted of five components with uncorrected sedimentation coefficients of 6.0, 27.0, 51.1, 80.8, and 125 S. Three additional cycles of centrifugation were carried out, using 40,000 rpm and 10,000 rpm for each cycle. The second 40,000 rpm pellet contained 11.0 mg of protein/g of yeast and 7.3 mg of RNA/g of yeast. It consisted of five components with uncorrected sedimentation coefficients of 9.8, 29.3, 50.6, 69.7, and 103 S. The third 40,000 rpm pellet contained 10.4 mg of protein/g of yeast and 8.1 mg of RNA/g of yeast, and consisted of six components with uncorrected sedimentation coefficients of 10.4, 28.9, 48.4, 61.9, 64.9 and 98.2 S. The fourth 40,000 rpm pellet was resuspended in 0.002 M KH_2PO_4-K_2HPO_4 (4:1), pH 6.0, without the $MgSO_4$. It contained 8.5 mg of protein/g of yeast and 7.3 mg of RNA/g of yeast, and consisted of five components with uncorrected sedimentation coefficients of 30.0, 49.5, 61.8, 64.5, and 79.0 S. The ribosomes prepared from standing culture, unlike those from aerobic culture, apparently did not require $MgSO_4$ for stability at pH 6.0.

The effect of substituting $MgCl_2$ for $MgSO_4$ on the 47 S particles from strain LK2G12 grown in a 48-hour standing culture was studied (6-18-69). The first 40,000 rpm pellet was resuspended in 0.020 M $MgCl_2$ and 0.002 M KH_2PO_4-K_2HPO_4 (4:1), pH 6.0. With $MgCl_2$, a large amount of the ribosomes was precipitated. The precipitate was removed by centrifuging at 10,000 rpm for 15 minutes. The first 40,000 rpm pellet (minus the precipitate formed due to change from $MgSO_4$ to $MgCl_2$) contained 7.5 mg of protein/g of yeast and 1.9 mg of RNA/g of yeast. In consisted of four components with uncorrected sedimentation coefficients of 8.1, 48.0, 82.8, and 130 S. The second 40,000 rpm pellet contained 4.5 mg of protein/g of yeast and 1.3 mg of RNA/g of yeast. It had three components with uncorrected sedimentation coefficients of 48.2, 88.1, and 139 S. The third 40,000 rpm pellet contained 3.5 mg of protein/g of yeast and 0.9 mg of RNA/g of yeast. There were three components with uncorrected sedimentation coefficients of 49.1, 85.1, and 123 S.

The effect of substituting $CaCl_2$ for $MgSO_4$ on the 47 S particles from strain LK2G12 grown in a 48-hour standing culture was studied (7-28-69). The first 40,000 rpm pellet was resuspended in 0.020 M $CaCl_2$ and 0.002 M KH_2PO_4-K_2HPO_4 (4:1), pH 6.0. With $CaCl_2$, an even larger amount of the ribosomes was precipitated. The precipitate was removed by centrifuging at 10,000 rpm for 15 minutes. The first 40,000 rpm pellet (minus the precipitate formed due to change from $MgSO_4$ to $MgCl_2$) contained 2.8 mg of protein/g of yeast and 0.25 mg of RNA/g of yeast. It consisted of four components with uncorrected sedimentation coefficients of 6.3, 25.1, 51.4, and 81.4 S. The second 40,000 rpm pellet contained 1.2 mg of protein/g of yeast and 0.13 mg of RNA/g of yeast. It had two components with uncorrected sedimentation coefficients of 24.0 and 51.4 S. The third 40,000 rpm pellet contained 0.44 mg of protein/g of yeast and 0.004 mg of RNA/g of yeast, and consisted of three components with uncorrected sedimentation coefficients of 25.1, 52.8, and 75.7 S. As expected, $CaCl_2$ was more effective than $MgCl_2$ in precipitating the ribosomes.

