type. We have shown that a bacterial species with DNA of the high GC type does nevertheless possess the means (activating enzymes and sRNA) to read coding units which do not contain G or C.

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References and Notes

1. Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid; sRNA, transfer (soluble)RNA; ATP and GTP, the 5'-triphosphate of adenosine and guanosine; the capital letters A, C, G, and U, are used for the nucleotides adenylic, cytidylic, guanylic, and uridylic acids, respectively, or their corresponding residues in polynucleotide chains. Figures in parentheses after (or under) the abbreviated polynucleotide names give the molar ratios of nucleoside diphosphates used in the preparation of the polymers with polynucleotide phosphorylase. Thus "polyUA (5 : 1)" means that the polynucleotide was prepared from a mixture of five parts of uridine diphosphate and one part of adenosine diphosphate. The converse would be true for polyAU (5 : 1). The analytically determined base ratios of the copolymers agreed closely with the input ratios of nucleoside diphosphate precursors.
2. A. J. Wahba, R. S. Miller, C. Basilio, R. S. Gardner, P. Lengyel, J. F. Speyer, *Proc. Natl. Acad. Sci. U.S.* **49**, 880 (1963); O. W. Jones, Jr., and M. W. Nirenberg, *ibid.* **48**, 2115 (1962).
3. S. Ochoa, *Federation Proc.* **22**, 62 (1963).
4. H. R. V. Arnstein, R. A. Cox, J. A. Hunt, *Nature* **194**, 1042 (1962); A. C. Griffin, and M. A. O'Neal, *Biochim. Biophys. Acta* **61**, 496 (1962); E. S. Maxwell, *Proc. Natl. Acad. Sci. U.S.* **48**, 1639 (1962); I. B. Weinstein and A. N. Schechter, *ibid.*, p. 1686; L. Marcus and H. O. Halvorson, *Federation Proc.* **22**, 302 (1963); I. B. Weinstein, *Cold Spring Harbor Symp. Quant. Biol.*, in press.
5. R. S. Gardner, A. J. Wahba, C. Basilio, R. S. Miller, P. Lengyel, J. F. Speyer, *Proc. Natl. Acad. Sci. U.S.* **48**, 2087 (1962).
6. A. J. Wahba, R. S. Gardner, C. Basilio, R. S. Miller, J. F. Speyer, P. Lengyel, *ibid.* **49**, 116 (1963).
7. American Type Culture Collection No. 8750.
8. G. B. Pinchot, *J. Biol. Chem.* **208**, 65 (1953).
9. P. Lengyel, J. F. Speyer, S. Ochoa, *Proc. Natl. Acad. Sci. U.S.* **47**, 1936 (1961).
10. M. Lubin and H. L. Ennis, *Federation Proc.* **22**, 302 (1963).
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Sonic Energy Effects in Bovine Serum Albumin Solutions

Abstract. *Bovine serum solutions exposed to high-frequency sound were examined by ultracentrifugal, electrophoretic, viscometric, conductivity, light scattering, and optical rotatory dispersion procedures. Parameters determined with treated material were the same as those determined with untreated albumin solutions except for slight differences in rotation, the dispersion constant, and in weight-average molecular weight.*

In contrast to DNA and collagen, which are highly anisometric molecules (*1–4*), the globular protein bovine serum albumin is strikingly insensitive to treatment with high-frequency sound. Doty *et al.* (*2*) and Nishihara and Doty (*3*) observed scissions in DNA in 1 minute and in collagen within 10 minutes when these substances were exposed to sound from a 50-watt, 9-kc Raytheon generator. The fragmentation of DNA is a nonrandom process and the strand is split preferentially near the midpoint (*2, 4*). The fragmentation of collagen results in shorter pieces which retain the three-stranded helical structure (*3*). Kanig and Künkel (*5*) reported turbidity, flocculation, and increased viscosity in bovine serum albumin and fibrinogen solutions treated with high-frequency sound. In contrast, Tolgyessy and Kovacs (*6*) found no change in viscosity but noted an increase in the conductivity of ovalbumin solutions exposed to ultrasound waves.

