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MACROMOLECULAR FRACTIONATION AND DETERMINATION

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SUMMARY

The specific objective of the research performed under this contract (NAS2-5754) was the characterization of biological materials supplied by Ames Research Center. Techniques of analytical ultracentrifugation were used, with the following results:

(1) The fatty acid synthetase activity of the 47 S particles in Saccharomyces cerevisiae, strain LK2G12, was high if the rupturing medium was 0.018 M $MgSO_4$ + 0.001 M $CaCl_2$ + 0.050 M KH_2PO_4 + 0.009 M $NaHCO_3$, pH 6.0; it was low if the rupturing medium was 0.020 M $MgSO_4$ + 0.0005 M $CaCl_2$ + 0.138 M KH_2PO_4 + 0.025 M $NaHCO_3$, pH 5.8. However, the 47 S particles could not be purified in these two salt solutions. With 0.005 M $CaCl_2$ to precipitate the ribosomes, differential centrifugation was used to purify the 47 S particles that had good fatty acid synthetase activity. The 47 S particles thus purified contained 67% protein, 31% RNA, and 2% polysaccharide. When other salt solutions were used for purification, the chemical composition of the particles was altered, and their enzymatic activity was greatly lessened.

(2) One sample of crystalline acetyl-CoA synthetase from S. cerevisiae, strain LK2G12, was characterized. The sedimentation coefficient was 10 S, and the molecular weight was 2.1 to 2.5 $\times$ 10^5, as determined by the Archibald technique.

(3) The ribosomes of thermophilic bacteria grown at 70°C required 0.002 to 0.005 M $MgSO_4$ for stability in solution at 4°C. The ribosomes consisted of five components with sedimentation coefficients--extrapolated to zero $MgSO_4$ concentration--of 35, 48, 65, 87, and 92 S, which corresponded to the relative molecular weights of 1, 1.6, 2.6, 3.6, and 4.2. However, the pattern of stabilized ribosomes varied with the $MgSO_4$ concentration in the growth medium and with the state of growth. In 0.001, 0.002, and 0.005 M $MgSO_4$, the ribosomes purified by differential centrifugation contained about 34% protein and 66% RNA and consisted of one predominant component, 92 S at 0.001, 65 S at 0.002, and 92 S at 0.005 M $MgSO_4$. In 0.00025 M $MgSO_4$, where the 92, 87, and 65 S had dissociated completely into 35 and 48 S particles, the 48 S particles purified by differential centrifugation contained 20% protein and 80% RNA, indicative of loss of protein. In late logarithmic growth, the predominant ribosomal components tended to dissociate into smaller ones up to 0.002 M $MgSO_4$ but not at 0.005 M $MgSO_4$. Solutions of ribosomes were not stable at 70°C.

(4) The buoyancy densities of thermophilic isolates (A, B, C, D, TE, and TD) were measured by CsCl gradient centrifugation to be within the range of 1.701 to 1.717 g/ml.

(5) Extracts obtained with 10% phenol at 4 to 36°C from the psychrophilic bacterium, Vibrio marinus, grown at 15°C, were analyzed. Of the two to four components present in these extracts, two were RNA. They had sedimentation coefficients of 17.4 and 22 S and were always present. A third component with a sharp boundary was probably DNA. A fourth component, which had a sedimentation coefficient of about 4.7 S, has not been identified. Only one sample was greatly affected by the temperature at which extraction was carried out; the others were not affected.

(6) Preliminary study was conducted on the ribosomes from V. marinus. One sample contained four components and the other five components, both at 15 and 30°C.

(7) Two samples of AMP-nucleosidase from Azotobacter vinelandii, strain OP, were characterized. The first sample had a sedimentation coefficient of 14.8 S and a molecular weight of 3.2 to 3.5 $\times 10^5$, as determined by the Archibald technique. Treatment with 0.1% sodium lauryl sulfate reduced the sedimentation coefficient to 2.4 S and the molecular weight to about 4.9×10^4. The uncertainty of binding with the detergent has not been resolved. The second sample had a molecular weight of about 2.6×10^5.

CONTENTS

TABLES

ILLUSTRATIONS

RESULTS

Purification of Fatty Acid Synthetase in Saccharomyces cerevisiae, Strain LK2G12

Earlier study by Klein et al.[1] has established that, in Saccharomyces cerevisiae, strain LK2G12, the fatty acid synthetase (FAS) activity was located in the 47 S particles with a diameter of about 30 mμ. Successful purification of the 47 S particles depended on the complete removal of ribosomes under conditions where the FAS activity remained stable. Preliminary experiments indicated that, in crude yeast extract, the simultaneous precipitation of the 47 S particles and the dissociation of the ribosomes into subunits by adding $(NH_4)_2SO_4$ to 1 M according to the procedure of Lynen et al.[2] was unsuccessful. Repeated precipitation with 1 M $(NH_4)_2SO_4$ resulted in complete dissociation of the 47 S particles into components with molecular weights less than 2×10^5, and a great loss of FAS activity occurred.

The following sections describe efforts to determine the conditions required for the stability of the FAS activity and to purify the 47 S particles under these conditions.

