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ELECTRON-TRANSFER-INDUCED ACIDITY/ BASICITY AND REACTIVITY CHANGES OF PURINE AND PYRIMIDINE BASES. CONSEQUENCES OF REDOX PROCESSES FOR DNA BASE PAIRS*

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Changes in the oxidation state of the DNA bases, induced by oxidation (ionization) or by reduction (electron capture), have drastic effects on the acidity or basicity, respectively, of the molecules. Since in DNA every base is connected to its complementary base in the other strand, any change of the electric charge status of a base in *one* DNA strand that accompanies its oxidation or reduction may affect also the *other* strand via proton transfer across the hydrogen bonds in the base pairs. The free energies for electron transfer to or from a base can be drastically altered by the proton transfer processes that accompany the electron transfer reactions. Electron-transfer (ET) induced proton transfer sensitizes the base opposite to the ET-damaged base to redox damage, i.e., damage produced by separation of charge (ionization) has an increased change of being trapped in a base *pair*. Of the two types of base pair in DNA, A–T and C–G, the latter is more sensitive to both oxidative and reductive processes than the former.

Proton transfer induced by ET does not only occur between the heteroatoms (O and N) of the base pairs (intra-pair proton transfer), but also to and from adjacent water molecules in the hydration shell of DNA (extra-pair proton transfer). These proton transfers can involve *carbon* and as such are likely to be irreversible. It is the A–T pair which appears to be particularly prone to such irreversible reactions.

KEY WORDS: electron transfer, oxidation/reduction, acidity/basicity, (de)protonation, purines, pyrimidines, DNA, base damage.

1. INTRODUCTION

It has long been recognized[1,2] that the degree of hydration of DNA is of great importance in influencing the type and the amount of damage inflicted upon this vital molecule by ionizing radiation.[3] This could, in principle, be interpreted in terms of varying contributions of the "direct" and the "indirect" (= via water radicals)[4] effects to the radiation damage. However, although the most obvious function of water is to serve as *proton* donor or acceptor, this simple if not trivial aspect has until recently found only limited attention. An analogous situation existed with respect to proton transfers *between* the bases in the base pairs, i.e., it was the radical chemistry of the *isolated* bases that was treated and little account was taken of the fact that in DNA the natural environment of a base, in addition to the water molecules in the hydration shell (located in the minor and the major groove), is its *complementary* base. The

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disregard of proton transfer reactions is understandable on the basis of the lack of knowledge, until recently, of the changes of the acidity or basicity, respectively, of the bases resulting from one-electron oxidation or reduction. This situation has changed, in so far as now the pK_a values of most of the base radical cations or (protonated) radical anions are known[5–8] or can at least be estimated.[9] The result is that the electron-transfer-induced changes in the acidity/basicity are so drastic that corresponding changes in the protonation and thereby charge state of the oxidized or reduced molecules in aqueous solution cannot be disregarded.[9,10] A further relevant aspect is that the *chemical* reactivity of a molecule (a radical) is generally different for its different protonation states. For example, radical *cations* usually show a *low* reactivity with O_2, in contrast to the *high* reactivity observed for *neutral* radicals and the even *higher* one for radical *anions*. It is thus evident that it is important to know the protonation state of the DNA base radicals in order to understand their reactivity.

