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Reevaluation of the Mode of Action of Streptolydigin in *Escherichia coli*: Induction of Transcription Termination In Vivo

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Growth of the permeable strain AS19 of *Escherichia coli* B is more sensitive to the antibiotic streptolydigin than is in vitro ribonucleic acid (RNA) synthesis. The in vivo chain elongation rates of *lacZ* messenger RNA and ribosomal RNA are not affected at 1.5×10^{-6} M, a concentration that reduces the growth rate threefold. The synthesis of large proteins is inhibited preferentially, and a considerable fraction of the polypeptides synthesized is unstable. The synthesis of complete β-galactosidase is inhibited relative to the synthesis of short, unstable polypeptides, which include the first 60 to 70 amino acids of β-galactosidase. The expression of the following polycistronic transcription units is strongly biased against promoter-distal genes: *trp, deo, rpoBC,* and *rrn*. The extent of polarity is proportional to the distance transcribed and to the streptolydigin concentration. Streptolydigin appears to destabilize active transcription complexes irreversibly irrespective of the type of transcript (messenger RNA, ribosomal RNA) and of transcription intensity. We suggest that streptolydigin leads to premature termination of transcription, resulting in release of incomplete transcripts and, thus, a decrease in overall messenger RNA concentration, which becomes limiting for protein synthesis, i.e., for growth.

The antibiotic streptolydigin interferes with bacterial ribonucleic acid (RNA) synthesis in vivo and in vitro (32, 34). Mutations to streptolydigin resistance are located in the β subunit of the RNA polymerase (13, 16, 32). Together with biochemical evidence (5, 34) this indicates that streptolydigin inhibits RNA synthesis by interaction with the RNA polymerase rather than by binding to the template as does actinomycin D (27). In contrast to rifampin, which acts on initiation of transcription (36, 41), streptolydigin has been shown to interfere with RNA chain elongation (5). Cassani et al. (5) also showed stabilization of the RNA-enzyme-deoxyribonucleic acid (DNA) complex in vitro when using purified RNA polymerase and T4 DNA; the inhibition of RNA chain elongation is reversible in vitro (34).

In attempts to apply these in vitro results in vivo, we realized that the effects of streptlydigin are more complex. Streptolydigin indeed blocks RNA chain elongation in vivo (29, 34); however—in striking contrast to the in vitro results (5)—active transcription complex (RNA-polymerase-DNA-nascent RNA) is not stabilized and reversibly inhibited but is irreversibly destabilized in vivo. At concentrations that partially inhibit growth, the drug induces a general polarity in gene expression irrespective of the transcript (messenger RNA [mRNA], ribosomal RNA [rRNA]) but has very little effect on RNA chain elongation rates. This type of polarity is apparently the result of drug-induced premature termination of transcription. The mechanism of action of streptolydigin, as well as the implications for its use in vivo, is discussed.

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MATERIALS AND METHODS

Bacterial strains. The strains used were as follows: AS19, *Escherichia coli* B carrying mutations that confer increased permeability to a large number of antibiotics (33), including rifampin (30) and streptolydigin (29); NF541, *E. coli* B AS19 *leu pyrB fuc*$^+$ (12); NF830, *E. coli* B AS19 *leu lacL37 lacUV5* (the double mutation in the *lac* promoter [34] rendering the *lac* operon insensitive to catabolite repression was

introduced into strain CM14 [*E. coli* B AS19 *leu lac*] by transduction with P1*vir* grown on strain CA8050 [*E. coli* K-12 HfrH *thi lacL37 lacUV5* obtained from John Scaife]); CM279, *E. coli* B AS19 *leu pyrB thyA deoB trpR* (the *trpR* mutation yielding constitutive expression of the *trp* operon was introduced into strain CM98 [*E. coli* B AS19 *leu pyrB thyA deoB*] by transduction with P1*vir* grown on strain PA2RTA [*E. coli* K-12 F⁻ *thi gal lacY tonA trpR argR strA* obtained from T. R. Lavallé], by selection for *deoB*⁺; *deoB* was reintroduced by selection for low-thymine requirement).

Growth conditions. Cells were grown at 37°C in A+B phosphate minimal medium (6) supplemented with 0.2% glucose and growth requirements at 10 to 20 μg/ml. Growth was measured with a PMQII Zeiss spectrophotometer as absorbance at 450 nm. Cultures of *leu* strains were always supplemented with leucine and isoleucine to obtain growth rates similar to those of wild-type cultures.

Radioactive labeling. (i) Stability of newly synthesized polypeptides. Stability of newly synthesized polypeptides was determined by pulse-labeling a culture, prelabeled with [^{14}C]leucine, with [^{3}H] leucine for 1 min followed by a chase with excess nonradioactive leucine, during which the loss of trichloroacetic acid-precipitable radioactivity was determined (for details see Fig. 7 in reference 11). The amount and half-life of unstable polypeptides were computed from the incorporation curve.

(ii) Size distribution of polypeptides and synthesis rate of the β and β′ subunits of RNA polymerase. Polypeptide distribution and subunit synthesis rates were determined by pulse-labeling cultures with [^{3}H]lysine (10 μCi/μg; 0.3 μg/ml) for 1 or 2 min. After a chase with 500 μg of lysine per ml, the cells were lysed in sodium dodecyl sulfate and mixed with appropriate amounts of a lysate of [^{14}C]lysine long-term-labeled control cells, and the polypeptides were separated on 12% (for size distribution) or 5% (for β and β′ analysis) sodium dodecyl sulfate-polyacrylamide gels. For details of gel electrophoresis and determination of radioactivity, see Johnsen et al. (18).

