Effects of Dihydrostreptomycin on Bacterial Ribosomes *in vivo*

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Herzog (1964) reported that ribosome preparations of *Escherichia coli* that had been grown in the presence of dihydrostreptomycin dissociated less readily in 1 mM-Mg²⁺ than did controls or preparations from streptomycin-resistant cultures treated with dihydrostreptomycin. This 'sticking effect', which was not observed in preparations from sensitive cultures to which the antibiotic was added at harvesting, seemed to indicate an effect on the physical state of ribosomes from sensitive organisms brought about by intracellular streptomycin. We thought that this effect could be utilized for the isolation of those ribosomes that had been altered and inactivated during treatment with the antibiotic *in vivo*, and therefore attempted to establish a quantitative relationship between the extent of this 'sticking effect' and the extent of growth inhibition. However, following the procedures that Herzog (1964) used, we found that the dissociation of 70S ribosomes in 1 mM-Mg²⁺ was a time-dependent process, and that demonstration of the 'sticking effect' in similar preparations from streptomycin-treated cultures was also time-dependent; i.e. these ribosomes dissociated more slowly than did controls.

The development of lytic methods for rapid release of cell extracts containing polyribosomes as well as 70S ribosomes and 'native' 50S and 30S subunits (Godson & Sinsheimer, 1967; Kohler, Ron & Davis, 1968; Friesen, 1968), and the demonstration that 70S particles derived from polyribosomes showed different sedimentation behaviour in 2 mM-Mg²⁺ from that of those produced by run-off conditions *in vivo* (Ron, Kohler & Davis, 1968), prompted us to re-examine Herzog's (1964) 'sticking effect'.

Using the method of Godson & Sinsheimer (1967), we prepared joint extracts of exponentially growing unlabelled control cultures (nine-tenths by dry weight) and cultures labelled with [¹⁴C]uracil for two generations before addition of 50 µg of dihydrostreptomycin/ml (one-tenth by dry weight), harvested together at the time when growth of the streptomycin-treated culture had just ceased (90–100% inhibition of growth rate; Kogut, Lightbown & Isaacson, 1965; Kogut & Harris, 1969). In this way we could compare directly the effects of treatment during growth and the effects of harvesting conditions. Addition of dihydro-

streptomycin during aerobic exponential growth leads to gradual diminution of heavier polyribosomes and their eventual accumulation as 70S particles (Kogut, 1969; Luzzatto, Apirion & Schlessinger, 1969) when growth ceases. According to Godson & Sinsheimer (1967), under the conditions used by Herzog (1964) loss of polyribosomes would occur during harvesting. Sucrose-density-gradient analysis of portions of the same extract after centrifugation in 10 mM-Mg²⁺ and in 2 mM-Mg²⁺, after brief treatment with ribonuclease (Ron *et al.* 1968), allows comparison of the original ribosome distribution between polyribosomes and 70S particles with their dissociation into 50S and 30S subunits in 2 mM-Mg²⁺.

Fig. 1 displays these ribosome patterns for two such joint extracts, i.e. control and streptomycin-treated, prepared from the same cultures. Fig. 1(a) shows the behaviour of an extract when the cultures were harvested under the conditions described by Herzog (1964), and Fig. 1(b) shows the behaviour of an extract from the same cultures, harvested 2 min later, under conditions that preserve polyribosomes, i.e. rapid cooling to 4°C (Godson & Sinsheimer, 1967). In the former the ribosomes from both control and streptomycin-treated cultures sedimented largely as 70S particles in 10 mM-Mg²⁺, whereas in 2 mM-Mg²⁺ the control behaved like run-off ribosomes, i.e. was mostly dissociated into 50S and 30S subunits. The extract prepared under the conditions of Godson & Sinsheimer (1967) gave a centrifugation profile in 10 mM-Mg²⁺ that shows loss of polyribosomes and accumulation of 70S particles in the fraction from the streptomycin-treated culture, but considerable presence of polyribosomes in the control portion, whereas in 2 mM-Mg²⁺ the fraction from the antibiotic-treated culture dissociated more than did the control.