Since sonic energy is used extensively to disrupt microorganisms (*7, 8*), it seemed worthwhile to inquire into the effects of the energy from high-frequency sound on globular proteins. A variety of procedures, such as ultracentrifugation, electrophoresis, viscosity, optical rotation, light scattering, and conductivity, have been used to examine sound-treated solutions of bovine serum albumin. Crystalline bovine serum albumin (Armour Pharmaceutical Co.) was dissolved in 0.1*M* KCl; the solution was placed directly in the steel cup of a 50-watt, 9-kc Raytheon magnetostriction generator and exposed to high-frequency sound without excluding oxygen for 120 minutes. The power output of the sonic oscillator was checked according to the procedure recommended by the manufacturer. Viscosity measurements were carried out at 20.0° ± 0.1°C (*9*). Protein concentrations were determined spectrophotometrically or by the Lowry procedure (*10*). In experiments carried out in a Model E Spinco centrifuge, control and treated samples were run simultaneously with wedge-window and normal cells under essentially identical conditions. The weight-average sedimentation coefficient $\bar{S}$ (*11*) was calculated from the rate of movement of the square root of the second moment of the entire gradient curve according to the method of Goldberg (*11*). Synthetic boundary experiments were carried out in a 12-mm cell with a 2° sector modified according to the method of Kegeles (*12*). Experimental procedures used in light scattering have been considered previously (*13*). A Rudolph automatic recording spectropolarimeter was used for optical-dispersion measurements.

Table 1. Comparison of bovine serum albumin exposed to 9-kc sound waves for 120 minutes with untreated bovine serum albumin. The results are averages of two to six experiments.

Physical parameters	Control	Treated
Intrinsic viscosity $[\eta]$ deciliters/gram	0.039	0.039
$(Kc/R_{90})_0$* $\times 10^5$	1.48	0.91
Weight-average molecular weight, M_w	68,000	110,000
Specific rotation, $[\alpha]_D$	—59.5	—56.0
Dispersion constant λ_c (mμ)	264	266
Mobility, $u \times 10^5$ cm^2 volt^{-1} sec^{-1}	—6.6	—6.6
$S^0_{20,w}$ from the second moment of the gradient curve	4.8	4.9
Refractive increment, $\frac{dn}{dc}$, in H_2O at 436 mμ; c in g/ml	0.1940	0.1940
Extinction coefficient $E^1\%_{208}$ deciliters/gram	6.45	6.45

* The limiting value of the scattering.

The results show (Table 1), despite the extensive exposure of the protein to sonic energy, a similarity in parameters determined from the treated and the nontreated samples. In electrophoretic patterns of sound-treated bovine serum albumin we observed a barely detectable shoulder moving more slowly than the main component. In synthetic-boundary studies, the differences in areas under the gradient curves obtained at 3000 rev/min and at 59,780 rev/min, after correction for radial dilution and the stretch of the rotor, reflect the removal from the boundary

region of rapidly sedimenting material. There was approximately 3 percent of such material in the treated sample. The shape of the curve from a graph of $\bar{S}$ plotted against total protein concentration depends on the distribution of particles according to size. If the distribution depends on concentration, there is an increasingly larger fraction of the lower molecular weight species at low concentrations. A linear relationship, a result incompatible with rapidly reversible interactions, existed. It should be emphasized that aggregates removed from the boundary region and which are not optically recorded are not included in the calculation of the weight-average sedimentation coefficient. Convincing qualitative evidence for highly aggregated material present in the treated bovine serum albumin but absent in the untreated was obtained by sedimentation in the ultracentrifuge with the "field relaxation method" (*14*).

Solutions of treated and untreated bovine serum albumin were dialyzed separately against equal volumes of 0.1*N* KCl in an attempt to demonstrate low molecular weight fragments resulting from treatment with high-frequency sound. The absorbency of the dialysates from the two solutions measured in the region of 250 to 280 mμ was identical. Peptides were not detectable in the dialysates by the Lowry procedure (*10*). The identical conductivity observed in solutions of treated and untreated bovine serum albumin provides further evidence for the absence of low molecular weight fragments When samples of treated and untreated albumin were centrifuged for 60 minutes at 105,000*g*, within the limits of sampling errors no difference in concentration distribution was observed, nor was there a detectable amount of sediment at the bottom of either tube.

Native bovine serum albumin is a compact relatively symmetrical molecule (*15*) containing either 17 or 18 disulfide groups (*16*). If no change in molecular weight is assumed, it is difficult to imagine any change in configuration which would substantially increase the capacity of the bovine serum albumin molecule to scatter light. The most plausible explanation for the effects of high-frequency sound appears to be aggregation. The protein forms aggregate in acid solution (*17*), on denaturation with urea (*18*), and on heating (*19*) owing to polymerization—one interpretation—by means of sulfhydryl-disulfide interchange. As ordinarily prepared, 68 percent of the bovine serum albumin molecules contain a free SH group (*16*). A second possibility, discussed by Waugh (*20*), is the reversible formation of fibrous aggregates. Since oxygen was not excluded, aggregation resulting from oxidation by free radicals formed during the sound treatment (*8*) provides a third plausible mechanism.

