Application of Isodesmic Reactions to the Calculation of the Enthalpies of H• and OH• Addition to DNA Bases: Estimated Heats of Formation of DNA Base Radicals and Hydrates

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In this work, we employ semiempirical and low-level ab initio molecular orbital theories with isodesmic/isogyric reactions to estimate the energetics of free radical reactions important to radiolytic damage to DNA. Results are presented (1) for H• and OH• addition reactions to the natural DNA bases, (2) for the addition of H• to the DNA base radical adducts so obtained, and (3) for the dehydration of the DNA base hydrates and regeneration of the undamaged bases. The technique employed sums the calculated enthalpy of an isodesmic reaction with the experimentally known reaction enthalpy of an associated reaction to estimate the enthalpies of the DNA base reactions of interest. The calculational techniques employed are the ROHF/6-31G* ab initio method and the ROHF/PM3 semiempirical method. For H• addition reactions, the ROHF ab initio method employing the 6-31G* basis set gives results that are in closest agreement with known experimental results. For the OH• addition processes, the ROHF/PM3 semiempirical technique results in predictions more in keeping with the experimental model systems. The values calculated are employed to estimate the heats of formation of various DNA base radicals and DNA base hydrates. Economical PM3 calculations of isodesmic reactions when combined with well-known experimental enthalpies of reaction are found to yield reliable thermodynamic quantities.

Introduction

High-energy radiation damage to water produces three major radicals: hydroxyl radicals, hydrogen atoms, and electrons. There is considerable evidence that the predominant reaction of •OH and H• with DNA model compounds is addition to a DNA base double bond, whereas the main mode of attack on the sugar phosphate backbone is hydrogen abstraction.[1,2] Hydrogen abstraction from the C5 methyl group of thymine also occurs, both in model compounds and in DNA itself.[3] Solvated electrons can add to the DNA bases to form anion radicals; when followed by irreversible protonation at a carbon atom, these radicals convert to the same species that are formed by H addition reactions.[1] With direct radiation damage[1] base anion radicals and base cation radicals are trapped at 77 K. Interestingly, in model compounds, the DNA base cation radicals undergo a water addition−deprotonation process to form, in effect, hydroxyl radical adducts.[1] One difference between the direct damage (electrons and holes) and attack from the water radicals (H•, $e^{\bullet -}$, •OH) is that the direct damage has more site specificity than damage from the water radicals. In addition there are alternative reaction paths for the ion radicals; for example, cation radicals often undergo deprotonation, resulting in a neutral radical.[1,2]

An understanding of the energetics of •OH and H• atom addition is important to a full understanding of the radiation damage to DNA. Direct application of molecular orbital calculations at a modest level of theory does not yield satisfactory results for this type of problem because it is widely recognized that Hartree−Fock theory gives poor results for direct calculation of bond dissociation energies.[4,5] However, at the single-determinant level, the energetics of isodesmic reactions (characterized by conservation of the number of like bonds in the reactants and products) are well predicted, due to a cancellation of errors.[4−7] If the reaction is also isogyric (characterized by conservation of electron spin), as most isodesmic reactions are, the accuracy of calculations is further enhanced. In an example actually considered in this work, the enthalpy change for reaction 1 (the bond enthalpy of the thymine methyl C−H bond) is sought employing the theoretically calculated enthalpy for the isodesmic process given in reaction 2 and the experimentally determined enthalpy for reaction 3.

$$\mathrm{T} \rightarrow \mathrm{UCH_2\bullet} + \mathrm{H\bullet} \tag{1}$$

$$\mathrm{T} + \mathrm{CH_2{=}CH{-}CH_2\bullet} \rightarrow \mathrm{UCH_2\bullet} + \mathrm{CH_2{=}CH{-}CH_3} \tag{2}$$

$$\mathrm{CH_2{=}CH{-}CH_3} \rightarrow \mathrm{CH_2{=}CH{-}CH_2\bullet} + \mathrm{H\bullet} \tag{3}$$

The enthalpy for reaction 1 is given by $\Delta H(1) = \Delta H(2)$[theory] + $\Delta H(3)$[experiment]. Clearly, the more similar the structures are in reaction 2, the more reliable the calculation is. In this manner, the enthalpy of the target reaction is determined by using the calculated enthalpy of an isodesmic reaction to adjust the experimental enthalpy of a properly chosen reaction. Recently this calculational technique has been successfully used to predict bond dissociation energies in a number of systems including peptides[7,8] and fluorocarbons.[9−15]

In previous work we have investigated the energetics of one-electron addition to and removal from the DNA bases[16,17] and to/from their hydroxyl radical and hydrogen atom adducts.[18] In this paper we extend our work to each step in the energetics of the following processes:

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$$\text{Base} + \text{H}\bullet \rightarrow \text{BaseH}\bullet \xrightarrow{+\text{H}\bullet} \text{BaseHH} \tag{4}$$

$$\text{Base} + \bullet\text{OH} \rightarrow \text{BaseOH}\bullet \xrightarrow{+\text{H}\bullet} \text{BaseHOH} \rightarrow \text{Base} + \text{H}_2\text{O} \tag{5}$$

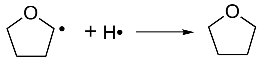
We find that the use of isodesmic reactions aids our estimates of these reaction enthalpies.

