1 Nucleoside models for the study of C5'-radicals fate

1.1 Introduction

The DNA

The technical aspects of life involve the complex chemical interactions that take place among several thousand different kinds of molecules found within any living cell. Of these, the deoxyribonucleic acid (DNA) is the master molecule in whose structure is encoded all of the information needed to create and direct the chemical machinery of life. Solving the structure of DNA was surely one of the most important biological discoveries of the 20th century. *James Watson* and *Francis Crick*, in their 1953 letter to *Nature*,[1] assembled pieces of a chemical puzzle that had been accumulating for more than 80 years.

DNA was already discovered in 1869 by a German doctor, *Friedrich Miescher*,[2] isolating a substance, he called "nuclein" from the nuclei of white blood cells. By 1900 the basic chemistry of nuclein had been understood. It was known to be a long molecule composed of three distinct chemical subunits: a five-carbon sugar, acidic phosphate and five types of nitrogen-rich bases (adenine, thymine, guanine, cytosine and uracil). By the 1920s, two forms of nucleic acid were differentiated by virtue of their sugar composition: ribonucleic acid (RNA) and deoxyribonucleic acid (DNA).[3,4] These forms were also found to differ slightly in base composition; thymine is found exclusively in DNA, whereas uracil is found only in RNA. The structure of the nucleosides was elucidated at the end of the 1940s.[5,6]. In 1950, *Erwin Chargaff* of Columbia University discovered a consistent one-to-one ratio of adenine-to-thymine (A-T) and guanine-to-cytosine (G-C) in DNA samples from a variety of organisms.[7] A few years later *Rosalind Franklin* obtained well-resolved X-ray diffraction photographs of DNA.[8] The diffraction patterns strongly suggested a helical molecule with a repeat of 34 angstrom (Å = 10^{-10} m) and a width of 20 Å. The structure *Watson* and *Crick* arrived at by manipulating paper and then metal models, was elegant in its simplicity. The DNA molecule they proposed is an α-helix and resembles a twisted ladder (Figure 1.1). The rails of the ladder, which run in antiparallel directions, contains alternating double units of deoxyribose sugar and phosphate. The aromatic nucleobases stack tightly on top one another, forming the rungs of the helical ladder. Each rung is composed of a pair of nucleotides (a base pair, bp) held together by hydrogen bonds (Figure 1.2). There are 10 bps *per* turn of the helix,

with an interplanar distance of 3.4 Å. In agreement with *Chargaff*'s observation, adenine always pairs with thymine and cytosine always pairs with guanine.[7] Thus, the nucleotide sequence on one half of the DNA helix determines the sequence of the other half.