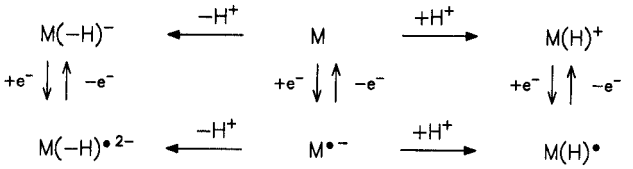
In DNA, due to the pairing of the bases, the protonation state of a base radical does not only depend on the "intrinsic" acidity or basicity of the radical but also on that of the complementary base in the opposite strand, which is the "natural" proton acceptor or donor (intra-pair proton transfer).[9] This is in contrast to the situation in aqueous solution where the proton exchange partner is always the same, i.e., bulk H_2O. In DNA the situation is really even more complex due to the fact that at least some of the O and N atoms between which exist intra-pair hydrogen bonds are involved in hydrogen bonds with water molecules in the hydration shell of DNA.[11–18] In the following, these bonds will be called extra-pair hydrogen bonds. In other words, in DNA both intra- and extra-pair proton transfers will have to be considered in order to be able to predict the protonation state and thereby the reactivity of a particular one-electron oxidized or reduced base. Obviously, this is not an easy task, particularly since the free energies of (de)protonation reactions depend strongly on the orientations of proton donor and acceptor.[19] In the following an attempt at predicting protonation states of base radicals in their base pairs will nevertheless be made, using essentially acidity/basicity and reactivity data obtained from aqueous phase studies[9] and combining these with structural data from solid (single crystal) phase accumulated[1,2,20–22] using ESR/ENDOR techniques. It is hoped that this approach, however crude it may be, will improve the understanding of the mechanisms of damage to DNA induced not only by ionizing radiation and high-energy light[23,24] but also by chemical oxidizing or reducing agents.

2. CHANGE OF ACIDITY/BASICITY RESULTING FROM REMOVAL/ ADDITION OF ONE ELECTRON FROM/TO A MOLECULE

Since electron and proton transfer are the simplest chemical reactions conceivable, the description and theoretical explanation of these processes[25–30] is a fundamental part of organic and inorganic physical chemistry. As is well known, proton transfer and electron transfer are intimately interrelated. For example, if a molecule M is protonated, its electron affinity (EA; it is a *gas* phase parameter) or one-electron reduction potential (E^1; it is a *solution* phase parameter*) is usually *in*creased; conversely,

*The superscript indicates that the potential refers to a *one*-electron transfer.

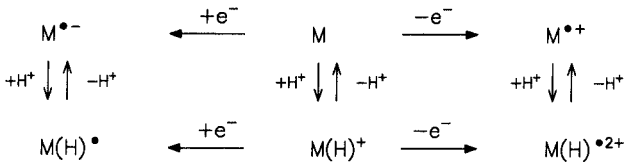
if the molecule *de*protonates, the electron affinity or reduction potential *de*creases; cf. eq. (1):*



(1)

$$EA(M(H)^+) > EA(M) > EA(M(-H)^-)$$

The reciprocal to the effect of (de)protonation on *electron* affinity is the effect of electron transfer (removal from or addition to a molecule) on *proton* affinity (= *gas* phase parameter) or (Brönsted) acidity/basicity (= *liquid* phase parameter). For example, if a molecule is one-electron oxidized, its *basicity* (proton *accepting* ability) *de*creases (or its *acidity* (proton *donating* power) *in*creases); conversely, electron addition to M (one-electron reduction) leads to an *enhancement* of its basicity, cf. scheme 2:



(2)

The order of acidities is predicted to be $M(H)^{\cdot} < M(H)^+ < M(H)^{\cdot 2+}$, or, expressed in terms of pK_a – values: $pK_a(M(H)^{\cdot}) > pK_a(M(H))^+ > pK_a(M(H)^{\cdot 2+})$.

In the following, examples will be presented that demonstrate the effect of electron removal or addition on the acidities/basicities of the nucleic acid bases and on some simple, mainly organic compounds.

3. THE NUCLEIC ACID BASES

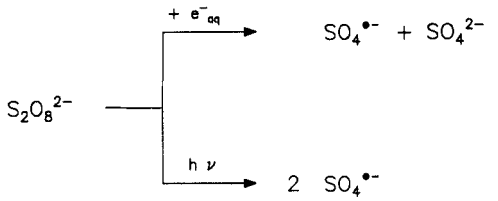
3.1. Electron Removal (One-Electron Oxidation)

3.1.1. Deoxyadenosine (A). Deoxyadenosine is not easily oxidized in aqueous solution. One-electron removal from this molecule requires* the use of the strong oxidant $SO_4^{\cdot -}$ ($E^0 = 2.5 - 3.1$ V/NHE[32])[9,33,34] or photolysis with 193 nm light.[35] The

*In this and the following equations (schemes), the dash preceding the H in the parentheses is to be read as "minus".

