

An Introduction to Radiation Induced Degradation of Biological Molecules in Aqueous Solutions

MANOHAR LAL

Chemistry Division

Bhabha Atomic Research Centre

Trombay, Bombay-400 085, India

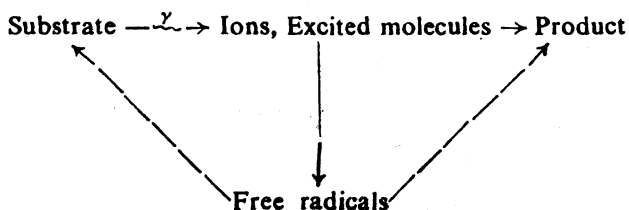
Radiation chemistry of aqueous systems is the chemistry of \dot{H} , $\dot{O}H$, e_{aq}^- , H_2O^+ and H_2O^* formed when a solute in aqueous solutions is exposed to ionising radiation. The pulse radiolysis technique has helped in the production, the detection and understanding of the reactions of primary species with solutes.

A great deal of data on radiation biochemical studies e.g. degradation of DNA, its constituents and their protection, radiation protection and sensitisation, generation of superoxide ion and their reactions has already been reported but a great deal still needs to be done for the understanding of radiation biology.

INTRODUCTION

Radiation Chemistry is mainly concerned with the chemistry of free radicals, ions and excited states which are initiated as a result of the interaction of matter with high energy particles and electromagnetic radiations. Looking back over the past half a century one finds that large number of significant contributions have emerged from the research in this field. The most recognised and striking example probably being the discovery of hydrated electron e_{aq}^- by Hart *et al.*¹

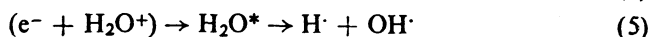
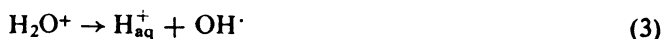
The first step is the deposition of energy (physical stage) ($\sim 10^{-16}$ – 10^{-14} sec) which leads to the formation of a large spectrum of ionised and excited species. This is followed by physico-chemical stage (10^{-14} – 10^{-12} sec) where in dissociation of molecules, ion molecule reaction and charge transfer reactions occur and finally the chemical stage (10^{-10} – 10^{-7} sec) where in thermalisation of low energy secondary electron occurs and the reaction of the primary transient species with substrates give rise to products.



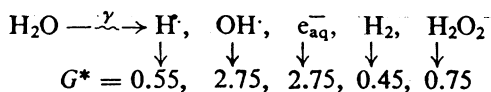
In aqueous solutions for example the energy is used for ionisation and excitation.



The H_2O^+ cation and H_2O^* excited water molecules converted within $\sim 10^{-12}$ sec into highly reactive species ($\text{H}\cdot$, $\text{OH}\cdot$ and e_{aq}^-) according to reactions (3-5)

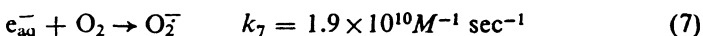
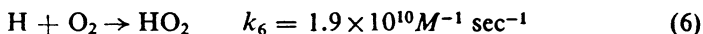


The overall reaction with the G -values of the species formed in water radiolysis at neutral pH can be written as follows. It is reaction of these



short lived species with substrates, which are of concern to radiation chemists.

In the presence of oxygen two more very important pH dependent species are generated and these species are found to be very damaging in biological systems.



pK value for equilibrium reaction 8 is ~ 4.7 .² The reactions of both O_2^- and HO_2 radicals with the substrate are very important.

The second important forward step was the development of time-resolved technique of pulse radiolysis.³ This allows production of large concentration of transient species in a very short interval of time ($\sim 10^{-6}$ – 10^{-9} sec) and the direct observation of short lived species. By the use of this technique a large variety of absolute rate constants have been measured and innumerable simple and complicated reaction mechanisms understood.

Today a lot has been achieved in the field of radiation chemistry and the modern radiation chemist has considerably broadened his base and uses radiation chemical methods more as a tool. The most interesting topics drawing the attention of radiation chemists are the structure of free radicals, reaction mechanisms redox processes, electron transfer

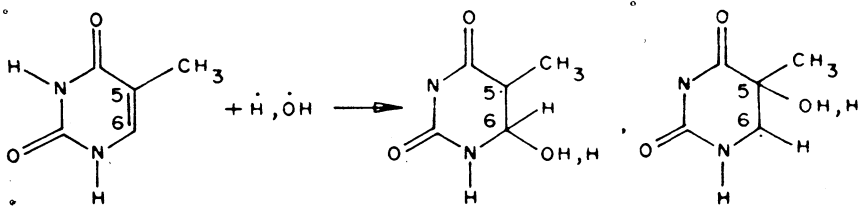
* G -value is defined as the number of molecules generated or destroyed per 100 ev of energy.

processes,⁴ radiation damage, radiation protection⁵ and sensitisation⁶ and understanding the free radical reactions in biological systems. It is rather difficult to cover all the topics in this small space. I shall touch upon few of them.