Methods of Calculation

The ROHF/6-31G* polarization basis set[19−21] and the ROHF/PM3 semiempirical method[22,23] implemented in the Gaussian 92 program[24] were used to fully geometry optimize the structures presented in this work on Cray C90 and IBM RS 6000 computers. Single-point calculations were performed employing the unrestricted Hartree−Fock theory.[25] The (*x*,*y*,*z*) coordinates are available in the Supporting Information. PM3 level calculations give room-temperature reaction enthalpies; however, ROHF/6-31G* ab initio calculations result in dissociation energies at absolute zero. To directly compare these different values, the dissociation energies at 0 K must be corrected for zero-point vibrational energies, as well as rotational and translational effects. These values must also be corrected to 298 K via frequency calculations. These corrections contribute only modest changes (0−2 kcal) to the isodesmic reactions considered in this work since the products and reactants have the same number and types of chemical bonds. These small corrections were estimated using the PM3 semiempirical method. This work considers gas-phase reactions only; solvation energy effects are not considered.

Results and Discussion

Energetics of •OH and H• Radical Addition to DNA Bases Using Isodesmic Processes. In an effort to further verify the validity of the isodesmic reaction approach, we have calculated the enthalpy involved in a termination reaction 6 involving tetrahydrofuran-2-yl.


(6)

This compound was chosen because it is a simple model for the sugar portion of DNA (the deoxyribose ring) and because the experimental C2−H bond dissociation energy is well-known (92 ± 2 kcal).[26] Direct calculations performed for reaction 6, i.e., for the homolytic bond dissociation energy, using ROHF/PM3 and ROHF/6-31G* methods, give poor results: 77 and 81 kcal, respectively.

However, the use of isodesmic/isogyric reactions results in calculated bond dissociation enthalpies for the tetrahydrofuran C2−H bond that lie within experimental error. In this case, enthalpies were calculated for the isodesmic reaction in which tetrahydrofuran-2-yl abstracts a hydrogen atom from ethane, ethanol, or cyclopentane (Figure 1). Experimental bond dissociation enthalpies for these three compounds are available.[27] Combination of the isodesmic reaction enthalpy with the appropriate known experimental bond dissociation energy gives the desired result. For example, when cyclopentane is used, our estimated reaction enthalpy for reaction 6 is −90.6 kcal for PM3 calculations and −93.7 kcal for 6-31G* calculations (Figure 1). It should be noted that the choice of the compound for the experimental bond dissociation energy has only a small effect on the final result, as long as the nature of the bond created is the same as that which is broken in the isodesmic H abstraction reaction.

Table 1 presents the calculated enthalpies for the isodesmic reactions used. Reactions I(a)−I(f) all correspond to H atom addition, at the C5 or C6 position of a pyrimidine [reactions I(a)−I(c)], at N3 of cytosine [reaction I(d)], and at C8 of the purines [reactions I(e), I(f)]. Reactions I(g)−I(n) correspond