†It is however possible to oxidize A with Tl(II) ($k = 1.3 \times 10^8\ M^{-1}s^{-1}$ for dAMP at pH $\approx$ 7).[31] As with deoxydytidine and thymine (see sections 3.1.3 and 3.1.4), the one-electron oxidant $Br_2^{\cdot -}$ ($E^0 = 1.6$ V/HNE) reacts with A with a rate constant $< 10^7\ M^{-1}s^{-1}$, which means that it is not possible to produce, in dilute solutions, the one-electron-oxidized species in times sufficiently short (i.e., 1 ms) to allow their study before they undergo (undesired) radical–radical reactions.

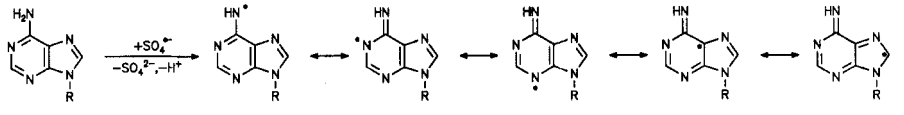
$SO_4^{\bullet -}$ radical, which can easily be produced from $S_2O_8^{2-}$ by reaction with e_{aq}^- or by photolysis (see Eq.(3)),



(3)

reacts with A with the rate constant $3.2 \times 10^9\,M^{-1}s^{-1}$.[9] In this reaction, the N^6-centered radical $A(-H)^{\bullet}$ ($\equiv A(N^6 - H)^{\bullet}$ is produced[9,33,34,36] by electron removal followed by deprotonation from N^6 of the resulting radical cation, $A^{\bullet +}$, see Eq. (4). Also in single crystals, $A^{\bullet +}$ deprotonates to give the neutral radical, $A(-H)^{\bullet}$, even at 10 K,[37-39] which is evidence for the large intrinsic driving force for this reaction.

The unpaired spin at N^6 is expected to be distributed also over the endocyclic nitrogens, as symbolized by the mesomeric structures II–III, and to some extent carbons 5 and 8 (IV, V):

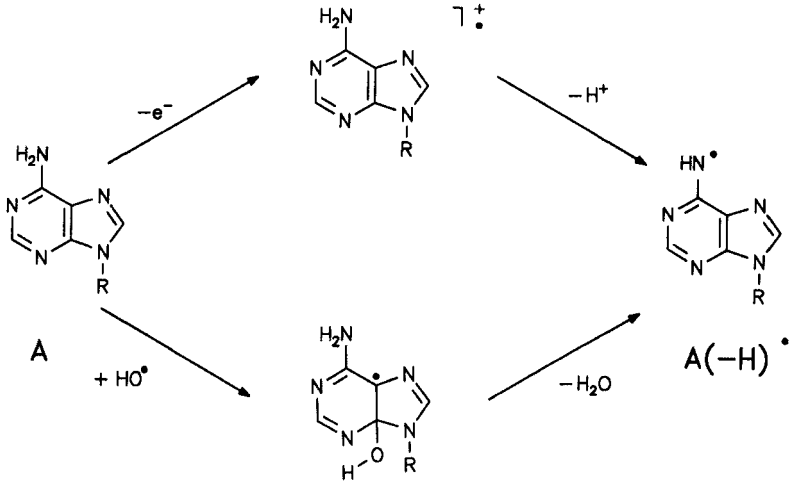


(4)

$A(-H)^{\bullet}$ ($\equiv A(N^6-H)^{\bullet}$)

Due to the unpaired spin density on the electron-affinic nitrogens,[38] $A(-H)^{\bullet}$ is predicted to be oxidizing, which has in fact been observed to be the case. The radical is reduced by *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD),[36,40,41] ascorbate, and thiols.[40]

