STABILITY OF RIBOSOMES FROM STREPTOMYCIN-EXPOSED ESCHERICHIA COLI

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Exposure of Escherichia coli to streptomycin resulted in a marked stabilization of their ribosomes to heat; ribosomes of streptomycin-resistant Escherichia coli were only stabilized slightly.

Phenotypic expression of bacterial sensitivity or resistance to streptomycin is manifested by differences in physical properties of ribosomes. When E. coli K 12 was exposed to streptomycin and the ribosomes were prepared from these bacteria, the particles were "stuck," i.e., they resisted dissociation into their 30 s and 50 s subunits upon lowering the concentration of Mg^{2+} in suspending media; ribosomes from a streptomycin-resistant mutant of K 12 did not become "stuck" (Herzog, 1964). Moreover, Leon and Brock (1967) recorded small differences between T_ms of E. coli ribosome preparations to which streptomycin was supplied in vitro when these ribosomes had been prepared from streptomycin-sensitive or -resistant bacteria.

Recording hyperchromicity at 260 mμ as a function of time of exposure of ribosomes to 52°C affords a measure of the relative stability of such ribosomes (Wolfe & Hahn, 1968). We report that ribosomes prepared from streptomycin-sensitive E. coli C-2 which had been exposed to streptomycin were more heat-stable at 52°C than were ribosomes from streptomycin-free control cultures (Fig. 1). Ribosomes from a streptomycin-resistant mutant of E. coli C-2 were intrinsically more stable than ribosomes from the sensitive parent strain; exposure of the mutant bacteria to streptomycin

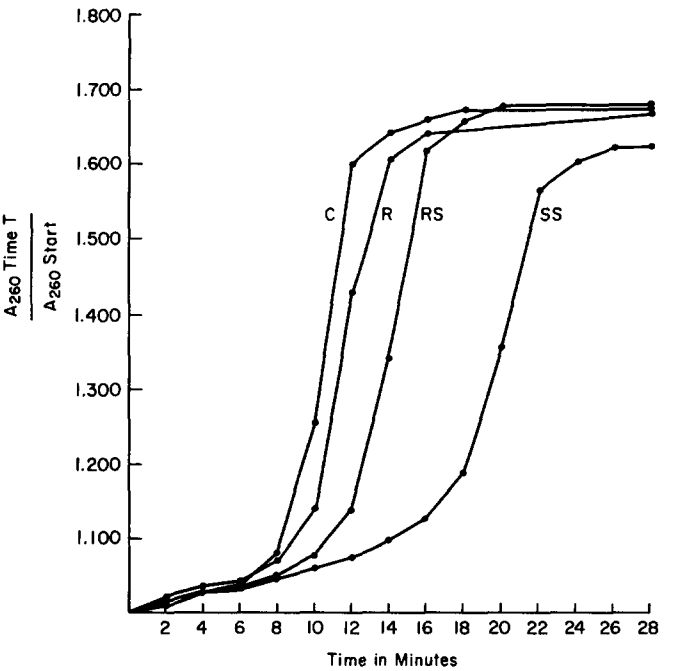

Fig. 1. Hyperchromic shifts (260 mμ) of E. coli ribosomes at 52° as a function of time.

Ribosomes were obtained from: C, streptomycin-sensitive E. coli C-2; R, a streptomycin-resistant mutant of E. coli C-2 (selected after ethyl methane sulfonate mutagenesis); RS, resistant E. coli C-2 which had been exposed to 40 μg/ml of streptomycin for 60 min during exponential growth; SS, sensitive E. coli C-2 equally exposed to streptomycin. Bacteria were grown in a mineral salts glucose medium containing 20 μg/ml of L-phenylalanine. Ribosomes were prepared by standard methods and hyperchromicities recorded in a Gilford Model 2000 instrument, using 3×10^{-2}M Tris buffer at pH 7.2, containing 4.6×10^{-4}M Mg^{2+} and 6.0×10^{-2}M K^{+} (Wolfe & Hahn, 1968). Ribosomes were micropipetted into preheated buffer solutions.

