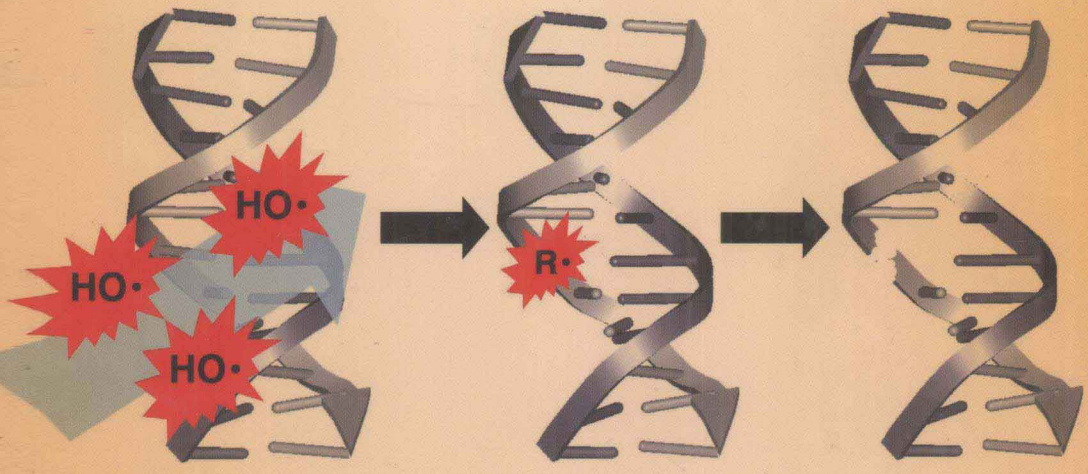
WILEY SERIES ON REACTIVE INTERMEDIATES IN CHEMISTRY AND BIOLOGY

Steven E. Rokita, Series Editor

RADICAL AND RADICAL ION REACTIVITY IN NUCLEIC ACID CHEMISTRY

Marc M. Greenberg, Editor



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Quinone Methides
Edited by Steven E. Rokita

Radical and Radical Ion Reactivity in Nucleic Acid Chemistry
Edited by Marc M. Greenberg

PREFACE TO SERIES

Most stable compounds and functional groups have benefited from numerous monographs and series devoted to their unique chemistry, and most biological materials and processes have received similar attention. Chemical and biological mechanisms have also been the subject of individual reviews and compilations. When reactive intermediates are given center stage, presentations often focus on the details and approaches of one discipline despite their common prominence in the primary literature of physical, theoretical, organic, inorganic, and biological disciplines. The *Wiley Series on Reactive Intermediates in Chemistry and Biology* is designed to supply a complementary perspective from current publications by focusing each volume on a specific reactive intermediate and endowing it with the broadest possible context and outlook. Individual volumes may serve to supplement an advanced course, sustain a special topics course, and provide a ready resource for the research community. Readers should feel equally reassured by reviews in their speciality, inspired by helpful updates in allied areas and intrigued by topics not yet familiar.

This series revels in the diversity of its perspectives and expertise. Where some books draw strength from their focused details, this series draws strength from the breadth of its presentations. The goal is to illustrate the widest possible range of literature that covers the subject of each volume. When appropriate, topics may span theoretical approaches for predicting reactivity, physical methods of analysis, strategies for generating intermediates, utility for chemical synthesis, applications in biochemistry and medicine, impact on the environmental, occurrence in biology, and more. Experimental systems used to explore these topics may be equally broad and range from simple models to complex arrays and mixtures such as those found in the final frontiers of cells, organisms, earth, and space.

Advances in chemistry and biology gain from a mutual synergy. As new methods are developed for one field, they are often rapidly adapted for application in the other. Biological transformations and pathways often inspire analogous development of new procedures in chemical synthesis, and likewise, chemical characterization and identification of transient intermediates often provide the foundation for understanding the biosynthesis and reactivity of many new biological materials. While individual chapters may draw from a single expertise, the range of contributions contained within each volume should collectively offer readers with a multidisciplinary analysis and exposure to the full range of activities in the field. As this series grows, individualized compilations may also be created through electronic access to highlight a particular approach or application across many volumes that together cover a variety of different reactive intermediates.

Interest in starting this series came easily, but the creation of each volume of this series required vision, hard work, enthusiasm, and persistence. I thank all of the contributors and editors who graciously accepted and will accept the challenge.

