REPLACEMENT OF THYMINE BY 5-ETHYLURACIL IN BACTERIOPHAGE DNA

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It has previously been shown that, under appropriate conditions, some (up to 18%) of the thymine residues in the DNA of thymine-dependent bacterial strains may be replaced by 5-ethyluracil (Piechowska & Shugar, 1965). Interest attaches to the use of this base analogue of thymine in that the 5-ethyl substituent, apart from its increased bulk, affects the pK_a of the base or nucleoside to only a small extent (Swierkowski & Shugar, 1966), and its base-pairing properties would not be expected to be altered, as is suspected to be the case for 5-halogeno uracils and other base analogues. The synthesis of 5-ethyldeoxyuridine (Swierkowski & Shugar, 1966) has now made it possible to study the incorporation of this base analogue into bacteriophage DNA, and to examine some of the properties of the resulting intact phage and its isolated DNA.

Initial trials were carried out with phage T3, the host cell being the thymine-dependent E. coli CR-34 strain. Phage synthesis in the presence of EtUdR[x] and Etu was examined in the following manner: The host cells were cultivated on a glycerol medium (Fraser & Jerel, 1953), in the presence of 10 μg/ml. thymine, to an optical density of 0.20 (1-2 x 10^8

[x] The following abbreviations are used in this text: Etu, 5-ethyluracil; EtU, 5-ethyluridine; EtUdR, 5-ethyldeoxyuridine; T, thymine; TdR, thymidine; Bru, 5-bromouracil; BrUdR, 5-bromodeoxyuridine; FUdR, 5-fluorodeoxyuridine.

cells/ml.). The cells were collected by centrifugation, washed twice with thymine-free medium, and suspended in freshly-warmed medium to an optical density of 0.2. The suspension was divided into five portions, one of which served as control; to the other four were added thymine or Etu at concentrations of 10 μg/ml. and EtUdR or TdR at concentrations of 20 μg/ml. Following 10 mins. incubation at 37° on a shaker, all five samples were innoculated with phage T3 at an infection multiplicity of 0.01. After 5 minutes adsorption without shaking, incubation was continued on a shaker at 37°, and samples withdrawn at various time intervals for measurements of phage titre.

The results of a typical experiment are illustrated in Fig. 1, and show that EtUdR is capable of supporting phage T3 synthesis in the

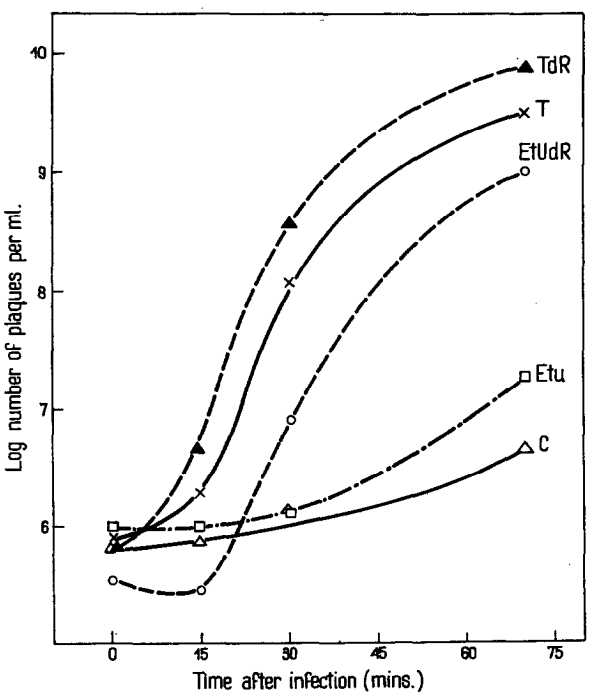

Fig. 1: Synthesis of bacteriophage T3 on E. coli CR-34 in the presence of thymine and thymidine, and the corresponding analogues 5-ethyluracil and 5-ethyldeoxyuridine (further details in text).

absence of thymine or TdR, albeit not as effectively as these latter; Etu is effective only to a small extent. Similar attempts were carried out with other T-phages, viz. T2, T4 and T7. Phage T7 was also found to be capable of utilizing EtUdR, but more effectively than phage T3, the yield of phage being almost identical to that obtained in the presence of thymine or TdR. The infectivity of both phages cultivated in the presence of EtUdR was similar to that of normal phage.

By contrast the T-even phages were capable of utilizing EtUdR only to a moderate extent. T-even phage synthesis was conducted in the presence of FUdR to inhibit thymidylate synthetase, which is known to be induced by T-even phages in thymine-dependent strains (Barner & Cohen, 1954). Under these conditions, phage T2 and T4 synthesis on E. coli CR-34 was relatively feeble as compared to T3 (also in presence of FUdR). Under the same conditions BrUdR supported T2 synthesis almost as effectively as TdR. It is therefore clear that the ability to utilize EtUdR differs between T2 and T3 which, as is known, exhibit appreciably different pathways of DNA synthesis (Stent, 1963).

Attempts to promote synthesis of phages T2 and T3 with EtUdR and E. coli B as host (in the presence of FUdR) were entirely negative, showing that the ability to utilize EtUdR is dependent also on the host cell.