(iii) Transcription time of the *rrn* transcription units. The transcription time of the *rrn* transcription units was determined by labeling with [^{3}H]adenine after rifampin addition and by analysis of the radioactivity in 5S rRNA by gel electrophoresis, as described by Molin (22) and Molin et al. (23).

(iv) Synthesis of 16S and 23S rRNA. Synthesis of 16S and 23S rRNA was determined by labeling 1-ml portions of cultures of strain NF541 with 1 μCi of [^{14}C]adenine (2 μCi/μg) for 4 to 10 min and extracting the radioactive RNA. After separation by electrophoresis, the bands of the various RNA species were localized by autoradiography, and the radioactivity was determined by scintillation counting (23).

(v) Residual RNA and protein syntheses. Residual RNA and protein syntheses after rifampin or streptolydigin addition were determined, as described by Pato and von Meyenburg (30) and Pato et al. (29), by labeling with [^{3}H]uridine and [^{14}C]proline and determining incorporation into trichloroacetic acid-precipitable material.

Enzyme activities. (i) Complete β-galactosidase and N-terminal auto-α fragment of β-galactosidase. Complete β-galactosidase and the N-terminal auto-α fragment of β-galactosidase (23) were determined as described earlier (11), although with some modifications (18). For induction, isopropyl-β-D-thiogalactoside was added at 1 mM.

(ii) *trp* operon enzymes. The *trp* operon enzymes were determined in lysates of the *trpR* strain KM279. Cells were harvested at intervals of half a doubling time, washed, and suspended in 0.1 M potassium phosphate buffer (pH 7.6). The cells were permeabilized by adding 0.05% Triton X-100 and freezing (G. Miozzari, unpublished data). The cell suspensions were adjusted to 2 to 4 mg of protein per ml. Protein was determined by the biuret method (14).

Determination of glutamine-dependent anthranilate synthetase (*trpE*) was by the method of Egan and Gibson (8). Fluorescence of the anthranilic acid formed was measured with an Aminco-Bowman fluorimeter at 400 nm (excitation at 338 nm). Indole 3-glycerolphosphate synthesis (*trpC*) was determined by the method of Wegmann and DeMoss (40). The tryptophan synthetase B (*trpB*) assay was as described by Tsai and Suskind (39).

(iii) Deoxyriboaldolase (*deoC*), thymidine phosphorylase (*deoA*), deoxyribomutase (*deoB*), and purine nucleoside phosphorylase (*deoD*). The four enzymes coded for by the *deo* operon (28, 38) (deoxyriboaldolase, thymidine phosphorylase, deoxyribomutase, and purine nucleoside phosphorylase) were assayed by the method of Munch-Petersen et al. (28). Cells were grown in the presence of thymidine (4 mM) to induce expression of the operon.

Antibiotics. Rifampin (Na salt) was a gift from Ciba-Geigy A/S, Copenhagen; streptolydigin was from J. Whitfield, the Upjohn Co., Kalamazoo, Mich.; and sodium nalidixate was from Winthrop Laboratories, Newcastle Upon Tyne, England. Chloramphenicol was purchased from Parke, Davis & Co., London. Stock solutions (1 to 4 mg/ml) of the antibiotics were prepared in water; for streptolydigin, the pH was adjusted to 8.0 with NaOH.

RESULTS

Growth in presence of low concentrations of streptolydigin. Addition of 25 μg of streptolydigin per ml to cultures of the permeable strain AS19 or its derivatives in glucose minimal medium resulted in an immediate 25% reduction of the growth rate, measured as optical density (Fig. 1); at higher concentrations, the inhibition was greater. The inhibition was immediate at all concentrations up to 5 μg/ml and did not change for at least 3 doublings of mass (Fig. 1). Similar effects on growth rate were observed after addition of streptolydigin to cultures growing in enriched media. In all cases the mass increase was exponential for several doubling times. A 50% inhibition of the growth rate occurred at a streptolydigin concentration of 0.7 to 1.0 μg/ml, i.e., 1.0×10^{-6} to 1.5×10^{-6} M.

RNA chain growth rates during partial

inhibition of growth by streptolydigin. The rate of chain elongation was determined (i) for mRNA from the *lacZ* gene and (ii) for rRNA.

(i) Strain NF830, harboring the catabolite repression-insensitive alleles *lacL37* and *lacUV5*, was challenged with 3 μg of streptolydigin per ml during growth in glucose minimal medium. After 1 doubling of mass (120 min), isopropyl-β-D-thiogalactoside was added to induce expression of the *lac* operon. To determine the transcription time of the *lacZ* gene, the approach described by Pato et al. (29) was used. Except for the antibiotics used it is identical to that of Leive and Kollin (20) and Jacquet and Kepes (17). Portions of the induced culture were placed in tubes containing either rifampin, to block further initiation of transcription, or a high concentration of streptolydigin (100 μg/ml), to block RNA chain elongation. The samples were incubated for 20 min at 37°C to allow completion and expression of nascent and finished *lacZ* mRNA or expression of finished *lacZ* mRNA alone.

