Escherichia coli and Bacillus subtilis Phage Deoxyribonucleic Acid-directed Deoxycytidylate Deaminase Synthesis in Escherichia coli Extracts*

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SUMMARY

A cell-free extract of Escherichia coli was induced by DNAs, prepared from various bacteriophages, to synthesize deoxycytidylate deaminase in vitro. With T4 DNA, only low levels of dCMP deaminase were synthesized. However, DNAs from two Bacillus subtilis bacteriophages, SP82 and SPSC, furnished very active templates for dCMP deaminase synthesis in the E. coli system. Each of these phages induces dCMP deaminase during infection of B. subtilis in vivo. Conversely, DNA from the B. subtilis bacteriophage SP3, which poorly induces dCMP deaminase in vivo, did not produce a dCMP deaminase synthesis in vitro. The synthesis of dCMP deaminase was shown to depend on both transcription and translation.

Recently we described the synthesis in vitro of several enzymes which are induced during infection of Escherichia coli by the bacteriophage T4 (1, 2). The cell-free system used in those experiments was prepared from uninfected E. coli. Virus-specific enzyme synthesis occurred in response to T4 DNA, and was dependent on both transcription and translation. The T4 enzymes made in vitro, the α- and β-glucosyl transferases and lysozyme, have been useful as representatives of specific classes of viral proteins, and various parameters of their synthesis have been investigated (1-4). Another T4-specific enzyme which is absent in uninfected E. coli is dCMP deaminase (5). Therefore, we have attempted to detect the appearance de novo of that enzyme after T4 DNA-directed protein synthesis.

The T4-induced dCMP deaminase appears to be a more complicated enzyme than those previously synthesized in the coupled system. The enzyme purified from T4-infected cells is a large protein, with a molecular weight over 100,000, and is subject to allosteric regulation by an activator, dCTP, and an inhibitor, TTP (6). The purified protein absolutely requires dCTP (or 5-hydroxymethyl-dCTP) for enzymatic activity, whereas TTP can completely inhibit deamination of dCMP. The dCMP deaminases induced by the Bacillus subtilis phages SP8 and SPSC are not subject to allosteric regulation, and hence might represent simpler proteins carrying the same catalytic activity as the T4 dCMP deaminase (7). Therefore, we compared the template activities of T4 DNA and the B. subtilis phage DNAs for the synthesis of dCMP deaminase. The use of B. subtilis phage DNAs also tested the efficiency of RNA and protein synthesis when the cell-free system was challenged by DNA templates which normally are not encountered in E. coli.

MATERIALS AND METHODS

The components of the cell-free system were prepared from E. coli 514 as previously described (1). Bacteriophage DNA was prepared according to Thomas and Abelson (8). Bacteriophage SP82 was a generous gift from Dr. E. Kahan (New York Public Health Research Institute), and the bacteriophages SP5C and SP3 were donated by Drs. M. Nishihara and H. V. Aposhian (University of Maryland School of Medicine). The bacteriophage SP82 was assumed to be essentially the same phage as SP8 (9).

Determination of dCMP Deaminase Activity—The estimation of dCMP deaminase activity after cell-free protein synthesis was complicated by the relatively large amounts of ATP remaining in the incubation mixtures. In order to eliminate substrate phosphorylation, we trapped the ATP remaining after protein synthesis with glucose and hexokinase. Aliquots (0.025 ml) of the protein synthesis reactions were mixed with 0.002 ml of a solution containing 0.5 mM glucose + chloramphenicol (1 mg per ml) + hexokinase (200 μg per ml) + 15% glycerol. After 2 min at 37°, 0.005 ml of substrate solution containing 0.5 mM dCMP-P (1 μCi per μmole) + 0.5 mM dCTP was added to each assay. After incubation for a suitable length of time, aliquots (0.002 ml) of the incubations were removed for analysis (10). PEI-cellulose thin layer sheets (Brinkmann MN-Polygram Cel 300 PEI) were previously spotted with 0.002 ml of a solution containing dCMP, dUMP, and dCTP at 0.05 μm each. The marker spots were allowed to air-dry, and the aliquots from...
the enzyme assays were applied on the same spots. The chromatograms were developed with 0.2 M sodium formate, pH 3.6. The marker spots were located with an ultraviolet lamp, cut out, and counted in a liquid scintillation spectrometer. The per cent conversion of dCMP to dUMP was determined and converted to picomoles of dUMP formed by the entire aliquot (0.025 ml) of the protein synthetic reaction. The radioactive substrate used in these experiments contained 18 picomoles per standard assay of material which comigrated with dUMP during PEI-cellulose chromatography. This substrate blank varied less than 1% and was subtracted from each experimental value. The precision of this assay was such that more than 2 pmol of dUMP over the substrate blank could be detected easily. Duplicate assays were reproducible to <5%. Purified T4 dCMP deaminase (kindly provided by Dr. J. Scocca) was assayed under the above conditions, and was found to be as active as when assayed according to Scocca, Panny, and Bessman (6). Neither the ingredients of the protein synthesis reaction, including untreated E. coli extracts, nor the components of the ATP trapping mixture inhibited the purified deaminase to any significant extent. We emphasize the importance of the ATP trapping procedure; incomplete removal of residual ATP resulted in rapid substrate phosphorylation as well as increased background radioactivity in the dUMP region of the chromatogram.