Internal Ionic Environment of the Strain LK2G12 Grown in an Aerobic Culture

The approximate internal ionic environment of the yeast grown in an aerobic culture was represented at 24 hours by 0.020 M $MgSO_4$ + 0.0005 M $CaCl_2$ + 0.138 M KH_2PO_4 + 0.025 M $NaHCO_3$, pH 5.8, and at 96 hours by 0.018 M $MgSO_4$ + 0.001 M $CaCl_2$ + 0.050 M KH_2PO_4 + 0.009 M $NaHCO_3$, pH 6.0. There was about a 50% decrease in K^+, Na^+, and PO_4 concentrations in the older culture, although Mg^{++} and SO_4 concentrations remained relatively unchanged. The two salt solutions were designated as complete salt solution I for 24-hour yeast and complete salt solution II for the 96-hour yeast, respectively.

Effect of Rupturing Media

When the yeast grown in an aerobic culture for 24 hours was ruptured in complete salt solution I, the FAS activity in the crude extract was poor--only about 5% of that when the yeast was ruptured in complete salt solution II (Table 1). Despite such an enormous difference in enzyme activity, the concentration of the 47 S particles appeared to be similar in the analytical ultracentrifuge (Figure 1). Apparently, the FAS activity of the 47 S particles was labile when the yeast was ruptured.

Table 1

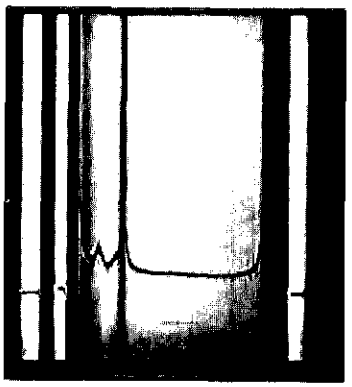
EFFECT OF RUPTURING MEDIA ON FAS ACTIVITY IN 56,600 × g SUPERNATANT (10 min) OF SACCHAROMYCES CEREVISIAE, STRAIN LK2G12

	Composition of Salt Solution (M)				
	$MgSO_4$	$CaCl_2$	KH_2PO_4	$NaHCO_3$	Total FAS Activity[a]
Complete salt solution I	0.020	0.0005	0.138	0.025	65 (29-103)[b]
Complete salt solution II	0.018	0.001	0.050	0.009	1238 (1054-1438)[c]

[a] Total FAS activity is the product of specific activity and total protein in the supernatant. Specific activity is defined as mμM of TPNA oxidized per minute per mg of protein.

[b] Average from 2 samples; range in parentheses.

[c] Average from 4 samples; range in parentheses.



(a) RUPTURED IN COMPLETE SALT SOLUTION I: 0.020 M $MgSO_4$ + 0.0005 M $CaCl_2$ + 0.138 M KH_2PO_4 + 0.025 M $NaHCO_3$, pH 5.8.

(b) RUPTURED IN COMPLETE SALT SOLUTION II: 0.018 M $MgSO_4$ + 0.001 M $CaCl_2$ + 0.050 M KH_2PO_4 + $NaHCO_3$, pH 6.0.

(Ultracentrifugal patterns were at 16 min after reaching 42,040 rpm. Phase plate angle was at 70°.)

FIGURE 1 ULTRACENTRIFUGAL PATTERNS OF 56,600 x g SUPERNATANT (10 min) OF STRAIN LK2G12 GROWN IN AEROBIC CULTURE FOR 24 hr.

To determine which ionic specie was responsible for maintaining the FAS activity in the 47 S particles, the 24-hour aerobic yeast was ruptured in 0.018 M $MgSO_4$ at pH 6.0, in 0.001 M $CaCl_2$ at pH 6.5, and in 0.050 M KH_2PO_4 + 0.009 M $NaHCO_3$ at pH 6.0, respectively. In all three cases, the FAS activities in crude yeast extract were substantially lower than those in the combination of all the salts. However, when solutions containing various concentrations of $MgSO_4$ were used as rupturing media, optimal FAS activity was obtained at about 0.010 M $MgSO_4$ (Figure 2A). The result with 0.010 M $MgSO_4$ was nearly as good as that with complete salt solution II. When solutions of $CaCl_2$ at various concentrations were compared, optimal FAS activity was obtained at 0.005 to 0.006 M $CaCl_2$ (Figure 2B), although the result was definitely not so good as that with 0.010 M $MgSO_4$. Surprisingly, using the mixture consisting of 0.010 M $MgSO_4$ + 0.005 M $CaCl_2$ as rupturing medium reduced the FAS activity considerably. In fact, any combination of $MgSO_4$ and $CaCl_2$ concentrations resulted in poor FAS activity in crude yeast extract. However, the addition of KH_2PO_4 + K_2HPO_4 tended to restore the enzyme activity in various mixtures of $MgSO_4$ and $CaCl_2$.

To yield highly active 47 S particles, it was necessary to rupture yeast either in an ionic environment such as complete salt solution II or in 0.010 M $MgSO_4$ or 0.005 to 0.006 M $CaCl_2$. Interestingly enough, the 47 S particles, unlike ribosomes, were not stable in 0.001 M $MgSO_4$ or 0.001 M $CaCl_2$.

Effect of Centrifugation at 56,600 × g on the Stability of 47 S Particles

The 47 S particles and their FAS activity in crude yeast extract were stable at 4°C for at least one week when complete salt solution II was used as rupturing medium. However, a substantial loss of the 47 S particles occurred when the yeast extract thus prepared was subjected to high-speed centrifugation (56,000 × g for 2 hr). In some cases, such loss occurred during the second cycle of centrifugation instead of the first cycle. In any event, the reduction of the 47 S particles was accompanied by a proportional loss of the FAS activity. Apparently, the ionic environment that protected the FAS activity when the yeast was ruptured caused the loss of the 47 S particles during high-speed centrifugation. After two cycles of centrifugation, the ribosomes were converted into ribosomal cores with a protein-to-RNA ratio of about unity.

