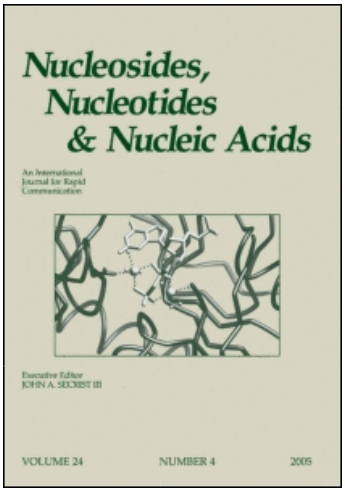

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Cytarabine-Induced Destabilization and Bending of a Model Okazaki Fragment

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CYTARABINE-INDUCED DESTABILIZATION AND BENDING OF A MODEL OKAZAKI FRAGMENT

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ABSTRACT: The effects of cytarabine on the structural and thermodynamic properties of an Okazaki fragment were investigated using UV hyperchromicity and 2D ^{1}H NMR. Cytarabine significantly decreased the stability of this model Okazaki fragment, decreasing the melting temperature from 46.8 °C to 42.4 °C at 1.33 x 10^{-5}M. Cytarabine also markedly increased the bend angle of the Okazaki fragment duplex from 20° to 42°. Changes to the structures and stabilities of Okazaki fragments may cause the biological effects of cytarabine.

Frequency of nuclear DNA replication concomitant with cell division is one of the principal distinguishing characteristics between normal and malignant cells. For this reason, DNA structures that occur only during the course of DNA replication, such as Okazaki fragments, provide appealing targets for the design of anticancer drugs. Interference with the machinery required for DNA replication is, in fact, the mechanistic basis for several anticancer agents that are currently widely used for the treatment of numerous malignancies. Cytarabine (1-β-D-arabinofuranosylcytosine), for instance, is an analog of deoxycytidine that is effective in the treatment of leukemia as a consequence of its facile uptake by transformed cells and its subsequent metabolism to the nucleoside triphosphate form that inhibits elongation of the lagging strand during DNA replication. Arabinosyl nucleosides are among the most potent nucleoside analogues available for the treatment of viral infections and cancer. Understanding how cytarabine interferes with elongation of the lagging strand during DNA synthesis requires information about the structures and stabilities of model Okazaki fragments with and without cytarabine substitution.

We report here that based on UV hyperchromicity and NMR spectroscopic data, cytarabine destabilizes and bends a model Okazaki fragment having a sequence derived from the genome of simian virus 40[1]. The sequence for the model Okazaki fragment is d(CAAAGATTCCTC):(gaggaATXTTTG) where deoxyribonucleosides are in upper case, ribonucleosides in lower case and X is either deoxycytidine (control) or cytarabine. 1-β-D-arabino-furanosylcytosine (cytarabine) was purchased from Sigma and was converted to a suitably protected 3'-O-phosphoramidite for incorporation into the RNA:DNA hybrid strand of using methods similar to those previously described[1]. UV Hyperchromicity and 2D NMR spectroscopy and structural refinement were done as previously described[1,2].

The effects of cytarabine substitution on the stabilities of the model Okazaki fragments were determined both by parametric fitting of individual UV hyperchromicity curves, and from the concentration dependence of the T_m. Cytarabine substitution significantly decreased the stability of the model Okazaki fragment with the cytarabine-substituted Okazaki fragment having a melting temperature of 42.4 °C compared to 46.8 °C for the control duplex, both at a concentration of 1.33 x 10^{-5} M. The decreased melting temperature for the cytarabine-substituded Okazaki fragment corresponds to a less favorable change in free energy for the melting transition of approximately1.2 kcal/mol at 37 °C. The local structure of the cytarabine-substituted Okazaki fragment is similar to that of the native sequence. Nonetheless, the global structure of the cytarabine-substituted fragment is very different resulting from a distinctly greater bend in the helical axis. The altered backbone parameters about the site of arabinosyl substitution that contribute to the increased bend angle are decreased values for the torsion angle delta and the amplitude of sugar pucker for the site adjacent to the cytarabine substitution and increased values for the torsion angle zeta and the amplitude of sugar pucker for the site of arabinosyl substitution.

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