RESULTS

Bacteriophage DNA Stimulation of dCMP Deaminase Synthesis—DNA from the bacteriophages T4, SP82, and SP5C stimulated dCMP deaminase synthesis, whereas DNA from the bacteriophage SP3 was nearly inactive (Table I). Each template DNA was added to a final concentration of 20 to 40 µg per ml in the protein synthetic reaction mixture, which was sufficient to saturate the system for amino acid incorporation (1, 2). The endogenous amino acid incorporation was stimulated 7- to 10-fold by each of the template DNAs, including DNA from the phage SP3. The amount of dCMP deaminase synthesized in response to the B. subtilis phage DNAs in vitro was proportional to the amount of enzyme induced by each phage 25 min after infection of B. subtilis (7). The bacteriophage SP3 induces very little dCMP deaminase in vitro (7), and DNA prepared from that phage was essentially inactive for dCMP deaminase synthesis in vitro.

T4 DNA was a poor template for dCMP deaminase synthesis. The amount of enzyme synthesis with T4 DNA was usually sufficient to convert 5 to 20 pmol of dCMP to dUMP under these conditions. This represented an increment of 30 to 100% over the background radioactivity (18 pmol of nonenzymatically formed dUMP have been subtracted from each experimental value; see “Materials and Methods”). The high precision of the PEI-cellulose analyses made differences of this magnitude significant, although these amounts of enzyme synthesis were near the limits of detection. The amounts of enzyme synthesis with SP82 DNA were usually sufficient to cause 5- to 15-fold increases in the radioactivity appearing in the dUMP spot after thin layer analysis.

DUMP production increases linearly for over 1 hour with the DNA-directed enzyme, synthesized in vitro, and in fact proportional to the input amount of cell-free reaction mixture.

Various inhibitors of transcription and translation were added and, in each case, T4 DNA and SP82 DNA-dependent dCMP deaminase synthesis was abolished (Table II). Furthermore, when incubated at 0° rather than at 37°, no dCMP deaminase was synthesized. The enzyme synthesis depends on the addition of ribosomes and the DEAE-cellulose protein fraction. There are no cells in the cell-free extracts, as determined by plating. We conclude that the appearance of dCMP deaminase activity resulted from DNA-directed RNA and protein synthesis in vitro.

Allosteric Regulation of dCMP Deaminases Synthesized in Vitro—Since the standard assay for dCMP deaminase contained dCTP at only 0.08 mM, the apparent dCMP deaminase synthesis in response to T4 DNA was measured in the presence

<table>
<thead>
<tr>
<th>Table I</th>
</tr>
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<tbody>
<tr>
<td><strong>Synthesis of dCMP deaminase in response to various bacteriophage DNAs</strong></td>
</tr>
</tbody>
</table>

Protein synthesis incubations were carried out as previously described (1). The magnesium ion concentration was 11 mM, and the ribosomes were at 8 mg per ml. After 30 min for protein synthesis at 37°, aliquots of 0.025 ml were assayed for dCMP deaminase activity with the ATP trap and the standard substrate concentrations (final dCMP-H and dCTP concentrations were 0.08 mM). The incubations for the dCMP deaminase reactions were for 60 min at 37°. Duplicate PEI-cellulose analyses were performed on each sample. The data are given as picomoles of dUMP formed per hour per assay. The PEI-cellulose analyses were repeated with 1.0 mM formic acid elution, and identical results were obtained (10). Leucine incorporation into protein was determined in parallel incubations, which included 14C-leucine at 25 µCi per µmole.