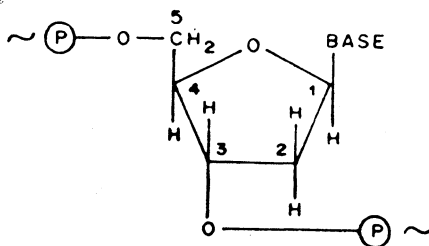
(a) Radiation Degradation of Nucleic Acids and its Constituents in Aqueous Solutions

Gamma and pulse radiolysis studies have contributed significantly to the present knowledge of the free radical degradation of nucleic acids. It is now well established that aqueous solutions of DNA when exposed to ionising radiations cause strand breaks, base, sugar and phosphate group damage in DNA and its eventual damage.¹⁰

Extensive studies have been carried out in the γ -irradiated bases e.g. thymine and uracil etc. and product yields determined. Many of the products formed reveal attack of OH and H radicals on 5 and 6 positions of these molecules which is believed to be responsible for DNA damage.¹¹



In the radiolytic degradation studies of DNA, a large variety of products are formed and identified during the strand breaking processes and radiolytic mechanism are proposed. On the basis of the results of the radiolysis of certain model systems e.g. ribose-5-phosphate (nucleotide) air saturated solutions, it has been concluded that the average of C(4)-C(5) bond are the cause of phosphate release in DNA.¹² Further studies on other model system e.g. di and trimethylphosphate¹² have been carried out and a lot more needs to be done in order to interpret the mechanism of DNA degradation.



Even though large amount of data is available on the free radical degradation of DNA, its constituents, bacteriophage and cells and it is generally believed that damage to DNA is the major cause of cellular death, the biological consequences of strand breaks are not yet identified. Only in bacteriophage it has unambiguously been identified that there is correlation between lethality and strand breaks. Such a correlation at cellular level has not yet been demonstrated. It would thus be important to undertake detailed and systematic investigations which could demonstrate the relevance of DNA strand breaks to the cellular systems.

(b) Radiation Protection and Sensitisation

The influence of thiols and disulphides on the radiation response of living systems has in the past been a subject of considerable interest. Most of the attention in the past has been focussed on the problem of protection of mammals against ionising radiations and with a view to develop certain drugs that could allow human beings to survive against lethal effects of radiations. Even though such studies were only of pharmacological interest, this stimulated considerable interest in radiochemical studies in naturally occurring thiols, thiopeptides and thio-enzymes in order to understand the radiolytic mechanism. This has now been established as a result of considerable amount of work in our and other laboratories. From the results and the reaction scheme postulated in our work⁶ and from the other work, mechanism for the radiation protection due to thiols has been established.

(i) Radical Scavenging

Since thiols are very reactive, the primary reactive species compete with the thiols and the target molecule, this diminishes the damage to the target as the primary species react with thiols. The process is termed as protection.

(ii) Repair by Hydrogen Transfer

The target molecules after reacting with primary reactive species are damaged. These are repaired by the transfer of hydrogen by the SH group of thiols as in reactions (9) and (10)



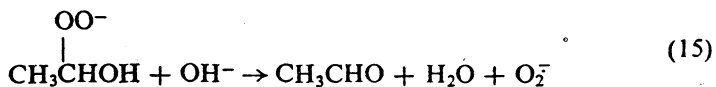
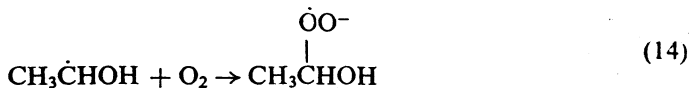
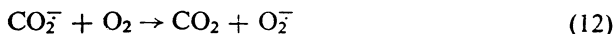
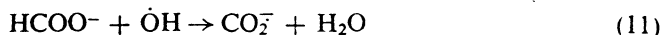
In the presence of oxygen, the target molecule gets converted to peroxy radical which cannot be repaired by the thiols. Thus the damage to the target molecule is fixed and this prevents regeneration of original target molecule. This is termed as the well known 'oxygen effect'⁷. Today many

more radiation protective agents e.g. vitamin E and C and superoxide dismutase⁸ are also being used as radiation protectors.

During the last few years the concept of hypoxic cell chemical radiosensitisers⁵ have emerged for the treatment of cancer. These sensitizers which include the use of drugs such as anesthetics, analgesics, tranquilisers and nitromidazoles are used in combination with conventional radiation therapy. A large variety of radiosensitizers have been tested in single cell systems and in experimental animal tumours. These radiosensitisers have proved to be of no use in controlled clinical tests. Recent studies from this establishment on chlorpromazine, promethazine which are used as common tranquilisers, trimeperazine and prochlorperazine have shown great promise as radiosensitisers for in vitro hypoxic cells and for fibrosarcoma and Sarcoma 180 A in Swiss mice¹⁰. Research in this field is continuing.

Generation of Superoxide Radical Anions

Superoxide radicals are generated in human systems and are implicated for a variety of disorders in human systems. Superoxide radicals are frequently generated enzymatically and valuable information has been gained by the enzyme generated O_2^- studies. Because of its limited pH range, it is not possible to study the reaction of its conjugate acid ($\dot{H}O_2$) with the substrates. Radiation chemical methods, where in oxygen saturated alkaline aqueous formate or ethanolic solutions are subjected to ionising radiation, give superoxide ions (O_2^-) or peroxy radicals ($\dot{H}O_2$).



Thus by the sequence of reactions 6, 7, 11 and 12 and 6, 7, 13, 14 and 15 all the primary species are converted into superoxide radicals. These methods can be used for the study of the reactivity of superoxide radicals with biologically important compounds e.g. DNA, enzymes, hyaluronic acid. The significance of superoxide radicals towards radiation biology is extremely important because of its ability to diffuse and react with

membranes, its capacity to inactivate enzymes and damage DNA. It would be of great interest to generate O_2^- by the above methods and investigate the reactions of O_2^- with biologically important molecules for understanding the overall role of O_2^- in biological systems.

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