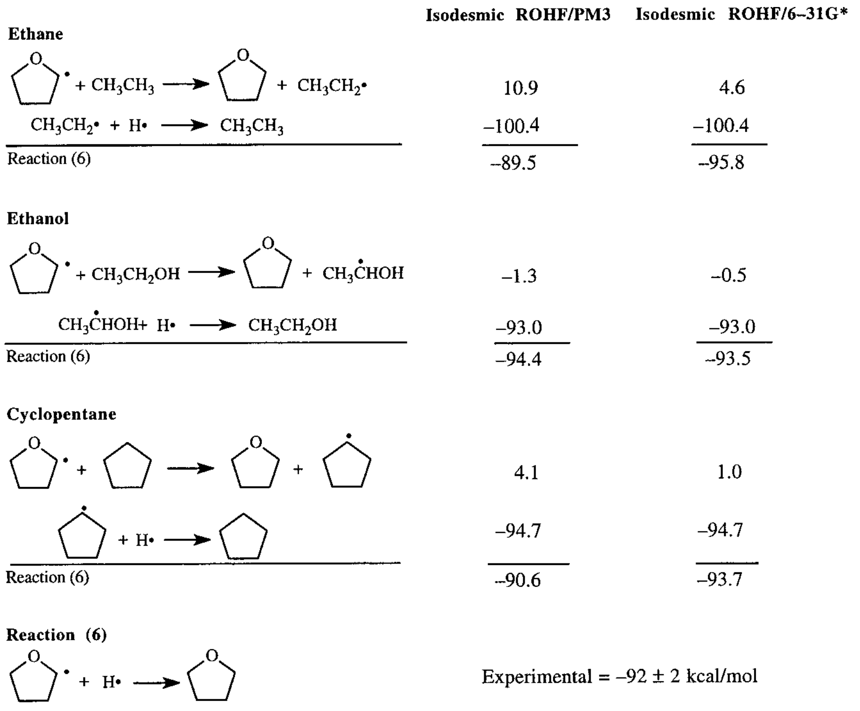


Figure 1. Illustration of the use of isodesmic reactions. In this case, the enthalpy of reaction (6) is desired. For each set of reactions shown, the enthalpy of the first reaction (which is isodesmic) is calculated using either the ROHF/PM3 method or the ROHF/6-31G* method, corrected for translational, rotational, and vibrational contributions at 298 K. These corrections are −1.12, 0.04, and 0.33 kcal for the three isodesmic reactions, respectively. The experimental enthalpy of the second reaction in each set is known to within ±1 kcal (ref 27) and shown in the figure. When the calculated enthalpy is added to the experimental enthalpy, a value for the energy of the target reaction is obtained. All of the results except one are within the experimental limits of 92 ± 2 kcal/mol.

TABLE 1: Calculated Enthalpies for Isodesmic Reactions (kcal/mol)[a]

	ROHF/PM3	UHF// ROHF/PM3	ROHF/6-31G*[b]	UHF// ROHF/6-31G*[b]	therm corr[c]
(a) $T + \bullet CH_2CH_3/CH_3\dot{C}H-CH_3 \rightarrow \bullet T(6)H + CH_2{=}CH_2/CH_3CH{=}CH_2$	8.4/10.2	6.7/8.9	9.9/9.1	8.6/8.0	1.5/1.2
(b) $C + \bullet CH_2CH_3/CH_3\dot{C}H-CH_3 \rightarrow \bullet C(5)H + CH_2{=}CH_2/CH_3CH{=}CH_2$	5.7/7.6	3.8/6.1	13.3/12.6	13.4/12.7	0.1/−0.1
(c) $C + \bullet CH_2CH_3/CH_3\dot{C}H-CH_3 \rightarrow \bullet C(6)H + CH_2{=}CH_2/CH_3CH{=}CH_2$	14.2/16.0	9.1/11.3	15.4/14.7	13.4/12.7	−0.2/−0.4
(d) $C + NH_2-CH_2\bullet \rightarrow \bullet C(3)H + NH{=}CH_2$	5.1	0.6	11.9	6.7	−0.3
(e) $A + \bullet NH-CH_3 \rightarrow \bullet A(8)H + HN{=}CH_2$	6.6	−0.3	11.7	7.7	−0.7
(f) $G + \bullet NH-CH_3 \rightarrow \bullet G(8)H + HN{=}CH_2$	4.9	−0.3	11.4	9.5	−0.8
(g) $G + \bullet C_6H_6OH \rightarrow \bullet G(4)OH + C_6H_6$	−8.6	−3.2	−8.9	−0.7	0.7
(h) $G + \bullet C_6H_6OH \rightarrow \bullet G(5)OH + C_6H_6$	−6.7	−2.9	−1.3	11.1	0.8
(i) $A + \bullet C_6H_6OH \rightarrow \bullet A(4)OH + C_6H_6$	−0.5	4.3	−0.8	10.0	−1.5
(j) $A + \bullet C_6H_6OH \rightarrow \bullet A(5)OH + C_6H_6$	2.3	4.2	8.8	12.7	0.2
(k) $T + \bullet C_6H_6OH \rightarrow \bullet T(6)OH + C_6H_6$	−14.5	−8.8	−22.0	−9.8	2.6
(l) $T + \bullet C_6H_6OH \rightarrow \bullet T(5)OH + C_6H_6$	−10.7	−4.6	−12.7	0.8	2.6
(m) $C + \bullet C_6H_6OH \rightarrow \bullet C(6)OH + C_6H_6$	−8.9	−6.9	−17.4	−5.8	1.1
(n) $C + \bullet C_6H_6OH \rightarrow \bullet C(5)OH + C_6H_6$	−14.3	−8.6	−12.4	0.9	1.3
(o) $UCH_2\bullet + CH_2{=}CH-CH_3 \rightarrow T + CH_2{=}CH-CH_2\bullet$[d]	2.5	2.1	−0.3	3.2	−1.1

[a] Optimized at ROHF/6-31G* and ROHF/PM3. [b] Including thermodynamic correction term from last column of this table. [c] Thermodynamic correction term (using UHF/PM3), includes translational, rotational, and vibrational contributions at 298 K. [d] Optimized at UHF.