The FAS activity in the 56,000 × g supernatant (10 min) when 0.005 M $MgSO_4$ was used as rupturing medium was about 50% of the enzyme activity in the same fraction when 0.010 M $MgSO_4$ was used as rupturing medium. However, in 0.005 M $MgSO_4$, only about 15% of FAS activity was lost after each cycle of centrifugation, compared with 50% loss in 0.010 M $MgSO_4$. In 0.005 M $MgSO_4$, the ribosomes were also converted into cores. The integrity of ribosomes required 0.001 M $MgSO_4$ (or 0.001 M $CaCl_2$).

The 47 S particles were relatively stable in 0.005 M $CaCl_2$, although about 40% of the FAS activity was lost after each cycle of centrifugation.

High-speed centrifugation also resulted in substantial loss of the 47 S particles in 0.018 M $MgSO_4$, in 0.001 M $CaCl_2$, and in 0.050 M KH_2PO_4 + 0.009 M $NaHCO_3$. In comparison, the yeast ribosomes were stable in 0.001 M $CaCl_2$, were converted into ribosomal cores in 0.018 M $MgSO_4$, and dissociated partially into subunits in 0.050 M KH_2PO_4 + 0.009 M $NaHCO_2$.

Precipitation of Ribosomes

The solubility of ribosomes was greatly reduced by a moderate increase in $CaCl_2$ concentration. In 0.005 M $CaCl_2$, at which concentration the 47 S particles were relatively more stable during high-speed centrifugation than either lower or higher concentrations of the salt, the 47 S particles were soluble to the extent of 2 to 3 mg/ml, whereas the ribosomes were insoluble.

Starting with crude yeast extract, both the 47 S particles and ribosomes were centrifuged down at 56,600 × g in 2 hours. The bulk of the ribosomes in the above pellet was rendered insoluble by resuspension with 0.005 M $CaCl_2$ and was removed by centrifuging at 16,300 × g for 20 minutes. However, complete removal of ribosomes generally required two to three cycles of centrifugation. Since about 40% loss of 47 S particles occurred after each cycle of centrifugation, it was desirable to limit the purification procedure to two cycles of centrifugation. A more effective way to precipitate the ribosomes was to employ 0.010 to 0.020 M $CaCl_2$ followed by dilution with distilled water to 0.005 M $CaCl_2$ before high-speed centrifugation. In purified preparations, only one component was observed in the analytical ultracentrifuge (Figure 3).

Characterization of 47 S Particles

The chemical composition of the purified 47 S particles when 0.005 or 0.010 M $CaCl_2$ was the rupturing and resuspension medium (after centrifugation at 56,600 × g) was about 67% protein, 31% RNA, and 2% polysaccharide (Table 2). The sedimentation coefficients were slightly dependent on concentration (Figure 4). From the extrapolated sedimentation coefficient of 50 S ($S^0_{20,w}$), specific volume of 0.70 ml/g, and reduced viscosity of 25 ml/g, the anhydrous molecular weight was calculated to be 5.2×10^6. The mean diameter and standard deviation of these particles was 30.5 ± 2.6 mμ, as determined by electron microscopy.

The chemical composition of the 47 S particles varied a great deal with the salt solution used for purification. The use of 0.005 M $CaCl_2$ containing 10% CH_3OH (v/v) resulted in a large reduction of RNA, and the anhydrous molecular weight of these particles was 3.6×10^6. The use of complete salt solution II to resuspend the first 56,600 × g pellet (2 hours) resulted in particles with an anhydrous molecular weight of 3.0×10^6, and the sedimentation coefficients of these particles were more concentration-dependent.

