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Brief Communication

Inhibition of DNA Chain Growth by α-2'-Deoxythioguanosine[1]

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SUMMARY

Mecca lymphosarcoma cells were incubated with [^{35}S]-α-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]-α-2'-deoxythioguanosine was found predominantly in the terminal nucleoside position of DNA chains, suggested that α-2'-deoxythioguanosine, once incorporated, terminates chain elongation.

Carcinostatic effects of the nucleoside analog α-TGdR[2] appear to be correlated with its incorporation into DNA (2, 3). Host toxicity of α-TGdR 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, α-TGdR may offer a unique tool for the study of DNA replication. Previous observations show that α-TGdR is incorporated predominantly into the terminal nucleoside position of DNA (2). This suggests that the incorporation of α-TGdR 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 α-TGdR in fact terminates the chain elongation, the incorporated α-TGdR will be found only in short oligonucleotides, which would 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 α-TGdR by centrifugation in alkali 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 Thirty-two hr later the cells were withdrawn and DNA was analyzed in an alkaline sucrose gradient.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 1*A*). In the experiment shown in Chart 1*B*, mice were injected with [^{3}H]TdR and [^{35}S]-α-TGdR simultaneously 8 hr prior to harvesting of the cells. An additional injection of [^{35}S]-α-TGdR 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

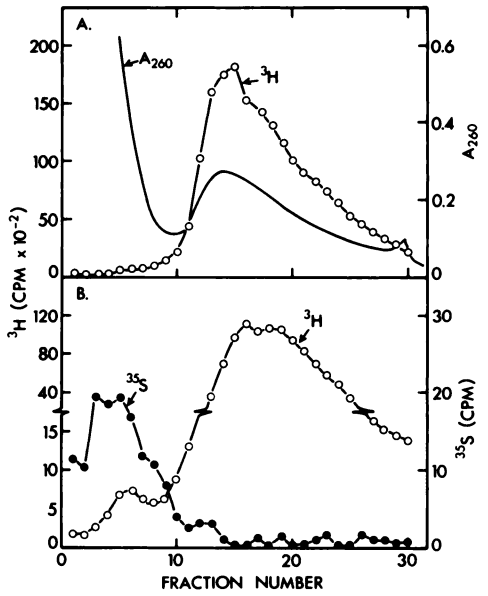

Chart 1. Incorporation of [^{3}H]TdR and [^{35}S]-α-TGdR into DNA of Mecca lymphosarcoma cells. In *A*, AKR female mice carrying 5-day-old Mecca lymphosarcoma cells were given injections of 20 μCi [^{3}H]TdR (360 mCi/mmole); 32 hr later the cells were removed, washed once with 0.15 M NaCl, and treated with 0.2 M NaOH-0.01 M EDTA (approximately 50 × 10^6 cells/ml) at 37° for 10 min. One ml was layered on a 5 to 20% sucrose gradient in 0.1 M NaOH-0.9 M NaCl and centrifuged in the Spinco SW25 rotor at 20,500 rpm for 15 hr at 4°. One-ml fractions were collected from the top of the tube. Cold 2 M HCl (1 ml) was added to each fraction. The precipitate that formed was collected onto glass fiber filters (Whatman GF) and the radioactivity was measured in a liquid scintillation counter. In *B*, the experimental conditions are the same as in *A*, except that mice were given injections of 20 μCi [^{3}H]TdR (360 mCi/mmole) and 2 μCi [^{35}S]-α-TGdR (0.8 mCi/mmole). Three hr later the same amount of [^{35}S]-α-TGdR was injected. Five hr later (8 hr from the 1st injection) the cells were collected and DNA was analyzed as described above. Sedimentation is from *left* to *right*.

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[2] The abbreviations used are: α-TGdR, α-2'-deoxythioguanosine; TdR, thymidine.