The time difference between the appearance of β-galactosidase activity in the samples with rifampin and the samples with streptolydigin corresponds to the transcription time for the *lacZ* portion of the *lac* operon; in exponentially growing *E. coli*, values of 65 to 75 S were found (Fig. 2A; 11, 29), corresponding to an RNA chain growth rate of ca. 50 nucleotides per s. For the streptolydigin-inhibited culture, the value was 75 to 80 s (Fig. 2B), only slightly longer than that of the control. Also, the kinetics of appearance of active β-galactosidase (measured in samples placed in chloramphenicol) were practically identical in the two cases; however, the rate of β-galactosidase synthesis was ca. sixfold lower in the inhibited curve.

(ii) The transcription time of the rRNA transcriptional unit was determined as described previously (22). The time at which 5S rRNA synthesis ceased after transcriptional initiation was blocked by rifampin was unchanged in the culture growing in presence of 3 μg of streptolydigin per ml as compared with the uninhibited control (Fig. 3). The transcription of the ca. 5,500-base-pair-long 16S-23S-5S rRNA units took 70 to 80 s, corresponding to a chain growth rate of 75 to 80 nucleotides per s (22, 23).

Polarity within the *lacZ* gene. Synthesis of the N-terminal part of the β-galactosidase polypeptide chain, the auto-α peptide fragment (60 to 70 amino acids), and of complete β-galactosidase (1,021 amino acids [10]) was measured in strain NF830 after inducton by isopropyl-β-D-thiogalactoside. The kinetics of accumulation of the two activities were identical in the control culture (Fig. 4A), except that the appearance of complete β-galactosidase was delayed 70 s. In Fig. 4B the kinetics are shown for cells growing in the presence of 3 μg of streptolydigin per ml. Complete β-galactosidase molecules started accumulating after a lag of 90 s, as in the control culture (Fig. 4A). A final accumulation rate of only 15% that of the uninhibited control was reached (see also Fig. 2A and B). However, auto-α activity accumulated as fast as in the control during the initial phase (between 0 and 2 min). At 1 μg of streptolydigin per ml, a similar, though less pronounced, discrepancy was found. The ratio between the initial rate of auto-α and β-galactosidase accumulation was ca. 1.7 at 1 μg of streptolydigin per ml and 6 at 3 μg/ml. Thus, the

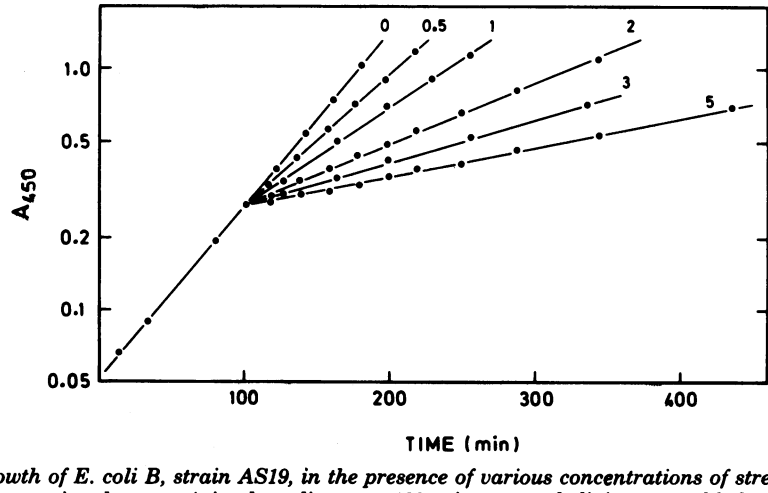

FIG. 1. *Growth of E. coli B, strain AS19, in the presence of various concentrations of streptolydigin. The strain was grown in glucose minimal medium; at 100 min, streptolydigin was added at the indicated concentrations (micrograms per milliliter). A_{450}, Absorbance at 450 nm.*

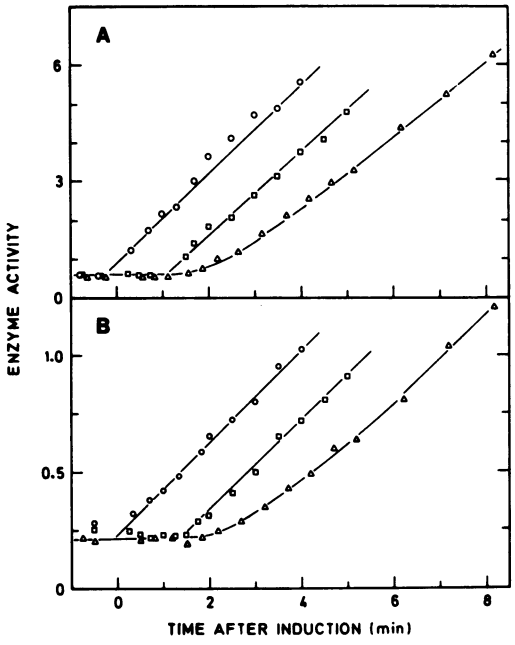

FIG. 2. *Initiation and termination of lacZ mRNA synthesis and synthesis of β-galactosidase. The lacL37 lacUV5 strain NF830 was grown in glucose minimal medium without (A) or with 3 μg of streptolydigin per ml (B); growth rates: 1.35 and 0.47 doublings per h. At time zero (absorbance at 450 nm [A_{450}]) = 0.500) isopropyl-β-D-thiogalactoside was added (1 mM); before and after induction, 0.5-ml portions were transferred to tubes containing rifampin (○), streptolydigin (□), or chloramphenicol (△) (final concentrations, 100 μg/ml), and incubated for 20 min at 37°C before determination of the β-galactosidase activity. Unit of enzyme activity: $E_{420}/60$ min, for a 1-ml culture at A_{450} = 1.000.*

presence of streptolydigin causes a drastic discrepancy between the rates of initiation and of completion of β-galactosidase, i.e., intracistronic polarity (11). The apparently unaffected rate of auto-α synthesis indicated that the drug did not affect initiation of transcription.