TABLE 2: Reactions of Known Enthalpy: Experimental and Calculated Enthalpies[a]

	expt ΔH	ROHF/PM3[b]	ROHF/6-31G*[c]	UHF// ROHF/PM3[b]	UHF// ROHF/6-31G*[c]	therm corr[d]
(a) $CH_2{=}CH_2 + H\bullet \rightarrow CH_3-CH_2\bullet$	-36.3 ± 0.4[e]	−51.2	−34.2	−54.1	−36.8	5.3
(b) $CH_3-CH{=}CH_2 + H\bullet \rightarrow CH_3-CH\bullet-CH_3$	-35.6 ± 0.7[e]	−53.0	−32.9	−56.3	−35.5	6.1
(c) $HN{=}CH_2 + H\bullet \rightarrow NH_2-CH_2\bullet$	-37 ± 5[e]	−52.4	−27.1	−54.2	−29.7	5.9
(d) $HN{=}CH_2 + H\bullet \rightarrow \bullet NH-CH_3$	-31 ± 5[e]	−45.1	−32.5	−47.2	−35.4	5.5
(e) $C_6H_6 + OH\bullet \rightarrow \bullet C_6H_6OH$	-16.5 ± 2[f]	−21.9	9.5	−31.9	−4.2	1.7
(f) $CH_2{=}CH_2 + OH\bullet \rightarrow \bullet CH_2-CH_2OH$	-30.7 ± 2[g]	−40.1	−14.3	−43.1	−14.4	2.5
(g) $CH_2{=}CH-CH_2\bullet + H\bullet \rightarrow CH_2{=}CH-CH_3$	-86.3 ± 1.4[h]	−85.5[i]	−73.9[i]	−76.9[j]	−57.5[j]	8.4

[a] kcal/mol. [b] Heat of formation differences. [c] Including thermodynamic correction term from last column of this table. [d] Thermodynamic correction term (using UHF/PM3) includes translational, rotational, and vibrational contributions at 298 K. [e] Calculated using enthalpies of formation from ref 27; ref 33; ref 30 [$\Delta H^\circ_{f,298}(NH{=}CH_2) = 21 \pm 4$ kcal/mol]. [f] Reference 32. [g] Calculated using enthalpies from: ref 32, eq 2; ref 27; ref 34 [$\Delta H^\circ_{f,298}(\bullet OH) = 9.43$ kcal/mol]. [h] Reference 27, pp 9−136. [i] ROHF//UHF. [j] UHF//UHF.

to OH addition at various positions of the purines and pyrimidines. No suitable compound of known bond dissociation energy could be found to associate with isodesmic reactions involving •G(8)OH or •A(8)OH; hence these intermediates were not investigated. Full geometry optimization of all base intermediates was performed at the ROHF/6-31G* level.

For comparative purposes, parallel studies were also performed with the semiempirical PM3 method at the ROHF level. The PM3 method has been shown to yield satisfactory heats of formations for all four natural DNA bases,[28] and calculations of bond dissociation energies involving various carbon-centered radicals at the ROHF/PM3 level are in fair agreement with experimental data.[29] All species considered in the isodesmic reactions investigated at ROHF/PM3 were fully geometry optimized at that level. The UHF results, however, usually were obtained from single-point calculations performed on the ROHF geometry optimized structures. (Full UHF geometry optimization performed on the isodesmic process involving T(6)OH• resulted in a difference of ca. 1 kcal/mol from the enthalpy calculated at UHF/ROHF.) However, because the effect of resonance on the geometry of the allyl radical is poorly predicted at the ROHF level, all reactants and products in reaction I(o) were fully optimized at UHF. These geometries were subsequently employed to perform single-point calculations at the ROHF level.

In the H addition reactions to the C5 and C6 sites of the pyrimidines, it is interesting to note that the nature of the compound chosen for the isodesmic reactions (propene or ethene) has only a small effect on the overall energetics, as long as the nature of the bonds created and destroyed is conserved. However, we find that the use of $CH_2{=}CH_2/\bullet CH_2-CH_2OH$ and $C_6H_6/\bullet C_6H_6OH$ in the isodesmic reactions involving •OH addition leads to significant variations in the enthalpies of the isodesmic processes (ca. 20 kcal/mol at ROHF and ca. 11 kcal/mol at UHF). These variations are mostly due to the destruction of the resonance in benzene upon formation of its hydroxyl adduct, but tend to vanish on calculating the base−H bond enthalpies, as discussed below.

The experimental bond dissociation enthalpies of the compounds to be used in determining the H• and •OH addition reaction enthalpies for the DNA bases are presented in Table 2. The experimental enthalpies for these reactions were combined with the corresponding calculated value for an isodesmic reaction (Table 1) to arrive at the estimated enthalpy of the target reaction. Reaction II(a) in this table was combined with the isodesmic reactions I(a)−(I)c of Table 1 to investigate H atom addition at the C5 and C6 positions of the pyrimidines. Reaction II(b) in Table 2 was used with these same reactions to evaluate the effect of structural changes of the compound of known bond energy on the overall energetics of the target reaction. Reactions II(c) and II(d) were employed to calculate the energetics of H• addition at N3 in cytosine and C8 in the purines (Table 1), respectively. Reactions II(e) and II(f) offer not only the possibility of calculating the bond enthalpies of the DNA bases' hydroxylated adducts (Table 1) but also the opportunity to investigate the effects of structural as well as electronic differences of the two compounds of known bond energy on the target reactions.

