MAGNETIC RESONANCE INVESTIGATIONS ON FREE RADICALS IN THE STUDY OF SOME RADIobiological AND BIOPHYSICAL PROBLEMS

SYNOPSIS

Electron Spin Resonance (ESR) and Nuclear Magnetic Resonance (NMR) techniques have been used to study the role of free radicals in radiobiological damage and, in carcinogenesis process. The major chemical changes induced by high energy radiation in molecules of a cell are believed to be mediated through primary water radicals, namely, hydrated electron, hydroxyl radical and hydrogen atom. Because these radicals have short life time (half life $\approx$ microsecond) at room temperature, their reactions with biomolecules have been studied in frozen systems. The mechanisms of modification (enhancement) of radiation damage by certain stable organic nitroxide free radicals and their use as molecular probes to understand mechanism of enhancement of radiation damage (radiosensitisation) by certain local anaesthetic drugs have been attempted by ESR. In addition, suitability of nuclear magnetic relaxation and electron spin resonance methods in the studies of physical complex formation of a chemical carcinogen with certain constituents of cells has been demonstrated.
Chapter I is a general introduction of the present work. The experimental techniques viz. electron spin and nuclear spin resonance used in the present work have been briefly described in Chapter II.

Chapter III deals with the radiation induced paramagnetic species trapped and identified by ESR in \( \gamma \)-irradiated frozen aqueous alkali hydroxide matrix at \( 77^\circ \text{K} \). These are electron (\( e_t \)), hydroxyl radical (\( \text{OH}_t \)) and oxygen atom anion (\( \text{O}_t \)).

During the present investigations, a new paramagnetic species has been observed and identified as molecular oxygen anion radical (\( \text{O}_2^\cdot \)). In addition, there has been a controversy regarding the nature of species giving rise to an esr signal on warming the \( \gamma \)-irradiated alkali hydroxide matrix from \( 77^\circ \text{K} \) to \( 178^\circ \text{K} \). The present results have doubted the interpretations of other investigators and the radical species in question has now been identified as the oxygen anion radical existing in the form of a complex with oxygen atom anion or ozone anion.

Furthermore, reactions of radiation induced electrons with deoxynucleic acid bases, nucleosides and nucleotides in frozen aqueous alkaline system at \( 77^\circ \text{K} \) and higher temperatures have been investigated. Results have

* subscript 't' denotes trapped species.
provided evidence to suggest that damage by electron attack is restricted to base moiety of nucleic acid constituents and corresponding base anions were detected at 77°K. These anions are, however, converted to purine and pyrimidine radicals at higher temperatures. The nature of base damage was unaffected but stability of anions and radicals was significantly reduced by addition of sugar and sugar phosphate groups on the base moiety. The amount of anions or radicals formed on the base were, however, not affected by sugar or sugar phosphate groups except in case of thymine. The radicals of sugar or phosphate groups were not detected in nucleosides and nucleotides suggesting absence of intranucleotide breakage. Significance of these results in radiobiological damage have been discussed.

Frozen systems are not suitable for studying hydroxyl radical reactions with solute since the trapped hydroxyl radicals do not participate in further reactions. The reactions of hydroxyl radicals generated in chemical systems (e.g. Ti$^{+3}$ - H$_2$O$_2$) were, therefore, investigated at room temperature by ESR flow system. The nature of species in this system entering chemical reaction with solutes was investigated. A complex of Ti$^{+3}$, 'n' molecules of solute and Ti$^{+4}$ has been suggested to be the reaction complex. Reactions of hydroxyl radicals and hydrogen atoms with some
biomolecules investigated by other workers have also been briefly described in this chapter, only with a view to indicate the nature of radicals formed.