Characterization of Ribosomes of Saccharomyces cerevisiae, Strain LK2G12

The ribosomes of Saccharomyces cerevisiae, strain LK2G12, contained protein, RNA, phospholipid, and polysaccharide. Their chemical composition was found to change with the age of the culture, which in turn depended on the supply of nutrient and the pH of the growth medium. Table II summarizes the chemical composition and physicochemical properties of the ribosomes of the strain grown under different conditions.

A culture medium containing 1% yeast extract, 2% peptone, and 2% dextrose was used in most of these experiments. Sufficient phosphoric acid was added to the above culture medium when pH 4.6 instead of pH 7.0 was used. The yeast cells were packed by centrifuging at 10,000 rpm for 15 minutes. An aliquot was diluted 1 to 2,000 with the salt mixture and counted in a hemacytometer.

The ribosomes were prepared by the following procedure: All operations were carried out at 4°C. The cells were washed first with distilled water and then with a salt mixture containing 0.001 M $MgSO_4$ and 0.002 M KH_2PO_4-K_2HPO_4 (1:1), pH 6.8. The washed yeast was resuspended in the salt mixture (2 ml per g) and ruptured twice in a French Press. The crude homogenate was centrifuged at 10,000 rpm for 30 minutes, and the residue was discarded. The yeast ribosomes were centrifuged down at 40,000 rpm for 90 minutes, resuspended in the above salt mixture, and cleared at 10,000 rpm for 15 minutes. They were centrifuged down in the same way two more times. The final volume of the ribosomes was so adjusted that 2 ml was equivalent to 1 g of yeast.

Protein was measured by the biuret reaction. RNA was extracted by heating with 5% trichloroacetic acid and was measured by the orcinol reaction. Phospholipid was extracted from lyophilized samples of ribosomes with choroform-methanol (2:1) and the phosphorus was measured by the 1-amino-2-naphthol-4-sulphonic acid reaction. Polysaccharide was released from lyophilized samples of ribosomes (by boiling with 30% KOH), precipitated with C_2H_5OH (1:1), dissolved in distilled water, and measured by phenol-sulfuric acid reaction.

For the physicochemical measurements, density was measured in a pycnometer of 5 ml capacity at 22.0-23.0°C. Specific volumes of the ribosomes were determined by the method of Lewis and Randall (Thermodynamics, p. 38, McGraw-Hill Book Co., Inc., New York, 1923). Viscosity was measured at 22.5 ± 0.001°C in a Cannon-Ubbelohde viscometer. Ultracentrifugal analyses were carried out in a Spinco ultracentrifuge (Model E). Electron micrographs were taken in a Hitachi Electron microscope (Model H10). Molecular weights of ribosomes were calculated by using the Svedberg equation and Stokes Law. A molecular weight of 4.1×10^6 was calculated for a sedimentation coefficient of 80 S, a reduced viscosity of 5.0 ml/g, and a specific volume of 0.67 ml/g.

Table II

PROPERTIES OF RIBOSOMES OF SACCHAROMYCES CEREVISIAE, STRAIN LK2G12

Growth Conditions pH	Hr	Yield of Cells (g/500 ml)	No. of Cells/g ($\times 10^{-9}$)	Conc. of Ribosomes in Cells (mg/g)	Chemical Composition (%) Protein	RNA	Phospholipid	Polysaccharide	Protein/RNA	Physicochemical Properties $[\eta]_{red}$ (ml/g)	Sp Vol (ml/g)	$S^{o}_{20,w}$	Molecular Weight ($\times 10^{-6}$) Ribosomes	r-RNA	r-Protein	Sample
Aerobic:																
7.0	7				54.6	45.3	1.2		1.21			83.7				12-6-67
	7	0.4	8.5	62.4	51.9	36.0	1.3	0.53	1.44	10.0	0.70	83.8	7.0	2.5	3.6	8-13-68
	8				57.8	38.0		0.73	1.52			92.0				1-24-68
	24	6.6	9.2	66.7	53.6	32.2	5.6	3.0	1.67	9.5	0.70	92.1	8.0	2.6	4.3	3-3-69
	48				60.0	31.3	1.7	3.0	1.92			88.8				1-11-68
	48*	9.6	16.0	39.5	57.2	26.5	4.1	9.1	2.16	11.1	0.72	90.2	8.8	2.3	5.0	11-20-68
	96	10.0	4.8	41.2	52.8	27.4	5.3	9.3	1.93	10.0	0.73	83.5	8.3	2.3	4.4	9-17-68
4.6→4.7	7	0.5	6.1	53.7	48.0	42.9	2.7	1.7	1.12	10.2	0.70	91.7	7.4	3.2	3.6	11-4-68
4.6→5.7	48	16.5	10.0	30.0	57.4	26.4	4.0	4.8	2.17	10.0	0.74	95.8	10.9	2.9	6.3	10-14-68
Semianaerobic:																
7.0	8				57.0	37.6			1.52			86.4				12-28-67
	48				52.4	30.8	3.0	9.0	1.70	9.0	0.72	87.5	7.5	2.3	3.9	10-18-66
	48				52.8	32.6	1.0		1.62			89.5				3-8-67
	48				54.6	32.4	1.0		1.69			87.5				2-15-67