The decrease of the rate of auto-α accumulation (Fig. 2B) indicated that the excess auto-α activity was metabolically unstable. On the basis of the data in Fig. 4B, a half-life of ca. 1 min was calculated by the method of Hansen et al. (11). During exponential growth in glucose minimal medium, 0 to 3% of the polypeptides synthesized at any time were metabolically unstable. When streptolydigin was present, the unstable fraction increased with increasing concentrations of the drug (8 and 15% at 1 and 3 μg of streptolydigin per ml, respectively). The unstable fraction decays with half-lives of 1 to 2 min (11), which agrees well with the half-life of the excess auto-α estimated above.

Size distribution of newly synthesized polypeptides. In exponentially growing cells, the rate of synthesis of any polypeptide corresponds to its rate of accumulation, neglecting the small amount of protein turnover (see above). This can be demonstrated by electrophoretic separation (on sodium dodecyl sulfate-polyacrylamide gels) of the polypeptides in a mixture of cells, part of them long-term-labeled with [^{14}C]lysine and chased and the other part pulse-labeled with [^{3}H]lysine (2 min) and chased for 1 min. When the labelings were performed under identical conditions of balanced, exponential growth, the $^{3}H/^{14}C$ ratios were identical for all sizes of polypeptides. However, when the polypeptides were pulse-labeled with [^{3}H]lysine during growth in the presence of streptolydigin, comparison with the ^{14}C-labeled control on the electropherogram revealed changes in the rate of synthesis of polypeptides of different sizes ($^{3}H/^{14}C$ ratio; Fig. 5).

At all streptolydigin concentrations (between 1 and 10 μg/ml; data for 5 μg/ml shown in Fig. 5), the rate of synthesis decreased with increasing size of the polypeptide. Over a molecular weight range of 10,000 to 150,000, the $^{3}H/^{14}C$ ratio fell approximately twofold at 1 μg/ml, four- to fivefold at 3 μg/ml, seven- to eightfold at 5 μg/ml (Fig. 5), and at least tenfold at 10 μg/ml. Although the decrease in the synthesis rate of polypeptides with increasing length was not smooth, the general trend indicates that strep-

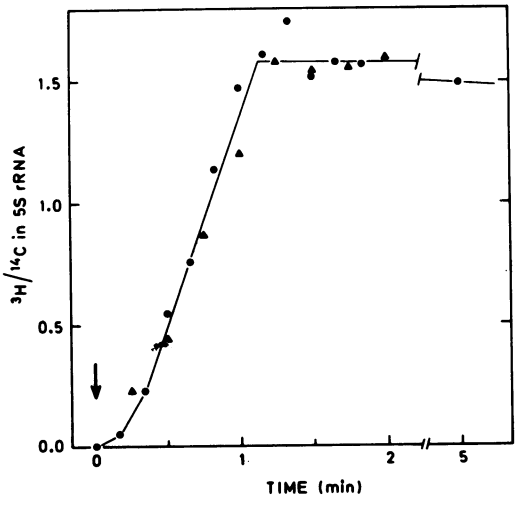

FIG. 3. *Residual synthesis of 5S rRNA (22, 23) after inhibition of transcription initiation by rifampin. Cultures of strain NF541 growing in glucose minimal medium in the absence (▲) or presence (●) of streptolydigin (3 μg/ml) were labeled with [^{3}H] adenine at time zero (A_{450} = 0.400); rifampin (100 μg/ml) was added simultaneously.*

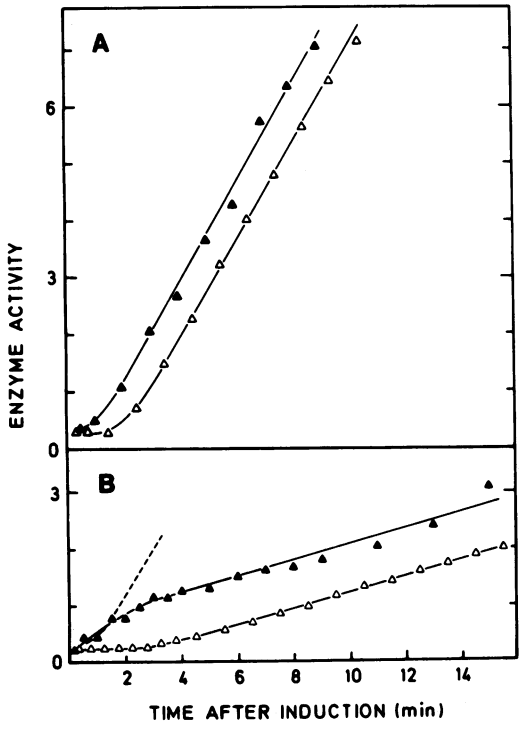

FIG. 4. *Induction kinetics of auto-α and β-galactosidase. Growth and enzyme units are as described in the legend to Fig. 2. (A) Control culture (1.33 doublings per h); (B) 30 min after addition of streptolydigin at 3 μg/ml (0.54 doublings per h). Induction with isopropyl-β-D-thiogalactoside (1 mM) was at time zero. Symbols: ▲, auto-α activity; △, β-galactosidase activity. The dashed line in (B) represents the induction kinetics of auto-α in the control culture (A).*