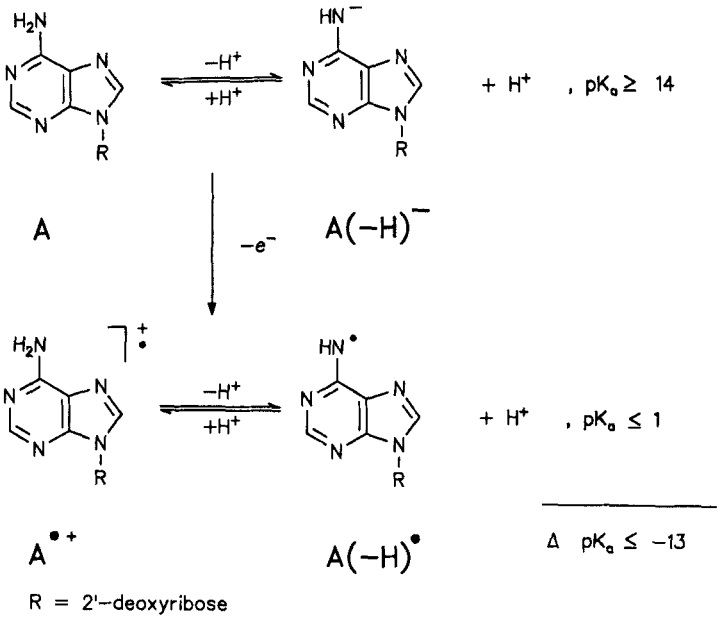
$A(-H)^{\bullet}$ is also produced by reaction in aqueous solution by the OH radical, via addition of $^{\bullet}OH$ at C4 of A followed by dehydration of the OH adduct, Eq. (5):[40,41]



(5)

However, this is not the *only* reaction of $OH^{\cdot}$. An additional $\approx 50\%$ of the OH radicals react by attachment at C8 followed by opening of the imidazole ring.[41]

It was found that the absorption spectrum of the species produced in aqueous solution by reaction with photochemically generated $SO_4^{\cdot -}$, identified as $A(-H)^{\cdot}$,[33,34,41] does not change from pH $\sim$ 11 down to pH $\sim$ 1, from which it was concluded[9] that the pK_a of $A^{\cdot +}$ is $\leqslant 1$.* Taking $pK_a(A^{\cdot +}) \leqslant 1$ and $pK_a(A) \geqslant 14$,[42] $\Delta pK_a(A/A^{\cdot +})$ results as $\leqslant -13$. This means that the acidity of the adenine moiety† is increased by $\geqslant 13$ orders of magnitude by its one-electron oxidation. This ΔpK_a corresponds to a differential driving force for deprotonation of the radical cation compared to its parent of $\geqslant 18$ kcal/mol.



(6)

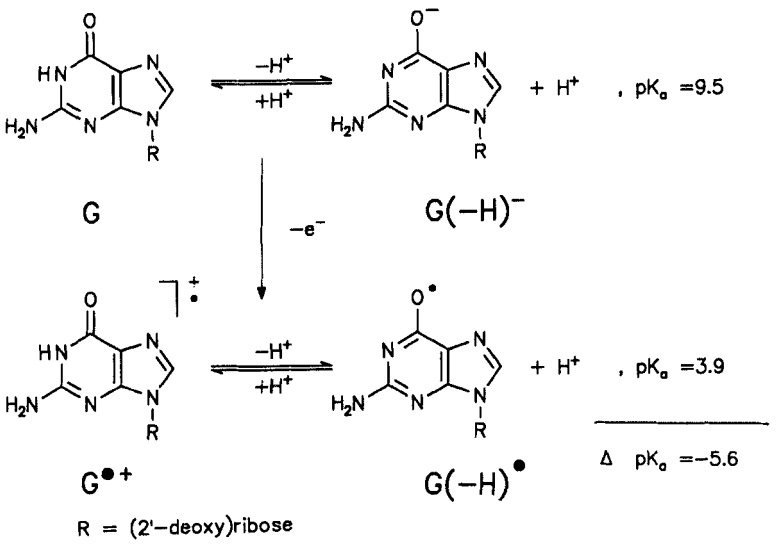
On the basis of this large driving force it is perhaps not surprising that $A^{\cdot +}$ deprotonates in crystals even at 10 K. It should, however, be borne in mind that in aqueous solution deprotonation involves proton transfer to a water molecule serving as a base, whereas in the crystal phase the proton acceptor is a neighboring molecule in the lattice.

