An Effect of Fluorophenylalanine on MS-2 RNA Replicase[1] (36309)

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The addition of 10 μg of fluorophenylalanine per ml during infection of *Escherichia coli* C-3000 with bacteriophage MS-2 in a chemically defined medium decreased progeny production 100-fold (1). Radioactive fluorophenylalanine was incorporated into the coat protein; two of the four phenylalanyl residues of the coat protein were accessible to fluorophenylalanine. More noninfectious particles and phage with reduced adsorption characteristics were produced. Modification of coat protein structure through fluorophenylalanine incorporation could account for these results or fluorophenylalanine could be incorporated into the RNA replicase and change its properties. The coat protein regulates the synthesis of RNA replicase (2). Therefore, the experiments reported in this paper were done to examine both the synthesis and biological activity of the RNA produced in infected cells growing in medium containing fluorophenylalanine. Incorporation of ^{32}P into progeny RNA was used to determine the synthesis of RNA and protoplast assays of infectious RNA which bypasses any involvement of the viral coat protein determined the functionality of the viral RNA.

Materials and Methods. ^{32}P-Orthophosphate (carrier free) was obtained from International Chemical and Nuclear Corporation, Irvine, CA. The synthetic medium 3XD described by Fraser and Jerrel (3) was used with the substitution of the amino acids and the supplementation of the medium with the vitamin mixture of Henderson and Snell (4). Growth procedures have been described previously (1).

The preparation and purification of ^{32}P-labeled MS2 and the extraction of RNA was as described by Godson and Sinsheimer (5). The preparation of protoplasts and the assay of infectious RNA was as described by Strauss and Sinsheimer (6). The procedure of Bishop, Claybrook, and Spiegelman (7) was used for electrophoresis of RNA on polyacrylamide gels which were scanned at 260 nm using a Gilford linear transport system.

Radioactivity of the gel slices was determined in a toluene based scintillation fluid containing 0.5% PPO (2,5-diphenyloxazole) and 0.01% POPOP (*p*-bis-2-(5-phenyloxazole)benzene) using a Packard liquid scintillation spectrometer.

Bacteriophage MS2 and its host *E. coli* Hfr C-3000 were kindly provided by A. J. Clark, University of California, Berkeley.

Results. The parental RNA of the RNA phages functions in the form of two replicative intermediates (8), but is not incorporated in detectable amounts into progeny (9, 10). To ascertain the effect of fluorophenylalanine on parental RNA conversion, MS2 labeled with ^{32}P in the RNA was used to infect host *E. coli* cells incubated with phenylalanine or fluorophenylalanine. After 45 min the RNA was extracted with phenol and analyzed by polyacrylamide gel electrophoresis. The results of a typical experiment (each experiment was duplicated) showed that in the phenylalanine-containing medium 75% of the parental RNA was degraded into low molecular weight forms while in the presence of fluorophenylalanine over 90% of the RNA

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remained as high molecular weight RNA. At shorter time intervals smaller amounts of the parental RNA had been converted to lower molecular weight forms. Thus in the presence of fluorophenylalanine the degradation of parental RNA is retarded.

The overall effect of fluorophenylalanine on the synthesis of phage RNA has been determined by measuring ^{32}P incorporation from phosphate into phage RNA produced in medium containing either phenylalanine or fluorophenylalanine. RNA was extracted using phenol and subjected to polyacrylamide gel electrophoresis. There was a slight decrease in the radioactivity incorporated into RNA migrating as whole phage RNA; a typical experiment showed 256,000 cpm in the presence of phenylalanine and 230,000 cpm in the presence of fluorophenylalanine. Qualitatively, fluorophenylalanine did not greatly reduce the synthesis of progeny RNA, but electrophoresis does not measure the fidelity of RNA produced.

The biological activity of the RNA can be measured by determining its infectivity in an infectious RNA assay. Samples of phage synthesized in the presence of phenylalanine or fluorophenylalanine during a 60 min incubation were isolated. The RNA was obtained from the purified phage and its biological activity determined in the protoplasts assay for infectious RNA. The amount of RNA isolated from each preparation was determined by absorbance measurements at 257 nm. When this value is divided into the number of phage progeny produced during the infectious RNA assay from a given sample of RNA, the number obtained reflects the "specific infectivity" of the RNA. Table I shows a 20-fold greater specific infectivity for RNA extracted from phage produced in the phenylalanine containing culture than was produced under the same conditions with fluorophenylalanine present in the culture medium. This experiment was done three times and equivalent reduction found in both the infectivity of the whole phage and in the isolated RNA. Thus, the biological activity of RNA synthesized in the presence of fluorophenylalanine is reduced.

