Deoxyuridine Triphosphate in Mammalian Cells*

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Short DNA fragments (Okazaki pieces), about 200 nucleotides in length, are formed during discontinuous DNA synthesis in mammalian cells. It has been suggested that some of the short fragments might be the result of excision of uracil from DNA.[1] With polyoma infected 3T6 cells it was shown that dUTP can replace dTTP during DNA synthesis, resulting first in the incorporation of uracil into DNA followed by excision of the pyrimidine by a glycosylase and cleavage of the strand by an endonuclease.[2] Furthermore, the drug hydroxyurea leads to an accumulation of short DNA fragments [3] and it seemed possible that this effect is the result of an increased intracellular dUTP pool. I have now analyzed the dUTP content of polyoma virus infected cells by a new method and determined the effect of hydroxyurea on the dUTP pool.

Appropriate cell extracts were first incubated with ^{14}C-dATP, 2 μM, poly d(A-T), 0.3 μg, and DNA polymerase I, 0.25 units, for 15 min at 37 °C as described by Lindberg and Skoog [4] for the assay of dTTP. The amount of ^{14}C incorporated into the d(A-T) polymer measures the sum of dTTP and dUTP added to the incubation mixture. Any incorporated uracil was excised with an excess of uracil glycosylase.[5] Alkaline hydrolysis with 0.3 M NaOH for 45 min at 37 °C then cleaved the sugar-phosphate backbone at the positions where uracil was present originally, resulting in the formation of short fragments of labeled poly d(A-T). The length of the fragments, determined by alkaline sucrose centrifugation, was an indirect measure of dUTP in the cell extract. From appropriate standard curves containing between 0.25 and 2 % of dUTP relative to dTTP the size of the dUTP pool could be determined.

With this method I have determined that cells infected with polyoma virus 26 h after infection (at the peak of DNA synthesis) contain amounts of dUTP corresponding to less than 0.5 % of the dTTP pool and that addition of hydroxyurea decreased this value (Table 1). These results do not support the hypothesis that a substantial part of Okazaki pieces formed either in the presence or absence of hydroxyurea originates from the incorporation of dUTP into DNA.

Table 1. Effect of hydroxyurea on dTTP and dUTP pools in polyoma virus infected 3T6 cells.

Time after addition of hydroxyurea (10 mM)	dTTP pool (pmol/μg of DNA)	dUTP pool % of dTTP	pmol/μg DNA
0	5.00	0.37	0.02
5	5.03	0.00	0.00
15	6.22	0.18	0.01
90	6.20	0.18	0.01

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