* The culture medium for this experiment contained 1% yeast extract, 1% peptone, and 1% dextrose. In all other experiments, the medium contained 1% yeast extract, 2% peptone, and 2% dextrose.

At pH 7 under aerobic conditions, the sedimentation coefficient was 83.8 S for the 7-hour ribosomes, 92.1 S for the 24-hour ribosomes, 88.8 S for the 48-hour ribosomes, and 83.5 for the 96-hour ribosomes. During logarithmic growth from 7 to 24 hours, the yield of the yeast increased from 0.4 to 6.6 g per 500 ml of culture medium. The size of the cells was unchanged, and the concentration of ribosomes was about 65 mg/g of yeast. The molecular weight of the ribosomes increased slightly from 7 to 8 x 10^6. There was an increase of protein, phospholipid, and polysaccharide. The molecular weight of the total ribosomal RNA (rRNA) was relatively unchanged (2.5-2.6 x 10^6).

After 48 to 96 hours under the same growth conditions, the yield of yeast had reached 10 g/500 ml of culture medium. During this period the yeast cells had ceased to multiply but had grown bigger instead. The ribosomes per gram of yeast were reduced to about 40 mg/g, but the content of ribosomes per cell remained about the same. The molecular weight of the ribosomes was about 8.5 x 10^6 and that of rRNA was 2.3 x 10^6.

More pronounced changes in the rRNA were observed when strain LK2G12 was grown at pH 4.6 under aerobic conditions. The molecular weight of the 7-hour ribosomes was 7.4 x 10^6 and that of the 48-hour ribosomes was 10.9 x 10^6. The molecular weights of the corresponding rRNA were 3.2 x 10^6 and 2.9 x 10^6, respectively.

The ribosomes obtained from strain LK2G12 grown under semianaerobic conditions were not different in chemical composition and physicochemical properties from those grown under aerobic conditions, with the exception that the former apparently did not require magnesium ions for stability at pH 6.

Based on the reduced viscosity of about 10 ml/g and specific volume of about 0.70 mg/g, the hydration of the ribosomes of strain LK2G12 was calculated to be about 3.2 g of water/g of ribosomes. The reduced viscosity of the ribosomes of strain LK2G12 was twice as high as the values reported for different species of yeast (Table III), the hydration of which was about 1.3 g of water/g of ribosomes. The reduced viscosity of the ribosomes of strain LK2G12 was, however, similar to the values for the ribosomes of pea seedling (11 ml/g)[9], rat liver (11 ml/g)[4], rabbit reticulocytes (8 ml/g)[10], and rat Novikoff hepatoma (9.8 ml/g)[6].

The ribosomes of strain LK2G12 tend to precipitate from solution at 4°C. In one sample (11-20-68), as much as 34% of the ribosomes was lost after 11 days, 24.5% of the protein was converted into amino acids, and 20% of the RNA was converted into compounds soluble in cold trichloroacetic acid. The chemical composition and physiocochemical properties of the ribosomes still in solution after storage were identical with those for freshly prepared ribosomes of the strain LK2G12.