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only produced a slight additional stabilization of their ribosomes (Fig. 1).

Withholding phenylalanine from cultures of E. coli C-2, a phenylalanine auxotroph, did not abolish the streptomycin-induced stabilization of ribosomes (Fig. 2). The degree of stabilization was proportional to the concentration of streptomycin supplied to phenylalanine-deprived cultures (Fig. 3). In such cultures, the loss of viability and the extent of ribosome stabilization both were functions of the streptomycin concentration.

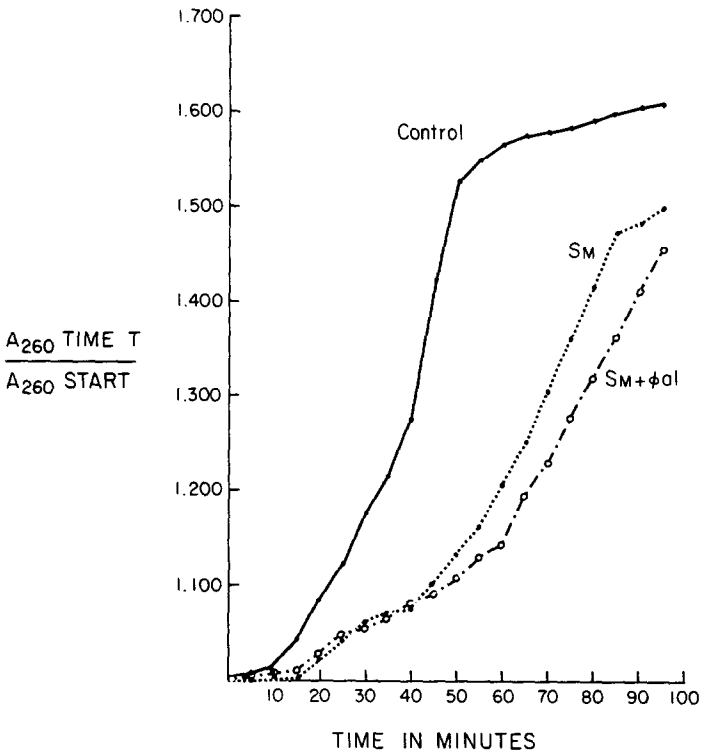

Fig. 2. Hyperchromic shifts (260 mμ) at 52° of ribosomes obtained from E. coli C-2 (a phenylalanine auxotroph) after exposure to 40 μg/ml of streptomycin in the presence (20 μg/ml) or absence of phenylalanine.

Bacteria were grown in the medium described under Fig. 1, collected, washed, resuspended in fresh medium without phenylalanine and incubated with forced aeration for 90 min. One aliquot of this suspension was maintained as control; a second aliquot, Sm, received streptomycin and both aliquots were further incubated for 4 hours; a third aliquot received streptomycin as well as phenylalanine, Sm+φal, and was incubated for one hour. Different incubation periods were employed in order to obtain an identical decline in the number of viable cells in each culture. Ribosomes were then prepared and hyperchromicities recorded as described above, using 3.0×10^{-2}M Tris buffer at pH 7.2, containing 1.3×10^{-3}M Mg^{2+} and 6.0×10^{-2}M K^{+}.

Our experiments and results on stabilization of ribosomes differ from those of Leon and Brock (1967) in that: (1) We studied ribosomes obtained from streptomycin-exposed bacteria while Leon and Brock supplied streptomycin to ribosome suspensions in vitro. (2) The quantities of free streptomycin carried over into our experimental ribosome suspensions are estimated to have been $<10^{-3}$ μg/ml while Leon and Brock were forced to

