# SINGLE ELECTRON TRANSFER REACTION STUDIES BY OPTICAL AND ESR SPECTROSCOPY
# ( A CASE STUDY OF NUCLEIC ACID BASES )

## ABSTRACT

BY

SUCHANDRA BHATTACHARJEE
DEPARTMENT OF CHEMISTRY
SCHOOL OF PHYSICAL SCIENCES
1997.

SUBMITTED

IN FULFILMENT OF THE REQUIREMENT OF THE DEGREE OF
DOCTOR OF PHILOSOPHY IN CHEMISTRY OF
NORTH EASTERN HILL UNIVERSITY, SHILLONG
INDIA.

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ABSTRACT

The Thesis, consisting of six chapters.deals with the results of an investigation on some chosen aspects of the nucleic acid bases.

Chapter 1 presents a brief general introduction pertaining to the work embodied in the thesis. It describes in general some of the important properties of the nucleic acid bases, the phenomenon of single electron transfer reactions - the spin trapping technique and the UV and ESR spectroscopy which has been employed in the present investigation.

Chapter 2 deals with a review of the work done so far and also the objective of the present work has been outlined. Damage to DNA has been a major area of research. This damage is primarily responsible for cancer, aging, cell deactivation etc.. Although, a lot of work on damage related studies have been done, the real cause of cell deactivation is still not precisely known and debate is going on. These studies have been mainly confined to DNA and its constituents, in the solid state or solution state, mainly the aqueous state. These studies have been undertaken at ;

(i) at 77K or even at 4K ,

(ii) Using a powerful source of irradiation e.g., -rays, pulse radiolysis, photoexcitation etc.,

(iii) Reaction with strong oxidising agent e.g., $SO_4^{\cdot -}$, $OH^{\cdot}$ etc.,

( iv) in aqueous phase.

Inspite of so much of work, the mother nature has illuded the scientific community and kept up to herself the greatest secret of all - the cause of cancer. We , therefore, felt strongly motivated to undertake the study with a different approach with a hope that our findings, no matter how trivial they may be, might help towards a wider understanding of the problem. We, therefore, confined our work ;

(i) at ambient temperature, because the human body is at that temp. Any damage at this temp. will be relevant to the system in vivo.

(ii) Not employing any powerful irradiating source of radical generation. Because the percentage of cancer deaths due to radiation exposure is very low.

(iii) By creating such conditions where initially only one electron is transferred and the chain of reactions begins.

(iv) By employing spin trapping technique to trap the short lived intermediates.

(v) Non-aqueous phase. It is known that some very fast two electron reactions in aqueous phase can proceed in two steps

involving one electron reaction in non-aqueous solvents. Moreover, the non-aqueous phase provide an ideal environment which mimics the interior environment of double helix of DNA.

(vi) If an electron transfer occurs under these conditions, does it follow an " Inner - sphere or Outer - sphere " mechanism.

Chapter 3, deals with the experimental details , which have been made use of.

This study is directed towards the UV Spectroscopic and Electron spin Resonance ( employing spin trapping ) studies of Single Electron Transfer ( SET ) processes involving the Nucleic Acid Bases in non-aqueous solvents at ambient temperature. The present project has been executed in two main parts.

Chapter 4, deals with the electronic spectroscopic studies of the nucleic acid bases in non-aqueous solvents. Literature survey revealed that so far the uv spectra of these base molecules in non-aqueous solvents has not been studied so far. Therefore, it became imperative to study the uv spectra and its associated properties in solvents of different polarity. The uv spectra of the nucleic acid bases in organic solvents of varying polarity have been successfully recorded. The phenomenon of tautomerism an important property associated with the nucleic acid bases

have been demonstrated to occur even at very low concentrations of the substrates in different solvents. In bases with two exocyclic groups ( e.g., guanine , cytosine and their corresponding nucleosides ) both kinds of tautomerism ; keto - enol and amino - imine have been shown to occur. Self - association of these bases have been successfully shown by uv, thus complimenting the findings by IR, NMR and X- ray Crystallography etc.,. We are reporting a band for the first time due to dimeric formation at ca. 225 nm in low polarity solvents. The relative associative capability have also been commented upon and found to be higher for the purine bases than the pyrimidine bases. This conclusion derived through UV studies are in agreement with those by IR and NMR.

Charge transfer complexes ,precursors to electron transfer processes has been satisfactorily demonstrated through the appearance of isosbestic points. These are indeed a positive indication for the feasibility of electron transfer processes to take place.

Chapter 5, the second major part of the project is the study of single electron transfer reactions by ESR ( employing spin trapping technique). The technique of spin trapping in ESR has been very successfully applied to trap the short lived intermediates formed as a result of transfer of a single electron from the nucleic acid bases to acceptors in

non-aqueous solvents.