Investigations, on modification of radiation damage by chemicals, carried out during the present work have been discussed in Chapters IV and V. Certain stable organic nitroxide radicals, such as, triacetoneamine-\(\text{N}-\text{oxyl}\) (TAN) have been known to sensitise bacterial and mammalian cells to radiation damage in the absence of oxygen. The mechanisms of sensitisation by TAN radicals has been investigated and the present results suggest that part of the sensitising effect of TAN radicals can be ascribed to their ability to react with sulfhydryl compounds e.g. cysteine, glutathione etc. and thereby reduce \(-\text{SH}\) levels in the cell which in turn will affect the radioprotective ability of cells. From other studies it has been suggested that TAN and \(O_2\) sensitise cells to radiation by reacting with the radicals formed on the cellular molecules (fixation hypothesis). The absence of sensitisation by TAN radicals in the presence of oxygen has been attributed to the relatively better efficiency of \(O_2\) to react with biomolecule radicals. Present results have, however, indicated that the ineffectiveness of TAN in presence of oxygen may partly be due to reaction of oxygen with TAN radicals.
In addition, the results of present experiments have also indicated the possible involvement of some radiation induced transients of TAN formed by reaction of primary water radicals in the sensitisation process through oxidation/reduction of the damaged molecules. Present results have also satisfactorily explained various other observations on sensitising effect of TAN radicals in bacterial system. For instance, absence of sensitisation in presence of ascorbic acid can be understood in terms of chemical reduction of nitroxide group in presence of the latter as investigated by ESR in this work.

Furthermore, possibility of using TAN radicals as molecular probe in various normal and pathological conditions to monitor the cell interior has been indicated and some results obtained on red blood cells have been discussed in Chapter IV.

Chapter V deals with the studies on mechanism of sensitisation of cellular systems towards radiation by certain membrane specific local anaesthetic drugs like procaine and tetracaine. In order to obtain information on alterations/disorganisations in the membrane by these sensitising drugs, nitroxide stable free radicals were used as molecular probes. Human erythrocyte membrane was used as model of membrane system and spin labels known to
specifically bind with proteins or attach/associate with lipids in the bilayer of membrane were used. The results obtained have shown that both procaine and tetracaine disorganise the erythrocyte membrane and the effect was reversible as the original membrane state could be obtained on removing the drugs from the membrane. These results thus explain the reported sensitisation of bacterial cells by these compounds only when present during irradiation and the lack of any effect observed once the drug was washed off before irradiation. Furthermore, present results have also indicated that although both the drugs penetrate lipid bilayer, procaine mainly localized in the outer polar region whereas tetracaine penetrated deeper into the interior (central region) of the bilayer. These observations were explained on the basis of the hydrophobic/hydrophilic characters of the drugs. The drug induced changes in lipid bilayer were also found to cause changes in the mobility of the membrane proteins. Implications of these observations in radiosensitisation of bacterial cells by the drugs have been discussed. A relation appears to exist between the ability of these drugs to produce disorganisation in the membrane and their anaesthetic potency. Present conclusions are in conformity with the fluorescence and nuclear magnetic resonance investigations by other workers.

In Chapter VI, usefulness of nuclear magnetic
relaxation and electron spin resonance methods in the study of physical molecular complexes has been demonstrated. Benzopyrene, B(a)P, is a widely distributed and potent carcinogen in environment. It has been suggested by other investigators that B(a)P is converted to some reactive intermediates at the target site which subsequently binds with the tissue components to give carcinogenic effect. A well known metabolite of B(a)P is 6-hydroxybenzopyrene which is easily oxidised to what has been identified as 6-oxo-benzopyrene radical. In the present study physical binding of this oxo-radical with DNA and caffeine (as purine representative) has been investigated. The oxo-radical is sparingly soluble in water but was relatively better solubilized in aqueous solution of DNA or caffeine. ESR studies have indicated that the radicals exist as dispersed monomers associated with DNA and with caffeine. NMR spin lattice and spin-spin relaxation times of protons of caffeine have given direct evidence that a part of the unpaired electron is transferred from the radical to the associated caffeine molecule. A $\pi - \pi$ complex between the oxo-radical and caffeine has been suggested in the present study. Theoretical considerations have however, shown that intermolecular charge transfer is not likely to be a major source of stabilization energy of the complex. Nevertheless the physical complexation between the active form of a carcinogen
with the vital molecules may be a pre-stage of their chemical binding. A knowledge on the ease of physical complexation between carcinogen intermediate and a particular target molecule may contribute to the understanding of chemical carcinogenesis.