Effects of Iodoamphenicol on Ribosome Assembly in Two Strains of Escherichia coli

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When the growth of Escherichia coli strain 15TP was inhibited by iodoamphenicol, three 'iodoamphenicol particles' accumulated with sedimentation coefficients of 25S, 33S and 45S. The 25S and 33S particles differ in sedimentation properties from equivalent ribosome precursor particles detected during pulse-labelling of exponentially growing cells. Inhibition of a mutant, strain 15-28 (defective in ribosome assembly), by iodoamphenicol resulted in the accumulation of 38S iodoamphenicol particles that are different from the particles made by the parent. The results support the contention that assembly of 50S ribosomal subunits by the mutant is altered at an early stage.

INTRODUCTION

One approach to the study of ribosome assembly in Escherichia coli is to use inhibitors to cause the bacteria to accumulate uncompleted ribosomes. For example, low concentrations of chloramphenicol cause the accumulation of ribonucleoproteins similar to ribosome precursors detected in pulse-labelled organisms (Osawa et al., 1969), while at higher concentrations 'chloramphenicol particles' accumulate which contain precursor forms of ribosomal RNA associated with ribosomal protein (Sykes et al., 1977a, b) but whose relationship to 'natural' precursors is less clear.

The present paper compares the effects of the synthetic antibiotic monoiodoamphenicol on ribosome synthesis in two strains of E. coli. In the parent, strain 15TP, ribosome assembly is normal; pulse-labelling identifies two sequential precursors to 30S ribosomal subunits and three to 50S ribosomal subunits (Butler et al., 1979, 1980). The mutant strain 15-28, derived from strain 15TP, has defects in ribosome assembly and function (MacDonald et al., 1967). Exponentially growing mutant organisms contain large quantities of '47S particles'. These are unusual ribosome precursors that lack three ribosomal proteins and contain mature, rather than precursor 23S rRNA (Markey et al., 1976; Butler et al., 1980). Synthesis of 47S particles proceeds as though they, rather than 50S ribosomal subunits, are the end-products of assembly; for example, pulse-labelling at 20 °C allows detection of three sequential precursors to 47S particles, each of which contains precursor 23S rRNA. In the experiments described below, monoiodoamphenicol was used as a further means of examining ribosome assembly in the two strains.

METHODS

The organisms, E. coli 15 thy pro (15TP) and the mutant (15-28) derived from it (MacDonald et al., 1967), were grown with aeration at 37 °C in minimal medium supplemented with Casamino acids, labelled with

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radioactivity and gently lysed as described previously (Butler et al., 1980). The monoiodoamphenicol (iodoamphenicol) used to inhibit growth was synthesized by the method of Rebstock (1950). The buffer used in the 15-30% (w/w) linear sucrose gradients was 10 mM-Tris/HCl, pH 7.4, containing 10 mM-magnesium acetate, 100 mM-KCl and 1 mM-spermidine. The gradients were centrifuged at 4 °C using a Spino SW50-1 rotor operated at 234 000 g for 2.75 h. Gradients were fractionated and the radioactivity of fractions was determined as in previous work (Markey & Wild, 1976). The procedures for extraction and subsequent electrophoresis of RNA in gels containing a gradient (2.5-7.5%, w/v) of polyacrylamide were also those used previously (Butler et al., 1980).

RESULTS

Effects of iodoamphenicol on macromolecular synthesis

Iodoamphenicol was added to portions of cultures in mid-exponential phase (generation times: strain 15TP, 40 min; strain 15-28, 110 min). After 2 min, [14C]uracil and [3H]lysine were added to monitor RNA and protein synthesis. In both strains protein synthesis was increasingly inhibited by higher drug concentrations (Table 1). RNA synthesis in the parent was slightly stimulated by 25 and 50 µg iodoamphenicol ml⁻¹ and at 100 µg ml⁻¹ was inhibited much less than protein synthesis. In the mutant, there was no stimulation of RNA synthesis but the inhibition was again less than that of protein synthesis. In both strains, the oversynthesis of RNA relative to protein increased with drug concentration but in the mutant the oversynthesis was less.