The critical role which oxygen plays in single electron transfer reactions have been observed in this system both by uv and ESR. To our knowledge only few such instances exist. The role of polar and hydroxylic solvents in solvating the charged species and thus diverting the reaction pathways have been clearly observed. Deprotonation of the base radical cation have been shown to be the major pathway in contrast to hydration reaction, under the present experimental condition. We have observed that deprotonation occurs from N1 in thymine and uracil, and in rare case a proton abstraction from methyl group of thymine has been observed. For cytosine, the NH2 group at C4 was observed to be the site of deprotonation. For adenine two sites of deprotonation were observed ; one at N1 and the other at N7. Reactions of bases particulary thymine with benzoyl peroxide under very mild condition led to the formation of benzoyloxyl and phenyl radical, both of which are known to act as damaging agent. In the case of nucleosides the primary radical site is the base and the site of free spin is transferred from the base radical to the sugar, a main cause of strand breakage and thus deactivation. Sugar derived radical could be obtained from ribose but not from deoxyribose.

The electron transfer proceeds by the so called " Outer

sphere Electron Transfer " mechanism.

Chapter 6, highlights the conclusions drawn out of the work and some facets of the scope of the work has also been projected.

The major achievements of this project derived from UV and ESR studies on the Nucleic acid bases are summarised below ;

(1) The uv spectra of the nucleic acid bases in organic solvents of varying polarity have been successfully recorded. To the best of our knowledge this is the first such studies.

(2) The phenomenon of Keto - Enol tautomerism plays a very significant role in mutagenesis. This property of the nucleic acid bases have been theoretically predicted and experimentally proved in some cases mainly by employing IR and NMR in aqueous solvents. We have successfully employed uv spectroscopy to demonstrate the occurance of tautomerism even at very low concentrations. In bases with two exocyclic groups ( e.g., guanine , cytosine and their corresponding nucleosides ) both kinds of tautomerism ; keto - enol and amino - imine have been shown to occur. To the best of our knowledge, this is the first and fairly comprehensive report of tautomerism using UV spectroscopy.

(3) Self - association of these bases have been reported, using IR, NMR and X- ray Crystallography etc. We have complimented these findings from uv studies. We are

reporting a band for the first time due to dimeric formation at ca. 225 nm in low polarity solvents. The relative associative capability have also been commented upon and has been generally found to be higher for the purine bases than the pyrimidine bases. This conclusion derived through UV studies are in agreement with those by IR and NMR.

(3) Charge transfer complexes are believed to be precursors to electron transfer processes. This has been very satisfactorily demonstrated . Charge transfer complexes through the appearence of isosbestic points were observed which are indeed a positive indication for the feasibility of electron transfer processes to take place.

(4) Examples involving the transfer of a single electron are limited . The debate involving single electron transfer processes vs polar pathways is still on. To this controversy, we have added another example in favour of " Single Electron Transfer " processes, involving nucleic acid bases in organic solvents.

(5) The technique of spin trapping in ESR has been very successfully applied to trap the short lived intermediates formed as a result of transfer of a single electron from the nucleic acid bases to acceptors in organic solvents.

(6) The role of oxygen in line broadening ( because of it being paramagnetic in the ground state )in ESR is well known. The crucial role which oxygen plays in free radical

chemistry is also well documented. But the critical role which oxygen plays in electron transfer reactions have come across in this system both by UV and ESR. To our knowledge only few such instances exist.

(7) The role of polar and hydroxylic solvents in solvating the charged species and thus diverting the reaction pathways have been clearly observed.

(8) Deprotonation of the base radical cation have been shown to be the major pathway in contrast to hydration reaction, under the present experimental condition. We have observed that deprotonation occurs from N1 in thymine and uracil, and in rare case a proton abstraction from methyl group of thymine has been observed. For cytosine, the NH2 group at C4 was observed to be the site of deprotonation. For adenine two sites of deprotonation were observed ; one at N1 and the other at N7.

(9) Reactions of bases particularly thymine with benzoyl peroxide under very mild condition led to the formation of benzoyloxyl and phenyl radical, both of which are known to act as damaging agent as a tumour promoter.

(10) Damage can be caused by strong oxidising agents like benzoyl peroxides and chloranil even under the mildest conditions.

(11) The primary radical site is the base and the site of free spin is transferred from the base radical to the sugar,

a main cause of strand breakage and thus deactivation.

(12) Sugar derived radical could be obtained from ribose but not from deoxyribose.

(13) The strong evidence for the charge transfer complexation suggests that the electron transfer proceeds through an " Outer - sphere " mechanism.

(14) 1,4 dioxan and tetrahydrofuran have been found to be the most suitable solvent for SET studies of these bases under the present experimental conditions.

We feel that the objective of the project has been quite satisfactorily met. We have developed a simple model for studying electron transfer reactions of the nucleic acid bases under mildest conditions without employing any irradiating source.