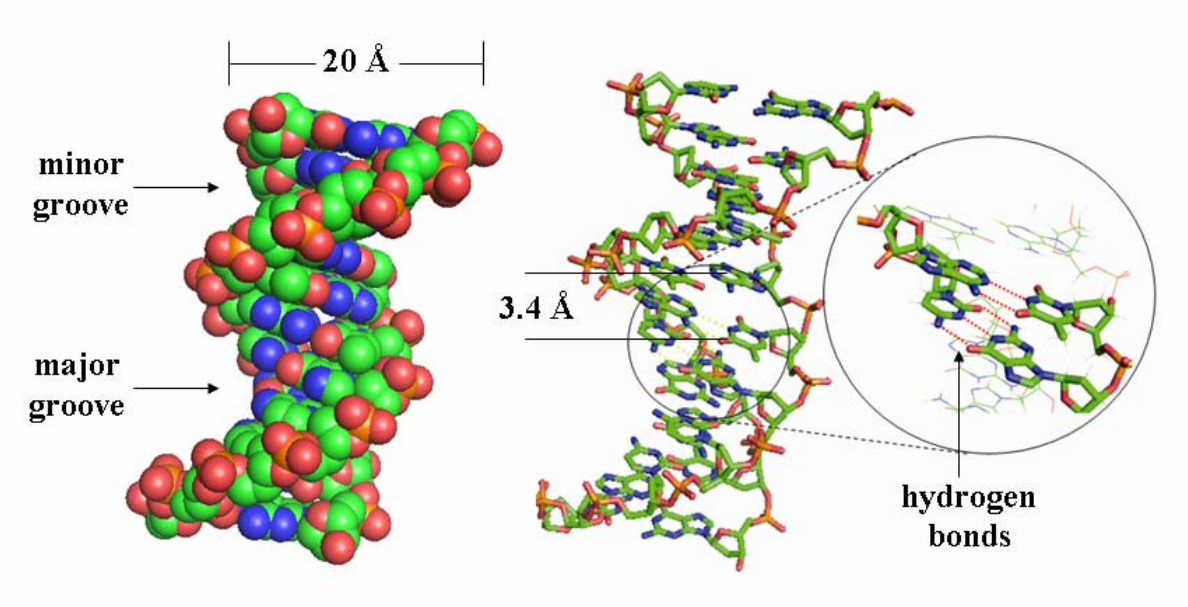


Figure 1.1 Structure of a B-DNA duplex in a "ball" (left) and "stick" (right) representation.

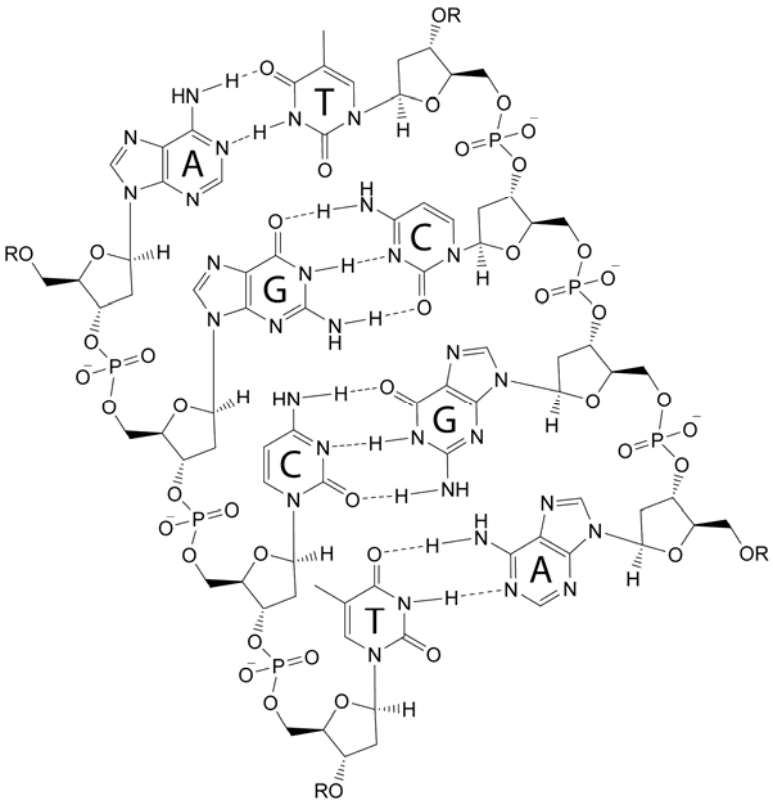
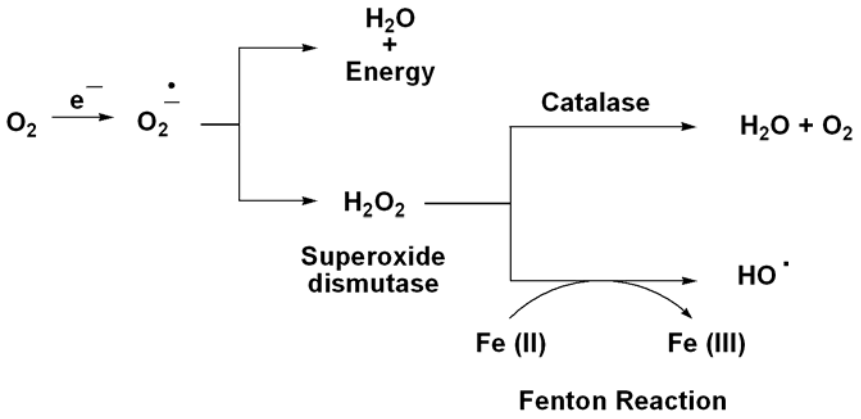


Figure 1.2 DNA structure. Structure of the nucleotides and the *Watson-Crick* hydrogen bonds between GC and AT.

