Valyl-tRNA Synthetase Modification-Dependent Restriction of Bacteriophage T4†

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A strain of Escherichia coli, CP 790302, severely restricts the growth of wild-type bacteriophage T4. In broth culture, most infections of single cells are abortive, although a few infected cells exhibit reduced burst sizes. In contrast, bacteriophage T4 mutants impaired in the ability to modify valyl-tRNA synthetase develop normally on this strain. Biochemical evidence indicates that the phage-modified valyl-tRNA synthetase in CP 790302 is different from that previously described. Although the enzyme is able to support normal protein synthesis, a disproportionate amount of phage structural protein (serum blocking power) fails to mature into particles of the appropriate density. The results with host strain CP 790302 are consistent with either a gratuitous inhibition of phage assembly by faulty modification or abrogation of an unknown role that valyl-tRNA synthetase might normally play in viral assembly.

Shortly after bacteriophage T4 infects Escherichia coli, the host valyl-tRNA synthetase is modified by the addition of a phage-coded peptide called τ (12). The phage gene vs at 56 kilobases on the standard T4 genetic map is responsible for this modification and presumably codes for the τ peptide (14). Bacteriophage T4 mutants that are deficient in the ability to modify the host synthetase grow well on typical E. coli laboratory strains. The biological role for modification is thus difficult to define.

We have nevertheless continued to study the valyl-tRNA synthetase modification because we feel that it might represent a unique kind of regulatory event to the bacteriophage T4, and that it might be a model for synthetase alterations in a number of biological systems (20).

Recent work in our laboratory has demonstrated that wild-type bacteriophage T4vs+ does not form plaques on a host strain of E. coli called CP 790302 (11). A variety of phage mutants deficient in modification of host valyl-tRNA synthetase, however, plaque normally on strain CP 790302. Previous genetic evidence had indicated that the valS0 and relA alleles in E. coli CP 790302 probably were responsible for this restriction phenomenon.

In this article, we present evidence that the inability of T4vs+ to form plaques on strain CP 790302 is due to a greatly reduced number of infectious centers, as well as to a reduced average burst size. In addition, the biochemical properties of the phage-modified valyl-tRNA synthetase in CP 790302 are different from those previously described for a T4-modified valyl-tRNA synthetase (19). Thus, we hypothesize that the novel valyl-tRNA synthetase, while continuing to aminoaacylate tRNAs and participate in protein synthesis, somehow interferes with viral development at a late stage in viral assembly; we do not know whether this postulated interference is direct or indirect.

MATERIALS AND METHODS

Bacteria and bacteriophage. Several strains of E. coli and bacteriophage T4 were employed in this study and are listed in Table 1. Cells were routinely grown in morpholinepro-

desulfonic acid TGYE medium prepared according to Neidhardt et al. (18). High-titer lysates of T4 were prepared on E. coli NP 4 as previously described (13), except that 20-ml lots were used. Lysates were titrated on strain NP 4 unless stated otherwise.

Preparation of cell-free extracts. Cell-free extracts were prepared as described previously (16), by sonication cultures on ice in phosphate buffer (0.006 M potassium phosphate, 0.006 M 2-mercaptoethanol [pH 7.2]). The protein concentration was determined by the method of Lowry et al. (10), and extracts were stored at 4°C; they were not used after 5 days.

Enzyme assays. The specific activity of valyl-tRNA synthetase aminoaacoylation was measured by attachment of [3H]valine to tRNA. Reaction mixtures contained 250 µg of tRNA, 100 µg of bovine serum albumin, 2 µmol of dipotassium ATP, 1 µmol of glutathione, 10 µmol of [2,3-3H]valine at 15 µCi/µmol, 5 µmol of KCl, 50 µmol of Tris base, and 5 µmol of MgCl₂, with a final pH of 7.2. A limiting amount of valyl-tRNA synthetase (cell-free extract) was added at 4°C to give a final volume of 0.5 ml. Blanks contained everything but enzyme. After 5 min of incubation at 37°C, the reaction was stopped by cooling quickly in an ice bath and adding 3 ml of cold 5% trichloroacetic acid. After 30 min, precipitates were collected on fiber glass filters and washed with 10 ml of 5% trichloroacetic acid and 5 ml of 67% ethanol. Filters were dried at 80°C for 30 min and counted in a Beckman LS-230 liquid scintillation counter with 5 ml of toluene base scintillation cocktail. One unit of synthetase specific activity is the amount of enzyme that catalyze the addition of valine to tRNA at the rate of 1 µmol/min under our assay conditions. Experiments measuring aminoaacoylation specific activity in CP 790302, when indicated, were performed as described above, except that a protective buffer containing 0.1 M Tris base, 0.001 M valine, 10% glycerol, and 4.2% 2-mercaptoethanol, with a final pH of 7.3, was used.