3.1.2. Deoxyguanosine (G). In aqueous solution, this compound is a weak acid with a pK_a of 9.5. On removal of one electron by use of the oxidants $SO_4^{\cdot -}$,[5] $Br_2^{\cdot -}$,[5]

*As in the case of $C^{\cdot +}$ (see section 3.1.3), this conclusion is of course based on the assumption that the optical absorption spectra of $A^{\cdot +}$ and $A(-H)^{\cdot}$ are sufficiently different (as they are in the case of G)[5] to enable their distinction by optical detection.

†Unless otherwise indicated the pK_a values of the parent bases refer mainly to the 2'-deoxynucleosides.[43]

or Tl(II)[5,31] or by 193 nm photoionization,[35] the radical cation is formed which has a pK_a of 3.9, as determined by time-resolved optical and conductance techniques.[5]

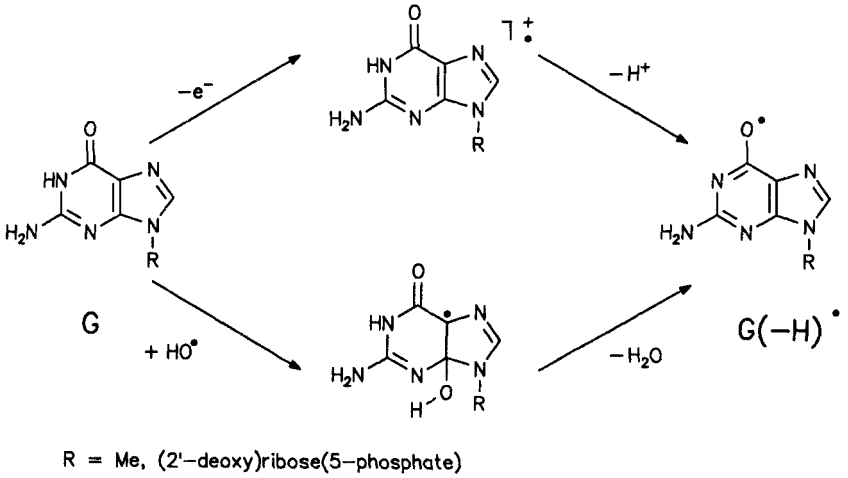


(7)

The difference in the pK_a values of the parent, G(pK_a = 9.5) and that of its radical cation $G^{\bullet+}$ (pK_a = 3.9) is −5.6. This means that removal of one electron from the molecule increases its acidity by 5.6 orders of magnitude.

Also in the crystal state, the radical cation has been found to deprotonate, even at ~10 K[44,45] which is certainly evidence for a high intrinsic driving force for the deprotonation. In the aqueous phase, the ΔpK_a of −5.6 corresponds to a driving force for deprotonation of the radical cation relative to that of the parent of 7.6 kcal/mol.

As in the case of the adenine system (Eq. (5)), the one-electron-oxidized molecule $G^{\bullet+}$ or $G(-H)^{\bullet}$ ($\equiv$ G(N1 − H)$^{\bullet}$) can also be obtained by reaction in aqueous solution with the OH radical, via addition at C4[46] followed by elimination of H_2O ($k = 5 \times 10^3\,s^{-1}$),[47] as shown in scheme 8:

