THEORETICAL INVESTIGATIONS ON THE PROPERTIES OF MODIFIED **DNA**

ABSTRACT

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A THESIS

SUBMITTED IN FULFILMENT OF THE REQUIREMENT
FOR THE DEGREE OF

Doctor of Philosophy

To

NORTH-EASTERN HILL UNIVERSITY
SHILLONG
INDIA
1995

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AN EXTENDED ABSTRACT

As the title of the thesis indicates, this work deals with applying the methods of quantum chemical theory to investigate certain salient properties and features of chemically modified DNA. Since the DNA system in its entirety as such is too large a system for these theoretical methods to treat, the approach employed here is to reduce the level of investigation to just the components of DNA, such as bases and nucleosides.

Chapter I serves as an Introduction to the thesis. In this Chapter, the backgound material pertenent to the thesis, the concepts of mutagenecity induced by both exogenous and endogenous agents and the consequent effects of mutagenicity are discussed. It is shown how important are the structural modifications for the successful induction of point mutations and highlights the various structural featurs to be studied in the following chapters.

In Chapter II, the validity of and principles employed in relation to quantum chemical study of modified DNA are examined and upheld. The various theoretical methodologies are briefly described, consisting of the AM1 and PM3 SCF-MO methods, the solvation model SM2, and the use of Class IV charges within the AM1 framework (the

CM1 charges). The Chapter goes on to describe some pertinent features of modified DNA, including base tautomerism, protic acid-base equilibria in bases and nucleosides, alkylation of nucleosides, pairing properties of normal, tautomeric, ionic, oxidised and alkylated bases, role of conformation for genotoxicity, and finally the stability, loss and repair of modified DNA nucleosides. The general theoretical approach employed here is also described in terms of the various theoretical indices used.

Chapter III is entitled "Protic Changes in DNA Bases and Nucleosides". Here, the three alternatives open for protic change are utilised, viz. tautomerism, protonation and proton abstraction. These three alternatives are first applied to the free (unmodified) nucleic acid bases G, A, C, T and U. The normal forms of these bases are identified as the stablest tautomeric forms, while energetically the most feasible tautomeric transitions are seen to involve shift of the concerned Watson-Crick proton to an adjacent nucleophilic site on the base. While the stablest tautomers and the most feasible transitions are correctly identified, the relative energetics of the processes involved does not fare well in comparison to the findings of experiment. Use of the solvation model AM1-SM2 furnishes a more realistic picture, but the relative heats of formation of the bases and the tautomeric transition energies are to a large extent the same as those predicted by the gas-phase model. Chapter III then goes on to deal with alkylated DNA nucleosides, drawing upon the physicochemical role of Watson-Crick proton shift for manifestation of mutagenicity. This shift can conceivably occur in two ways, viz. by tautomerism or by deprotonation. The AM1 and MNDO methods are used to calculated the possibilities open for tautomerism

in alkylated DNA bases, which study largely excludes the role of tautomerism for manifestation of mutagenicity in these alkylated systems. The AM1 method is further used to explore the possibilities open for Watson-Crick deprotonation. Theoretical indices for facility of Watson-Crick proton abstraction indicate a good demarcation between mutagenic and non-mutagenic alkylated nucleosides. By use of the solvation AM1-SM2 model, a remarkably good correlation is arrived at between theoretically predicted proton acidity and experimental pK_a values (both acidic and basic spanning the whole gamut of protic acid-base equilibria in nucleic acid systems). Using the theoretically predicted pK_a values, a correct classification into mutagenic and non-mutagenic types of alkylated nucleosides is achieved, also providing the prediction that the N^3-methylguanine system would be mutagenic.

Chapter IV is entitled "Pairing Properties of Modified DNA Bases". The accurate PM3 SCF-MO methodology is deemed as the best semiempirical strategy to employ here in order to study pairing between nucleic acid bases. Here, the types of DNA modification considered include tautomers and ions of free nucleic acid bases, alkylated bases and C^8-oxidised purines. The tautomeric forms G^*, A^*, C^*, and T^* are seen to pair favourably with non-complementary bases such as to induce base-pair transitional mutations. The complementary base-pairs corresponding to the normal situation are, however, not as well-favoured. This suggests that tautomerism of DNA bases could well supply a ground for the induction of spontaneous mutations of DNA. The protonated forms of nucleic acid bases are also noted to pair favourably with both complementary and non-complementary

bases, and the net inference is that these cationic forms would tend to stabilise the double-helix at low pH. The deprotonated forms, pertaining to appreciably high pH, pair more or less in the double-helical configuration with non-complementary bases, giving a potentially mutagenic situation.