Serum blocking experiments. Serum blocking power was determined by using the standard endpoint assay of De Mars (6) and procedures thoughtfully provided by M. Snyder. Phage lysates were prepared by adding T4vs+ or T4vs2, at a multiplicity of infection (MOI) of 7, to 20 ml of E. coli NP 4 or CP 790302 broth cultures grown at 30°C to a density of 2 x 10⁶ cells per ml. After 90 min of incubation at 30°C with aeration, cells were lysed by the addition of chloroform. 
Lysate samples were incubated with T4 antiserum in serum blocking buffer at 48°C for 12 h to allow a complete reaction. The residual neutralizing activity in each sample was measured by adding a known concentration of purified (22) T4Vs+ tester phage and determining the inactivation after 48 min of incubation at 46°C. PFU produced on E. coli G(λ) by surviving tester phage in the lysate samples were compared with PFU produced by surviving tester phage in samples containing a known concentration of CsCl-banded T4 rII (rdf deletion). Tester phage (T4Vs+) form plaques on indicator strain G(λ), but T4 rII does not. Thus, serum blocking power concentrations in the lysates can be converted to phage equivalents by using standard curves that relate PFU produced by tester phage to phage equivalents of T4 rII. Standard curves were run in each experiment.

RESULTS

Restriction of T4Vs+ by CP 790302. Workers in our laboratory have demonstrated that E. coli CP 790302 is restrictive for T4Vs+ but permissive for T4Vs2 (11). A pair of E. coli strains (NF 536 and NF 537), which contain a different valS+ allele than CP 790302, permit T4Vs+ and vs- mutants to form plaques efficiently. Consequently, the restriction appears to be dependent upon the valS+ allele employed in CP 790302. We have verified this by P1vir transduction of valS+ into CP 790302 (11), as well as by crosses with a number of Hfr strains (P4X, K.L5, K.L16-99, and Hfr H). In all cases, T4Vs+ formed plaques on the valS+ recombinants at a high frequency. In contrast, his+, metB+, and pyrB+ recombinants, either alone or in combination, continued to restrict T4Vs+ plaque formation (data not shown). The importance of the relA allele to the restriction phenomenon is more ambiguous. Although we were unable to directly select relA+ recombinants either via P1vir transduction or Hfr crosses, a related strain, E. coli NP 910212, which contains the same valS+ allele as CP 790302 but is relA+, allowed formation of minute plaques with T4Vs+.

Infection and virus progeny production. Five minutes after T4Vs+ or T4Vs2 phage were added to actively growing cultures of E. coli CP 790302, approximately 80% of the phage were adsorbed, both cultures experienced a decrease in optical absorbance, and fewer than 1% of the bacteria survived either infection (data not shown). Adding T4Vs+ to lawns of CP 790302, however, failed to produce plaques, whereas T4Vs2 formed plaques efficiently on the E. coli strain (11). This phenomenon was further investigated by using single-cell burst and one-cell growth experiments.

When a culture of CP 790302 was infected with T4Vs+ and unadsorbed phage were neutralized with antiserum, plating infectious centers on the permissive host E. coli B (NP 4) resulted in approximately 25% (15 of 66) as many plaques as when the infection was conducted with T4Vs2. Ninety minutes after T4Vs2 addition to CP 790302, an average of more than 200 PFU per infectious center were observed. Fewer than 10 PFU per infectious center were produced after infection with T4Vs+ (Table 2). Chloroform addition to effect cell lysis did not increase the burst sizes. Furthermore, T4Vs+ phage produced on CP 790302 formed plaques with equal efficiency on CP 790302 and a standard indicator strain, NP-4 (data not shown).