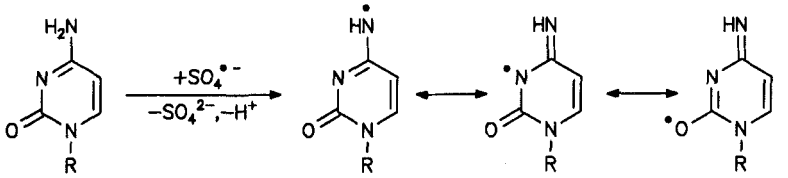


(8)

Thus, with the two purines, A and G, *direct* oxidation (ionization) and *indirect* oxidation (via the OH radical) lead to the same radicals. However, the OH radical does not *only* add at C4 (leading to one-electron oxidation of the purine) but also at C8, in which case opening of the imidazole ring may occur yielding radicals which are reducing, or, alternatively, oxidation of the radical to yield 8-hydroxyguanine derivatives.[9,47]

Concerning the deprotonated radical cation, $G(-H)^{\cdot}$, this species is strongly oxidizing.[46] Its reduction potential at pH 7, E_7^1, can be estimated to be $\geqslant 1.2$ V/NHE.*

3.1.3. Deoxycytidine (C). In aqueous solution, C can be oxidized rapidly only with $SO_4^{\cdot -}$. Not sufficiently reactive are $Br_2^{\cdot -}$, $N_3^{\cdot}$ or Tl(II). Ionization can also be achieved with 193 nm light.[35] The reaction of $SO_4^{\cdot -}$ with C has been suggested[33] to lead to an anilino-type radical formed by one-electron oxidation followed by deprotonation from the exocyclic nitrogen:



(9)

C $\qquad$ $C(-H)^{\cdot}$ $(\equiv C(N^6-H)^{\cdot})$

R = 2'-deoxyribose

On the basis of pulse radiolysis experiments with conductance detection,** the lifetime of $C^{\cdot +}$ at pH 5.2 is $\leqslant 200$ ns.

As seen from the mesomeric structures, the unpaired electron resides with the electron-affinic heteroatoms (N^4, N3, O^2). The radical $C(-H)^{\cdot}$ is therefore expected to be oxidizing, as is experimentally observed.[33,51] On the pulse radiolysis time scale (< 1 ms), $C(-H)^{\cdot}$ does not react with oxygen.[33]

By generating the radical via $SO_4^{\cdot -}$ produced by 248 nm laser photolysis of aqueous solutions containing $S_2O_8^{2-}$, it was found that the absorption spectrum[33] of the radical does not change in the pH range from 11 to 4. Below pH $\sim$ 4 changes did occur.[9] If it is assumed that the absorption spectrum of the neutral radical, which exists at pH $\sim$ 6,† is different from that of the radical cation, these observations mean that the pK_a of the radical cation of C is less than 4. Since the pK_a of the parent, C, is > 13, $\Delta pK_a(C/C^{\cdot +}) \leqslant -9$, which means that the Brönsted acidity of C increases by $\geqslant 9$ orders of magnitude upon one-electron oxidation.

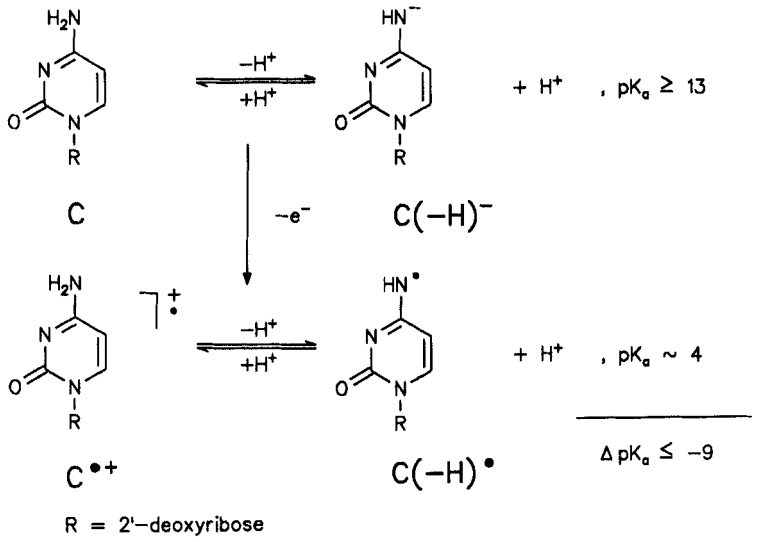
Deprotonation of $C^{\cdot +}$ from the NH_2 function is also observed to take place in the

*Based on the value published by Jovanovic and Simic[49] and using an improved number (1.08 V/NHE)[50] for the potential of the reference compound.