STEVEN E. ROKITA

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INTRODUCTION

More than a century ago, observations were reported in a period of less than five years from distinct fields that would comprise a large portion of the basis of radical mediated DNA damage. X-rays were discovered in 1895 and used to treat tumors shortly thereafter. In addition, by the turn of the twentieth century the Fenton reaction was reported and in 1900 Gomberg proposed the formation of the first carbon-centered radical. These seemingly disconnected reports were ultimately determined to overlap significantly. X-rays were found to kill tumor cells by oxidatively damaging DNA. Hydroxyl radical, which is produced from hydrogen peroxide in the Fenton reaction, was found to be a primary reactive species responsible for DNA damage mediated by gamma radiolysis. Finally, the connections are completed by the formation of radicals in DNA. The carbon-centered (and other) DNA radicals are produced from hydroxyl radical by hydrogen atom abstraction, as well as by addition to π-bonds.

The complexity of this chemistry becomes immediately apparent upon considering the size and heterogeneity of DNA, as well as the variety of experimental conditions (e.g., O_2, redox active metal ions, hydrogen atom donors) under which damage is induced.

Despite the complex nature of oxidative DNA damage, a broad range of scientists using a variety of physical and analytical methods have made significant contributions to our understanding of this chemistry during the latter half of the twentieth century. Clemens von Sonntag, a leader in this field, summarized much of this research in his seminal book *The Chemical Basis of Radiation Biology* (1987). By using ionizing radiation in conjunction with various techniques such as mass spectrometry and EPR spectroscopy, scientists were able to identify products, observe reactive intermediates, and proffer mechanisms of nucleic acid damage. Recent contributions describing significant advances in computational aspects of radiation damage, as well the direct

effects of ionizing radiation, the role of reactive species other than hydroxyl radical produced by ionizing radiation, the effects of electron affinic radiosensitizing agents on DNA radical chemistry, and the exciting realization of the role of low energy electrons in DNA damage, are described in this book.

Investigations in which ionizing radiation is used to initiate DNA damage face the limitation that they lack control over which reactive intermediates are produced or they cannot control where nucleic acids are damaged. The advent of solid phase oligonucleotides synthesis, modern mass spectrometry methods capable of analyzing biopolymers directly (e.g., MALDI-TOF MS and ESI-MS), and the assimilation of biochemical techniques such as gel electrophoresis and the utilization of nucleic acid modifying enzymes provided chemists with the wherewithal to probe oxidative DNA damage with greater precision. By utilizing these tools in conjunction with chemical synthesis of oligonucleotides containing modified nucleotides, chemists were able to simplify studies on nucleic acid damage and in the process uncover mechanistic complexities and unrecognized reaction pathways. In addition, the application of these and other state-of-the-art techniques facilitated elucidating the pathways for electron transfer in DNA that can be initiated directly or indirectly by ionizing radiation, as well as the utilization of DNA damage as a means for understanding protein–DNA interactions, the mechanisms of drugs and other species that target DNA, and the utilization of DNA damage as a chemical sensor. Experts on the respective topics also review advances in each of these areas in this compilation.

I am grateful to all of the contributors to this book. Their research and that of others described within have provided a deeper understanding of this biologically and technologically significant area of science and have also provided the basis for future investigations and applications of radicals in nucleic acid damage in general. Finally, I want to thank Professor Steven Rokita for having the vision and motivation to establish *Reactive Intermediates in Chemistry and Biology,* as well as for inviting me to participate in this project.

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CONTENTS

1

THEORETICAL MODELING OF RADIATION-INDUCED DNA DAMAGE

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1.1. INTRODUCTION

Ionizing radiation causes a variety of damages to DNA in living systems. Thus, the understanding of radiation-induced chemical processes leading to specific damage in DNA is of substantial biological importance.[1–10] Radiation ionizes each component of DNA (i.e., base, sugar, phosphate) and the surrounding water molecules in a random fashion and produces a cascade of secondary electrons, most of which are below 15 eV and are designated as low-energy electrons (LEE). LEE are produced in a large quantity (4×10^4 per MeV energy deposited)[11] along the tracks of the ionizing radiation and have been shown to result in direct DNA damage in model systems in the seminal work of Sanche and co-workers, who found that LEEs create single- and double-strand breaks (SSBs and DSBs) in DNA through dissociative electron attachment (DEA).[12–18] These findings having been substantiated by the work of others.[19–22] Only a small fraction of LEE result in DNA damage, because most electrons are thermalized and either recombine with positive charge ("hole") or are captured by the pyrimidines [thymine (T) and cytosine (C)], resulting in radical anion formation. During ionization events, all the bases are randomly ionized and "holes" (radical cations) are formed, which travel within DNA toward the base having lowest ionization potential, the order being guanine (G) < adenine (A) < cytosine (C) $\approx$ thymine (T).[2] Therefore, in a randomly ionized DNA double strand, the hole tunnels or hops from one base to

