Stability of *Escherichia coli* Membrane Proteins During Chloramphenicol Treatment

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It is shown that there is no degradation of previously formed inner and outer membrane proteins of *Escherichia coli* during 1 h of chloramphenicol treatment.

Bacterial protein synthesis is completely inhibited by moderate concentrations of chloramphenicol. This drug apparently binds to the 50S ribosomal subunit, inhibiting the enzyme peptidyl transferase (10). In addition, chloramphenicol treatment of *Escherichia coli* cells has other consequences that are less well understood. These include the uncoupling of ribosomal ribonucleic acid (RNA) and protein syntheses in rel+ strains (9) and the increase in permeability of organisms to actinomycin D (7).

The stimulation of RNA synthesis in the absence of protein production is generally thought to be the consequence of amino acid sparing by chloramphenicol (9), and the increase in permeability has been suggested to be the result of cell wall distortion by the excess accumulation of RNA (7).

Evidence is available, however, that the state of the cell envelope influences nucleic acid synthesis. ("Cell envelope" in this report refers to the entire cell wall of *E. coli*, the cytoplasmic and outer membranes as well as the peptidoglycan layer.) Glaser et al. found that inhibition of phospholipid biosynthesis resulted in simultaneous arrests of deoxyribonucleic acid, RNA and protein syntheses (4). Mutants of *E. coli* that are defective in initiation or replication of the chromosome have been shown to have cell envelope protein deficiencies (5, 12). Dworsky and Schaechter, studying the effect of rifampin on the attachment of deoxyribonucleic acid to the cell membrane, (presumably cytoplasmic), concluded that RNA polymerase plays a role in maintaining this attachment (3). Cold-shocked cells, suffering some unspecified membrane damage, lost the "stringent" control of RNA synthesis (1). We have observed that in certain rel+ strains of *E. coli*, unbalanced RNA synthesis may be stimulated by perturbing the cell envelope with polar solvents (6).

Therefore the possibility exists that stimulation of RNA synthesis is also a consequence of cell envelope damage. Matzura and Broda found that 30 min of chloramphenicol treatment of *E. coli* CP78 cells resulted in alterations of the cell envelope, as evidenced by permeability to actinomycin D (7).

We examined whether certain cell wall proteins are preferentially degraded during exposure to chloramphenicol. Two 400-ml cultures of *E. coli* CP 78 were grown aerobically in minimal salts medium (2) supplemented with 0.3% (wt/vol) glucose; histidine, arginine, and threonine each at 50 μg/ml; and with either [3H]leucine or [14C]leucine, each at 0.125 μCi (30 μg/ml). At a cell density of about 5 × 10⁷/ml, each culture was divided into halves. The [3H]-labeled organisms and one of the [14C]-labeled cultures were poured onto crushed ice and kept at 4°C for 1 h. The other [14C]leucine-labeled culture received chloramphenicol at 100 μg/ml (final concentration) and was incubated at 37°C for 1 h with shaking. This fraction too was then poured onto crushed ice and chilled. Each of the four fractions was washed once at 4°C. The chloramphenicol-containing cells were combined with one of the untreated tritium-labeled culture, and the untreated [14C] cells were also mixed with the other untreated [3H] culture. Membranes from these two mixtures were prepared by the method described by Osborn et al. (8), except that ribonuclease and deoxyribonuclease (10 μg/ml each) were added to the sonically treated spheroplasts. The total membrane proteins were solubilized for 20 min at 70°C in the presence of 1% (wt/vol) sodium dodecyl sulfate and 0.5% (vol/vol) mercaptoethanol. Aliquots were subjected to electrophoresis on 7.5% polyacrylamide gels containing sodium dodecyl sulfate. The gels were cut into 1-mm slices, and the radioactivity of each slice was determined in a liquid scintillation spectrometer. The results are shown in Fig. 1 and Table 1.

From Fig. 1 we conclude that during the arrest of protein synthesis by chloramphenicol, no selective breakdown occurs among the proteins of the cell envelope. However, these data do not exclude the possibility of uniform breakdown among the membrane proteins. In Table 1...
we show that chloramphenicol treatment did not increase the ratios of radioactivity of untreated to chloramphenicol-exposed proteins. Thus we cannot show any breakdown of membrane proteins during 1 h of exposure to chloramphenicol.

It appears that the alteration in the permeability barrier of E. coli during chloramphenicol treatment is not the result of loss of preexisting cell envelope proteins. It could be the consequence, rather, as suggested (7), of cell wall distortion by the accumulating RNA, or perhaps of continued extension of the cell envelope in the presence of chloramphenicol, the new envelope material being deficient in proteins (11). Lastly, it could also come about as a manifestation of the activity of autolytic enzymes, in the absence of growth and protein turnover. Further experimentation will be needed to resolve this question.

LITERATURE CITED