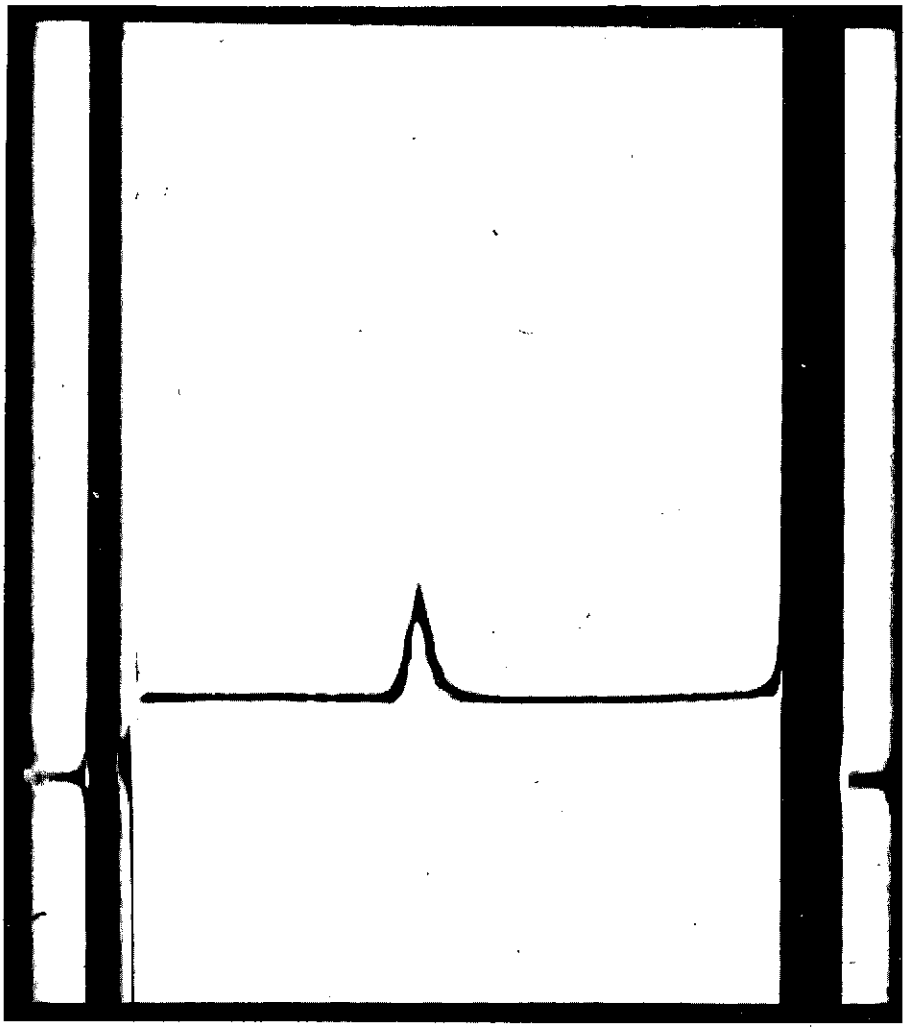


FIGURE 3 ULTRACENTRIFUGAL PATTERN OF PURIFIED 47 S PARTICLES IN 0.005 M $CaCl_2$ (Ultracentrifugal Pattern Was at 16 min After Reaching 42,040 rpm. Phase Plate Angle Was at 70°.)

Table 2

PROPERTIES OF 47 S PARTICLES OF *SACCHAROMYCES CEREVISIAE*, STRAIN LK2G12

Sample	Rupturing Medium	Removal of Ribosomes	Resuspension Medium	Yield (mg/g of yeast)	Chemical Composition (%)			Physiochemical Properties				FAS Sp. Act.[a]
					Protein	RNA	Polysaccharide	$[\eta]_{red}$ (ml/g)	Sp. Vol. (ml/g)	$S^0_{20,w}$	Mol. Wt. (x 10^{-6})	
8-31-71	0.005 M $CaCl_2$	2 cycles of centrifugation with 0.005 M $CaCl_2$	0.005 M $CaCl_2$	0.61	68	30	2	25	0.70	50.0	5.2	1320
9-14-71	0.005 M $CaCl_2$	2 cycles of centrifugation	0.005 M $CaCl_2$	0.72	64	36	1					
8-9-71	0.005 M $CaCl_2$	3 cycles of centrifugation	0.010 M $CaCl_2$	0.20	69	28	3					
3-30-71	0.005 M $CaCl_2$ + 10% CH_3OH	3 cycles of centrifugation	0.005 M $MgSO_4$	0.10	78	2	20	11	0.72	48.5	3.6	321
11-17-70	0.010 M $CaCl_2$	3 cycles of centrifugation: 1st cycle with 0.010 M $CaCl_2$; 2nd cycle with 0.050 M K_2PO_4 + 0.009 M $NaHCO_3$ + 0.018 M $MgSO_4$ + 0.001 M $CaCl_2$; 3rd cycle with 0.010 M $CaCl_2$	0.010 M $CaCl_2$	0.25	84	16	--	6.6	0.70	52.4	3.0	258

[a] Specific activity of fatty acid synthetase is defined at mμM of TPNH oxidized per minute per mg of protein.