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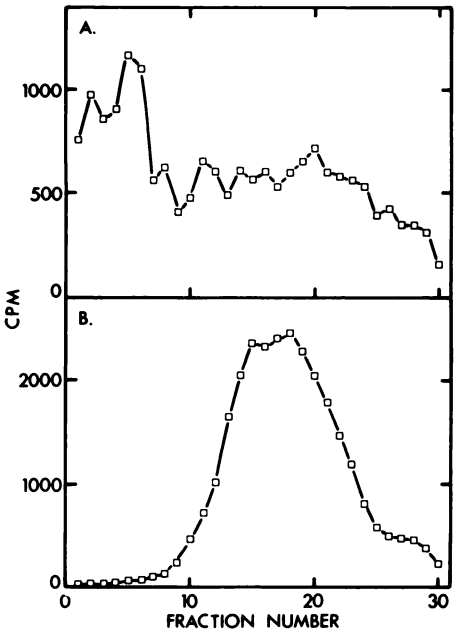

Chart 2. Sedimentation profiles of DNA pulse labeled with [^{3}H]TdR before and after chase. Mecca lymphosarcoma cells were withdrawn from mice 5 days after transplantation and washed once with 0.15 M NaCl. Cells (30×10^6) were incubated with 10^{-6} M [^{3}H]TdR (2 Ci/mmole) for 1 min at 37° in 10 ml Fisher's medium without serum. The culture was cooled in ice and divided into 2 equal portions. One portion of the cells was lysed in 0.7 ml 0.2 M NaOH-0.01 M EDTA, as described in the legend of Chart 1. The other was resuspended in 5 ml Fisher's medium without serum but supplemented with 10^{-6} M TdR and incubated at 37° for 10 min. The cells were spun down, washed once with Fisher's medium, and lysed in 0.7 ml 0.2 M NaOH-0.01 M EDTA. The lysates were analyzed by centrifugation in alkaline sucrose gradients, as described in the legend of Chart 1. Sedimentation is from *left* to *right*.

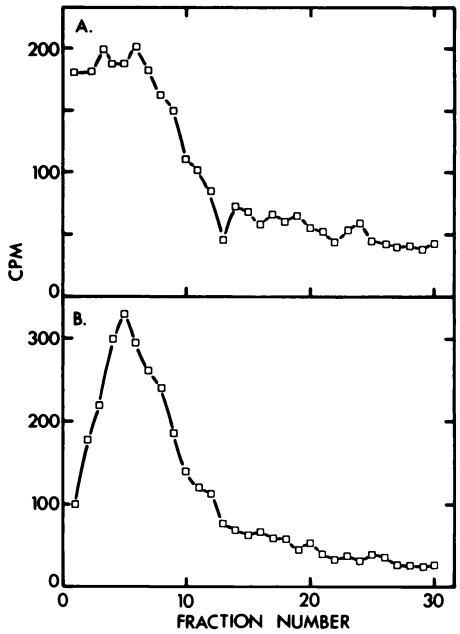

Chart 3. Sedimentation profiles of DNA pulse labeled with [^{35}S]-α-TGdR before and after chase. Cells were incubated with 10^{-6} M [^{35}S]-α-TGdR (4.75 mCi/mmole) as described in the legend of Chart 2, except that the number of cells was increased to 190×10^6, and pulse and chase periods were extended to 3 and 15 min, respectively, to ensure a significant amount of incorporation of the label into DNA. Sedimentation is from *left* to *right*.

the formation of short pieces of DNA chains terminated by [^{35}S]-α-TGdR. A small peak of ^{3}H radioactivity in the slow-sedimenting region may represent [^{3}H]TdR incorporated into these fragments prior to the chain termination. The major part of ^{3}H radioactivity, however, sedimented in the region of bulk DNA, indicating that the cellular capacity for DNA synthesis was not grossly affected by [^{35}S]-α-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 [^{3}H]TdR or [^{35}S]-α-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 [^{3}H]TdR showed that the radioactive peak was in the slow-sedimenting region after the pulse (Chart 2*A*) but was found in the region of bulk DNA after the chase (Chart 2*B*). Similar observations have been reported with various mammalian systems and taken as evidence for the discontinuous model of DNA synthesis, which involves an initial formation of short, replicative intermediates.

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

These results, together with the previous observation that α-TGdR is present predominantly in the terminal nucleoside position, show that α-TGdR is incorporated into short, nascent DNA fragments and terminates the chain growth. Since the incorporation of α-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 α-TGdR is probably due to the α configuration of the sugar moiety, because β 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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