ANALYSIS OF THE pH-DEPENDENT EQUILIBRIUM RELATIONSHIP BETWEEN THE GUANINE OXIDATION PRODUCTS 5-GUANIDINOHYDANTOIN AND IMINOALLANTOIN

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Oxidation of 2'-deoxyguanosine (dG) results in the major two-electron product 8-oxo-7,8-dihydro-2'-deoxyguanosine (dOG) that can undergo further oxidation to other products. One such product is 5-guanidinohydantoin (dGh) that is highly mutagenic and has been found in vivo. The ring architecture of dGh is similar to allantoin, an oxidation product of uric acid. Allantoin establishes equilibrium between two constitutional isomers in solution. Therefore, we hypothesize that dGh could establish a similar equilibrium with its constitutional isomer iminoallantoin (dIa).

Studies were conducted to monitor the interconversion of dGh and dIa in the nucleoside and oligodeoxynucleotide (ODN) contexts. Synthesis of dGh nucleoside was achieved by literature methods at pH 4.0. The purified nucleoside samples were incubated at pH values ranging from 4.0 to 10.5, followed by HPLC analysis. This analysis observed two distinct products with identical masses and different retention times that were found to have pH dependency in their abundance. Support for the early eluting peaks as dGh and the late eluting peaks as dIa was established via a set of ^{1}H and ^{13}C NMR studies at pH 6.0 and 10.5, conditions that favor dGh and dIa, respectively. These studies identified dGh to be the isomer at low pH (< 10.0) and dIa to be the isomer at high pH (> 10.0). In the ODN context, the dGh and dIa interconversion was monitored by following the pH dependency in DNA polymerase insertion opposite the lesion. Because dGh has H-bonding properties similar to thymidine, the polymerase should insert dATP opposite; while dIa has H-bonding properties similar to cytosine and the polymerase should insert dGTP opposite. The different base pairing characteristics were found to change as a function of pH, in which dATP was preferentially inserted at pH > 8.0 and dGTP was preferentially inserted at pH > 8.0. In the DNA context, the equilibrium transition pH is shifted to a lower value compared to the nucleoside context, in which dGh was favored at pH < 8.0, and dIa was favored at pH > 8.0. The biological significance of these results is discussed with respect to the mutation profiles expected for dGh/dIa in vivo.