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Thermally Induced Intracellular Alteration of Ribosomal Ribonucleic Acid[1]

LEONARD J. ROSENTHAL[2] AND JOHN J. IANDOLO
Division of Biology, Kansas State University, Manhattan, Kansas 66502

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Heating at 55 C causes the intracellular degradation of ribosomes of *Staphylococcus aureus* MF-31. The 30*S* subunit of heated cells appears to be selectively attacked. Analysis of ribonucleic acid (RNA) showed that 16*S* RNA was destroyed and that the secondary structure of 23*S* RNA was altered.

A rapid decay of ribonucleic acid (RNA) has been observed (1, 3) when living cells are exposed to elevated temperature. Both messenger RNA (1, 8) and ribosomal RNA (2, 9) appear to be involved and have been reported to be extensively degraded as a result of heat shock. However, little is known regarding the specificity of susceptibility of the ribosomal RNA species to turnover in vivo. We have investigated this area and have found a potentially interesting system for further study.

Cultures of *Staphylococcus aureus* MF-31 were grown and heated as previously described (3). Cell-free extracts were prepared by suspending normal and heat-treated cells in a lytic mixture consisting of tris(hydroxymethyl)aminomethane (Tris) buffer (9) containing 10^{-2} M magnesium acetate (TM-2), 0.89% of NaCl, 12 μg of Lysostaphin per ml (supplied by P. A. Tavormina, Mead Johnson & Co., Evansville, Ind.), 5 μg of deoxyribonuclease per ml (Worthington Biochemical Corp.), and 0.2% sodium deoxycholate. Both cell suspensions were incubated on a rotary shaker (100 rev/min) at 25 C for 10 to 20 min. The ribosomes were harvested from the lysates by centrifugation at 97,000 × *g* for 3 hr. Any remaining cellular debris was removed by low speed centrifugation at 27,000 × *g* for 10 min. Ribosomes were dissociated into 50 and 30*S* particles either by suspending the pellets in Tris buffer containing 10^{-4} M magnesium acetate (TM-4) or by overnight dialysis against TM-4 buffer.

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[2] Present address: The John Collins Warren Laboratories of the Huntington Memorial Hospital of Harvard University, Massachusetts General Hospital, Boston, Mass. 02114.

RNA was obtained from the crude extracts and whole cells by a modified phenol-dupanol method originally described by Kirby (4). The extracted RNA was dissolved in a minimal volume of Tris-acetate buffer, and polyacrylamide gel electrophoresis was performed as described by Loening (5). The gels were scanned at 260 nm in a Gilford model 240 spectrophotometer equipped with a gel transport.

Tritium labeled 23*S* RNA (uracil-6-^3H; Schwarz BioResearch Inc.) from heated and control cells was purified by sucrose gradient centrifugation. Formaldehyde titration of the 23*S* RNA was performed by the method described by Penniston and Doty (7) and ribonuclease hydrolysis by the method of Sypherd and Fansler (10).

The specific site involved in thermally induced ribosome degradation was investigated by centrifuging derived ribosome preparations through 5 to 20% linear sucrose gradients (Fig. 1). The control sample contained both 50 and 30*S* particles, whereas the heat-treated preparation was essentially devoid of the 30*S* subunit. Subsequent experiments have shown that the degradation of 30*S* particles varies from 85 to 100%, whereas the 50*S* particles (Fig. 1) appear intact.

This unusual pattern of degradation prompted us to investigate the effect of heat on both major species of ribosomal RNA. Polyacrylamide gel electrophoresis was used to fractionate normal and heated RNA preparations from both crude extracts and whole cells. These data are presented in Fig. 2. Both molecular species of RNA were found in the extracts from untreated cells, but in the heated preparation 16*S* RNA was highly degraded and present only as a very small region. When experiments were conducted in vitro to determine if ribonuclease were associated with

these preparations (6), solubilization of label was not observed. On the other hand, the addition of exogenous ribonuclease or a 105,000 × *g* supernatant fraction from normal cells resulted in complete degradation of 16*S* and 23*S* RNA, regardless of whether the ribosome preparation was preheated. Therefore, if ribonuclease affected the degradation of 16*S* RNA, other unknown internal control measures protected 23*S* RNA from attack. Nevertheless, such an association in vivo may be responsible for the loss of 16*S* RNA during heating, even though Neu and Heppel (6) suggested that ribonuclease was present in the 30*S* ribosome as a result of adsorption during isolation.