<table>
<thead>
<tr>
<th>Template</th>
<th>Leucine incorporation</th>
<th>dCMP deaminase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>73</td>
<td>&lt;2</td>
</tr>
<tr>
<td>T4 DNA</td>
<td>612</td>
<td>10</td>
</tr>
<tr>
<td>SP82 DNA</td>
<td>705</td>
<td>180</td>
</tr>
<tr>
<td>SP5C DNA</td>
<td>573</td>
<td>64</td>
</tr>
<tr>
<td>SP3 DNA</td>
<td>540</td>
<td>4</td>
</tr>
</tbody>
</table>

**Table II**

**Effect of inhibitors of RNA and protein synthesis on dCMP deaminase synthesis**

Incubations for enzyme synthesis and dCMP deaminase estimation were as described above Table I. Rifampicin, actinomycin D, chloramphenicol, and puromycin were added before the incubation for protein synthesis to final concentrations of 20 µg per ml, 30 µg per ml, 100 µg per ml, and 10⁻⁴ M, respectively. Each inhibitor at that final concentration completely inhibited DNA-dependent amino acid incorporation.

<table>
<thead>
<tr>
<th>Template</th>
<th>Inhibitor added</th>
<th>dCMP deaminase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>&lt;2</td>
</tr>
<tr>
<td>SP82 DNA</td>
<td>None</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>Rifampicin</td>
<td>&lt;2</td>
</tr>
<tr>
<td></td>
<td>Actinomycin D</td>
<td>&lt;2</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol</td>
<td>&lt;2</td>
</tr>
<tr>
<td></td>
<td>Puromycin</td>
<td>&lt;2</td>
</tr>
<tr>
<td>T4 DNA</td>
<td>None</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Rifampicin</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Actinomycin D</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Puromycin</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

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of increased dCTP. The effect of the inhibitor TTP was also measured. As shown in Table III, the activity of deaminase synthesized in response to SP82 DNA was not influenced by either dCTP or TTP, whereas the dCMP deaminase synthesized in response to T4 DNA was stimulated by dCTP. No large inhibition by TTP was observed (6). In some cases, the addition of high levels of dCTP appeared to depress the activity of the SP82 DNA-directed dCMP deaminase. However, since the added dCTP was slowly converted to dCDP and dCMP, those inhibitions probably reflected dilution of the labeled substrate. The response of the T4 DNA-directed dCMP deaminase to dCTP and TTP was never absolute, in that massive stimulation or complete inhibition did not occur. Furthermore, added dCTP was not able in any experiment to cause the T4 DNA-directed deaminase to convert more than 20 pmols of dCMP to dUMP during a 1-hour incubation with the standard 0.025-ml aliquot of the protein synthetic reaction.

**Optimum Mg**$\text{++}$ Concentration for dCMP Deaminase Synthesis—The synthesis of the T4 enzymes previously detected in the coupled system was highly sensitive to the magnesium ion concentration. Each of the early T4-specific proteins was synthesized optimally at 11 mM Mg$^{++}$, whereas lysozyme synthesis was optimal at 15 mM. The synthesis of dCMP deaminase in response to T4, SP82, and SP5C DNA was measured at various magnesium ion concentrations. Enzyme synthesis in response to T4 DNA and SP5C DNA was optimal at 11 mM Mg$^{++}$, whereas SP82 DNA-dependent dCMP deaminase synthesis was optimal at somewhat higher magnesium ion concentrations (Fig. 1). In some experiments, SP82 DNA gave a magnesium profile which peaked at 11 mM Mg$^{++}$ but contained a large shoulder at 10 mM. The Mg$^{++}$ profile for T4 DNA-directed dCMP deaminase was nearly identical with that previously observed for T4 DNA-directed β-glucosyl transferase synthesis (3).

**DISCUSSION**

These experiments show that low levels of T4-specific dCMP deaminase can be detected after DNA-dependent protein synthesis in the E. coli coupled system. These results are meaningful only because of the high precision of the PEI-cellulose analyses, and the fact that in those analyses dCDP and dCMP do not interfere with the estimation of substrate conversion to dUMP. The DNAs from the B. subtilis bacteriophages SP82 and SP5C are similar to T4 DNA for stimulation of amino acid incorporation into protein, yet the amounts of dCMP deaminase synthesized in response to those B. subtilis phage DNAs are much higher. Thus, a "foreign" DNA can be transcribed and translated with a high degree of fidelity in the E. coli system. The detailed quantitative determination of dCMP deaminase synthesis with T4 DNA suggests that higher amounts of enzyme synthesis might be expected. As seen in Table IV, several enzymes are synthesized to the extent of 0.1% of the total protein made in vitro. These calculations are not more accurate.