TABLE I. Specific Infectivity of RNA Isolated from MS2 Grown in the Presence of Phenylalanine and Fluorophenylalanine.[a]

Type of RNA	Specific infectivity (pfu/A_{257})
Phenylalanine grown	7.8×10^{14}
Fluorophenylalanine grown	4.0×10^{13}

[a] Two 50 ml cultures of *E. coli* C-3000 were grown in 3XD medium to an absorbance of 0.12 at 630 nm. The amino acids, phenylalanine and fluorophenylalanine, were added at a concentration of 10 μg/ml concomitant with phage infection at a multiplicity of 10. The incubation was continued for 60 min. Bacteria and debris were removed by centrifugation at 10,000*g* for 20 min, and 17 g $(NH_4)_2SO_4$ was added per 50 ml of supernatant solution. After one hour at 4°, the precipitate, containing most of the phage particles, was collected by centrifugation, suspended in 1 ml of TE buffer (0.01 *M* Tris buffer pH 7.5 and 0.01 *M* EDTA) and dialyzed overnight against the same buffer. The dialysate was then layered on a 5–20% linear gradient of sucrose containing TE buffer and centrifuged for 5 hr at 25,000 rpm in a Spinco SW25 rotor. The peak fractions (A_{260}) were pooled and dialyzed against 0.15 *M* sodium acetate. The RNA was isolated from purified phage by phenol extraction and diluted to a concentration of 1×10^{10} molecules per ml as determined by absorbancy measurements. To infect protoplasts with RNA 0.4 ml of protoplast stock solution was added to 0.4 ml RNA solution in 0.05 *M* Tris buffer (pH 7.0). After 30 sec at 37°, 3.2 ml of PAM medium (6) at 37° was added with mixing. The infected protoplasts were incubated at 37° for 150 min and then opened by freezing and thawing three times. The resulting phage was titered.

Discussion. Hummler and Wecker (11) found structurally altered poliovirus particles produced by fluorophenylalanine incorporation; the particles retained the gross morphology, diameter, and shape of mature virus, but the capsid was permeable to phosphotungstate and ribonuclease. Such changes were not observed with fluorophenylalanine containing MS2 (1).

The normal population of the RNA phages contain only about 15% infectious particles in the crude lysate (12). The proportion of noninfectious particles in a preparation has been increased by several methods. The series of suppressor mutations studied by Zinder's group (13) produce defective parti-

cles and the lesion may be in different parts of the replicative cycle (14). Thus su3 mutants (15) produce less RNA replicase while su11 mutants produce excessive amounts of the replicase. The su3 group of mutants have a modification in the cistron directing synthesis of the coat protein (16). The coat protein regulates *in vitro* translation of non-coat protein genes and represses at the level of transcription (17). Fluorophenylalanine incorporation into the coat protein could disturb the regulation of the replication cycle and this could account for the previous observation (1). Herrmann, Schubert, and Rudolph (18) have shown that empty shell envelopes are formed in the absence of viral RNA; however, the experiment in Table I showed that fluorophenylalanine reduced the specific infectivity of the RNA and not just the quantity produced.

Defective particles may have modifications in their RNA. Growth of MS2 in the presence of 5-fluorouracil reduces specific infectivity 80% (19) and two classes of progeny are produced. Both contain fluorouracil and the lighter one contains less RNA, is noninfectious, does not adsorb, and has a normal coat protein (20). About 2/3 of the 5′ end of the RNA molecule is present and the RNA is more active in *in vitro* coat protein synthesis but RNA replicase is not formed (21).

While incorporation of the fluorophenylalanine into the maturation protein could be responsible for the decreased adsorption to host bacteria (1), this could not modify the infectiousness of the isolated RNA as observed in Table I. Likewise, incorporation of fluorophenylalanine into coat protein could modify the quantity of RNA made but not its infectivity. A possible mechanism for production of RNA with decreased infectivity would be that incorporation of fluorophenylalanine into the RNA replicase produces an enzyme more likely to make errors in the fidelity of RNA production.

Speyer, Karam, and Lenny (22) demonstrated that DNA polymerase has a role in base selection during replication since certain T4 mutants of gene 43 are very mutagenic (a mutator gene). Thus, changes in the enzyme can produce changes in the DNA produced. This might be the situation with the RNA phages.

Summary. Fluorophenylalanine decreases the degradation of parental RNA and only slightly reduces the incorporation of $^{32}P_i$ into phage RNA. The infectivity of the RNA produced is markedly reduced suggesting that fluorophenylalanine incorporation into the RNA replicase may produce an enzyme more likely to make errors in the fidelity of RNA production.

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