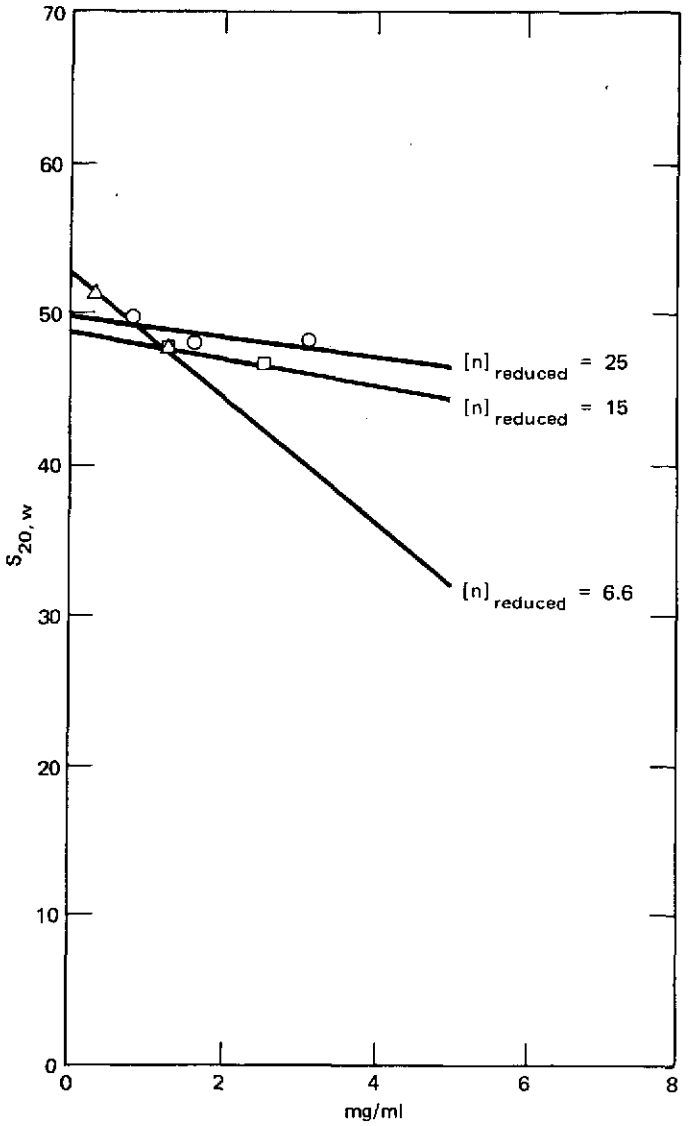


FIGURE 4 DEPENDENCE OF SEDIMENTATION COEFFICIENT ($S_{20,w}$) ON CONCENTRATION FOR 47 S PARTICLES OF STRAIN LK2G12 GROWN IN AEROBIC CULTURE FOR 24 hr

Molecular Weight of Acetyl-CoA Synthetase of *Saccharomyces cerevisiae*, Strain LK2G12

One sample of crystalline acetyl-CoA synthetase prepared from *S. cerevisiae*, strain LK2G12, was studied. The sedimentation coefficient was 10 S at 3.5 mg protein/ml in 0.10 M KH_2PO_4-K_2HPO_4 (pH 7.4) + 1×10^{-4} M ATP. Molecular weight was determined by the Archibald technique as modified by Trautman.[3] The values were 2.1×10^5 for a partial specific volume of 0.72 ml/g and 2.5×10^5 for 0.73 ml/g.

Purification of Ribosomes from Thermophilic Bacteria

Requirement of $MgSO_4$ for Growth

The thermophilic bacteria (YTP or TPA) grew well at 70°C in a medium containing 3 g/ℓ Bacto-Tryptone, 3 g/ℓ sodium pyruvate, and 0.06 M KH_2PO_4-K_2HPO_4 (36:24), if 0.00025 to 0.005 M $MgSO_4$ was included. Optimal growth required 0.002 M $MgSO_4$, and growth in the above medium containing 0.0001 M $MgSO_4$ was slow.

Stability of Ribosomes

The requirement of $MgSO_4$ for stability of ribosomes in solution was determined by analytical ultracentrifuge. In this study, the ribosomes were prepared by suspending the thermophilic bacteria in a given concentration of $MgSO_4$ plus 0.002 M KH_2PO_4-K_2HPO_4 (1:1), rupturing the suspension in a French Press, and centrifuging down insoluble material at 10,000 rpm (30 minutes in Sorvall rotor HG-4, 16,300 × g).

For the thermophilic bacteria during logarithmic growth, the 10,000-rpm supernatant consisted of four components when the rupturing medium contained 0.00025 M $MgSO_4$ and six to seven components when the extraction medium contained 0.001 to 0.005 M $MgSO_4$. The various components were identified by their relative positions in the ultracentrifuge patterns and by their sedimentation coefficients extrapolated to infinite dilution. The slowest two components (6 S and 26 S)--the concentrations of which apparently were not affected by the $MgSO_4$ concentration in the extraction medium--were not ribosomes. The two components preceding the two slowest ones were ribosomes, as evidenced by the fact that at higher $MgSO_4$ concentration their concentrations decreased substantially as faster components were formed.

During late logarithmic growth, the 10,000-rpm supernatant (30 minutes) consisted of four components at 0.0001 to 0.001 M $MgSO_4$ and seven components at 0.002 to 0.005 M $MgSO_4$. As was the case with logarithmic growth, the two slowest components were not affected by change in concentration of $MgSO_4$ in the rupturing media, but the ribosomes consisted of two components in 0.0001 to 0.001 M $MgSO_4$ and five components in 0.002 to 0.005 M $MgSO_4$.

Sedimentation Coefficients of Ribosomes

Table 3 summarizes the sedimentation coefficients of ribosomes from thermophilic bacteria grown at 70°C in media containing 0.00025, 0.001, 0.002, and 0.005 M $MgSO_4$ and ruptured in media containing the same $MgSO_4$ concentration plus 0.002 M KH_2PO_4-K_2HPO_4 (1:1). The sedimentation coefficients at zero $MgSO_4$ concentration were obtained by extrapolation. The relative size of the five ribosomal components during logarithmic or late logarithmic growth were calculated by the Svedberg equation to be approximately 1, 1.6, 2.6, 3.6, and 4.2. The five ribosomal components may be designated as A, B, C, D, and E in order of increasing molecular weight. Components A and B were not monomers and dimers but were two basic ribosomal particles. Component C apparently consisted of one A and one B, Component D of two As and one B, and Component E of one A and two Bs. When it was present, Component D was always at low concentration compared with Component E. The dissociation of E would give one C and one B or two Bs and one A.

Profile of Ribosomes

During logarithmic growth in a medium containing 0.001 to 0.005 M $MgSO_4$, one ribosomal component was predominant. It was 98 S at 0.001 M, 76 S at 0.002 M, and 105 S at 0.005 M $MgSO_4$. Components 98 S and 105 S were the fastest, and the 76 S component was the third fastest.

During late logarithmic growth in a medium containing a concentration of $MgSO_4$ as high as 0.001 M or 0.002 M, the predominant ribosomal components tended to dissociate into smaller ones. However, with 0.005 M $MgSO_4$ in the growth medium, the 106 S component remained at high concentrations.