![Graph showing optimum Mg$^{++}$ concentration for dCMP deaminase synthesis](http://www.jbc.org/)

**Fig. 1.** The reaction mixtures for enzyme synthesis were the same as described in Table I. Mg$^{++}$ was added as magnesium acetate. After 30 min at 37° for enzyme synthesis, aliquots of 0.025 ml were assayed for dCMP deaminase activity with the standard assay and a 60-min incubation. The 100% values for dCMP deaminase synthesis directed by T4 DNA, SP5C DNA, and SP82 DNA were 21, 72, and 246 pmoles, respectively.

**Table III**

<table>
<thead>
<tr>
<th>Template</th>
<th>Additions</th>
<th>dCMP deaminase activity I</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4 DNA</td>
<td>None</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Plus dCTP</td>
<td>15</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Plus TTP</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>SP82 DNA</td>
<td>None</td>
<td>230</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>Plus dCTP</td>
<td>220</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Plus TTP</td>
<td>260</td>
<td>72</td>
</tr>
</tbody>
</table>

**Table IV**

<table>
<thead>
<tr>
<th>Template</th>
<th>Enzyme</th>
<th>% total</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4 DNA</td>
<td>β-Glucosyl</td>
<td>0.1–0.2</td>
</tr>
<tr>
<td></td>
<td>Transferase</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lysozyme</td>
<td>0.05–0.10</td>
</tr>
<tr>
<td></td>
<td>dCMP deaminase</td>
<td>0.0001</td>
</tr>
<tr>
<td>SP82 DNA</td>
<td>dCMP deaminase</td>
<td>0.08</td>
</tr>
</tbody>
</table>
than a factor of 5, yet it is clear that the amount of active T4
dCMP deaminase resulting from RNA and protein synthesis
is quite low. There are at least two explanations for this phe-
omenon. There could be selection against the transcription or
expression of the T4 dCMP deaminase activity may not readily
occur. Consistent with this interpretation is the fact that the
dCMP deaminase resulting from RNA and protein synthesis
transcriptional discrimination would be interesting in light of the observations
concerning the specificities of E. coli and T4-specific σ factors
(13). However, recent experiments have shown that mRNAs
extracted from T4-infected E. coli direct the synthesis of β-
glucosyl transferase and dCMP deaminase in the same apparent
ratio as does T4 DNA, and this ratio is much higher than is
observed in vivo.2 An alternative explanation for the low level
of dCMP deaminase is that the T4 DNA directs the synthesis
of large amounts of the correct polypeptide subunits, but that
there are difficulties inherent in the cell-free system in assemb-
ing of large amounts of the correct polypeptide subunits, but that
there are difficulties inherent in the cell-free system in assemb-
ling what may be a multimeric protein (6). Presumably because
of the low concentrations of newly synthesized proteins, the
subunit interactions which we assume are important for the full
expression of the T4 dCMP deaminase activity may not readily
occur. Consistent with this interpretation is the fact that the
T4 enzyme synthesized in vitro behaves only qualitatively as the
native enzyme purified from infected cells, for the responses to
dCTP and TTP are weak (6).

The dependences of dCMP deaminase synthesis on the mag-
nesium ion concentration are similar to those previously reported
(1-4). T4 DNA and SP5C DNA stimulate dCMP deaminase
synthesis maximally at 11 mM Mg++, the identical Mg++ level
for optimal T4 DNA-dependent α- and β-glucosyl transferase
synthesis. However, the magnesium ion profile for SP52 dCMP
deaminase synthesis is shifted toward higher Mg++ concentra-
tions, and resembles that observed for T4 DNA-directed lyso-
zyme synthesis (2). Although some experiments have been
directed toward understanding the Mg++ profiles in the T4
DNA-primed system, the molecular basis for this observation is not understood (14). It may be significant that, of all of the
enzymes synthesized in vitro with various bacteriophage DNAs,
only the SP52 dCMP deaminase and the T4 lysozyme represen-
t enzymes which, during infection, are not shut off following DNA
replication (15).

2 P. Loewen, personal communication.

These experiments increase the available marker enzymes
which can be synthesized in vitro in response to bacteriophage
DNA. However, T4 DNA-directed dCMP deaminase synthesis
is only marginally useful, as detailed quantification of slightly
altered levels of enzyme are difficult. The experiments with
B. subtilis bacteriophage DNAs are promising, in that factors
which affect transcription of those DNAs in vivo by interacting
with the DNA could be studied in vitro in this heterologous sys-
tem (16).

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