The data presented in Fig. 1 and 2 indicated that neither the 50*S* subunit nor 23*S* RNA were affected by exposure to elevated temperature. However, the temperature used in this study (55 C) was in the melting range for rRNA and

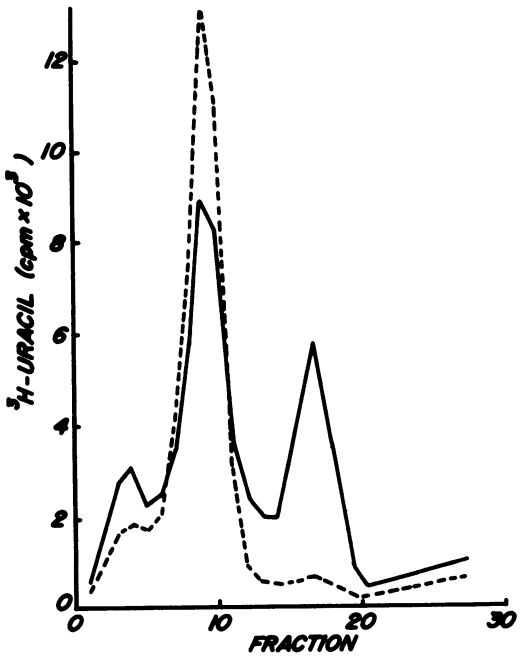

FIG. 1. *Absence of 30S ribosomal particles in heated preparations of S. aureus MF-31. The culture was labeled for 16 hr at 37 C with 0.1 μCi/ml (3.1 Ci/mmole) of uracil-6-^{3}H. Ribosomes were extracted as described and centrifuged in the SW-36 rotor for 4.5 hr at 30,000 rev/min through 5 to 20% sucrose gradients in TM-4. The fractions collected were made up to 5% with trichloroacetic acid and the resulting precipitates were deposited on fiber-glass filters and dried. The samples were then counted by liquid scintillation. Symbols: unheated cells, solid line; heated cells, dashed line.*

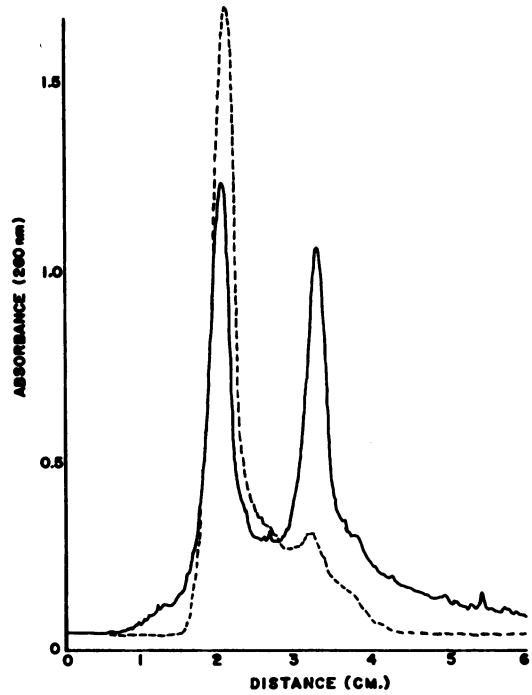

FIG. 2. *Absence of 16S rRNA in heat-treated cells of S. aureus MF-31. Polyacrylamide gel electrophoresis was carried out for 120 min at 5 ma/gel. Symbols: unheated cells, solid line; heat-treated cells, dashed line.*

could have caused configurational changes. The extent of secondary structural differences in normal and heated 23*S* RNA was estimated by examining susceptibility to hydrolysis by pancreatic ribonuclease (Fig. 3A). Normal 23*S* RNA contained 25.2% resistant areas of helical configuration, whereas the heated preparation was 16.6% resistant. The number of paired bases was also estimated by titrating the free amino groups of normal and heated 23*S* RNA with ^{14}C-labeled formaldehyde. These data are presented in Fig. 3B and confirm the ribonuclease resistance observed in Fig. 3A. Heated 23*S* RNA was shown to have fewer areas of hydrogen bonding. The data revealed that the control RNA bound 88.5% of the total formaldehyde bound by the heated RNA. Similarly, when the extent of ribonuclease digestion was compared, the degree of RNA digested in the control was 89.5% of the RNA digested in the heated preparation. Consequently, it appears that during heat shock unwinding of 23*S* RNA occurred, which on cooling and reannealing resulted in a net loss of helical configuration.