**Conditions: argon-saturated aqueous solution containing 1 mM 2'-deoxycytidine, 50 mM $S_2O_8^{2-}$, 0.1 M tert-butanol: pulse length 100 ns and DC detection.

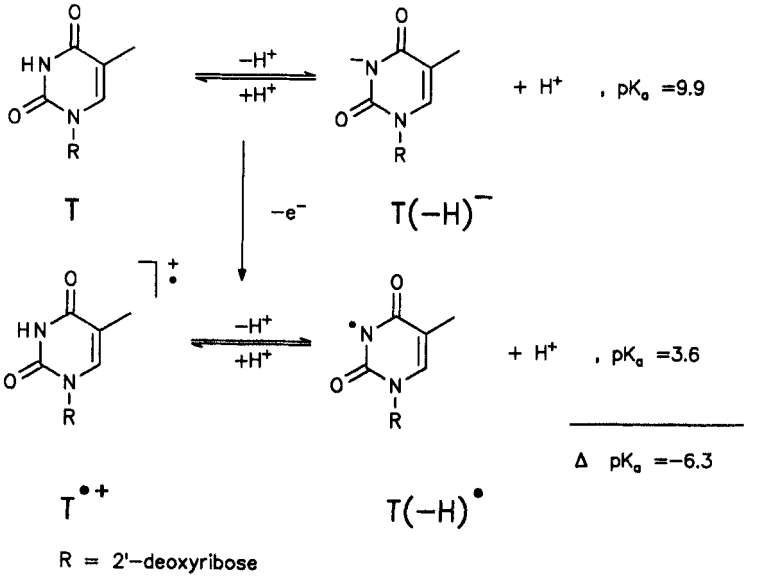
†This statement is based on the results of conductance experiments at pH 5–6 using 0.2 mM 2'-deoxycytidine (to minimize buffering) in a solution otherwise identical to that described above.

crystal state.[1,52–54] This is a further example demonstrating that the fundamental reactivity of the nucleic base radicals is to a large degree *in*dependent of their environment.



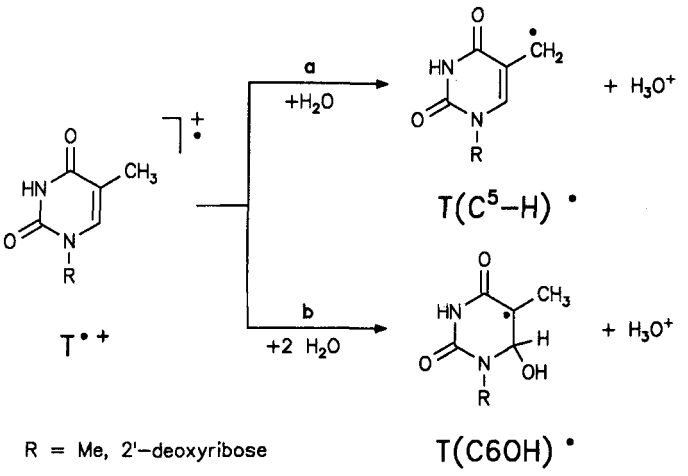
(10)

3.1.4. Thymidine (T). As adenosine and cytidine, thymidine requires the strong one-electron oxidant $SO_4^{\bullet -}$ to abstract an electron from this pyrimidine base.[6,33] Time-resolved (pulse radiolysis) experiments with optical and conductance detection have shown that the radical cation $T^{\bullet +}$ *reversibly* deprotonates at N3 to give the N3-centered neutral radical, T(N3 − H)$^{\bullet}$ (≡ T(−H)$^{\bullet}$). The pK_a of the radical cation is 3.6. Since the parent, T, has a pK_a of 9.9, it is evident that one-electron removal from the molecule leads to a drastic increase in its acidity.