Purification of Ribosomes

The ribosomes in the 10,000-rpm supernatant described in the preceding section were purified by three cycles of centrifugation, each cycle run at 40,000 rpm for 60 minutes in a Spinco rotor 50 (56,600 $\times$ g) and 10,000 rpm for 15 minutes in a Sorvall rotor HG-14 (16,300 $\times$ g). The pellet spun down after the high-speed centrifugation was resuspended in the original rupturing medium; the precipitate after the low-speed centrifugation was discarded each time. The slowest three components, 6 S, 26 S, and 36 S--the slowest ribosomal component--were completely removed after three cycles of centrifugation. Table 4 summarizes the chemical composition and sedimentation coefficients of the purified ribosomes. Figure 5 shows the ultracentrifugal patterns of these ribosomes. At 0.001, 0.002, or 0.005 M $MgSO_4$ plus 0.002 M KH_2PO_4-K_2HPO_4 for each $MgSO_4$ concentration, the purified ribosomes contained about 34% protein and 66% RNA but consisted of two to four components, of which one was predominant--98 S at 0.001 M $MgSO_4$, 80 S at 0.002 M $MgSO_4$, and 108 S at 0.005 M $MgSO_4$. The sedimentation coefficients were 92 S, 65 S, and 92 S, respectively, at zero $MgSO_4$ concentration. At 0.00025 M

Table 3

SEDIMENTATION COEFFICIENTS OF RIBOSOMES IN 10,000-rpm SUPERNATANT (30 min) PREPARED FROM THERMOPHILIC BACTERIA (Log and Late Log Growth)[a]

$MgSO_4$ Conc. In Growth and Extraction Media	Sedimentation Coefficient ($S^{\circ}_{20,w}$)	
	Log Growth	Late Log Growth
0.005	40, 58, 76, 87, 105	36, 54, 76, 85, 106
0.002	37, 57, 76, 87, 107	33, 51, 68, 77, 95
0.001	37, 54, 68, 87, 98	34, 51
0.00025	36, 49	34, 47
0 (by extrapolation)	35, 48, 65, 87, 92	34, 46, 62, 73, 88

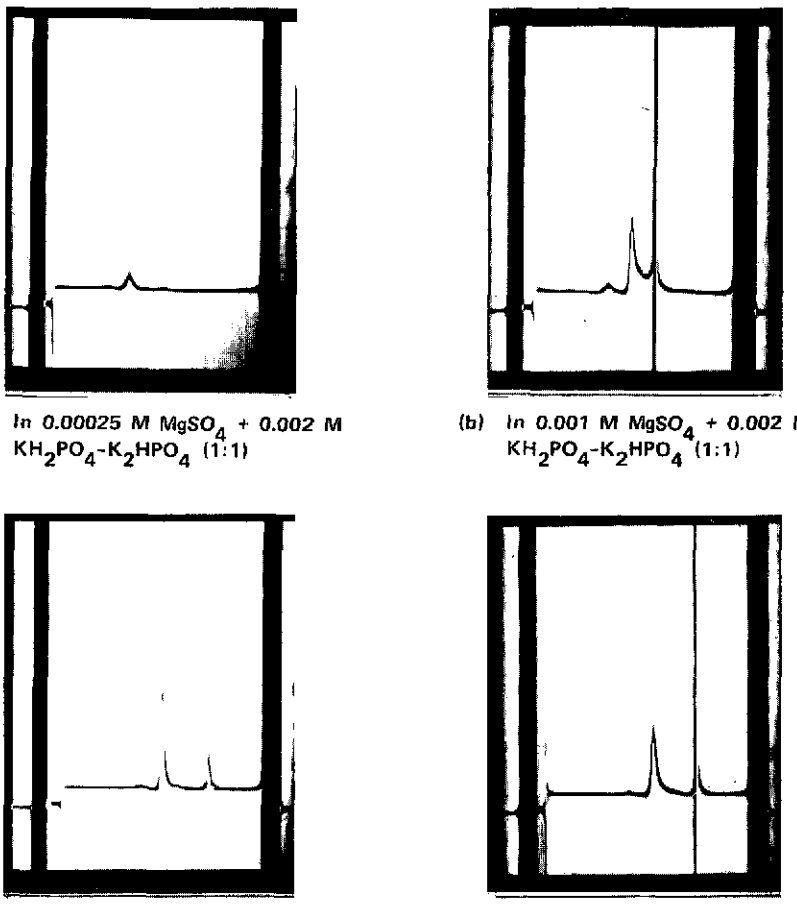
[a]Data compiled from Quarterly Reports 11 and 12.

Table 4

CHEMICAL COMPOSITION AND SEDIMENTATION COEFFICIENTS OF RIBOSOMES PURIFIED FROM THERMOPHILIC BACTERIA (Log Growth)

Sample	$MgSO_4$ Conc. in Growth and Extraction Medium	Chemical Composition (%)		Sedimentation Coefficient $S^{o}_{20,w}$
		Protein	RNA	
11-07-72	0.005	32.4	67.6	75, __, 108[a]
9-01-72	0.002	35.2	64.8	63, 80,[a] __, 108
12-08-72	0.001	34.7	65.3	54, 68, 87, 98[a]
9-13-72	0.00025	20.0	80.0	53
	0 (by extrapolation)			48, 65, 87, 92

[a] Predominant components.