tolydigin creates a strong bias against the synthesis of longer polypeptide chains. Together with the observed instability of newly synthesized polypeptides it indicates the induction of a general polarity of gene expression.

Expression of individual genes in polycistronic operons. The synthesis rates of the β and β′ subunits of RNA polymerase (molecular weights, 155,000 and 165,000, respectively), for which the genes (*rpoB* and *rpoC*) are linked in a transcriptional unit (9), were determined by radioactive labeling and separation of the subunits by electrophoresis. The differential rate of the β′ subunit synthesis, the product of the promoter-distal gene, decreased strongly with increasing streptolydigin concentration (Table 1), in contrast to a modest effect on β-subunit synthesis. Degrees of polarity between the *rpoB* (β) and *rpoC* (β′) genes of 1.6, 2.2, and 3.0 were found at 0.5, 1, and 2 μg of streptolydigin per ml, respectively.

Streptolydigin also affects the expression of the *trpC* and *trpB* genes of the *trp* operon (15) more strongly than the expression of the promoter-proximal *trpE* gene (Table 1). At 5 μg of streptolydigin per ml, the relative ratio *trpE/trpC* was 2 and the ratio *trpE/trpB* was 2.5 (compared with a relative ratio of 1.0 in the absence of streptolydigin), reflecting the degree of polarity within the *trp* operon induced by streptolydigin.

The expression of the four genes of the *deo* operon (2, 28) was measured under conditions of induction by thymidine (Table 1). Again the expression of the promoter-distal genes was depressed relative to that of the proximal gene *deoC* during growth in the presence of streptolydigin. Frequencies of initiation of transcription of the various operons that could be derived from the differential rates of synthesis of the different proteins will be discussed later.

Synthesis of 16S and 23S rRNA, the products of the *rrn* transcriptional units (30, 37) was determined by labeling with radioactive precursors for short periods and electrophoretically separating the extracted rRNA (see Materials and Methods). The synthesis rate of 23S rRNA was reduced relative to transcription of the promoter-proximal 16S rRNA gene when the cells were grown in the presence of streptolydigin; at concentrations of 1 and 2 μg/ml, the ratios (16S rRNA molecules/23S rRNA molecules) were 1.6 to 2.0 and 2 to 2.5, respectively, relative to the ratio in the inhibited control.

Stability of the transcription complex during complete inhibition of RNA synthesis by streptolydigin in vivo. Removal of streptolydigin was used to study the transcription complex. To provide the background necessary to understand experiments involving both streptolydigin and rifampin, we first determined to what extent streptolydigin action was reversible and how rapidly streptolydigin and rifampin acted.

The instantaneous rate of RNA synthesis in *E. coli* AS19 dropped to 10% within 15 s after addition of 100 μg of streptolydigin per ml. This delay of full inhibition was reflected in a minor, residual RNA synthesis recorded in the presence of streptolydigin (Fig. 6A, filled squares). When streptolydigin was removed by filtration and washing of the cells at 3 min, RNA synthesis resumed with a lag of 1 min (Fig. 6A). After 10 min, the rate reached about 50% of the control rate. Presumably because of incomplete removal of streptolydigin, the restarted culture did not reach the control rate. When rifampin (and nalidixic acid, to prevent DNA synthesis [30]) was added together with streptolydigin, the residual RNA synthesis was less than that with streptolydigin alone (Fig. 6A). The subsequent removal of streptolydigin at 3 min did not lead to re-