In a mixture of dimers and monomers, 62 percent of dimers would be required for a weight-average molecular weight of 110,000, a value inconsistent with the results from ultracentrifuge measurements. With a sample containing 59 percent dimers, Bro, Singer, and Sturtevant (*17*) observed a distinctly bimodal boundary. A polymer having an average weight of 1×10^6, comprising 2 to 4 percent of the treated sample, is consistent with our observations. A portion of the aggregated material was removed from the boundary region and was not optically recorded in sedimentation-velocity studies. Other procedures employed in studying the treated samples did not remove these larger aggregates from consideration, and the results reflect the presence of aggregates. The small shoulder, amounting to less than 5 percent of the total area, seen in the electrophoretic patterns would appear to represent some polymer formed during the exposure to high-frequency sound. Extensive fragmentation, even where reaggregation of fragments occurred, would be expected to result in distinct and more striking qualitative differences in ultracentrifugal and moving-boundary electrophoretic properties.

The changes in the optical rotatory properties of the treated albumin were slight enough to be considered within experimental error. The data shown in Table 1, however, are the average of a number of experiments. Because the specific rotation in all cases decreased and the dispersion constant (λ_c) was greater after treatment, we are led to conclude that the small changes observed were indeed real. Steinrauf and Dandliker (*19*) observed similar small changes in optical rotation, $[\alpha]_D$, during the polymerization of bovine serum albumin with heat.

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References and Notes

1. A. R. Peacocke and H. K. Schachman, *Biochim. Biophys. Acta* **15**, 198 (1954); H. S. Rosenkrantz and A. Bendich, *J. Am. Chem. Soc.* **82**, 3198 (1960); A. J. Hodge and F. O. Schmitt, *Proc. Natl. Acad. Sci. U.S.* **44**, 418 (1958).
2. P. Doty, B. H. McGill, S. A. Rice, *Proc. Natl. Acad. Sci. U.S.* **44**, 432 (1958).
3. T. Nishihara and P. Doty, *ibid.*, p. 411.
4. D. Freifelder and P. F. Davison, *Biophys. J.* **2**, 235 (1962).
5. K. Kanig and H. Künkel, *Z. Physiol. Chem.* **309**, 166 (1957).
6. G. Tolgyessy and P. Kovacs, *Magy. Kem. Folyoirat* **62**, 327 (1956); *Chem. Abstr.* **53**, 483 (1959).
7. W. B. Hugo, *Bacteriol Rev.* **18**, 87 (1954); E. L. Hess and H. D. Slade, *Biochim. Biophys. Acta* **16**, 346 (1955); R. Davies, *ibid.* **33**, 481 (1959).
8. D. E. Hughes and W. L. Nyborg, *Science* **138**, 108 (1962).
9. E. L. Hess and A. Coburе, *J. Gen. Physiol.* **33**, 511 (1950).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
11. R. Goldberg, *J. Phys. Chem.* **57**, 194 (1953).
12. G. Kegeles, *J. Am. Chem. Soc.* **74**, 5532 (1952).
13. I. Komine, H. D. Slade, E. L. Hess, *Arch. Biochem. Biophys.* **89**, 245 (1960).
14. G. Kegeles and C. L. Sia, *Federation Proc.* **22**, 290 (1963).
15. J. T. Edsall, in *The Proteins*, H. Neurath and K. Bailey, Eds. (Academic Press, New York, 1953), p. 721.
16. R. Cecil and J. R. McPhee, *Advan. Protein Chem.* **14**, 255 (1959).
17. P. Bro, S. J. Singer, J. M. Sturtevant, *J. Am. Chem. Soc.* **80**, 389 (1958).
18. W. Kauzmann and R. G. Douglas, *Arch. Biochem. Biophys.* **65**, 106 (1956).
19. L. K. Steinrauf and W. B. Dandliker, *J. Am. Chem. Soc.* **80**, 3833 (1958).
20. D. F. Waugh, *Advan. Protein Chem.* **9**, 388 (1954).
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Separation of Microsomal RNA into Five Bands during Agar Electrophoresis

Abstract. *Microsomal RNA from rabbit livers and lymph nodes separate into five major bands during agar-gel electrophoresis. The electrophoretic method may be used either as an analytical or preparative tool. The 33S and 19S peaks of microsomal RNA from sucrose-gradient zone centrifugation divide into two bands each during simple agar electrophoresis.*

The simple technique of electrophoresis in agar devised by Gordon (*1*) has served to identify, separate, and prepare many substances from complex mixtures. Although Uriel *et al.* (*2*) showed RNA electrophoresis in agar and that RNA could be identified by pyronine staining, Bachvaroff, Yomtov, and Nikolov (*3*) first applied the method to the separation of RNA into