(a) In 0.00025 M $MgSO_4$ + 0.002 M KH_2PO_4-K_2HPO_4 (1:1)

(b) In 0.001 M $MgSO_4$ + 0.002 M KH_2PO_4-K_2HPO_4 (1:1)

(c) In 0.002 M $MgSO_4$ + 0.002 M KH_2PO_4-K_2HPO_4 (1:1)

(d) In 0.005 M $MgSO_4$ + 0.002 M KH_2PO_4-K_2HPO_4 (1:1)

(Ultracentrifugal patterns were at 8 min after reaching 42,040 rpm. Phase plate angle was at 70°.)

FIGURE 5 ULTRACENTRIFUGAL PATTERNS OF PURIFIED RIBOSOMES FROM THERMOPHILIC BACTERIA GROWN AT 70°C AND GROWN AND RUPTURED IN MEDIA CONTAINING THE SAME $MgSO_4$ CONCENTRATION

$MgSO_4$ plus 0.002 M HK_2PO_4-K_2HPO_4 (1:1), the purified ribosomes contained 20% protein and 80% RNA and consisted of one component with a sedimentation coefficient of 53 S, which was 48 S at zero $MgSO_4$ concentration. The altered chemical composition in 0.00025 M $MgSO_4$ was probably due to a loss of protein from the ribosomes during high-speed centrifugation.

Effect of Heating on Purified Ribosomes

In an exploratory experiment (9-14-72), the effect of heating the ribosomes was studied. The ribosomes were prepared from the thermophilic bacterium grown in a medium containing 0.002 M $MgSO_4$. After two cycles of centrifugation, the ribosomes consisted of one component with an uncorrected sedimentation coefficient of 67.3 S (Run 939). Heating to 75°C for five minutes and cooling to 20°C resulted in the formation of a large mass of insoluble material, which was removed by low-speed centrifugation; the remaining soluble material consisted of a component with an uncorrected sedimentation coefficient of 50.5 S (Run 941). Heating a mixture consisting of 8 parts of purified ribosomes and 2 parts of 40,000-rpm supernatant (100 minutes) also resulted in a heavy precipitate; what remained soluble was one component with an uncorrected sedimentation coefficient of 49.8 S (Run 940).

The 10,000-rpm supernatant (30 minutes) from the various samples, regardless of whether the thermophilic bacteria were in logarithmic or late logarithmic growth, contained significant amounts of DNA but without the characteristic sharp boundary normally observable in the analytical ultracentrifuge for other bacteria. After treatment with deoxyribonuclease, the 10,000-rpm supernatant from one sample (10-19-72) consisted of essentially the same four components, with uncorrected sedimentation coefficients of 4.5, 23.6, 32.2, and 44.3 S (Run 950), as those in the untreated sample (Run 946; values of 4.1, 21.0, 29.4, and 40.4 S).

Buoyancy Density of DNA from Thermophilic Bacteria

Table 5 summarizes the buoyancy densities of DNA in samples of thermophilic bacteria measured by CsCl gradient centrifugation at 42,040 rpm and 20.0°C for about 16 hours. The DNA from Micrococcus lysodeikticus served as a marker. The weight fraction of the CsCl was 0.560, and the density of the CsCl was 1.701 g/ml at 20.0°C. The mean buoyancy density was 1.7275 g/ml (range of 1.720 to 1.735) for the M. lysodeikticus DNA.

RNA from Psychrophilic Bacterium Vibrio marinus

Table 6 summarizes ultracentrifugation analysis of RNA extracted with 10% phenol at 4 to 30°C from samples of V. marinus grown at 15°C. The RNA solutions, in 0.15 M sodium acetate and 0.05 M sodium chloride plus 0.01 M EDTA at pH 5.1, consisted of two to four components. The two components with sedimentation coefficients ($S^{\circ}_{20,w}$) of approximately

Table 5

BUOYANCY DENSITIES OF DNA FROM THERMOPHILIC BACTERIA

Sample	Band Position (cm from center of rotation)	Buoyancy Density (g/ml)	Run
1. Thermophilic isolate A	6.725	1.705	837
M. lysodeikticus	6.905	1.725	
2. Thermophilic isolate B	6.77	1.710	838
M. lysodeikticus	6.96	1.731	
3. Thermophilic isolate C	6.685	1.701	851
M. lysodeikticus	6.86	1.720	
4. Thermophilic isolate D	6.83	1.717	843
M. lysodeikticus	7.02	1.735	
5. Thermophilic isolate E	---	---	850
M. lysodeikticus	6.89	1.724	
6. Thermophilic isolate TE	6.80	1.713	862
M. lysodeikticus	6.97	1.733	
7. Thermophilic isolate TD	6.825	1.716	872
M. lysodeikticus	6.91	1.726	
8. Thermophilic isolate YTG-2	6.73	1.705	897
M. lysodeikticus	6.93	1.728	