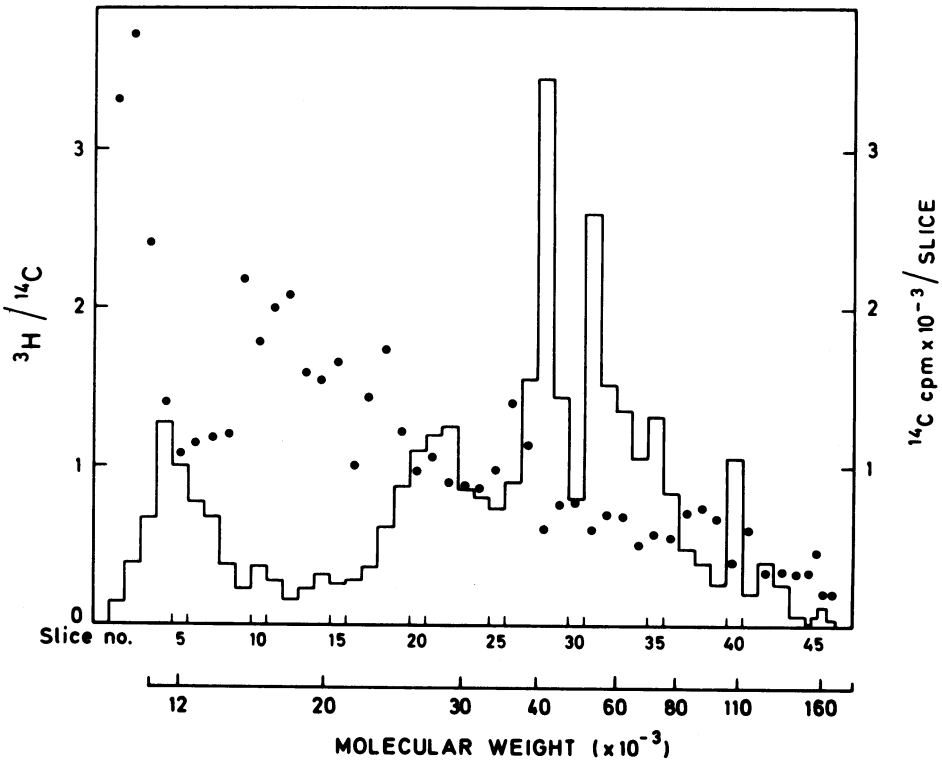

FIG. 5. *Separation, by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, of polypeptides synthesized during growth in the presence of streptolydigin (5 μg/ml). Strain NF541 grown for 100 min in glucose minimal medium containing 5 μg of streptolydigin per ml was labeled for 2 min with [^{3}H]lysine. After a 1-min chase with 500 μg of nonradioactive lysine per ml, the cells were lysed and mixed with a lysate of [^{14}C]lysine-labeled control cells. Gel electrophoresis and determination of the radioactivity were as described in the text. The profile of the polypeptide from the control cells is shown (——; ^{14}C counts per minute) together with the $^3H/^{14}C$ isotope ratio (●).*

sumption of RNA synthesis (Fig. 6A). The capacity for residual RNA synthesis—expressed after the addition of rifampin alone (completion of nascent RNA chains; Fig. 6A)—was not conserved by the streptolydigin treatment; in fact, a short treatment efficiently eliminated all further completion of RNA chains.

Since the presence of rifampin and nalidixic acid might have an adverse effect on the resumption of RNA chain elongation after removal of streptolydigin, the pattern of residual protein synthesis was analyzed in analogous experiments (Fig. 6B). After addition of rifampin, residual protein synthesis could be monitored by labeling with radioactive amino acids; addition of streptolydigin and rifampin together gave a 45% lower translational yield, reflecting translation of preexisting mRNA only; addition of streptolydigin (100 μg/ml) alone resulted in a similar reduction, the only difference being a slight escape synthesis. Removal of streptolydigin by filtration and washing of the cells at 3 min resulted in a gradual resumption of protein synthesis (Fig. 6B). However, when rifampin was added when the streptolydigin was removed, no increase in residual protein synthesis occurred (Fig. 6B). The same result occurred when rifampin was present from the time of streptolydigin addition (Fig. 6B). The 45% unexpressed capacity (nascent mRNA to be completed in presence of rifampin) was lost and could not be recovered after removal of streptolydigin.

Thus, high concentrations of streptolydigin affected the transcription complex irreversibly; in vivo RNA synthesis (rRNA and mRNA) could only resume when initiation of transcription was permitted. The preexisting transcription complexes appeared to have lost their activity.

DISCUSSION

Susceptibility of *E. coli* to streptolydigin. In the permeable strain AS19, 50% growth inhibition was observed at a streptolydigin concentration of 1×10^{-6} to 1.5×10^{-6} M. This is in striking contrast to the concentration of 1×10^{-5}

TABLE 1. *Differential rate of synthesis of polypeptides coded by polycistronic operons*[a]

Streptolydigin concn (μg/ml)	*rpoBC*		*trpEDCBA*			*deoCABD*			
	β subunit (*rpoB*)	β′ subunit (*rpoC*)	Anthranilate synthetase (*trpE*)	Indole 3-glycerol phosphate synthetase (*trpC*)	Tryptophan synthetase subunit B (*trpB*)	Deoxyriboaldolase (*deoC*)	Thymidine phosphorylase (*deoA*)	Deoxyribomutase (*deoB*)	Purine nucleoside phosphorylase (*deoD*)
0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
0.5	0.95	0.57							
1.0	0.80	0.37				1.5	1.13	1.05	0.68
1.5			0.66	0.34	0.32				
2.0	0.60	0.21				2.1	1.37	1.05	0.78
5.0			0.36	0.18	0.14				

[a] *rpoBC*: Strain NF541 was grown in glucose minimal medium. At an absorbance at 450 nm of 0.500, streptolydigin was added. After further growth for 25 to 50 min, the cells were labeled with [^{3}H]lysine for 2 min and then chased. The cells were lysed and mixed with a lysate of a [^{14}C]lysine-labeled control culture. After electrophoresis, the isotope ratios $^3H/^{14}C$ in the β and β′ bands were determined and divided by the $^3H/^{14}C$ ratio of total protein in the samples. These normalized differential rates of synthesis are given in the table. The actual differential rate of β or β′ synthesis in the control culture is 0.005 to 0.006 (21). *trpEDCBA*: Strain CM279, a *trpR* derivative of strain AS19, was grown for 2.5 doubling times at the indicated streptolydigin concentrations. The activity of the 3 enzymes was determined at intervals of 0.5 doubling time, and the differential rates of enzyme synthesis were calculated as: rate of enzyme synthesis/rate of total protein synthesis. The values for the control culture are set arbitrarily at 1.0. *deoCABD*: Strain AS19 was grown for 3 doubling times on glucose minimal medium at the indicated streptolydigin concentrations in presence of thymidine (4 mM). Samples for enzyme determinations were withdrawn at intervals of half a doubling time. The differential rate estimates are based on 5 measurements and are set arbitrarily at 1.0 for the control culture.

to 1.5×10^{-5} M required for 50% inhibition of RNA synthesis in vitro (5, 32, 34). The discrepancy between sensitivity of growth and in vitro transcription indicates that streptolydigin acts differently in the cell and in the test tube.

