Inhibition of DNA Chain Growth by $\alpha$-$2'$-Deoxythioguanosine

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SUMMARY

Mecca lymphosarcoma cells were incubated with $[^{35}S]$-$\alpha$-$2'$-deoxythioguanosine for 8 hr and DNA was analyzed in alkaline sucrose gradients. $^{35}S$ radioactivity was found exclusively in a low-molecular-weight fraction. Pulse-chase experiments showed that $^{35}S$-containing DNA fragments formed during the pulse were not incorporated into high-molecular-weight DNA following the chase. These results, together with the previous observation that $[^{35}S]$-$\alpha$-$2'$-deoxythioguanosine was found predominantly in the terminal nucleoside position of DNA chains, suggested that $\alpha$-$2'$-deoxythioguanosine, once incorporated, terminates chain elongation.

Carcinostatic effects of the nucleoside analog $\alpha$-TGrD appear to be correlated with its incorporation into DNA (2, 3). Host toxicity of $\alpha$-TGrD is relatively low because this nucleoside is not phosphorylated to a significant level in bone marrow cells (5).

Aside from the potential value in cancer chemotherapy, $\alpha$-TGrD may offer a unique tool for the study of DNA replication. Previous observations show that $\alpha$-TGrD is incorporated predominantly into the terminal nucleoside position of DNA (2). This suggests that the incorporation of $\alpha$-TGrD may terminate the chain growth by preventing further addition of nucleotide residues. Replication of DNA in mammalian cells, as in microorganisms, has been shown to involve an initial synthesis of low-molecular-weight precursor molecules (replication fragments or "Okazaki fragments"), which are later joined by ligase activity (see Ref. 1). If $\alpha$-TGrD in fact terminates the chain elongation, the incorporated $\alpha$-TGrD will be found only in short oligonucleotides, which fail to form high-molecular-weight DNA. In the experiments reported below we tested this possibility by analyzing the size of DNA synthesized in the presence of $\alpha$-TGrD by centrifugation in alkaline sucrose density gradients.

Mecca lymphosarcoma cells were grown in ascitic form in AKR female mice by transplantation i.p. of $10^7$ cells/mouse at weekly intervals. The synthesis of DNA in control Mecca cells in vivo was tested by injecting $[^{3}H]$TdR into mice carrying 5-day-old Mecca cell growths. Thirty-two hr later the cells were withdrawn and DNA was analyzed in an alkaline sucrose gradient. The distribution of radioactivity corresponded to the bulk DNA (Chart 1A). In the experiment shown in Chart 1B, mice were injected with $[^{3}H]$TdR and $[^{35}S]$-$\alpha$-TGrD simultaneously 8 hr prior to harvesting of the cells. An additional injection of $[^{35}S]$-$\alpha$-TGrD was made 3 hr later to increase the level of incorporation of this analog. It can be seen that $^{35}S$ radioactivity was confined to a small-molecular-weight fraction. This is compatible with
the formation of short pieces of DNA chains terminated by [³⁵S]-a-TGdR. A small peak of ³H radioactivity in the slow-sedimenting region may represent [³H]Tdr incorporated into these fragments prior to the chain termination. The major part of ³H radioactivity, however, sedimented in the region of bulk DNA, indicating that the cellular capacity for DNA synthesis was not grossly affected by [³⁵S]-a-TGdR under the experimental conditions used.

In order to obtain further information on the nature of the slow-sedimenting fraction, pulse-chase experiments were performed. For this purpose, 5-day-old Mecca cell implants were incubated in vitro briefly (1 or 3 min) with either [³H]Tdr or [³⁵S]-a-TGdR, the medium was changed, and the incubation continued in the absence of the label. Alkali sucrose gradient analysis of DNA from the control cells that were incubated with [³H]Tdr showed that the radioactive peak was in the slow-sedimenting region after the pulse (Chart 2A) but was found in the region of bulk DNA after the chase (Chart 2B). Similar observations have been reported with various mammalian systems and as evidence for the discontinuous model of DNA synthesis, which involves an initial formation of short, replicative intermediates.

Pulse labeling of cells with [³⁵S]-a-TGdR also resulted in the formation of slow-sedimenting material (Chart 3A). However, the subsequent chase did not lead to a shift of the radioactivity peak to a faster-sedimenting fraction (Chart 3B), indicating that the short fragments containing a-TGdR are unable to link together to form high-molecular-weight DNA.

These results, together with the previous observation that a-TGdR is present predominantly in the terminal nucleoside position, show that a-TGdR is incorporated into short, nascent DNA fragments and terminates the chain growth. Since the incorporation of a-TGdR into DNA is observed only in those cells capable of phosphorylating this analog to the triphosphate level (3—5), the reaction is probably catalyzed by DNA polymerase. The failure to form additional phosphodiester bonds with the terminal a-TGdR is probably due to the α configuration of the sugar moiety, because λ anomer (β-2'-deoxythioguanosine) has been found to be incorporated into the internal, nucleotide positions of DNA (2). Thus slow-sedimenting material formed in the presence of α-TGdR is presumed to be incomplete replicative intermediates. Studies of these fragments may be of value in understanding the nature and process of formation of precursor molecules for DNA replication.

Several nucleotide analogs, such as 2',3'-dideoxy-TTP and 3'-amino-ATP are known to inhibit DNA synthesis catalyzed by DNA polymerase in vitro through a mechanism similar to that reported here (1). A particular interest in α-TGdR as an inhibitor for DNA synthesis is related to...
its metabolism in different cells, i.e., it is phosphorylated in certain tumors but not in normal bone marrow cells, thus exhibiting a selective toxicity toward these tumors (2–5).

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