Oxidation of DNA: H-abstraction from the sugar unit

During cellular metabolism of oxygen to water in the mithocondria, a small fraction of the oxygen is reductively converted into superoxide as a by-product.[9] Through complex biochemical processes, superoxide may be converted into various reactive oxygen species (ROS), e.g. hydroxyl radicals (•OH), H_2O_2, $^1O_2$, *etc*. These ROS and in particular the highly diffusible •OH are known to cause chemical modifications on DNA through the formation of strand breaks and nucleobase modifications.[10]



Scheme 1.1 Formation of superoxide, its enzymatic deactivation and the generation of •OH by a Fenton reaction.

DNA damage and strand breaks may also be induced through other environmental influences such as ionizing radiation, photooxidation and naturally occurring or synthetic chemical mutagens.[11]

Maintaining the integrity of DNA is essential for every living organism. To this end evolution designed specific enzymes. Repair and protection of DNA is consequently performed by DNA repair enzymes via several routes. A majority of cells possess defence mechanisms against the harmful effects of ROS. The superoxide dismutase and catalase systems (Scheme 1.1) are able to quench radicals and other oxidising species. The endogenous gluthatione (a γ-glutamylcysteinylglyticine tripeptide, GSH) or the exogenous vitamins are smaller molecules that can drastically reduce the amount of ROS before they can damage the DNA. Lesions formed in DNA can be repaired by mismatch repair enzymes, error-prone repair enzymes, nucleotide excision repair process (NER) or base excision repair process (BER). The latter two are accomplished by several enzymes which recognise and

repair the DNA lesion. A simplified example of their working principle is illustrated in Figure 1.3. However, deleterious genetic alterations may accumulate in cells with age through errors in repair and recombination when the oxidative damages are poorly repaired. In addition, the level of repair enzymes may decrease with the age of the cell. DNA damage can eventually cause cell death or the modification of its functionality, as in the case of tumor cells. Thus, there is a great interest in the study of the mechanisms of DNA damage formation and effects on the cell and the repair systems involved in these processes. In addition, DNA damage is used in medical applications, i.e. in cancer therapies. Many cytostatic cancer drugs target the DNA of cells inducing apoptosis through DNA damage.

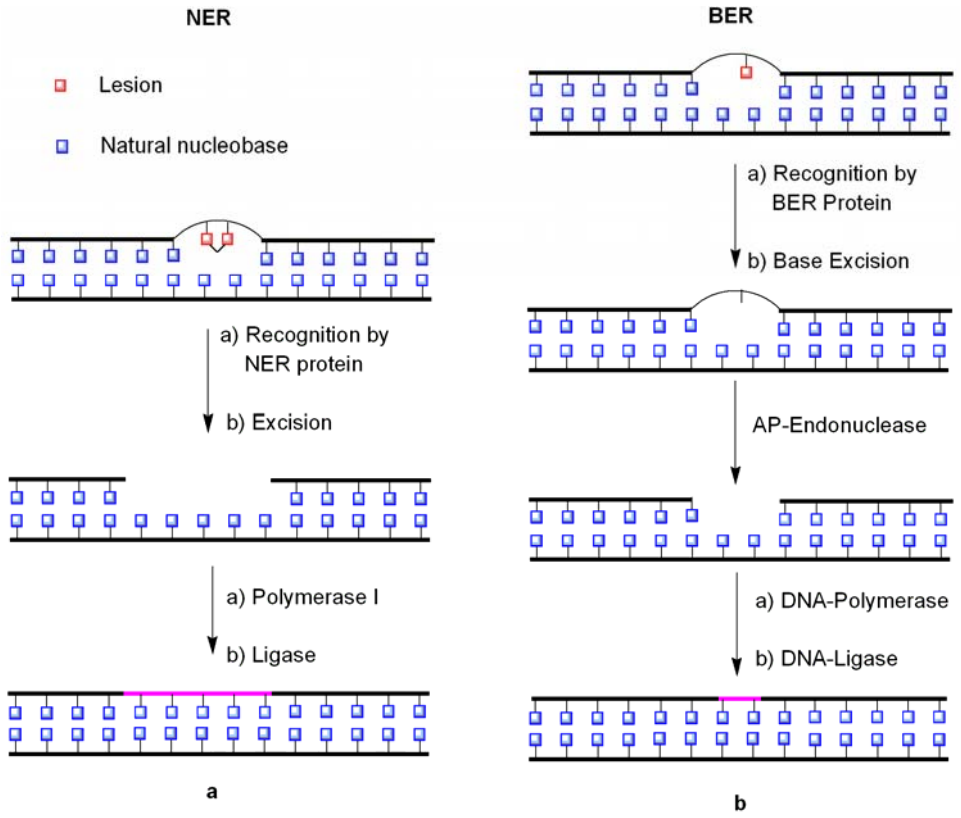


Figure 1.3 Schematic working principle of NER and BER processes.