Apparent lack of effect on RNA chain elongation rate. At 3 μg/ml (5×10^{-6} M), at which the cell growth rate was reduced threefold, practically no effect on RNA chain elongation could be detected either for rRNA or for *lacZ* mRNA. The reduction was at most 15% and could not account for the drastic reduction of the growth rate. Furthermore, all RNA polymerases that finished *lacZ* or *rrn* transcription transcribed at the same rate, as indicated by an abrupt increase in the rate of completion of *lacZ* mRNA at 80 s after induction (Fig. 2B) and the immediate halt of residual 5S rRNA synthesis at 75 s after rifampin addition. If there was heterogeneity in chain growth rates, gradual changes in completion of these transcripts should have been found. The small effect on RNA chain elongation rates in vivo at 5×10^{-6} M is in accordance with in vitro data, which show only a 15 to 20% inhibition of RNA synthesis at this concentration (5). This indicates that streptolydigin probably is not being concentrated in the cell.

Induction of a gradient in gene expression. The presence of low concentrations of streptolydigin during growth leads to polarity in gene expression.

The measurements of β-galactosidase and auto-α activity (Fig. 4A and B) and also the observation of a general trend of decreased synthesis of long polypeptides (differential rate of β-galactosidase synthesis; gel electrophoresis: Fig. 5) show the occurrence of intracistronic polarity (11). The synthesis of the promoter-proximal part of the *lacZ* mRNA, which reflects initiation of transcription, was apparently unchanged when cells grew in the presence of 5×10^{-6} M streptolydigin, as evidenced by an unchanged rate of synthesis in the N-terminal part of β-galactosidase, the auto-α activity (Fig. 4). Thus, growth inhibition by streptolydigin was probably not due to a preferential inhibition of initiation of transcription.

The polarity observed was not limited to intracistronic polarity. In all of the polycistronic operons tested, polarity, i.e., a gradient in gene expression, was induced by streptolydigin. Furthermore, at high drug concentrations a complete, rapid, and irreversible inactivation of active transcription complexes occurred. As a measure of the effect of different drug concentrations, we computed "half-ways" (in analogy to mRNA half-life) for actively transcribing RNA polymerases, i.e., the distance from the promoter at which 50% of the transcription complexes

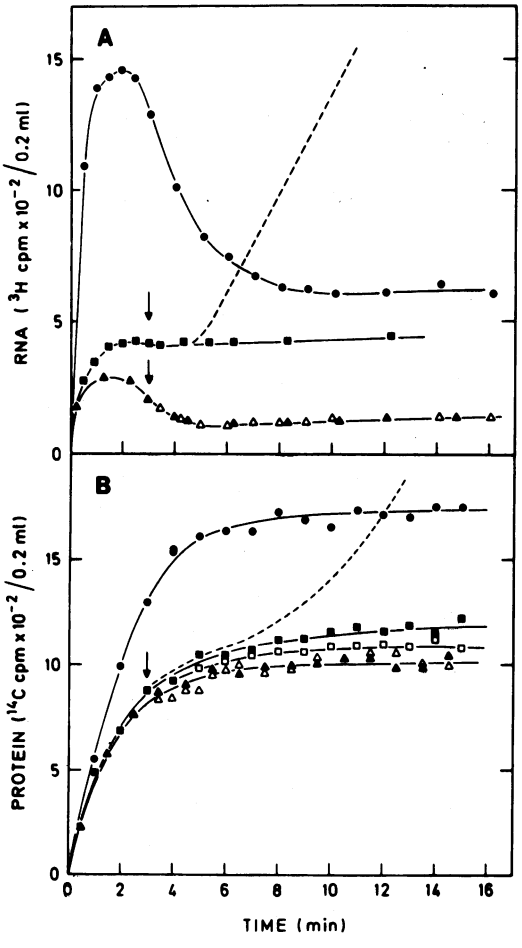

FIG. 6. *Residual RNA and protein synthesis after rifampin and streptolydigin treatment and recovery of RNA and protein synthesis after streptolydigin removal. Portions of a culture of strain AS19 in glucose minimal medium at an absorbance at 450 nm of 0.500 were labeled with [^{3}H]uridine (A) or [^{14}C]proline (B) at time zero and treated as follows. (A) ●, Addition of rifampin (40 μg/ml) and nalidixic acid (20 μg/ml) at time zero; ▲, addition of rifampin (40 μg/ml), nalidixic acid (20 μg/ml), and streptolydigin (100 μg/ml) at time zero; △, as for ▲ but with streptolydigin removed at 3 min; ■, addition of streptolydigin (100 μg/ml) and nalidixic acid (20 μg/ml) at time zero; -----, as for ■ but with streptolydigin removed at 3 min. (B) ●, Addition of rifampin (40 μg/ml) at time zero; ▲, addition of rifampin (40 μg/ml) and streptolydigin (100 μg/ml) at time zero; △, as for ▲ but with streptolydigin removed at 3 min; ■, addition of streptolydigin (100 μg/ml) at time zero; -----, as for ■ but with streptolydigin removed at 3 min; □, as for ----- but with rifampin (40 μg/ml) added at 3 min.*