Table III

PROPERTIES OF RIBOSOMES OF DIFFERENT SPECIES OF YEAST

Specie	Growth Condition	Chemical Composition (%) Protein	RNA	Physicochemical Properties $[\eta]_{red}$ (ml/g)	Sp Vol (ml/g)	$S^{o}_{20,w}$	MW ($\times 10^{-6}$)	Authors
Saccharomyces cerevisiae (Bakers)	pH 4	58	42	5.0	0.67	80	4.1	Chao & Schachman (1956)[2]
Schizosaccharomyces pombe	log	59	41	4.5	0.65	83	3.8	Lederberg & Mitchison (1962)[8]
Saccharomyces cerevisiae (Bakers)	not defined		42	4.9	0.66	79.9-82.7	-	Lansink (1964)[7]
Saccharomyces fragilis 610 x Saccharomyces dobzhanskii (1974) Saccharomyces lactis (Y-14)	pH 5.2, log	45	55	not determined	0.62	78.3-83.2	3.4	Bruening (1965)[1]
Saccharomyces fragilis	log	-	-	4.6	not determined	80.4	3.95	Cotter et al. (1967)[3]

Characterization of Ribosomes of Other Yeasts

The ribosomes from commercial baker's yeast, Red Star and Fleischmann, and from Saccharomyces lactis, strain Y14, were prepared and characterized by the same procedures used for strain LK2G12 and described above. The results are summarized in Table IV. The chemical composition as well as the physicochemical properties of these ribosomes were quite different from each other and also different from those of strain LK2G12. Apparently, the variation of ribosomes among different species and strains of yeast was too pronounced to be accounted for by the difference in growth conditions alone. The reduced viscosity values of these ribosomes, however, were more similar to those of strain LK2G12 than to those reported for different species of yeast (Table III).

Effect of Freezing Ribosomes

Ribosomes freshly prepared from S. cerevisiae, strain LK2G12, and from S. lactis, strain Y14, were frozen and compared with unfrozen ribosomes. The results are summarized in Table V. Freezing rendered a large portion of the ribosomes insoluble. The ratio of protein to RNA was higher for the insoluble material than for the unfrozen ribosomes. Freezing also solubilized some protein and RNA. The ribosomes that remained in solution after being frozen had an unchanged sedimentation coefficient, a somewhat smaller specific volume, and a reduced viscosity of about 5 ml/g. The molecular weight of the ribosomes after being frozen was about half that of the unfrozen ribosomes. The diameters of the two types of ribosomes from strain LK2G12 were measured by electron microscopy. When the samples were air-dried, the diameter of the unfrozen ribosomes was 28.5 ± 2.5 mμ and that of the frozen ribosomes was 26.1 ± 4.1 mμ. When the samples were freeze-dried, however, the diameters were about the same (30.6 ± 2.8 mμ vs 30.0 ± 2.4 mμ).

Effect of Sucrose Density Gradient Centrifugation on Ribosomes of Strain LK2G12

The ribosomes of strain LK2G12 from a 24-hour culture were prepared (6-3-69). The yield of ribosomes was 59.2 mg/g of yeast. Their chemical composition was 57.4% protein, 21.8% RNA, 7.3% phospholipid, and 4.1% polysaccharide. The ribosomes were layered on top of 10-34% continuous sucrose density gradient, which was cushioned on 60% sucrose. All sucrose solutions contained the salt mixture of 0.001 M $MgSO_4$ + 0.002 M KH_2PO_4-K_2HPO_4 (1:1), pH 6.8. After centrifugation at 25,000 rpm (63,581 x g) for 4 hours in a swinging bucket, one fraction remained in the sucrose gradient. There was also a pellet with specific gravity greater than 60% sucrose. Both fractions were resuspended in the salt solution.