Chapter IV continues to apply the same approach to protonated and deprotonated forms of alkylated guanines and thymines. Five different alkylated bases are taken into consideration, which include N^7-methyl-, O^6-methyl- and N^3-methylguanines together with O^2-methyl- and O^4-methylthymines. The criteria for mutagenicity are formulated to be (a) loss of the Watson-Crick protons at biological pH, and (b) removal of steric hindrance from exocyclic O-alkyl groups so as to adopt the anti conformer. Pairing with formally complementary and non-complementary bases is studied with reference to steric and energetic requirements for successful base-mismatching. In the cases involving protonated bases, pairing with complementary bases is indicated as favourable within the double--helical configuration, which would lead to no mutations. On the other hand, the deprotonated systems pair most favourably only with non-complementary bases, which would lead to a mutagenic situation. The role of the exocyclic O-alkyl groups is clearly seen where the syn conformers cannot adopt a Watson-Crick alignment while pairing with non-complementary bases, whereas the anti conformers can. All in all, this study corroborates the two structural and conformational criteria mentioned above as necessary for succesful base-mismatching in alkylated guanines and thymines.

The last part of Chapter IV deals with C^8-oxidised purines. The tautomeric possibilities are explored, from which the keto (oxo) form is clearly preferred over the enol form. Next, the rotation of the modified base moiety around the glycosidic bond is studied in detail, leading to an assignment of about 5 kcal/mol for the anti to syn barrier, the orientation being with respect to the sugar moiety, where the syn conformers alone would be potentially mutagenic. Finally, the syn conformers of the two bases 8-oxoguanine and 8-oxoadenine are paired with adenine and guanine respectively in their normal conformers. It is seen that only 8-oxoguanine pairs favourably within the range of double- helical aligment, while 8-oxoadenine does not, thereby predicting that the former would be mutagenic (leading to a transversion) while the other would not, being easily recognised and repaired.

Chapter V is entitled "Stability and Repair of Modified DNA Nucleosides". The point here is to relate chemical or <u>in vitro</u> stability to the <u>in vivo</u> biological phenomena of enzymatic repair of DNA lesions. It has been established that N-alkylated nucleosides suffer facile depurination, while O-alkylated lesions are susceptible to hydrolytic dealkylation. These have bearing upon the choice of repair mechanism for each class, where the N-alkylated nucleosides are repaired by glycosylase enzymes which cleave the glycoside bond, while O-alkylated nucleosides are repaired by alkyltransferase enzymes which remove only the alkyl group. Five alkylated DNA nucleosides are subjected to study here, being the same as those used in Chapter III and IV. The in vitro chemical stability is here expressed in terms of lability of the alkyl groups present, and of strength of the

glycosidic linkage. The effects of external pH, of alkylation and the site of alkylation, and of the presence/absence of the Watson-Crick protons are all taken into consideration through a consideration of the appropriate structure for each situation. Appropriate indices for alkyl group lability and for glycoside bond strength are framed for use here.

It is found that deglycosylation would tend to precede dealkylation as experiment goes to indicate in vitro, regardless of nucleoside type. Here, lowering of pH through introduction of the Watson-Crick protonated species is found to significantly destabilise both the alkyl group bond and the glycoside bond. Cationic N^7-methyldeoxyguanosine is found to be the most susceptible of all these systems to depurination. The O- alkylated nucleosides are predicted to have stable glycoside linkages similar to the unmodified DNA nucleosides. Upon restricting the study to biological pH through choice of the appropriate forms of each system, a transition state approach is attempted to yield clues to in vitro stability and in vivo repair mechanisms via their respective activation energies. The conclusion is that N^7-methyldeoxyguanosine would be repaired by a glycosylase enzyme, while O^6-alkyldeoxyguanosine would undergo alkyltransferase repair.