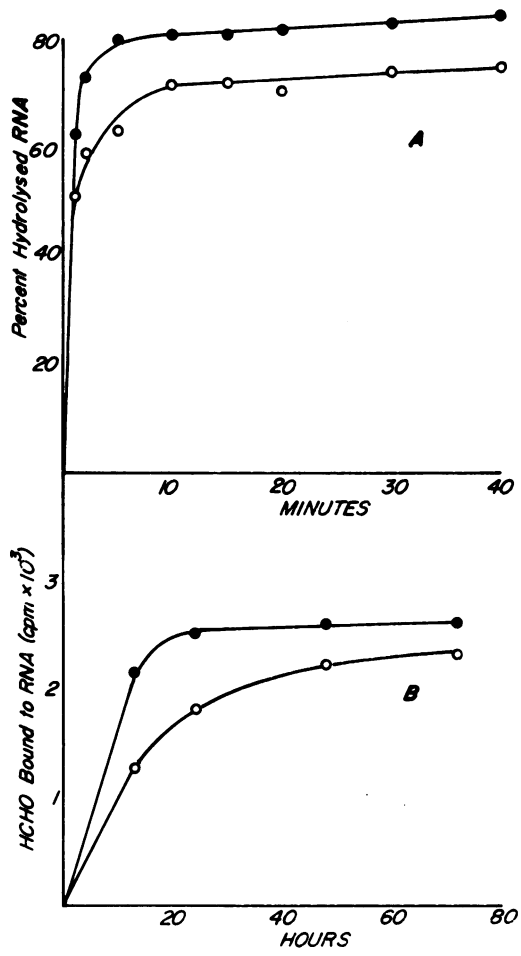

FIG. 3. *Variation in the helical configuration of 23S RNA as a result of heating suspensions of S. aureus MF-31 at 55 C for 15 min. Curve A represents the relative susceptibilities of 23S RNA to digestion by pancreatic ribonuclease. Curve B shows the extent to which ^{14}C-formaldehyde is bound to 23S RNA. After phenol extraction of ribosomes, 23S RNA was purified by centrifugation through a 5 to 20% linear sucrose gradient for 16 hr at 25,000 rev/min in the Spinco SW25.1 rotor. Symbols: RNA from unheated cells, ○; RNA from heated cells, ●.*

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LITERATURE CITED

1. Byfield, J. E., and O. H. Scherbaum. 1967. Temperature effect on protein synthesis in a heat synchronized protozoan treated with actinomycin D. Science 158:1504–1505.
2. Haight, R. D., and Z. J. Ordal. 1969. Thermally induced degradation of staphylococcal ribosomes. Can. J. Microbiol. 15:15–19.
3. Iandolo, J. J., and Z. J. Ordal. 1966. Repair of thermal injury of *Staphylococcus aureus*. J. Bacteriol. 91:135–142.
4. Kirby, K. S. 1956. A new method for the isolation of ribonucleic acids from mammalian tissue. Biochem. J. 64:405–408.
5. Loening, U. E. 1967. The fractionation of high-molecular weight ribonucleic acid by polyacrylamide-gel electrophoresis. Biochem. J. 102:251–257.
6. Neu, H. C., and L. A. Heppel. 1964. Some observations on the latent ribonuclease of *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. 51:1267–1274.
7. Penniston, J. T., and P. Doty. 1963. Reaction of formaldehyde with soluble ribonucleic acid. Biopolymers 1:145–164.
8. Schiebel, W., T. G. Chayka, A. DeVries, and H. P. Rusch. 1969. Decrease of protein synthesis and breakdown of polyribosomes by elevated temperature in *Physarum polycephalum*. Biochem. Biophys. Res. Commun. 35:338–345.
9. Sogin, S. J., and Z. J. Ordal. 1967. Regeneration of ribosomes and ribosomal ribonucleic acid during repair of thermal injury to *Staphylococcus aureus*. J. Bacteriol. 94:1082–1087.
10. Sypherd, P. S., and B. S. Fansler. 1967. Structural transitions in ribonucleic acid during ribosome development. J. Bacteriol. 93:920–929.