Table 6

ULTRACENTRIFUGE ANALYSES OF RNA PREPARED FROM VIBRIO MARINUS

Sample	Extraction Temperature (°C)	RNA Conc. (mg/ml)	Sedimentation Coefficient ($S_{20,w}$)	Run
125		0.55	15.3, 19.5	848
121	4	0.85		852
	8	1.18	16.1, 19.4	853
	12	1.05		854
	16	1.16	15.3, 19.8	855
127	15	1.46		
128	8	1.86	17.0, 21.8	867
	11	2.14	16.2, 21.1	868
	16	1.94	16.6, 21.4	870
130	15	2.74	15.8, 20.5	873
129	23	3.40	4.4, 15.9, 20.2	874
129	30	3.10	3.8, 15.8, 20.4	875
130	36	2.41	16.7, 20.7	876
130	15	2.74	17.9, 23.3	877
136	15	11	4.7, 8.8, 12.9, 15.1	908
	23	11	4.4, 9.7, 15.1, 18.1	911
	29	11	4.6, 13.3, 16.0, 20.2	910
	36	11	4.7, 8.5, 11.2, 12.7, 15.3	909
141	15	7.5	4.4, 10.1, 14.1, 17.0	934
	23	7.5	4.8, 11.0, 14.2, 17.3	935
	30	7.5	4.4, 11.0, 14.7, 17.8	936
	36	7.5	4.5, 11.8, 14.4, 17.8	937
142	15	7.5	4.7, 10.6, 14.6, 17.7	951
	36	7.5	4.6, 11.7, 14.5, 18.2	952

17.4 and 22 S were present in all preparations; these were the RNA components. In some preparations, an unusually sharp boundary with uncorrected sedimentation of 8.8 to 10.1 S for 15°C extracts was probably DNA. In only one preparation (Sample 136), the concentrations of the four components were found to vary with the temperatures at which extraction was conducted. From 15 to 29°C, the reduction of the concentration of each component coincided with an increase in the uncorrected sedimentation coefficient for the same component; at 36°C, however, the solution of nucleic acids consisted of five components, which were too heterogeneous to be identified with the corresponding components at a lower temperature.

Ribosomes from Vibrio marinus

Table 7 summarizes ultracentrifugation analyses of ribosomes from two samples of V. marinus grown at 15°C. For each sample, aliquots of the bacteria were maintained separately at 15 and 30°C for 40 minutes, after which the ribosomes were partially purified by the procedure of Pace and Campbell.[4] In 0.01 M $MgCl_2$ plus 0.01 M Tris HCl at pH 7.3, the ribosomes consisted of four to five components that were apparently unaffected by the two temperatures used in treating the bacteria.

Table 7

ULTRACENTRIFUGE ANALYSES OF RIBOSOMES PREPARED FROM VIBRIO MARINUS

Sample	Extraction Temperature (°C)	Chemical Composition (mg/ml) Protein	RNA	Sedimentation Coefficient $S_{20,w}$	Run
132	15	6.8	15	57.7, 77.2, 93.1, 120.9	895
	30	6.8	15	57.2, 75.9, 96.8, 116.1	896
133	15	17.3	17.8	38.3, 53.2, 68.1, 92.8, 112.7	898
	30	17.3	17.8	34.6, 51.2, 69.1, 99.6, 119.0	899

Molecular Weight of AMP-nucleosidase from Azotobacter vinelandii

Two samples of the AMP-nucleosidase from Azotobacter vinelandii, strain OP, were studied in the analytical ultracentrifuge. Table 8 summarizes the results of this study. The first sample (1-27-71) appeared as a single component in the analytical ultracentrifuge. The sedimentation coefficient extrapolated to zero protein concentration ($S^{o}_{20,w}$) was 14.8 S. The concentration of the enzyme used for this study was too low for viscosity measurements. Hence, the sedimentation coefficient obtained above was not used for molecular weight calculation. Instead, molecular weight was determined by the Archibald technique as modified by Trautman.[3]

Table 8

CHARACTERIZATION OF AMP-NUCLEOSIDASE FROM AZOTOBACTER VINELANDII

Sample	Salt Solution	Protein Concentration (mg/ml)	Sedimentation Coefficient ($S_{20,w}$)	Molecular Weight	Run No.
1-27-71	0.1 M Tris HCl + 0.002 M AMP, pH 8.0	3.90	13.5		797
		2.43	13.8		784
		1.56	15.0		772
		1.22	14.3		785
		0	14.8 ($S^0_{20,w}$)		
				3.2×10^5	786
	0.1 M Tris HCl, pH 8.0	3.90	13.9		790
				3.3×10^5	791
	0.1% sodium lauryl sulfate + 0.01 M dithiothreitol	2.98	2.45		797
		1.49	2.25		798
		0	2.4 ($S^0_{20,w}$)		
				4.9×10^4	796
3-10-71	0.1 M Tris HCl + 0.002 M AMP, pH 8.0	5.89	10.5, 13.2		788
				2.6×10^5	789

By this method, the molecular weight of AMP-nucleosidase was calculated to be 3.2×10^5 in 0.1 M Tris HCl + 0.002 M AMP, and 3.3×10^5 in 0.1 M Tris HCl. Apparently, 0.002 M AMP was not necessary for the integrity of the enzyme. Treatment of the enzyme with 0.1% sodium lauryl sulfate in the presence of 0.01 M dithiothreitol converted it completely into subunits. The subunits have a sedimentation coefficient of 2.4 S and an apparent molecular weight of 4.9×10^4. The number of subunits in the enzyme was most likely six, although the uncertainty regarding the binding of the subunits with sodium lauryl sulfate was not resolved.

The second sample (3-10-71) appeared less homogenous, because there was a slower component in addition to the main component. The molecular weight was calculated to be 2.6×10^5.

RECOMMENDATION

It is recommended that data obtained for the 47 S particles of Saccharomyces cerevisiae, strain LK2G12, and those for the ribosomes of thermophilic bacteria be submitted for publication.

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