MOLECULAR MECHANISMS OF RADIATION INDUCED DNA DAMAGE: H-ABSTRACTION AND β-CLEAVAGE

L. PARDO,* R. OSMAN, J. BANFELDER, A.P. MAZUREK and
H. WEINSTEIN

*Department of Physiology and Biophysics, Mount Sinai School of Medicine of the
City University of New York, New York, N.Y. 10029, USA*

Quantum mechanical simulations of hydrogen abstraction by hydroxyl radical from methanol and ethanol yield barriers that agree very well with those measured experimentally. Analysis of the multiconfigurational wavefunction indicates that the strength of the C–H bond is the electronic parameter that has a major contribution to the barrier for H-abstraction. Similar analysis applied to 2-deoxy-D-ribose shows that the strength of a C–H bond together with the steric accessibility of the hydrogen determine that H_4' is the most susceptible hydrogen for abstraction by a hydroxyl radical. Quantum mechanical simulations of β-cleavage show that a concerted mechanism in which a water molecule assists in the bond breaking process is more likely than a S_N^1 mechanism. However, the polar transition state suggests that the environment of the DNA and the surrounding water will have an important effect on the reaction.

KEY WORDS:

INTRODUCTION

Radiation damage to biological systems comprises a diverse set of phenomena.[1] Of special interest is the damage induced in DNA, as many biological consequences can be traced to this specific event. Because ionizing radiation acts indiscriminantly on all molecules in a treated sample, radiation effects on DNA must be considered as resulting from both indirect and direct actions. Through direct actions,[2] the energy is deposited directly in the DNA molecule, while in indirect action the ionizing radiation forms reactive species (OH radical and H radical) in the surrounding environment, and these diffuse towards DNA and interact with it. The radical H adds primarily to double bonds in the bases and exhibits very little reactivity toward the sugar. The OH radical can react with the bases through addition to double bonds and through hydrogen abstraction and the formation of carbon based radicals on the sugar ring.[3] The cleavage of a β-bond and the elimination of the 3′-phosphate leads to a single strand break of DNA[4] and is an important consequence of radical formation on C_4' of the sugar.

We summarize here the results of a study of the mecahnism of hydrogen abstraction by the OH radical, followed by strand breaking. The reactions were simulated computationally and the energetic parameters were evaluated with quantum mechanical methods.

---

*Permanent address: Department of Biostatistics, Faculty of Medicine, Universidad Autonoma de Barcelona, Bellaterra, Barcelona, Spain.

RESULTS

Mechanism of hydrogen abstraction

To define the theoretical tools and determine their reliability in the quantitative description of the key mechanism of hydrogen abstraction by a hydroxyl radical, we first studied the reaction on the model systems methanol and ethanol. These molecules were chosen as models because the energies of activation of hydrogen abstraction reactions from these molecules are available from experiments:[6] 1.59 Kcal/mol for methanol and 0.54 Kcal/mol for the α hydrogen in ethanol. The energy barriers for hydrogen abstraction calculated at the MP4SDQ/6-311G**//HF/6-31G level are in good agreement with these data: 1.36 Kcal/mol for methanol and 1.09 Kcal/mol for H_α of ethanol. The calculated value for H_β is 2.56 Kcal/mol, but the experimental value is not available.[6] The simpler MP2/6-31G*//HF/6-31G scheme produces similar results i.e., 1.57, 1.22 and 2.85 Kcal/mol respectively, and can be used to calculate the hydrogen abstraction in realistic models of deoxyribose. Results of the computations point to the strength of the C-H bond as a major contribution to the energy of activation, and provide an explanation for the differences in the barriers of H abstraction from C_α of methanol and C_α and C_β of ethanol.

Hydrogen Abstraction from 2-deoxy-D-ribose

The electronic determinants defined above, taken together with a steric accessability parameter, provide reliable predictors for hydrogen abstraction on the 2-deoxy-D-ribose. Calculations of the bond strength for all hydrogen atoms reveal H_4' as the most susceptible for abstraction. The steric accessibility parameter shows that H_4' is also the most exposed in a B-DNA double strand.[5] These findings indicate that the reactive site of a deoxyribose that is most susceptible to hydrogen abstraction, is the H_4'. Simulations of the hydrogen abstraction of H_4' by OH radical show that the abstraction is assisted by a hydrogen bond between the hydroxyl radical and O_3'. This makes the energy of activation -2.3 Kcal/mol if isolated reactants are considered as the reference, or 5.3 Kcal/mol if the hydroxyl radical hydrogen bonded to O_3' is considered as the reference. The energy of the reaction is -27.2 Kcal/mol in a system where the resulting water is hydrogen bonded to O_3' and the radical is centered on C_4'.

β-cleavage

Our simulations of the second step in the strand breaking process were based on quantum mechanical studies of β-cleavage of the phosphodiester bond carried out on models of deoxyribose with a radical on C_4'. Results from these studies suggest that the mechanism need not proceed through the formation of a carbocation on C_3' and a phosphate anion in a S_N^1 mechanism. Rather, the mecahnism is more likely to proceed in a concerted fashion in which a water molecule bound to the DNA near the O_3' oxygen assists in the cleavage to yield directly the products. The calculated energy of activation is 36.91 Kcal/mol and the energy of reaction is -4.1 Kcal/mol if deoxyribose with a radical on C_4' plus a water molecule are considered as reference. Analysis of the charge distribution of the transition state shows a partial charge of -0.691 electron units in the O_3'-H moiety which is the leaving group, and 0.628 electron units on C_4' which is the atom attacked by the water molecule. This very polar structure must be strongly stabilized by its environment provided by DNA ions and water. To

obtain a realistic description of the energetics of H abstraction and β-bond cleavage, it therefore becomes necessary to evaluate the energetics of the process in the electrostatic field generated by the various components of the DNA system, and to include these effects and the polarization of the DNA molecules in the quantum mechanical descriptions.

References

1. C. von Sonntag (1987) The Chemical basis of radiation biology. Taylor and Francis, London-New York-Philadelphia.
2. R. Osman, L. Pardo, J. Banfelder, A.P. Mazurek, L. Shvartzman, R. Strauss and H. Weinstein (1990) Molecular mechanism of radiation induced DNA damage: H-addition to bases, direct ionization and double strand break. *Free Radical Research Communications* In press.
3. R. Osman, W.J. Clark, A.P. Mazurek and H. Weinstein (1989) Theoretical studies of molecular mechanisms of DNA damage induced by hydroxyl radicals. *Free Radical Research Communications.*, 6 131.
4. G. Behrens, G. Koltzenburg and D. Schulte-Frohlinde (1982) Model reactions for the degradation of DNA-4' radicals in aqueous solution. Fast hydrolysis of α-alkoxyalkyl radicals with a leaving group in ß-position followed by radical rearrangement and elimination reactions. *Z. Naturforsch.*, 37c, 1205.
5. C.J. Alden and S. Kim (1979) Solvent-accessible surfaces of Nucleic acids. *Journal of Molecular Biology.*, 132, 411.
6. R. Atkinson (1985) Kinetics and mechanisms of the gas-phase reactions of the hydroxyl radical with organic compounds under atmospheric conditions. *Chemical Reviews*, 85, 69.

Accepted by Prof. G. Czapski