Trans-dominant effect of infection with T4Vs+. Virus production was examined when T4Vs+ and T4Vs2, each at an MOI of 4, were added simultaneously to cultures of CP 790302. An 80% decrease in progeny virus production was observed at 90 min after this co-infection compared with virus production during T4Vs2 infection alone (Table 2).

Neidhardt and Earhart (19) reported the appearance of heat-stable valyl-tRNA synthetase activity in extracts of bacteriophage T4Vs+ - or T6-infected NP 29 cultures. The T-odd bacteriophage were examined similarly and lacked the ability to thermally stabilize the temperature-sensitive synthetase of NP 29. Their data suggest that modified enzyme is formed after bacteriophage T4 or T6 infection of E. coli. However, our results demonstrate that, unlike T4 infection, T6 and T7 infections of CP 790302 result in plaque formation (Table 2).

Bacteriophage macromolecular synthesis and assembly. The accumulation of macromolecules in CP 790302 cells during T4 infection was analyzed by measuring the incorporation of 3H-labeled precursors into trichloroacetic acid-precipitable material. The rate and amount of DNA, RNA, and protein

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>T4Bc+</td>
<td>This laboratory</td>
<td>Wild type; normal modification of VRS*</td>
</tr>
<tr>
<td>T4Vs1</td>
<td>This laboratory</td>
<td>Missense mutant; unusual modified VRS</td>
</tr>
<tr>
<td>T4Vs2</td>
<td>This laboratory</td>
<td>Amber mutant; no modified VRS</td>
</tr>
<tr>
<td>T4Vs2</td>
<td>This laboratory</td>
<td>Spontaneous revertant of T4Vs2; modified VRS</td>
</tr>
<tr>
<td>RF01</td>
<td>M. Snyder</td>
<td>rdf deletion</td>
</tr>
<tr>
<td>E. coli</td>
<td>This laboratory</td>
<td>Wild-type E. coli B; indicator strain</td>
</tr>
<tr>
<td>NP 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>This laboratory</td>
<td>Wild-type E. coli KB</td>
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<tr>
<td>NP 2</td>
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<td></td>
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<tr>
<td>E. coli</td>
<td>This laboratory</td>
<td>valS*</td>
</tr>
<tr>
<td>NP 29</td>
<td>G. Björk</td>
<td>F- pyrB his metB relA valS+ rpsL ampA (valS+ by P1 from NP 910212)</td>
</tr>
<tr>
<td>E. coli CP 790302</td>
<td>G. Björk</td>
<td>F- recA stra valS+ pyrB trpA</td>
</tr>
<tr>
<td>910212</td>
<td>M. Snyder</td>
<td>Lambda lysogen; restrictive host for T4rII</td>
</tr>
<tr>
<td>E. coli G(λ)</td>
<td>G. Björk</td>
<td>leu valS+ relA</td>
</tr>
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<td>E. coli NF 536</td>
<td>G. Björk</td>
<td>leu valS+ relA</td>
</tr>
<tr>
<td>E. coli NF 337</td>
<td>G. Björk</td>
<td>leu valS+ relA</td>
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* VRS, Valyl-tRNA synthetase.