The lighter ribosomes were subjected to two additional cycles of centrifugation in order to remove the sucrose. The yield of this fraction was 5.4 mg/g of yeast. Its chemical composition was 44.4% protein, 40.7% RNA, 1.8% phospholipid, and 1.5% polysaccharide. The

Table IV

PROPERTIES OF RIBOSOMES FROM OTHER YEASTS

Specie	Yield of Cells (g/500 ml)	No. of Cells/g (x 10^{-9})	Conc.of Ribosomes in Cells (mg/g)	Chemical Composition (%)				Protein /RNA	Physicochemical Properties						Sample
				Protein	RNA	Phospho-lipid	Polysac-charide		$[\eta]_{red}$ (ml/g)	Sp Vol (ml/g)	$S^{0}_{20,w}$	Molecular Weight (x 10^{-6}) Ribo-somes	r-RNA	r-Protein	
S. cerevisiae (Red Star)			18.3	50.0	37.0	6.3	6.2	1.35	8.8	0.73	82.5	7.7	2.9	3.9	5-8-68
S. cerevisiae (Fleischmann)		14.6	21.4	58.8	19.3	5.8	9.8	3.11	15.7	0.71	90.2	10.0	1.9	5.9	1-6-69
S. lactis, strain Y14, 48-hr growth at pH 7.0	10.0	31.0	51.8	51.2	28.0	5.1	2.8	1.81	10.5	0.75	92.2	11.0	3.1	5.6	2-17-69

Table V

EFFECT OF FREEZING ON RIBOSOMES FROM SACCHAROMYCES CEREVISIAE AND SACCHAROMYCES LACTIS

	Conc. in Cells (mg/g)	Chemical Composition (mg/g of yeast)					Physicochemical Properties			
		Protein	RNA	Phospholipid	Polysaccharide	Protein /RNA	$[\eta]_{red}$ (ml/g)	Sp Vol (ml/g)	$S^{o}_{20,w}$	Molecular Weight (x 10^{-6})
Saccharomyces cerevisiae, strain LK2G12										
Unfrozen ribosomes	66.7	35.8	21.5	3.7	2.0	1.67	9.5	0.70	92.1	8.0
Frozen ribosomes	26.6	13.3	13.5	0.05	0.8	0.99	5.0	0.65	92.5	4.6
Insoluble after frozen	30.0	17.0	5.1	1.6	0.8	3.33				
Soluble after frozen		2.6	1.5							
Saccharomyces lactis, strain Y14										
Unfrozen ribosomes	51.8	27.0	11.6	4.7	2.7	2.33	10.5	0.75	92.2	11.0
Frozen ribosomes	14.4	5.4	6.0	0.04	1.1	0.90	5.2	0.67	92.0	5.0
Insoluble after frozen		20.0	4.4			4.55				

sedimentation coefficient of the ribosomes purified by sucrose density gradient centrifugation was 90.0 S. The specific volume was 0.70 ml/g, the reduced viscosity was 6.0 ml/g and the molecular weight was 6.1×10^6. The yield of the pellet with specific gravity greater than 60% sucrose was 2.5 mg/g of yeast. Its chemical composition was 40.0% protein, 32.0% RNA, 5.7% phospholipid, and 6.7% polysaccharide.

The lighter ribosomes purified by sucrose density gradient centrifugation were examined by electron microscopy. The diameters of the ribosomes were 32.0 ± 3.1 mμ for the air-dried samples and 32.0 ± 2.6 mμ for the freeze-dried samples.

After heat denaturation, the ribosomes did not become heavier in sucrose density gradient centrifugation. In fact, ribosomes before and after denaturation moved to the same position in 1.5 M sucrose after spinning at 30,000 rpm (73,499 x g) for 2 hours.

Characterization of Acetyl-CoA Synthetase from Strain LK2G12

The acetyl-CoA synthetase (12-23-68) extracted from strain LK2G12 by 0.5 M potassium phosphate at pH 7.5 appeared mainly as one component with uncorrected sedimentation coefficient of 6.5 S in the analytical ultracentrifuge, but there was a small amount of 34.4 S component. The same enzyme (2-14-69) prepared in 0.01 M Tris buffer at pH 7.5 showed four components with uncorrected sedimentation coefficients of 3.4, 9.7, 13.2, and 27.5 S.