The accumulation of 'iodoamphenicol particles'

A culture of strain 15TP was incubated with 50 µg iodoamphenicol ml⁻¹ for 15 min with [3H]uracil present. An extract was made and centrifuged after mixing with an extract of strain 15TP grown in the presence of [32P]phosphate. Inhibition by iodoamphenicol led to the appearance of three components with sedimentation coefficients of about 25S, 33S and 45S (Fig. 1a). These particles were compared with the ribosome precursors detected by pulse-labelling. For this, an exponentially growing culture of strain 15TP was labelled with [3H]uracil for 50 s; a second culture was grown for 30 min in the presence of [32P]phosphate and 50 µg iodoamphenicol ml⁻¹. Extracts were made, mixed and centrifuged. The 3H radioactivity profile (Fig. 1b) shows a 'p3OS-1' precursor particle to the smaller ribosomal subunit and two of the precursors ('p50S-1' and 'p50S-2' particles) to the larger subunit. There are clear differences in sedimentation properties between the p30S-1 precursor particle and the 25S iodoamphenicol particle, as well as between the p50S-1 precursor and the 33S iodoamphenicol particle. In other experiments (results not shown), RNA extracted from isolated iodoamphenicol particles was characterized by gel electrophoresis. The 25S iodoamphenicol particle contained exclusively the precursor form of 16S rRNA; the RNA from 33S iodoamphenicol particles was in the 23S region of a gel, where it was equally divided between precursor and mature forms. The 45S iodoamphenicol particles contained 23S rRNA, very largely in the mature form.

With the mutant strain 15-28 (Fig. 1c), a 32P-labelled 'reference' sedimentation profile (derived from exponentially growing organisms) shows 70S ribosomes, 47S particles and material with the sedimentation properties of 30S ribosomal subunits (Butler et al., 1980). After inhibition for 30 min by 50 µg iodoamphenicol ml⁻¹, and with [3H]uracil present, two of the newly-made components were indistinguishable in sedimentation properties from 30S ribosomal subunits and 47S particles. However, there was also considerable synthesis of a third species with a sedimentation coefficient of about 38S. The RNA of 38S particles was 23S rRNA, very largely in the precursor form. It is apparent from Figs 1a and 1c that 33S (parent) and 38S (mutant) iodoamphenicol particles differ appreciably in sedimentation properties. This was directly confirmed by sedimentation of mixed extracts.
Effects of iodoamphenicol on ribosome synthesis

Table 1. *Oversynthesis of RNA by two strains of E. coli during inhibition by iodoamphenicol*

Strains were grown to $A_{450} 0.4$. To portions (10 ml) of each, iodoamphenicol was added, followed 2 min later by $[^{14}C]$uracil [0.04 μCi (1.48 kBq); 10 μg ml$^{-1}$] and carrier-free $[^{3}$H$] $lysine [final specific activity about 2.5 μCi (92.5 kBq); 50 μg ml$^{-1}$]. Samples (0.5 ml) were taken after 40 min for measurement of $^{14}$C and $^3$H radioactivity in material insoluble at 0°C in 5% (w/v) trichloroacetic acid.

<table>
<thead>
<tr>
<th>Iodoamphenicol concn (μg ml$^{-1}$)</th>
<th>Strain</th>
<th>RNA synthesis* (a)</th>
<th>Protein synthesis* (b)</th>
<th>Ratio (a)/(b)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>15TP</td>
<td>25</td>
<td>75</td>
<td>1.7</td>
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<td></td>
<td></td>
<td>50</td>
<td>38</td>
<td>2.9</td>
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<td></td>
<td>100</td>
<td>21</td>
<td>3.9</td>
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<tr>
<td></td>
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<td>74</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>72</td>
<td>1.9</td>
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<tr>
<td></td>
<td></td>
<td>100</td>
<td>47</td>
<td>2.8</td>
</tr>
</tbody>
</table>

* Percentage of incorporation by uninhibited control.