The results for phage T3 synthesis in the presence of EtUdR on E. coli CR-34 indicated appreciable incorporation of this analogue. Phage synthesis was therefore carried out under these conditions on a larger scale and the phage purified and concentrated as described by Miyazawa & Thomas (1965) and collected by centrifugation at 60,000 g. Phage DNA was then isolated by the phenol method according to Frenkel (1963). The DNA was hydrolyzed in concentrated formic acid (Wyatt, 1955) and the bases separated by two-dimensional thin-layer chromatography on MN-300G cellulose as described by Randerath (1965), using the following solvents:

I, methanol-conc. HCl-H_2O (70:20:10, v/v/v); II, butanol-H_2O-conc. NH_4OH (87:8:5, v/v/v), which gave an excellent separation of 5-ethyluracil from thymine. The bases were eluted as described by Randerath & Randerath, 1965) with 0.02N HCl and concentrations determined spectrally. The results demonstrated that 66% of the thymine residues were replaced by Etu.

For purposes of comparison, phage T3 was also prepared on a larger scale in the presence of BrUdR and the resulting DNA isolated and hydrolyzed in the same manner. Complete separation of Bru in this case required the use of a third solvent system, ethyl acetate-H_2O-formic acid (60:35:5, v/v/v). In this way it was shown that replacement of thymine by Bru occured to the extent of 61 mole %.

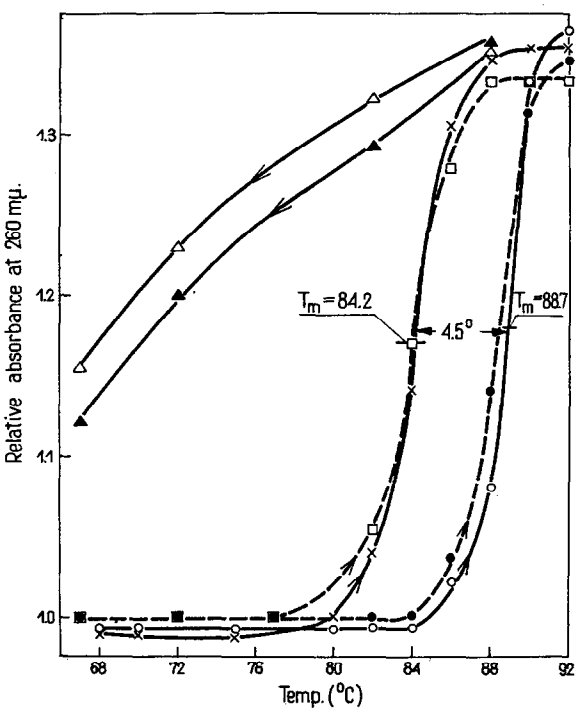

Fig. 2: Thermal profiles in SSC solvent of phage T3 DNA:
o-o-o-o, heating profile for normal DNA.
x-x-x-x, heating profile for DNA containing 66% Etu residues in place of thymine.
▲-▲-▲-▲, cooling profile for normal DNA.
Δ-Δ-Δ-Δ, cooling profile for Etu-DNA.
●-●-●-●, second heating profile for normal DNA.
□-□-□-□, second heating profile for Etu-DNA.

The temperature profiles of the Etu-DNA and Bru-DNA were then compared with those of normal phage T3 DNA, with the results shown in Fig. 2, for Etu-DNA and normal DNA, in SSC (0.15M NaCl + 0.015M sodium citrate). It will be seen that the temperature hyperchromicity of both DNA samples is the same, as are also the breadths of the profiles. But the T_m of Etu-DNA is 4.5° lower than that of the control, thus indicating a lower degree of stability. This decrease in stability was unaltered at a 10-fold lower salt concentration, and is in agreement with the lower stability of the twin-stranded poly-(A + EtU) as compared to poly-(A + rT) (Swierkowski & Shugar, 1966).

By contrast phage T3 Bru-DNA, in 0.1 SSC, exhibited a temperature profile with a T_m 2.8° higher than that of normal phage T3 DNA. With SSC as solvent this difference in T_m, and stability, disappeared, in agreement with reported findings on synthetic polynucleotide analogues (Inman & Baldwin, 1962), mammalian DNA (Kit & Hsu, 1961) and bacterial DNA (Szybalski & Menningman, 1962).

From Fig. 2 will be seen that at elevated temperatures Etu-DNA appears to renature more slowly than normal DNA during the cooling cycle. However, in separate experiments conducted under more optimal conditions, at 65°, this difference was considerably decreased. Renaturation was complete in all instances, as estimated from the reappearance of the original hypochromicity and, as shown in Fig. 2, from the fact that a second heating cycle gave an identical profile and T_m value. The behaviour of Bru-DNA was fully analogous. A comparison of the kinetics of renaturation of Etu-DNA and Bru-DNA with normal phage T3 DNA demonstrated that the rate for Etu-DNA was slightly less than that for normal DNA, while that for Bru-DNA was, in turn, slightly less than that for Etu-DNA. The small differences in rates of renaturation imply that the bulkier ethyl group does not introduce any appreciable steric hindrance to formation of a helical structure.

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