Oxidative DNA damage can be produced by the oxidation of nucleobase or sugar units. More than 80 modified purines and pyrimidines resulting from oxidative damage have been identified. This base damage threatens the genomic integrity and is the origin of lethal effects or mutations in DNA. Damage to deoxyribose leads to the loss of one base and/or a strand break on DNA. Both kinds of damage eventually result in lethal lesions, especially when an oxidative process produces a double-strand break (DSB), that is two single strand breaks

(SSBs) on opposite strands. Furthermore a few examples of damage that involve both, the nucleobase and the sugar unit, are known. Cyclopurine and cyclopyrimidine lesions are observed among the decomposition products of DNA when exposed to ionising radiations or to certain antitumor agents.[12-19] Two examples, described later in this Chapter 1, are the 5',6-cyclo-5,6-dihydrothymidine and the 5',8-cyclo-2'-deoxyadenosine. They possess an additional bond between the C6 position of pyrimidine or C8 position of purine and the C5' position of the 2'-deoxyribose.

All the sugar degradation processes are initiated by H-abstractions, since the aliphatic nature of deoxyribose does not favour oxidation by electron abstraction as in the case of the nucleobases. Subsequently, the generated sugar free radical can repair itself by hydrogen atom abstraction from glutathione (GSH), leading to modifications of the sugar unit (DNA-damage), or to strand-breakage.[11,14,20] Although all seven hydrogen atoms of deoxyribose are belived to be reactive toward oxidising species and free radicals, not all are equally likely to be abstracted from DNA. The H-abstraction pathway is determined by the helical structure of DNA and on the relative orientation of the oxidant to the sugar. According to the structures obtained by X-ray crystallography of several oligonucleotides, the 5'-, 4'- and 1'-positions of B-form double strand DNA (dsDNA) are accessible from the minor groove, while the 3'- and 2'-positions are accessible from the major groove. One 5'-hydrogen atom (*pro-S*) points directly into the minor groove; the other points away from the backbone (see Figure 1.4).[21]

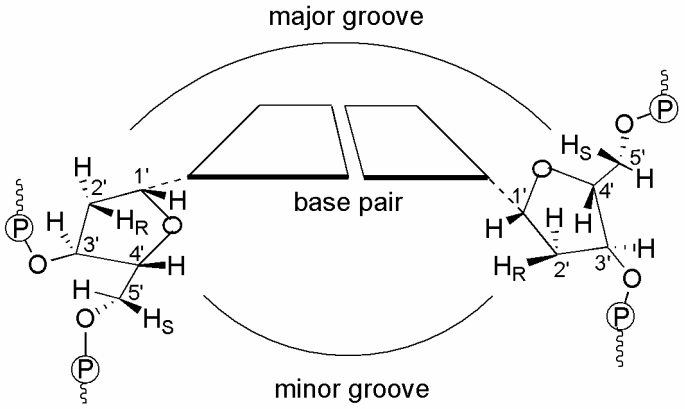


Figure 1.4 All seven C-H bonds of deoxyribose are prone to oxidation, but four point into the minor groove (indicated by wedge-shaped bonds), and three into the major groove (H_R and H_S refer to the absolute configuration – *pro-R* and *pro-S* – of hydrogen atoms at C2' and C5').