Also displayed in Table 2, as a test for the predictive nature of the various levels of calculation, are various calculated values for the enthalpies of the reactions shown. The calculated enthalpies of the reactions presented in Table 2 suggest that the ROHF/6-31G* level of calculation is in good agreement with the experimental data for the H atom addition reactions, while the experimental enthalpies of OH• addition appear to be best represented at the ROHF/PM3 level. Reaction II(g) of

TABLE 3: DNA Base •OH and •H Addition Enthalpies Using the Isodesmic Method[a]

	ROHF/PM3	ROHF/6-31G*[b]
T + •H → •T(6)H	−26.1	−27.2[c]
C + •H → •C(5)H	−28.7	−23.7[c]
C + •H → •C(6)H	−20.3	−21.6[c]
C + •H → •C(3)H	−32	−25[c]
A + •H → •A(8)H	−24	−19[c]
G + •H → •G(8)H	−26	−20[c]
C + •OH → •C(6)OH	−25.5[c]	−33.9
C + •OH → •C(5)OH	−30.8[c]	−28.9
T + •OH → •T(6)OH	−31.0[c]	−38.5
T + •OH → •T(5)OH	−27.2[c]	−29.2
G + •OH → •G(4)OH	−25.1[c]	−25.4
G + •OH → •G(5)OH	−23.2[c]	−17.8
A + •OH → •A(4)OH	−17.0[c]	−17.3
A + •OH → •A(5)OH	−14.2[c]	−7.7
UCH_2• + •H → T	−83.8[c]	−86.6

[a] kcal/mol. [b] Using values with thermodynamic corrections derived from Table 1. [c] Preferred values.

Table 2 is a radical recombination reaction involving the allyl radical. Because it is not isogyric and because of the problems with the allyl radical mentioned earlier, the enthalpy of this reaction was calculated using the UHF fully optimized reactants and products. As can be seen, with UHF optimized geometries this reaction is calculated best at the ROHF/PM3 level. However, the ROHF/6-31G*, UHF/ROHF, and UHF/UHF calculations are all in poor agreement with experiment.

Base−OH• and Base−H• Bond Enthalpies of the DNA Base Intermediates. Upon combining the results presented in Table 1 with the experimental bond enthalpies of Table 2, we obtain our estimates of the DNA base−H• and base−OH• bond enthalpies (Table 3). On the basis of the previously observed better agreement of the bond enthalpies calculated at ROHF/6-31G* and ROHF/PM3 with the experimental results in Table 2, only calculations at these levels are reported in Table 3. We find that addition of the •OH radical to the pyrimidines (−25.5 to −31.0 kcal/mol) is more exothermic than addition to the purines (−14.2 to −25.1 kcal/mol). This likely results from the significant geometry reorganization observed in the purines upon C4 and C5 adduct formation.[35]

Although not reported in Table 3, the base−OH bond enthalpies calculated at the ROHF/PM3 level from isodesmic reactions combined with reaction II(f) (Table 2) lie within ca. 4 kcal/mol from those, reported in Table 3, which were obtained from isodesmic reactions combined with reaction II(e); this shows a moderate effect of structural and electronic differences of the compounds of known bond dissociation energy on the base−OH bond enthalpies, calculated at this level. Hydrogen atom addition reactions occur with a smaller energy range than OH• addition reactions (ca. 10 vs 17 kcal/mol), with those additions to the pyrimidines being slightly more exothermic than H• addition to the purines at C8.

Thymine Methyl Group C−H Bond Energy. As stated earlier, the experimentally observed preferred sites of •OH attack on the pyrimidines are the C5 and C6 carbon atoms. However, it has been shown in thymine that some abstraction could occur at the methyl group,[36] resulting in the formation of UCH_2•. Using the methods of this work, we find that the methyl group C−H bond energy in thymine is 84−87 kcal/mol (Table 3, last reaction). UCH_2•, which is an allyl type radical, appears to be neither strongly reducing nor strongly oxidizing as experimentally shown[36] and theoretically predicted.[18]

Also, the UCH_2• radical is not expected to be a hydrogen-abstracting agent in DNA because the UCH_2−H bond energy is weaker than any other DNA C−H bond. This C−H bond also appears weaker than the S−H bond of a typical thiol (ca. 87 kcal/mol for CH_3SH). Therefore, healing of the damaged base is unlikely to occur through H atom transfer from a thiol; that is, radioprotection by hydrogen atom transfer from a thiol to UCH_2• should not occur. Recent ESR work performed in our laboratory[37] suggests that an alternative route for UCH_2• to react is by attack at the C6 position on thymine to form a dimer radical species. This dimer radical, unlike •UCH_2, is experimentally found to be repaired through hydrogen atom transfer reaction from thiols.

Base Products and Regeneration of the Natural DNA Bases: Reduction of H and OH Adduct DNA Base Radicals. The radical adducts presented in Table 3 are known to undergo reduction and subsequent dehydration to regenerate the natural DNA bases.[38] In an effort to determine the ease of reduction of these radical adducts, π electron spin densities were calculated at the UHF level with the INDO method on the ROHF/PM3 geometry optimized base adducts shown in Figure 2. The site of highest spin density is indicated in each structure. Reduction, through H atom addition, occurs, presumably, at this site.[39] These results led us to calculate the enthalpies of H atom addition for a selected set of reactions, shown in Table 4. The isodesmic approach described above was used, with ethanol as a model compound in the formation of the H atom adducts.