<table>
<thead>
<tr>
<th>Infection</th>
<th>Virus produced per infectious center (PFU)</th>
<th>% Reduction</th>
</tr>
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<tbody>
<tr>
<td>T4Vs+</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>T4Vs2</td>
<td>250</td>
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</tr>
<tr>
<td>T4Vs+ plus T4Vs2</td>
<td>57</td>
<td>80</td>
</tr>
<tr>
<td>T6</td>
<td>139</td>
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<tr>
<td>T4Vs+ plus T6</td>
<td>19</td>
<td>86</td>
</tr>
<tr>
<td>T4Vs2 plus T6</td>
<td>340</td>
<td>0</td>
</tr>
<tr>
<td>T7</td>
<td>119</td>
<td></td>
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</table>

* Cells were grown at 30°C to a density of 5 × 10^8 cells per ml, and identical cultures were infected with one or more strains of virus each at a MOI of 4. After 10 min, unadsorbed phage were inactivated with phage antiserum. Five minutes later, each culture was diluted and plated on wild-type indicator strain E. coli NP 4 to determine infectious centers. Cultures were diluted, and after 90 min, samples were removed and plated on the indicator strain. Experimental details were described by Adams (1).

* The data are expressed as the percent reduction in virus produced (per infectious center) during mixed infection compared with the corresponding single infection with T4Vs2 or T6.
accumulated during restrictive infection (T4v5+, T4v5s2RF01) was comparable to that measured during infections in which virus production was normal (T4v5s2, T4v5x1) (data not shown).

In additional experiments, viral proteins were pulse-labeled (under conditions that diminished labeling of host proteins) and analyzed by electrophoresis in sodium dodecyl sulfate and by autoradiography (2, 3). A pair-wise comparison of proteins from cultures of CP 790302 infected with T4v58 or T4v5s2 revealed no major differences in autoradiographic intensities. The temporal appearance of proteins was similar in both infections (data not shown).

Phage antigen synthesis was determined by measuring serum blocking power in lysates derived from E. coli cultures infected with T4v58 or T4v5x2. The ratio of PFU to serum blocking power measured in the lysate of T4v58-infected CP 790302 (2.4 \times 10^8 PFU/3.3 \times 10^4 serum blocking power [sbp] = 7.2%) is only one-fifth the ratio measured in the lysate of T4v58-infected CP 790302 (2.2 \times 10^8 PFU/6.0 \times 10^3 sbp = 36.5%). Although infectious virus concentration was low in the restrictive infection, the lysate contained 55% as much serum blocking power as was measured in the lysate from the permissive infection.

Lysates of CP 790302 cultures infected with T4v58 or T4v5s2 were sedimented on cesium chloride density gradients (Fig. 1). Although two peaks of serum blocking power were observed for both lysates, a greater proportion of the material derived from the permissive infection sedimented at a density typical of assembled phage particles. Plaque assays verified the presence of infectious virus. Most of the material from the nonpermissive infection sedimented at the density of protein. These studies suggest that phage maturation in T4v58-infected CP 790302 cells is restricted at the position of virus assembly. The following experiments were done in an attempt to identify the cause of the restriction.

Aminoacylated tRNA levels. The in vivo levels of aminoacylated tRNA were measured by the peridate method (5). The fraction of total tRNA^am that was aminoacylated was 87% in uninfected, 80% in T4v58-infected, and 72% in T4v5s2-infected cultures of CP 790302. The high level of charging of tRNA^am in each culture is consistent with aminoacylation levels reported by Comer and Neidhardt (5), who used cultures of uninfected and T4v58-infected E. coli B.

Protein synthesis and virus production at high temperature in T4-infected cultures. Table 3 compares protein synthesis in uninfected and T4v58-infected cultures of CP 790302, NP 910212, and NP 29 at 30°C and when the cells were shifted to 43.5°C. The three uninfected, temperature-sensitive strains were unable to synthesize protein at 43.5°C because they contained a thermolabile valyl-tRNA synthetase. Thermal stabilization of the temperature-sensitive enzyme occurred after T4 infection of NP 29, and protein synthesis continued when the infected cells were shifted to 43.5°C. In contrast, protein synthesis remained depressed at 43.5°C in T4v58-infected cultures of CP 790302 and NP 910212. These E. coli strains harbor the same valS allele, which is different from that in NP 29 (8, 11).

These data are consistent with data obtained by comparing average burst sizes on these strains (Table 3). Virus production in T4v58-infected NP 29 cells was the same at both 30 and 43.5°C. However, T4v58-infected NP 910212 produced progeny virus at 30°C but not at 43.5°C.