Characterization of a Microsomal Fraction of Strain LK2G12

Fractionation of crude yeast homogenate was carried out by centrifuging down three successive pellets at (1) 2,500 rpm (1,020 x g) for 5 minutes, (2) 10,000 rpm (12,100 x g) for 30 minutes, and (3) 40,000 rpm (96,562 x g) for 90 minutes. The first pellet contained cell debris and a microsomal fraction. The microsomal fraction was obtained from the first pellet by three cycles of centrifugation at 2,500 rpm for 1 minute and at the same speed for 15 minutes. The residue coming down at the 1-minute centrifugation was discarded. The yield of microsomes was 28.9 mg/g of yeast from a 48-hour aerobic culture (1-22-69). Their chemical composition was 50.2% protein, 3.1% RNA, 2.9% phospholipid, and 1.3% polysaccharide. After treatment at 4°C overnight with 0.4% sodium deoxycholate (DOC) in 0.05 M KH_2PO_4, 0.009 M $NaHCO_3$, 0.018 M $MgSO_4$, and 0.001 M $CaCl_2$, pH 6.8, a residue was centrifuged down at 10,000 rpm for 15 minutes. The dry weight of the residue was 15.7 mg/g of yeast. Its chemical composition was 82.9% protein, 2.3% RNA, 2.1% phospholipid, and 1.4% polysaccharide. The material extracted by 0.4% DOC contained protein equivalent to 1.8 mg/g of yeast and RNA equivalent to 0.50 mg/g of yeast. Ultracentrifugal analysis showed one major component with a sedimentation coefficient of about 77 S and a minor component with 117 S. These ribosome-like particles released from the microsomal fraction by 0.4% DOC probably had a sedimentation coefficient less that that of the free ribosomes.

Characterization of Phosphoribulokinase and Carboxydesmutase

The phosphoribulokinase of Thiobacillus thioparus (12-10-68), at 1.7 mg/ml of protein in 0.25 M potassium phosphate, pH 7.0, appeared as one component with a sedimentation coefficient of 18.4 S. The same enzyme of Thiobacillus neapolitanus at 0.5 mg/ml of protein in 0.25 M potassium phosphate appeared as one component with a sedimentation coefficient of 12.7 S. The carboxydesmutase of T. neapolitanus (12-18-68) at 1.1 mg/ml of protein in potassium phosphate showed three components with sedimentation coefficients of 2.0, 9.0, and 14.0 S.

Attempts were made to purify phosporibulokinase and carboxydesmutase from T. neapolitanus. An extract of the bacteria (6-23-69) was prepared in 0.05 M Tris buffer, pH 7.5, according to the procedure of MacElroy et al. (Arch. Biochem. Biophys. 127, 310, 1968). The extract was centrifuged at 40,000 rpm for 6 hours. The pellet thus obtained was resuspended in 0.05 M Tris buffer and again centrifuged at 40,000 rpm for 30 minutes to remove insoluble material. Two additional cycles of centrifugation were carried out. The first 40,000 rpm pellet contained 26.4 mg of protein/g of bacteria and 5.6 mg of RNA/g of bacteria, and consisted of six components with uncorrected sedimentation coefficients of 6.5, 9.4, 16.8, 18.8, 23.5, and 48.1 S. The second 40,000 rpm pellet contained 22.0 mg of protein/g of bacteria and 4.2 mg of RNA/g of bacteria. It had six components with uncorrected sedimentation coefficients of 5.4, 10.3, 16.4, 19.7, 24.1, and 49.0 S. The third 40,000 rpm pellet contained 7.8 mg of protein/g of bacteria and 0.3 mg of RNA/g of bacteria, and showed two components with uncorrected sedimentation coefficients of 19.2 and 24.2 S.