In this case, the isodesmic reaction used for each base is given by reaction 7. The experimental bond disssociation energy for

$$\text{BaseOH}\bullet + CH_3CH_2OH \rightarrow \bullet CH_2CH_2OH + \text{BaseHOH} \quad (7)$$

reaction 8 is 99.4 kcal/mol.[26] (The calculated bond dissociation energy at the ROHF/6-31G* level and enthalpy at the ROHF/PM3 level are 88.3 and 88.4 kcal/mol respectively.)

$$CH_3CH_2OH \rightarrow \bullet CH_2CH_2OH + H\bullet \quad (8)$$

TABLE 4: Estimated Enthalpies for H Atom Addition to DNA Base Adducts[a]

	isodesmic method		raw calculated		
	ROHF/PM3	ROHF/6-31G*[b]	ROHF/PM3	ROHF/6-31G*[c]	therm corr[d]
C(6)OH• + H• → C(6)OH(5)H	−95.2	−93.4	−84.2	−82.3	0.0
C(5)OH• + H• → C(5)OH(6)H	−85.8	−92.4	−74.8	−81.0	−0.2
T(6)OH• + H• → T(6)OH(5)H	−89.7	−89.4	−78.7	−77.9	−0.3
T(5)OH• + H• → T(5)OH(6)H	−87.9	−93.2	−76.9	−81.9	−0.2
G(4)OH• + H• → *cis*-G(4)OH(5)H[e]	−92.5	−86.7	−81.5	−75.1	−0.4
G(4)OH• + H• → *trans*-G(4)OH(5)H[e]	−72.8	−76.4	−61.8	−64.7	−0.5
G(5)OH• + H• → *cis*-G(5)OH(4)H[e]	−90.8	−93.0	−79.8	−81.4	−0.5
G(5)OH• + H• → *trans*-G(5)OH(4)H[e]	−67.8	−77.5	−56.8	−65.6	−0.8
A(4)OH• + H• → *cis*-A(4)OH(5)H[e]	−93.5	−86.6	−82.5	−77.2	1.9
A(4)OH• + H• → *trans*-A(4)OH(5)H[e]	−68.6	−68.4	−57.6	−58.9	1.7

[a] kcal/mol. [b] Including thermodynamic correction term from last column of this table. [c] No thermodynamic correction applied. [d] Thermodynamic correction term (using UHF/PM3) to reaction 7 in text, includes translational, rotational, and vibrational contributions at 298 K. [e] Reference 35.