Fig. 1. Formation of 'iodoamphenicol particles'. (a) To a culture (20 ml) of strain 15TP at $A_{450} 0.4$, iodoamphenicol was added to a final concentration of 50 μg ml$^{-1}$. After 2 min, $[^{3}$H$] $uracil [5 μCi (185 kBq); 5 μg ml$^{-1}$] was added; after 15 min, an extract was made. Another extract was prepared from a culture (25 ml) of strain 15TP grown for three generations to $A_{450} 0.4$ with $[^{32}$P$] $phosphate (5 μCi ml$^{-1}$). Portions of each extract were mixed and centrifuged. (b) Two cultures of strain 15TP were grown to $A_{450} 0.4$. One then received $[^{3}$H$] $uracil [20 μCi (740 kBq); 0.5 μg ml$^{-1}$] for 50 s, and an extract was prepared. To the other was added 50 μg iodoamphenicol ml$^{-1}$ and, 2 min later, $[^{32}$P$] $phosphate [3.5 μCi (129.5 kBq) ml$^{-1}$]; after 30 min with iodoamphenicol, an extract was made. A mixture of the extracts was centrifuged. (c) To a culture (20 ml) of strain 15-28 at $A_{450} 0.4$, 50 μg iodoamphenicol ml$^{-1}$ was added, then, after 2 min, $[^{14}$C$] $uracil [1 μCi (37 kBq); 5 μg ml$^{-1}$]. After 30 min, an extract was made. Another extract was made from a culture (25 ml) of strain 15-28 grown for three generations to $A_{450} 0.4$ with $[^{32}$P$] $phosphate (5 μCi ml$^{-1}$). Portions of each extract were mixed and centrifuged. △, $^{14}$C radioactivity; ○, $^{32}$P radioactivity; ●, $^{3}$H radioactivity.
DISCUSSION

The parent strain of E. coli studied accumulates iodoamphenicol particles presumably because the drug, like others with similar effects (e.g. Hosokawa & Nomura, 1965; Holmes & Wild, 1967), inhibits RNA synthesis less than protein synthesis, so that the assembly of ribosomes becomes limited by the availability of particular ribosomal proteins. Limited availability of some ribosomal proteins may also be important in determining the overall rate of ribosome synthesis in exponentially growing organisms, although rate-limiting conformational changes similar to those found in vitro may also be involved (Schlessinger, 1974).

Inhibition by iodoamphenicol has been reported to cause production of '32S' ribonucleoprotein particles (Pongs & Messer, 1976). The present experiments confirm this but show that there is also accumulation of particles with sedimentation coefficients of about 25S and 45S. The 32S particles were thought to be similar to a precursor to the larger ribosomal subunit isolated from exponentially growing cells (Pongs & Messer, 1976; Nierhaus et al., 1973). However, the 33S particles described in the present paper do not co-sediment with the equivalent particle detected by pulse-labelling; similarly the 25S particles have no counterpart in pulse-labelled organisms. In addition, although the 45S iodoamphenicol particles and the pulse-labelled 'p5OS-2' precursor have similar sedimentation properties this is no proof of identity. The 23S rRNA in 45S particles is very largely 'mature'; during the imbalanced assembly caused by iodoamphenicol, maturation events may occur out of normal sequence.

In a given medium the mutant strain has a higher RNA content than its parent (MacDonald et al., 1967); this may explain why several antibiotics tested (in unpublished experiments by P. D. Butler) failed to imbalance RNA and protein synthesis further and why the effects of iodoamphenicol are less marked than with the parent strain. During inhibition of the mutant by iodoamphenicol, two ribonucleoprotein particles accumulate that have the same sedimentation properties as 30S subunits and 47S particles. The third (a '38S' particle with precursor 23S rRNA) has no counterpart in the exponentially grown parent and also differs from the 'equivalent' 33S iodoamphenicol particle of the parent strain. That the two strains respond differently shows that the genetic lesion that results in altered ribosome synthesis continues to operate phenotypically during inhibition. This, in turn, implies and supports previous arguments (Butler et al., 1980) that the mutation in strain 15-28 acts at an early stage in ribosome assembly.

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REFERENCES


