NOTES

Identity of Genes Coding for Soluble and Structural Dihydrofolate Reductases in Bacteriophage T4

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By recombination between bacteriophage T4 wh2, a dihydrofolate reductaseless mutant, and T6, I have prepared T4 whT6, a T4 strain which codes for the T6-specific soluble dihydrofolate reductase. This strain has the heat sensitivity of T6, not T4, which provides direct evidence that the wh gene codes for both the soluble dihydrofolate reductase and the structural dihydrofolate reductase which is a constituent of T-even phage tail plates.

The enzyme dihydrofolate reductase is a structural element of the tail plates of T-even bacteriophages, as shown in recent work of Kozloff et al., although its precise function has not yet been determined (4). T-even phage genomes code for a soluble dihydrofolate reductase, which helps to maintain the increased rate of thymidylate biosynthesis which is observed after T-even phage infection (1, 5-7). Since it is unusual to find an enzyme which plays two such diverse roles, one might well ask whether the structural dihydrofolate reductase is the same molecular entity as the soluble dihydrofolate reductase. The following results of Kozloff et al. (4) suggest that the two enzymes are the same. (i) T4 wh mutants, defective in the structural gene for the soluble reductase, show no enzyme activity in purified tail plates. (ii) Infectivity of phage T4D is lost upon incubation with either reduced nicotinamide adenine dinucleotide phosphate (NADPH) or reduced nicotinamide adenine dinucleotide (NADH). According to Kozloff et al., this represents reduction in situ of dihydropteroyl-hexaglutamate, a pteridine compound present in tail plates and which is presumably bound therein by the structural reductase (3, 4). In line with this idea, the relative rates of phage inactivation by NADH and NADPH are similar to the relative activities of the reduced pyridine nucleotides as substrates for the soluble reductase.

On the other hand, several observations of Kozloff et al. suggest that the structural reductase is quite different from the soluble reductase. (i) NADPH inactivation is reversible by nicotinamide adenine dinucleotide phosphate (NADP^+), suggesting that reduction of the bound pteridine in situ is reversible, although we have been unable to demonstrate significant reversibility of the soluble enzyme in solution (8). (ii) T4 wh1, a mutant unable to induce the soluble reductase, is inactivated by NADPH, which is difficult to explain if phage inactivation results from catalytic activity of the structural enzyme in situ. (iii) T4D infectivity is lost also upon incubation with NADP^+, which Kozloff et al. interpret in terms of oxidation in situ of the dihydropteridine; however, the soluble enzyme has no activity as a folate reductase, in either direction (8). (iv) Inactivation of T4D is more rapid at pH 5.0 than at pH 7.3; whereas dihydrofolate reductase of animal origin is optimally active at acidic pH values, the soluble enzyme has essentially no activity at pH 5 (5).

In this communication, I describe an approach to the following question: are the soluble and structural dihydrofolate reductases coded for by the same gene? If they are, then this should establish beyond reasonable doubt that they are, in fact, the same molecular entity. The approach derives from an observation of Kozloff et al. (4): an unsuppressed wh amber mutant phage had much greater heat stability than either the suppressed mutant or wild-type T4D. Since the dihydrofolate reductase of Escherichia coli is much more heat-stable than the phage-coded soluble reductases (8), Kozloff et al. cited this as evidence that, in the absence of a phage-coded reductase, a developing phage can incorporate the corresponding host cell enzyme into its tail plate. Regardless of the validity of this interpretation,
the observation does suggest that the heat stability of a phage particle is determined, at least in part, by the heat stability of its structural dihydrofolate reductase.

The wh gene in T4 codes for soluble dihydrofolate reductase (1). The soluble reductases of T-even phages differ considerably in heat stability, with the T4 enzyme being the most stable and the T6 enzyme being the most labile (5). If the wh gene codes for a structural element, then it might well affect the heat stability of a phage particle. On the other hand, if the wh gene product is not a structural element, then the wh gene should have no control over the heat stability of a phage particle.

As a specific way of posing the above question experimentally, I prepared, by recombination between T4 and T6 followed by extensive backcrossing against T4, a T4 phage which codes for a T6 soluble reductase. If the soluble reductase is the same as the structural reductase, then the heat stability of this T4 whT6 recombinant should be characteristic of T6, rather than T4.

A rationale for preparation of the recombinant is presented in Fig. 1. T4 wh2, a dihydrofolate reductaseless mutant isolated in this laboratory (6), and wild-type T6, originally obtained from Lloyd Kozloff, were used to infect mixedly E. coli B in nutrient broth, at multiplicities of nine T4 and one T6 particle per cell. The resultant lysate was mixed twice in succession with excessive amounts of E. coli B/3, 4, 7, a T4-resistant strain. Most phages with the attachment specificity of T6 are removed by this step, whereas phages with the attachment specificity of T4 (i.e., gene 37) remain unadsorbed. A portion of the lysate was used to infect E. coli S/6, a T6-resistant strain, to remove any phenotypically mixed particles (e.g., T4 phage with a T4 wh gene but bearing a T6 structural reductase). The resultant lysate was plated out under the selective conditions of Hall, Tessman, and Karlström (2), and a non-halo-forming plaque was picked (wh mutants form plaques surrounded by white halos under these conditions). A stock prepared from this plaque

FIG. 1. Summary of the isolation of T4 whT6.

FIG. 2. Stability of soluble dihydrofolate reductases and phage infectivity. (A) Heat inactivation of dihydrofolate reductase preparations, determined as described previously (5). (B) Inactivation of dihydrofolate reductase preparations by urea, also determined as described previously (5). (C) Heat inactivation of phage particles. Single-plaque isolates of T6, T4BO1, and T4 whT6 were diluted in 0.02 M sodium phosphate buffer (pH 7.0) to titers of about 10⁶ per ml and placed in a water bath at 60 C for the indicated periods of time before dilution and plating, with E. coli B as the plating host and 37 C as the temperature for incubation of the plates.
was subjected to four successive backcrossings against T4 wh2, at input ratios of one recombinant to nine T4 wh2. After each backcross, a non-wh phage stock was selected for subsequent manipulation. From the number of crosses and the input ratios used, one can calculate that the average amount of T6 genetic material remaining after the final backcross (exclusive of the whT6 gene which was selected for) is but one part in 10^6. Since T4 probably contains no more than 200 genes, it seems safe to conclude that all extraneous T6 genetic material, exclusive of the wh gene, has been removed in the final T4 whT6 preparation. Since the original isolation of T4 wh2 involved four backcrosses against T4BO1, we can state that T4 whT6 is genetically identical to T4 BO1, except for the wh gene.

Figure 2 (A and B) shows that the soluble reductase induced by T4 whT6 is the T6 enzyme, as shown by the criteria of heat stability and inactivation by urea. Figure 2C shows that phage particles of T4 whT6 are inactivated at 60°C at a rate identical to that of T6 and quite distinct from that of T4. Thus, the wh gene does affect the heat stability of phage particles, a clear indication that both soluble and structural dihydrofolate reductases are coded for by the same gene. Unexpectedly, the relative heat stabilities of T4 and T6 particles are the reverse of what might be predicted from the relative stabilities of the soluble reductases. This implies that the properties of the enzyme are changed considerably when it is bound into the rigidly organized matrix of the tail plate. The apparent discrepancies between the soluble and structural reductases, outlined in the second paragraph of this note, seem to be further manifestations of the same effect. Whatever the nature of these changes, it is clear that the structural and soluble reductases are both coded for by the wh gene.

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LITERATURE CITED