Properties of valyl-tRNA synthetase in uninfected and T4v58-infected temperature-sensitive and wild-type E. coli strains. We examined valyl-tRNA synthetase activity in extracts of uninfected and T4v58-infected CP 790302 cells to determine whether the host enzyme was actually modified. Extracts of uninfected, temperature-sensitive cells are de-
absence of a high-molecular-weight complex in infected CP 790302 further implies that modification after T4vs⁻ infection of these cells is unusual, and that, in turn, leads to an altered interaction of the modified synthetase with tRNA.

**DISCUSSION**

A previous report from this laboratory demonstrated that bacteriophage T4 with the wild-type vs gene is unable to form plaques at 30°C on E. coli CP 790302 (11). Phage with various amber, missense, and deletion mutations in the vs gene plate efficiently on this strain. The restriction is attributable to infectious centers that exhibit a dramatic reduction in burst size or fail to produce even a single infectious virus particle.

Despite differences in the production of PFU, the accumulation of DNA, RNA, and protein in the two infections is similar as measured by precursor accumulation. The kind of protein synthesized also appears to be similar in the restrictive and permissive infections; their temporal appearance is also the same. However, although substantial amounts of serum blocking power are synthesized in the restrictive infection, only a small fraction is assembled into infectious virus particles. The remainder sediment with a density of protein on CsCl gradients. These results indicate, therefore, that virus maturation is blocked at some position before complete assembly.

Interestingly, bacteriophage T6 forms plaques on CP 790302. Since the phage is thought to modify valyl-tRNA synthetase, and since simultaneous infection with T4vs2 (Table 2) does not result in substantially reduced burst sizes, then the inference can be drawn that the τ peptide in this T-even phage is structurally different from the one in bacteriophage T4. This conclusion is analogous to the observations of Moen et al. (15) that bacteriophage T4 and T6 specify a different repertoire of tRNA molecules. The ability of T7 to plate on CP 790302 is consistent with the observation of Neidhardt and Earhart (19) that the T-odd phage do not modify E. coli valyl-tRNA synthetase. Thus, they are phenotypically like T4vs2.

Earlier studies in this laboratory (17) had indicated that, during modification of the valyl-tRNA synthetase in E. coli, the τ peptide is buried in the cleft between the two globular domains of the enzyme. The aberrant modification of the enzyme in CP 790302 described in this paper may produce an enzyme with a different conformation and, therefore, an altered interaction with other elements in the infected cell. The amino acid composition and molecular weight of the τ peptide (16) are similar to those of a number of nucleic acid binding proteins, such as histone H2A (9) and the cro protein of bacteriophage lambda (21).

That the modified enzyme from T4vs⁻ infected CP 790302 fails to sediment as a high-molecular-weight complex on sucrose density gradients is one demonstrable example of an altered interaction with a cellular component. This alteration, however, was not detectable by any change in the overall in vivo charging level of tRNAval in these cells. Our studies do not reveal more subtle alterations that may exist in the charging of different valyl-tRNA species or in the relative rates of tRNA charging.

The precise manner in which valyl-tRNA synthetase modification arrests virus development in CP 790302 is not understood at this time. The unusual modification apparently occurs during T4vs⁻ infection of NP 910212, but tiny plaques are produced presumably because the stringent response in this strain somehow reverses the restriction.

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**FIG. 2.** Sucrose density gradient centrifugation of valyl-tRNA synthetases. E. coli cultures grown at 30°C were left uninfected or were infected with T4 vs⁺ at an MOI of 7 for 10 min. Cell-free extracts were prepared from the cultures, and 0.2 ml of each preparation was layered separately onto linear 5 to 20% sucrose density gradients. These were centrifuged in an SW50.1 rotor at 4°C for 10 h at 40,000 rpm. The gradients were collected from the bottom, and alternate fractions were assayed for specific activity of valyl-tRNA synthetase aminoacylation. Closed squares represent arginyl-tRNA synthetase specific activity, used as a marker in the assay. The high-molecular-weight form of the modified enzyme is due to its association with tRNA.
LITERATURE CITED


