Response of a Phage Modification Factor to Enhanced Production of Its Target Molecule†

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Escherichia coli cells with plasmids bearing the valS gene were constructed from the Clark-Carbon collection. Their increased valyl-tRNA synthetase (EC 6.1.1.9) activity was not matched by commensurate production of a modifying peptide during T4 infection. Thus, phage vs gene expression is set to modify the normal amount of valyl-tRNA synthetase activity.

Bacteriophage T4 contains a gene, vs, that codes for a peptide, τ, which is added to host valyl-tRNA synthetase (VRS) immediately after infection. The physiological function of modification is unknown (6, 10, 12, 16). Bacteriophage mutants deficient in τ synthesis plate normally on Escherichia coli under laboratory conditions; however, a T4vs- restrictive host, E. coli CP79032, has been identified in which modification is abnormal and few viral progeny are produced. Interestingly, vs gene mutants plate normally on this host (9, 18).

Strains of E. coli that overproduce VRS would be valuable to a study of its modification. As an example, coordinated regulation of the vs gene, as well as the influence of different levels of modified VRS on phage development, could be investigated.

The Clark-Carbon collection of E. coli cells containing ColE1 plasmids ligated to different fragments of the E. coli genome (5) was crossed to temperature-sensitive E. coli NP910212 (F- recA strA valS (ts) pyrB). Colony growth at 43.5°C identified cells that acquired a hybrid ColE1-valS plasmid and temperature stable VRS.

Four independent, temperature-stable strains were selected and called B1, B2, B3, and B4. These strains simultaneously became resistant to colicin E1 in contrast to the colicin-sensitive recipient NP910212. Additionally, the B strains became wild type for pyrB (ura+), presumably due to the proximity of the pyrB locus to valS (0.89 cotransduction frequency with valS) (21). The argF gene maps between valS and pyrB and is presumably included in the hybrid plasmid. The valS, pyrB, and argF genes, respectively, code for VRS, aspartate carbamoyltransferase (EC 2.1.3.2), and ornithine carbamoyltransferase (EC 2.1.3.3).

The four B strains were grown at 37°C, and cell extracts were prepared by sonication of cultures in phosphate buffer. The aminocacylation-specific activity of VRS in each extract was measured by attachment of [3H]valine to tRNA, using the standard assay described by Müller and Marchin (13) (Table 1). Enzyme-specific activity in the B strains is elevated as much as 3.7-fold when compared with enzyme-specific activity measured in extracts of wild-type E. coli NP4 and NP2 (valS) grown at 37°C. Strains containing temperature-sensitive VRS typically do not exhibit in vitro aminocacylation activity (7).

Additionally, aspartate carbamoyltransferase and ornithine carbamoyltransferase activities were measured by procedures described by Adair and Jones (1). Table 1 indicates 3.4- to 5.3-fold-elevated levels of aspartate carbamoyltransferase-specific activity in the B strains when compared with wild-type E. coli. This, however, comparable increases were observed for both valS- and pyrB-coded enzyme activities in the B strains. These require expression of the wild-type valS and pyrB alleles located on the hybrid plasmids, since the recipient NP910212 cells are mutant in both alleles. Extracts of NP910212 exhibited low levels of aspartate carbamoyltransferase activity, presumably because these cells contain only the mutant pyrB allele. The ornithine carbamoyltransferase-specific activities measured in cell extracts of the B strains were comparable with those observed in NP2 and NP910212, which are wild type for argF.

We isolated from strain B4 a single plasmid species (pB4) with a molecular weight of 17.5 × 106 measured by agarose gel electrophoresis and comparison with known molecular weight markers. Since Col E1 DNA has a molecular weight of 4.2 × 108 (4), an estimate of the size of the inserted fragment is 13.3 × 106 or 20 kilobases. This is in agreement with the average size of the sheared E. coli DNA fragments (9.9 × 106 ± 3.5 × 106) used in constructing the Clark-Carbon hybrid plasmid bank. It is also similar to the 22.2-kilobase chromosomal DNA insert measured in pB2 (20). The average size of the E. coli genetic map is 41 kilobases/min, so the inserted fragment in pB4 is estimated to have a genetic length of 0.49 min. Since strain B4 simultaneously acquired the ability to grow at 43.5°C and the pyrB marker, the length of the insert does not include enough DNA to code for additional known E. coli markers distal to valS or pyrB. The recently mapped regulatory gene sbaA is between argF and valS (2) and should therefore be included in the insert.