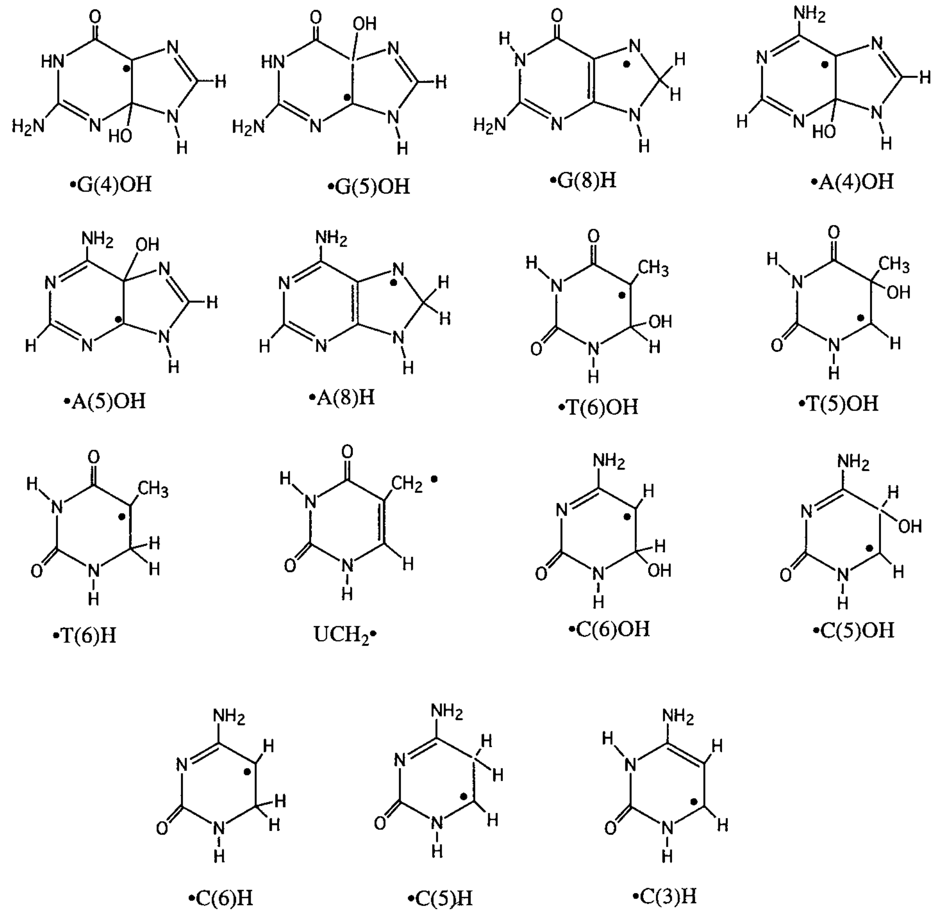


Figure 2. Structures and symbols for DNA base •OH and H• adduct intermediates. The unpaired electron is placed at the position of highest π-spin density as determined by calculations at the UHF level with the INDO method, on the ROHF/PM3 geometry optimized adducts.

By summing the calculated enthalpy for the isodesmic reaction 7 with the experimental enthalpy of −99.4 kcal /mol for the reverse of reaction 8, the estimated enthalpy of the target reaction 9 is obtained; the results of this sum are given in the first and second columns of Table 4, for two different methods of calculation for the isodesmic reaction 7. The direct ROHF/6-31G* and ROHF/PM3 results for reaction 9 are also presented in Table 4 for comparison.

$$\text{BaseOH}\bullet + \text{H}\bullet \rightarrow \text{BaseHOH} \qquad (9)$$

The results indicate that all H• additions are energetically very favorable, with cis additions to the purines being more favorable than the trans additions, as observed in a previous paper.[35] The PM3 and the 6-31G* results, employing isodesmic reactions, are in reasonable accord.

Base Products and Regeneration of the Natural DNA Bases: Dehydration of DNA Hydrates. The isodesmic, model compound reaction of known enthalpy and target reaction employed in the investigation of the dehydration energetics of DNA base hydrates are reactions 10−12, respectively.

$$\text{BaseHOH} + CH_2{=}CH_2 \rightarrow \text{Base} + CH_3CH_2OH \qquad (10)$$

$$CH_3CH_2OH \rightarrow CH_2{=}CH_2 + H_2O \qquad (11)$$

$$\text{BaseHOH} \rightarrow \text{Base} + H_2O \qquad (12)$$

The experimental enthalpy for reaction 11 is 10.9_5 kcal/mol.[27] Table 5 shows the results of the calculated values for target reaction 12, which result from summing the calculated enthalpy for reaction (10) with the experimental enthalpy for reaction 11.

The regeneration of the natural base considered in these reactions is energetically favorable (see Table 5). For the purines, loss of water from the trans isomer is far more favored than from the cis isomer. This result is likely due to the fact that the trans isomer undergoes a relatively small conformational change upon dehydration, whereas the cis isomer requires considerable structural rearrangement from the “butterfly” shape of the adduct to the planar geometry of the natural base.[35] The cis form of the purines also shows the greatest difference (about 10 kcal in magnitude) between the two levels of calculation, whereas the other structures show an average difference of only ca. 1.6 kcal in magnitude between the calculational levels. The highly strained nature of the cis structures appears to provide a difficult test for these calculations.

Heats of Formation of the Various DNA Base Radical Intermediates and Molecular Products. The enthalpies of the reactions reported here can be employed to estimate the heats of formation of the species investigated in this work. Enthalpies of formation of the radical intermediates shown in Table 3 are simply computed from the literature values for the heats of formation of the DNA bases (A, 49 ± 2; T, −79 ± 1; G, 0.5; C, −14 ± 2 kcal/mol),[28] the hydrogen atom (52.1 kcal/mol), or the hydroxyl radical (9.43 kcal/mol)[34] and the overall reaction enthalpies in Table 3. The enthalpies of formation are reported in Table 6. The enthalpies of formation of the hydrates of the DNA bases have been determined from the PM3 enthalpies of the reactions given in Table 5. We report the Table 5 derived values because these have the best experimental value for the reaction of known enthalpy (the dehydration of enthanol). In addition, the values from Table 5 depend only on the reaction shown in the table, whereas the enthalpies of formation derived

TABLE 5: Estimated Dehydration Enthalpies of DNA Base Hydrates Using the Isodesmic Method[a]

	ROHF/PM3[b]	ROHF/6-31G*[b,c]
C(6)OH(5)H → C + H_2O	−2.6	−0.3
C(5)OH(6)H → C + H_2O	−6.7	−6.5
T(6)OH(5)H → T + H_2O	−2.5	0.2
T(5)OH(6)H → T + H_2O	−8.2	−5.3
cis-G(4)OH(5)H → G + H_2O	−5.7	−15.5
trans-G(4)OH(5)H → G + H_2O	−25.3	−25.8
cis-G(5)OH(4)H → G + H_2O	−9.3	−16.9
trans-G(5)OH(4)H → G + H_2O	−32.3	−32.4
cis-A(4)OH(5)H → A + H_2O	−12.8	−23.8
trans-A(4)OH(5)H → A + H_2O	−37.7	−42.0

[a] kcal/mol. [b] The experimental dehydration energy of ethanol employed is 10.9_5 kcal/mol. [c] Includes a thermodynamic correction term (using UHF/PM3) to isodesmic reaction 10, including translational, rotational, and vibrational contributions at 298 K.