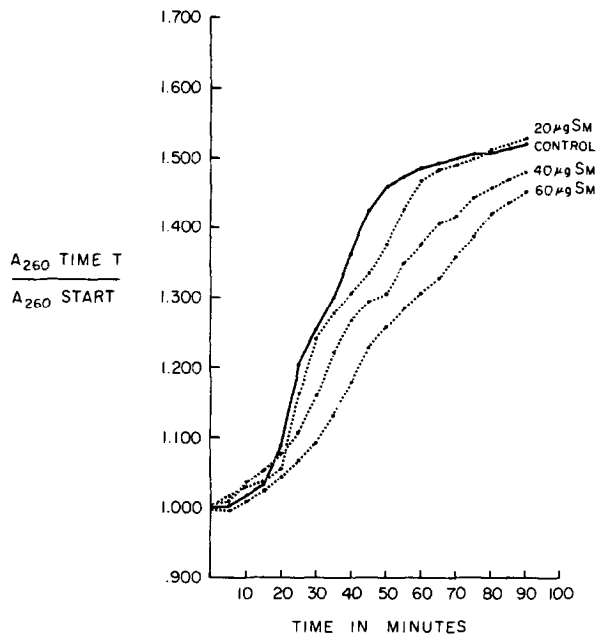

Fig. 3. Hyperchromic shifts (260 mμ) of E. coli C-2 ribosomes at 52° obtained from phenylalanine-free cultures that had been exposed for 4 hours to concentrations of streptomycin indicated in the Figure.
Ribosomes were prepared and hyperchromicities recorded as above, using 3.0×10^{-2}M Tris buffer at pH 7.2, containing 7.0×10^{-4}M Mg^{2+} and 6.0×10^{-2}M K^{+}.

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supply at least 100 μg/ml of streptomycin in order to demonstrate increased heat stability of ribosomes. (3) The streptomycin-induced stabilization of ribosomes which we report is indicated by marked differences between the time-O.D. (260 mμ) profiles of samples from controls and from streptomycin-exposed cultures. The melting curves and intrapolated T_m values of Leon and Brock, on the other hand, only indicate small differences between the responses of ribosomes to added streptomycin and similarly small differences between the values for ribosomal control preparations from sensitive or resistant bacteria.

We interpret our present findings, as well as Herzog's (1964) observation of "stuck" ribosomes, both consequential to exposing sensi-

tive E. coli to streptomycin, as results of physiological or biochemical events which are caused by the antibiotic in vivo and increase the physical stability of ribosomes. Recent in vitro experiments of Kaji and Tanaka (1968) indicate the formation of ribosome-streptomycin-messenger RNA complexes. If such complexes were also formed in streptomycin-exposed bacteria, it would explain observations of lethal RNA synthesis in such bacteria (Stern et al., 1966), as well as the in vivo stabilization of ribosomes reported here and by Herzog (1964).

It is worthy of consideration that stabilized and "stuck" ribosomes are incapacitated and can no longer participate in a ribosome cycle in vivo necessary for sustained protein biosynthesis (Schlessinger et al., 1967) according to which 70 s ribosomes must dissociate into their 30 s and 50 s subunits after completion of the biosynthesis of a protein molecule and return to a reservoir of free subunits from which new reactive complexes are assembled. Streptomycin-exposed E. coli is progressively incapacitated to synthesize protein (Hahn et al., 1962; Stern et al., 1966).

REFERENCES

Hahn, F. E., Ciak, J., Wolfe, A. D., Hartman, R. E., Allison, J. L. & Hartman, R. S., Biochim. Biophys. Acta 61, 741 (1962).
Herzog, A., Biochem. Biophys. Res. Comm. 15, 172 (1964).
Kaji, H. & Tanaka, R., Fed. Proc. 27, 459 (1968).
Leon, S. A. & Brock, T. D., J. Mol. Biol. 24, 391 (1967).
Schlessinger, D., Mangiarotti, G. & Apirion, D., Proc. Nat. Acad. Sci. U.S. 58, 1782 (1967).
Stern, J. L., Barner, H. D. & Cohen, S. S., J. Mol. Biol. 17, 188 (1966).
Wolfe, A. D. & Hahn, F. E., In preparation (1968).