The valS gene was subcloned into pBR322 after partial EcoRI digestion of pB4. The DNA was used to transform temperature-sensitive E. coli CP790302. In vitro VRS-specific activities in these transformants were comparable with those measured in the B strains (N. J. Olson, Ph.D. thesis, Kansas State University, Manhattan, 1983). Skogman and Nilsson observed a total eightfold increase in VRS-specific activity in E. coli transformants when the valS gene was subcloned into pBR322 after partial Sau3A digestion of pB2 (20). Various increases in enzyme-specific activities have been observed by cloning the aiaS gene (10-fold)(19), glnS

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TABLE 1. Enzyme-specific activities in cell extracts of E. coli strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>VRS aminocaylation*</th>
<th>Aspartate carbamoyltransferase*</th>
<th>Ornithine carbamoyltransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>0.0120</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>B2</td>
<td>0.0130</td>
<td>0.64</td>
<td>1.33</td>
</tr>
<tr>
<td>B3</td>
<td>0.0167</td>
<td>0.50</td>
<td>1.75</td>
</tr>
<tr>
<td>B4</td>
<td>0.0170</td>
<td>0.41</td>
<td>0.67</td>
</tr>
<tr>
<td>NP2</td>
<td>0.0061</td>
<td>0.12</td>
<td>1.14</td>
</tr>
<tr>
<td>NP4</td>
<td>0.0046</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>NP910212</td>
<td>0</td>
<td>0.05</td>
<td>1.56</td>
</tr>
</tbody>
</table>

* Cell extracts were prepared from cultures grown at 37°C in morpholinepropanesulfonic acid TGYE broth medium (14).

** Cell extracts were prepared from cultures grown at 37°C in morpholinepropanesulfonic acid minimal medium plus tryptophan (14). Uracil was omitted to ensure derepressed conditions and maximum enzyme synthesis. Strain NP910212 (valS ts) pyrB was grown at 32°C in morpholinepropanesulfonic acid minimal medium plus tryptophan and uracil.

† ND, Not determined.

gene (15-fold)(8), and tyrS gene (100-fold)(3) into pBR322. This variation may suggest different types of aminocayl tRNA synthetase regulation.

E. coli GM49 contains only the genomic, wild-type valS allele and a normal ColE1 plasmid. Strains GM49 and B4 were infected with T4BC- at a multiplicity of 8. Samples were removed at various times after infection and assayed for virus-modified VRS activity by the criterion of urea stability (11, 13, 17). The VRS activity in GM49 (0.0061 umol/min per mg) was predictably and substantially modified by 10 min after infection (Fig. 1). In B4, however, with a higher VRS activity (0.0170 umol/min per mg), only 40% of the VRS molecules were converted to the modified, urea-resistant form. This amount of modified VRS activity (0.0068 umol/min per mg) did not substantially increase during the course of phage development. Inability to modify the full complement of VRS in B4 appeared to have no effect on the plating efficiency or plaque morphology of T4BC- at 25, 37, or 43.5°C (data not shown).

Expression of the vs gene appears to be regulated in the sense that production of the τ peptide is set to modify the amount of VRS that T4 encounters in a normal E. coli cell. This result is consistent with data obtained by Neidhardt and Earhart (15), who failed to detect additional modification capacity by mixing extracts of T4-infected and uninfected cells and assaying for in vitro modification. Similarly, we have failed to find free τ molecules in extracts prepared from restrictive infections of E. coli CP790302 with T4vs+.

Finally, although B4 bears a valS (ts) allele on the genome and presumably produces a temperature-sensitive VRS activity, the modification of which would be undetectable by in vitro activity measurements, we think it is not modified, because its modification reduces virus production in both E. coli NP910212 and CP790302 (18). As previously stated, virus production in B4 appears to be normal. The manner and position of the apparent regulation of the vs gene and synthesis of the peptide are currently under investigation in our laboratory.

We thank Thomas R. Johnson for technical assistance with the experiments measuring phage modification.

LITERATURE CITED


FIG. 1. Modification of VRS during T4 infection. Cultures of E. coli were infected with T4BC-. Samples were withdrawn at the indicated times after infection and assayed for valine aminoacylation activity before and after treatment with 4 M urea. Urea-resistant activity was converted to percent modification for strains GM49 (●) and B4 (○).