The effect of using 0.02 M $MgSO_4$ and 0.05 M Tris buffer, pH 7.5, on the two enzymes of T. neapolitanus (7-7-69) was studied. The first 40,000 rpm pellet contained 24.4 mg of protein/g of bacteria and 8.0 mg of RNA/g of bacteria. It consisted of eight components with uncorrected sedimentation coefficients of 5.2, 9.0, 16.1, 18.7, 24.2, 31.1, 47.3, and 60.7 S. The second 40,000 rpm pellet contained 15.6 mg of protein/g of bacteria and 3.9 mg of RNA/g of bacteria. There were seven components with uncorrected sedimentation coefficients of 9.8, 16.1, 19.2, 24.5, 32.2, 50.6, and 64.4 S. The third 40,000 rpm pellet contained 12.0 mg of protein/g of bacteria and 2.2 mg of RNA/g of bacteria, and consisted of five components with uncorrected sedimentation coefficients of 16.2, 19.3, 24.1, 31.9, and 41.8 S.

A sample of highly purified phosphoribulokinase from Thiobacillus neapolitanus (8-8-69) was prepared in 0.20 M potassium phosphate at pH 7.0. It contained one main component with a sedimentation coefficient of 12.7 S at 0.5 mg of protein/ml of 0.20 M potassium phosphate.

Characterization of Membrane Fragments of Halobacterium cutirubrum

The membrane fragments of H. cutirubrum in 3.4 M NaCl and at pH 9.4 showed two boundaries in the analytical ultracentrifuge. A major component

had a sedimentation coefficient of 3.9 S after correction for viscosity and specific gravity of the 3.4 M NaCl. A minor component had a sedimentation coefficient of about 8.9-9.3 S.

Characterization of Fatty Acid Synthetase from Rat Mammary Gland

The fatty acid synthetase, freshly prepared from rat mammary gland (12-11-68) and suspended in 0.25 M potassium phosphate containing 0.001 M EDTA and 0.01 M dithiothreitol, appeared as one component with a sedimentation coefficient of 13.6 S.

CsCl Density Gradient Centrifugation of Bacterial DNA

Preparations of DNA from bacteria were submitted to CsCl centrifugation (sp gr = 1.701) at 42,040 rpm (102,750 x g) for about 20 hours at 20.0°C. The DNA from Micrococcus lysodeikticus had a band with buoyancy density of 1.691 g/ml; the DNA from Proteus mirabilis had a band with buoyancy density of 1.693 g/ml. Three samples of DNA prepared from an unidentified bacterium were also examined. There were three bands for Sample No. 6 with buoyancy densities of 1.684, 1.700, and 1.716 g/ml; three bands for Sample No. 46 (old cells) with buoyancy density of 1.684, 1.697, and 1.716 g/ml; and one band for Sample No. 46 (new cells) with buoyancy density of 1.716 g/ml.

Characterization of CH_3-NH_3 Polymer

Samples of CH_3-NH_3 polymer were dissolved in methanol. The first sample (W-2) had a sedimentation coefficient of 1.02 S at 17.9°C. The second (N-1) had a sedimentation coefficient of 0.81 S at 18.4°C. The third (N-5) had sedimentation coefficients of 0.50 S for 10 mg/ml at 21.6°C, 0.66 S for 7 mg/ml at 20.7°C, and 0.67 S for 10 mg/ml at 19.2°C.

Electron Microscopy of Pseudomonas saccharophila

The technique of negative staining with ammonium molybdate (1%) followed by shadowing with uranium, as described by Wang and Lundgren (J. Bacteriol. 97, 947, 1969), was used. The bacteria from a 16-hour culture appeared quite flat. In contrast, the bacteria in a 4-hour N-starved culture, in the presence of either air or pure oxygen, appeared to be bigger and at the same time quite thick at the central portion. The material that caused the bulging of the bacteria upon drying was probably poly-β-hydroxybutyric acid as described by Wang and Lundgren. The contour of the polymer coincided with the vacuole-like structures seen in sections. The bacteria were fixed in freshly prepared formaldehyde as described by Peters and Ashley (J. Cell Biol. 33, 53, 1967), embedded in Maraglas by the procedure described by Freeman and Spurlock (J. Cell Biol. 13, 437, 1962), cut into 500-Å sections, and stained by lead citrate as described by Reynolds (J. Cell Biol. 17, 208, 1963).

RECOMMENDATION

We recommend that the research reported herein be continued.

Fu-Chuan Chao

Fu-Chuan Chao, Ph.D.
Biophysical Chemist

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