apparently lost their activity at a given drug concentration. The data in Table 2 were obtained by plotting the degrees of polarity, determined for the various genes and operons, versus the length of the respective DNA stretch. The half-way appears to be inversely proportional to the streptolydigin concentration. Actually, streptolydigin is an alternative agent for transcriptional mapping, analogous to the technique using actinomycin D, described by Bleyman et al. (3).

From the degree of polarity and the observed differential rates of synthesis of the different gene products (see Results), one can obtain relative estimates of the frequency of transcription initiations as follows: differential rate of synthesis × growth rate × degree of polarity. It was found that the initiation frequency of the operons *rpoBC, deo,* and *rrn* (unpublished data), was not reduced at 1.0 μg of streptolydigin per ml, but, rather, was somewhat increased relative to the control culture. On the other hand, the rate of initiation of the *trp* operon decreased, as reflected by the low rate of synthesis of the promoter-proximal gene *E* product (Table 1). These findings are reminiscent of a shift-up situation: stimulation of rRNA synthesis and depression of *trp* operon expression (31).

We conclude that the growth rate was reduced because of the preferential reduction of the rate of synthesis of complete polypeptides coded for by large genes and promoter-distal genes in polycistronic operons due to decreased synthesis of the respective mRNA's.

With respect to the application of streptolydigin for in vivo studies, we want to emphasize that this drug should only be used at high concentrations, which block transcription totally.

TABLE 2. *Half-way of actively transcribing RNA polymerase at different streptolydigin concentrations*[a]

Streptolydigin concn (μg/ml)	Half-way (base pairs)
0.5	7,000
1.0	4,000
1.5	2,800
3.0	1,800
5.0	1,000

[a] Half-way defined as the stretch of DNA in number of base pairs after transcription of which half the initially active RNA polymerases apparently have lost their activity. All values are ±15%. Sizes of genes (number of base pairs) used for the estimation of the half-ways: *rpoC* (β′ subunit), 4,200; *trpD,* 1,730; *trpC,* 1,250; *trpB,* 1,250; *deoA,* 2,000; *deoB,* 1,270; *deoD,* 630; 23S rRNA, 3,400; and *lacZ,* 3,000.

At low, partially inhibitory concentrations, the induction of transcriptional polarity leads to disproportionate expression of otherwise coordinately controlled genes. Serious, "artifactual" changes of cell physiology, such as increased frequency of initiation of DNA replication (19) and preferential inhibition of cell division (K. von Meyenburg, unpublished data), may result.

Mechanism of inactivation of transcribing RNA polymerase by streptolydigin. The stabilization ("freezing") of the transcription complex by high concentrations of streptolydigin observed in vitro (5) could not be reproduced in vivo; rather, we found a rapid, complete, and irreversible loss of active transcription complexes. Only when reinitiation of transcription was permitted could RNA synthesis restart after a short period of blocking by streptolydigin (Fig. 6A and B). We suggest that such destabilization also occurs at low concentrations of streptolydigin. At a given streptolydigin concentration, there would be a certain probability (inversely proportional to the half-way) that an engaged RNA polymerase loses its activity, releasing incomplete RNA, which gives rise to incomplete, metabolically unstable polypeptide fragments (Table 1; Fig. 4).

Since the polarity was observed in various operons, including the *rrn* transcription units, we consider it unlikely that streptolydigin induced transcriptional misreading, which would have yielded nonsense codons in the mRNA and subsequent premature termination of transcription under the influence of rho (1, 7) and/or endonucleolytic attack on mRNA (25, 26).

RNA polymerases inhibited by the drug could be "pushed off the track" by a following uninhibited enzyme molecule. The finding that the polarity is independent of the transcriptional intensity on a given gene or operon argues against such an explanation. At a growth rate of 1.4 doublings per hour, the initiation frequency on an *rrn* transcription unit was about 30/min; on the fully induced *lac* operon it was approximately 10 times lower, and on the *rpoBC* operon it was approximately 30 times lower (calculations based on the rRNA content of the cells and differential rate of synthesis of β-galactosidase and β and β' subunit of RNA polymerase [Table 1]).

A direct destabilizing effect of streptolydigin on the DNA-RNA polymerase-nascent RNA complex can be envisaged; however, this also seems unlikely, since in the purified in vitro system stabilization of the complex and reduced chain growth has been reported (4, 5).

Additional elements seem to be responsible for the expression of streptolydigin-induced polarity effects in vivo. We propose that binding of streptolydigin to the RNA polymerase in the transcription complex renders the polymerase more susceptible to interaction with a termination factor(s), leading to release of an unfinished RNA chain and core enzyme from the template. In preliminary experiments with a crude, coupled in vitro system (42), this induction of polarity by streptolydigin was demonstrated.

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