Observation of a 'sticking effect' in ribosome preparations from streptomycin-treated cultures therefore seems to depend on the method of harvesting and preparing ribosomal extracts applied to the control as well as to the treated cultures. Only where conditions of growth or harvesting lead to 'run-off' in control cultures can a sticking effect be demonstrated. On the other hand, where no run-off conditions are deliberately imposed during harvesting the streptomycin-treated cultures apparently do contain some run-off 70S

Fig. 1. Effect of dihydrostreptomycin treatment during growth and of harvesting conditions on the distribution and behaviour of intracellular ribosomes. Exponential-phase culture of *Escherichia coli* C600 in mineral salts-glucose-casamino acids (casein hydrolysate) medium was divided in two. One was treated with dihydrostreptomycin (50 $\mu\text{g/ml}$) after two generations of growth in [^{14}C]uracil (0.0086 $\mu\text{Ci}/\mu\text{g}$; 15 $\mu\text{g/ml}$), and the other was grown with 15 μg of unlabelled uracil/ml. Approximately nine-tenths of the unlabelled control and one-tenth of the labelled culture (total 6 mg dry wt.) were rapidly mixed and harvested at the time when the growth rate of the treated culture had declined to less than 10% of the control value. Crude extracts of the mixed cultures, prepared as described by Godson & Sinsheimer (1967), were divided. One portion of each was layered on 4.0 ml of a linear 15–30% (w/v) sucrose gradient above a 0.5 ml 50% (w/v) sucrose 'cushion', all in medium containing 10 mM-tris-HCl buffer, pH 7.3, 10 mM- MgSO_4 and 6 mM-KCl, and centrifuged immediately for 45 min at 50 000 rev./min in the SW 50 rotor of a Beckman Spinco L2 centrifuge at 5°C. Another portion of each extract was treated for 5 min with ribonuclease (0.1 $\mu\text{g/ml}$) and layered on similar sucrose gradients in medium containing 2 mM- MgSO_4 instead of 10 mM- MgSO_4 and centrifuged for 2 h under the same conditions. Gradients were fractionated by piercing the bottom of each tube and collecting 6-drop fractions in parallel sets of tubes; the middle 2 drops of each fraction were taken for measurement of E_{260} in a spectrophotometer; the other 4 drops were treated with 10% (w/v) trichloroacetic acid after the addition of 200 μg of carrier RNA/fraction, and the precipitate was washed and its radioactivity counted in a Nuclear-Chicago end-window gas-flow counter. (a) Cultures harvested as described by Herzog (1964); (b) cultures harvested as described by Godson & Sinsheimer (1967). (i) Profile after 45 min spin in 10 mM- Mg^{2+} ; (ii) profile after 2 h spin in 2 mM- Mg^{2+} . —, E_{260} (representing unlabelled controls); $\triangle-\triangle$, c.p.m./fraction (representing labelled treated cultures).

monoribosomes, as judged by their dissociation in 2 mM- Mg^{2+} .

According to the model of streptomycin action proposed by Luzzatto, Apirion & Schlessinger (1968, 1969), i.e. that the antibiotic inhibits at the initiation stage and produces 'stuck initiation complexes', no run-off 70S ribosomes should appear

in streptomycin-treated cultures when growth inhibition is complete. This does not seem to be the case. In addition, earlier studies on the gradual diminution of polyribosomes and accumulation of 70S particles in relation to inhibition of growth by dihydrostreptomycin (Kogut, 1969) had already led to the conclusion that the effect of this anti-

biotic on ribosomes *in vivo* (Dubin, 1964; Engelberg & Artman, 1964; Kogut & Harris, 1969) results in slowing, but not in complete inhibition, of translational movement subsequent to initiation. This is also in agreement with the model proposed by Modolell & Davis (1969) on the basis of their studies *in vitro*.

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Dubin, D. T. (1964). *J. molec. Biol.* **8**, 749.

Engelberg, H. & Artman, M. (1964). *Biochim. biophys. Acta*, **80**, 256.

Friesen, J. D. (1968). *J. molec. Biol.* **32**, 183.

Godson, N. & Sinsheimer, R. L. (1967). *Biochim. biophys. Acta*, **149**, 476.

Herzog, A. (1964). *Biochem. biophys. Res. Commun.* **15**, 172.

Kogut, M. (1969). *J. gen. Microbiol.* **57**, 25.

Kogut, M. & Harris, M. (1969). *Eur. J. Biochem.* **9**, 42.

Kogut, M., Lightbown, J. W. & Isaacson, P. (1965). *J. gen. Microbiol.* **39**, 165.

Kohler, R., Ron, E. & Davis, B. D. (1968). *J. molec. Biol.* **36**, 71.

Luzzatto, L., Apirion, D. & Schlessinger, D. (1968). *Proc. natn. Acad. Sci. U.S.A.* **60**, 873.

Luzzatto, L., Apirion, D. & Schlessinger, D. (1969). *J. molec. Biol.* **42**, 315.

Modolell, J. & Davis, B. D. (1969). *Nature, Lond.*, **224**, 345.

Ron, E., Kohler, R. & Davis, B. D. (1968). *J. molec. Biol.* **36**, 83.