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the next, finally localizing on guanine to form the guanine radical cation ($G^{\bullet+}$).[1–3,7–9] Ionization radiation also induces holes on the sugar–phosphate backbone site that lead to two competitive reactions: (i) deprotonation of the sugar cation radical at a carbon site resulting in the formation of neutral sugar radicals at carbon C_1' to C_5' sites[23–28] and (ii) transfer of hole to a nearby base in DNA.[28]

Since most sugar radicals lead to DNA strand breaks, sugar radical formation in DNA becomes of crucial importance to the biological consequences of radiation. Recently, it has been found that irradiation of DNA by a high LET (linear energy transfer) radiation, a high-energy argon ion beam,[23] produced a far higher yield of sugar radicals than was found by a low LET radiation, γ-irradiation. The authors report that the excess sugar radicals were created within the track core.[23] The energy density in the track core is high and results in ionizations and excitations in close proximity. For this reason, it was hypothesized that excited states of radical cations might result in the neutral sugar radicals in the core of the ion track. To test this hypothesis, recent experiments in our laboratory were performed on the photoexcitation of guanine and adenine radical cations ($G^{\bullet+}$, $A^{\bullet+}$) in DNA model systems.[24–27] It was found that excited DNA base cation radicals formed high yield of sugar radicals which confirmed the proposed hypothesis.[24–27]

While strand breaks are biologically significant, it is combinations of DNA damages known as multiple damage sites (MDS) that are the most lethal type of DNA damage. Such combinations of single- and double-strand breaks and base damages with 10 base pairs are known to lead to irreparable damage because of the loss of local structural information. High-LET radiations (α particles, atom ion beams, neutrons) are found to be about 10 times more damaging than the low-LET radiations such as β particles, X rays, and γ rays in the production of such damage.

From the above discussion, it is evident that ionization and excitation are the initial events in DNA damage. As the damage unfolds from these initial events, the processes may become complex in nature; however, the simplicity of the initial events allow for a clear understanding of these initial processes. Thus ionization, excitation, and electron addition to DNA bases have been extensively treated by theoretical calculations using a variety of methods with density functional theory (DFT) perhaps the most useful to large systems. The advent of substantial computing power and the availability of inexpensive computational resources[29] allows the application of more sophisticated level of theoretical calculation such as TD-DFT, Møller–Plesset perturbation theory (MP2), CCSD(T), and CASPT2 that can shed light on the underlying chemical processes controlling the DNA damage. A close agreement between theory and experiment is expected, given an appropriate use of theory. In this review we present recent investigations that employ theory to aid understanding of DNA base and sugar radical formation, via ionization, excitation, and electron attachment to DNA. Specific topics include (1) ionization energies and electron affinities of bases and base pairs, (2) excited states of radical DNA base cations and their roles in leading to sugar radicals, (3) the role of excited states of DNA base anion radicals in the formation of LEE (low-energy electron)-induced DNA single-strand breaks, (4) the nature of hole delocalization in adenine stacks systems including the usual stability of the dimer radical cation $(A_2)^{\bullet+}$ and its importance to the unusual long-range hole transfer within

A stacks in DNA, and (5) the prototropic equilibria found for the guanosine radical cation, which also modulates hole transfer in DNA.

1.2. DIRECT EFFECT OF IONIZING RADIATION IN RADICAL ION FORMATION

As described in the Introduction, the direct interaction of ionizing radiation with DNA initially creates "hole" (cation radical) in DNA and ejects an electron that is usually captured as an anion radical in DNA. Electron spin resonance (ESR) spectroscopy studies show that for γ-irradiated salmon testes DNA at 77 K, the relative amounts of the observed initial ion radicals are: 35% guanine radical cation ($G^{\bullet+}$) with a small amount (< 5%) of adenine radical cation ($A^{\bullet+}$) with nearly equal amounts of thymine and cytosine radical anions ($T^{\bullet-}$, $C^{\bullet-}$) summing to ~45%.[30, 10] The remaining fraction of 10–15% is made up of neutral radicals primarily on the sugar–phosphate backbone.[30, 10] The minimum energy required to form a radical cation is estimated from the ionization potential (IP), while the energy of formation for the anion radical is estimated from the electron affinity (EA) of the corresponding DNA base, sugar, and phosphate. The determination of these fundamental properties are of substantial importance, and much effort has been expended in this area.