TABLE 6: Heats of Formation of the Various DNA Base Radicals and Hydrates[a,b]

hydrogen adducts	ΔH_f° [c]	hydroxyl adducts	ΔH_f° [c]	DNA base hydrates	ΔH_f° [d,e]
•T(6)H	−54(2)	•C(6)OH	−30(9)	C(6)OH(5)H	−69(9)
•C(5)H	14(5)	•C(5)OH	−35(3)	C(5)OH(6)H	−65(4)
•C(6)H	17(2)	•T(6)OH	−101(8)	T(6)OH(5)H	−134(8)
•C(3)H	13(8)	•T(5)OH	−97(7)	T(5)OH(6)H	−129(4)
•A(8)H	82(7)	•G(4)OH	−15(2)	*cis*-G(4)OH(5)H	−52(6)
•G(8)H	33(8)			*trans*-G(4)OH(5)H	−32(3)
		•G(5)OH	−13(6)	*cis*-G(5)OH(4)H	−48(9)
				trans-G(5)OH(4)H	−25(6)
		•A(4)OH	41(2)	*cis*-A(4)OH(5)H	4(7)
				trans-A(4)OH(5)H	29(4)
		•A(5)OH	44(7)		
		UCH_2•	−47(3)		

[a] kcal/mol. [b] Estimated absolute error is likely ±5 kcal. [c] Propagated error (in parentheses) is based on range of PM3 and 6-31G* calculations (Table 3) and errors in reported bond energies (Table 2). [d] Heat of formation calculated using isodesmic reaction enthalpies from PM3 values in Table 5 and known heats of formation of water and DNA base. Values calculated from Table 4 reactions are 4 kcal more negative. [e] Propagated error (in parentheses) is based on: estimated error in calculated isodesmic reaction enthalpy, the error in the ΔH_f for the DNA base, and the error in the enthalpy of the dehydration of ethanol (±0.5 kcal).

from the reactions in Table 4 must also use the enthalpies of formation of the radical intermediates given in Table 3. Even so, the PM3-derived entahlpies of formation using the reactions in Table 4 were only 4 kcal more negative in enthalpy than those derived from Table 5. In addition, the enthalpies of formation derived from 6-31G* calculations for the reactions in Tables 4 and 5 were in reasonable agreement with the PM3 results, with an average difference of about 4 kcal between the PM3-derived values and the 6-31G*-derived values. The four resulting individual results are given in the Supporting Information. The fact that agreement is found using two independent approaches and at two levels of calculation gives us some confidence in the reported enthalpies of formations.

Conclusions

In this work, isodesmic reactions have been employed to estimate the energetics of the reactions of H• and OH• addition to the DNA bases, of the •H atom addition reactions to the radical OH adducts formed, and finally, of the dehydration of the resulting hydrates. Each step in the overall process (reaction 5) starting with OH• addition is exothermic. Note that the overall process simplifies to •OH + •H → H_2O, so that the overall energetics should indicate a highly exothermic process. The experimental value for this process (which is the negative of the bond dissociation energy of water) is −119.2 kcal/cal. For each of the PM3 results, we find −122.7 kcal/mol for the overall process, whereas the ROHF/6-31G* results give −127.1 kcal/mol. This provides a check for consistency and favors the PM3 results. The energetics of the last step (reaction 12) of the overall process suggests that none of the purine hydrates will be stable against dehydration. It is interesting to note that the 4,5 hydrates have not been isolated from G and A after •OH attack, as might be expected from the large exothermic dehydration enthalpies.[18] The pyrimidine hydrates show greater thermodynamic stability; in agreement, thymine hydrates, which are readily formed by photohydration, have been isolated.[40−42]

We find that using isodesmic reactions provides an economical and convenient way to estimate reaction energetics without resorting to higher level ab initio methods. In our use of the isodesmic reaction technique, we find that the identity of the compound of known enthalpy of reaction appears to have small to moderate effects on the overall energetics of the target reaction. But, of course, we expect that the best enthalpy of reaction for the target reaction will result when the reaction of known enthalpy is experimentally well characterized and, also, when there is a close similarity between the experimental and target structures. We also observe that for H• addition reactions the most reliable results are obtained using the 6-31G* basis set at the ROHF level of calculation, whereas the ROHF/PM3 technique appears to be preferable for OH• addition processes.

Finally, we believe that one of the most important aspects of this work is that economical PM3 calculations of isodesmic reactions when combined with well-known experimental enthalpies of reaction can yield reliable thermodynamic quantities.

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Supporting Information Available: Coordinates (*x*,*y*,*z*) for geometry-optimized species at ROHF/6-31G* and ROHF/PM3 as well as tables of calculated total energies, thermodynamic corrections, and heats of formation (25 pages). Ordering information is given on any current masthead page.

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