In the formation of a strand break, which of the seven hydrogen atoms is abstracted is the key question in elucidating mechanisms of nucleic acid degradation. *Osman* and co-workers calculated the energy of hydrogen abstraction initiated by the hydroxyl radical for the positions 1', 2', 3', and 4' of the model sugar 2-deoxy-*D*-ribose and found that the theoretical probability of abstraction is correlated with C-H bond strength.[22] They also determined that, aside from the C2'-H, the abstraction of every hydrogen bond requires a similar amount of energy, presumably because the resulting radical is stabilized by the α-oxygen atom. When similar studies were performed on double-stranded B-DNA, solvent accessibility became an additional critical factor. In this case the hydrogen of C1' is virtually "hidden" by the local bulkiness, whereas the C4'-H and the C5'-H are significantly more exposed to solvent, making them much more likely to be abstracted.[23,24]

Another important point is that not all oxidation events lead to immediate strand cleavage. Several pathways result in the formation of metastable sugars or sites that can be hydrolysed *via* base-catalyzed reactions. Furthermore, the formation of a radical on the sugar unit can lead to stable nucleotide modifications by interaction with adjacent nucleobases of the same DNA strand (*intra*-strand cross-linking) or with the counter strand (*inter*-strand cross-linking). In addition, as in the case of the above-mentioned cyclopurines and cyclopyrimidines, the reaction can be *intra*-molecular. In fact, it has been verified that the C5' radical, initially generated by hydrogen abstraction, intramolecularly attacks the double bond of the nucleobase moiety to form a cyclonucleotide as the final product after oxidation or reduction (see also Scheme 1.6, pag. 18).[25-27]

Here I briefly describe the chemistry of radicals formed by hydrogen abstraction from the sugar unit of DNA. The cases of the five sugar positions will be discussed focusing first on the C5'-H abstraction. The C5' radical generation and study of its fate represent the topic of Chapter 1. The molecules used by researchers to abstract hydrogen atoms from the sugar unit with a different degree of selectivity and/or the technique they used for the generation and detection of the intermediate and final products will only be described in this thesis for the C5' position. The reader is referred to excellent reviews for more details on this topic.[11,14,20,28]

C1' radicals

The C1'-centered radical **1.1** can be generated by hydrogen abstraction through the interaction of DNA, oligonucleotides or nucleosides with ionizing radiation (γ-radiolysis), metal complexes (Mn-TMPyP, Cu(*o*P)$_2$ and anticancer drugs such as enediynes). Photolysis of photoreactive groups that are specific C1' radical precursors have been used by *Greenberg* and *Chatgilialoglu* for kinetic and product studies.[29-31] In addition the photoreaction of 5-halouracil-containing oligonucleotides gives rise to C1'- (and C2'-) centered radicals. The latter photoreaction has been extensively used in different fields of research as proliferation-marker[32] or as electron acceptor in DNA excess electron transfer studies,[33-35] and as DNA-structure probe.[36] An example of radical generation via photoreaction of halouracil is reported for the C2'-radical in Scheme 1.3. More recently, *Sevilla* and co-workers reported the generation of sugar radicals using UV-A / visible photo-exitation of the guanosine radical cation ($G^{\bullet+}$). According to the authors, selective radical generation is possible when optimised reaction parameters are employed. Specifically C1'-radicals have been observed in dsDNA under exposure to light (310-480 nm), whereas other radicals have been formed with longer wavelengths of irradiation (C3'- and C5'-radicals).[37]

The C1' radical **1.1** abstracts a hydrogen atom from a thiol to give a mixture of β-anomer **1.2** (repairing reaction), and α-anomer **1.3** (Scheme 1.2). The latter belongs to a class of nucleotides that have been shown to be premutagenic *in vitro* when generated during γ-radiolysis of DNA under anaerobic conditions.[38,39] In the presence of oxygen or metal complexes, radical **1.1** leads to abasic site damage, through the formation of the instable peroxyradical **1.4** resulting in the formation of a 2-deoxyribonolactone residue **1.6** and the corresponding free base BH **1.7**.[11] This lesion is alkaline-labile and results in strand scission with formation of 3'-phosphate **1.8**, 5'-phosphate **1.10** and 5-methylene furanone (5-MF) **1.9**. In a biological environment, oxygen and GSH trapping of C1' radicals are competitive processes, due to the μM oxygen concentration (but high rate constant of 1×10^9 M^{-1} s^{-1}) in the nucleus. Once the peroxyl radical is formed, the C1' peroxyl radical **1.4** expels a superoxide radical anion with a rate constant of ca. 2×10^4 s^{-1}. The resulting C1' cation **1.5** leads to the formation of the ribonolactone much faster than it is trapped by GSH. The H-abstraction from the thiol to give the hydroperoxide, has in fact a rate constant ≤ 400 M^{-1} s^{-1}. Direct oxidation to cation **1.5** is also observed in the presence of metal complexes.