Theoretical calculations of molecular structures of bases in their neutral and ionized radical states, their spin density distributions, and their IPs and EAs provide valuable information that aid interpretations of experiment. In Figure 1.1, the molecular structures of guanine (G), adenine (A), thymine (T), cytosine (C), uracil (U) (present in RNA), and sugar moiety are shown.

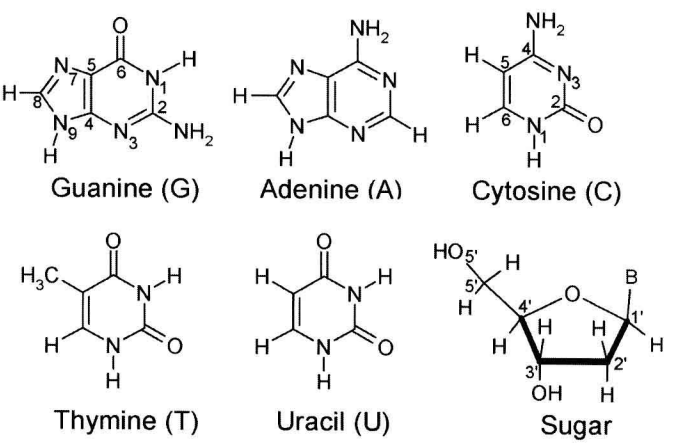

FIGURE 1.1. Molecular structures of guanine (G), adenine (A), cytosine (C), thymine (T), uracil (U) and sugar moiety, present in DNA/RNA. In the sugar moiety, B shows the site of base attachment.

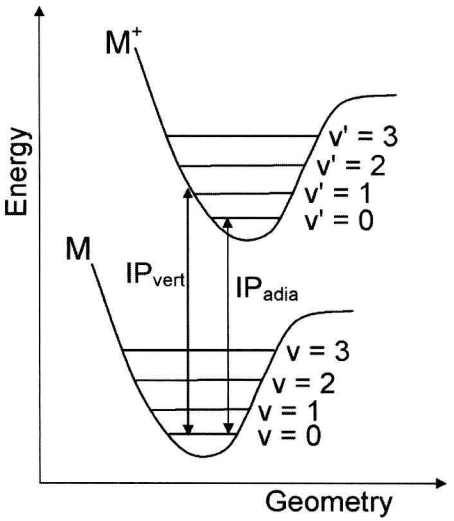

FIGURE 1.2. Diagram showing the definitions of vertical ionization potential (IP_{vert}) and adiabatic ionization potential (IP_{adia}).

1.2.1. Ionization Potential of DNA Bases and Base Pairs

The vertical ionization potential (IP_{vert}) of DNA bases G, C, A, and T in the gas phase has been measured experimentally using photoelectron spectroscopy by Hush and Cheung[31] while the corresponding adiabatic ionization potential (IP_{adia}) values were measured by Orlov et al.[32] using photoionization mass spectrometry in the gas phase. Recently, Kim and co-workers[33] reported the ionization potential of thymine (T) using the high-resolution vacuum ultraviolet mass-analyzed threshold ionization (VUV-MATI) spectroscopy. The ionization potential of a neutral molecule M is the energy required to remove an electron from the molecule. In Figure 1.2, theoretical estimates of the IP_{vert}, IP_{adia} are shown. If the energies are zero point energy (ZPE)-corrected, the ionization potentials are referred to as ZPE-corrected (IP_{zero}). Another quantity, the nuclear relaxation energy (NRE), calculated as the difference between IP_{vert} and IP_{adia}, is of interest because it provides an additional energetic barrier to "hole" transfer within DNA. Figure 1.3 shows the experimental IPs of A, T, G, and C along with their NRE energies. Using different theoretical methods, the gas-phase ionization potential of DNA bases were also calculated.[34–41] A comparison of the theoretically calculated IP values of G, A, C and T along with their corresponding experimental values are presented in Table 1.1. In Table 1.1, we see that both theory and experiment predict the same order of ionization potential of DNA bases as G < A < C < T.

The adiabatic ionization potentials of adenine and cytosine have been studied using CCSD(T)/6-311++G(3*df*, 2*p*) level of theory,[38] and the corresponding values are in an excellent agreement with those calculated using experiment, see Table 1.1. Recently, Cauët et al.[39] used the MP2 method to calculate the vertical and adiabatic ionization potentials of DNA bases. In their study, they added another polarization function (α_d)