(11)

In addition to the reversible proton transfer from and to N3 (Eq. (11)), there is an *ir*reversible deprotonation from the methyl group at C5 and also a nucleophilic attack of a water molecule at C6 followed by deprotonation to give the (oxidizing) 6-hydroxy-5,6-dihydrothymid-5-yl radical (T(C6OH)$^{\bullet}$), as shown in Eq. (12):[6]

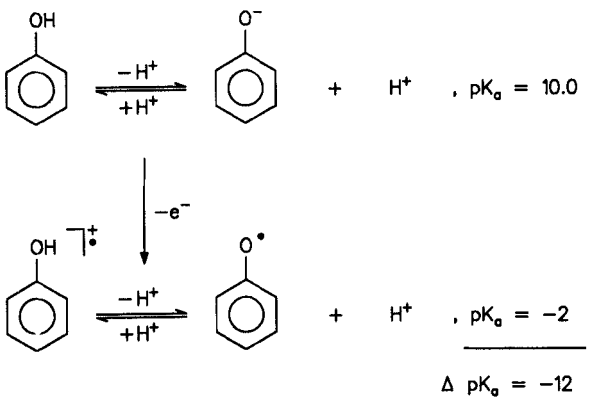


(12)

The deprotonation from the methyl group of T to give the allyl-type radical (Eq. (12a)) is the dominant reaction of the radical cation in the solid state[1,2,20,22,39,55] so there is again an independence of reactivity on the environment (crystalline/liquid). However, it is worth mentioning that the deprotonation product *from N3* of the radical cation of T, T(N1 − H)$^{\bullet}$ (see Scheme 11), has so far not been seen in irradiated single crystals or matrices.*

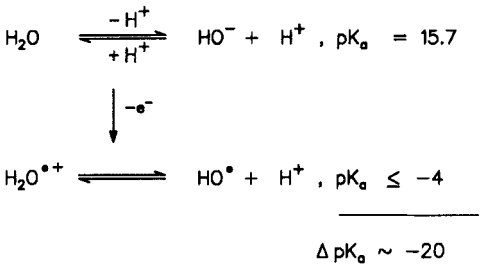
3.1.5. Other compounds. The increases in acidity as a result of one-electron oxidation described above for the nucleic acid bases are, of course, not restricted to this class of compounds but reflect a general phenomenon. A further example of this principle is the phenol system. Phenol is a weak acid with a $pK_a = 10.0$. One-electron oxidation produces the radical cation which in neutral aqueous solution deprotonates immediately to give the phenoxyl radical. Using electron-paramagnetic resonance, it was found that the pK_a of the phenol radical cation is -2.[58] The increase in acidity on one-electron removal from phenol to yield the radical cation thus is by 12 orders of magnitude, which corresponds to an enhancement of the driving force for deprotonation of 16.4 kcal/mol.

*The radical cations of uracil and thymine have been postulated to be formed in CF_3Cl.[56] However, on the basis of a comparison of the reported coupling constants with those[57] of the neutral radicals (formed by deprotonation of the radical cations from N1) as determined in aqueous solution, the species in CF_3Cl may as well have been the neutral radicals.



(13)

To take an example from inorganic chemistry, in the case of water, which is a very weak acid (pK_a = 15.7), one-electron oxidation (by ionization) is expected to yield $H_2O^{\bullet+}$, a species which has so far not unambiguously been identified, which is due to its short lifetime. $H_2O^{\bullet+}$ loses H^+ by transfer to a neighboring (H-bonded) water molecule to give $^{\bullet}OH$. Assuming that the rate of this process is $\geqslant 10^{14}\,s^{-1}$ and that the rate of the reverse reaction is $10^{10}\,M^{-1}\,s^{-1}$, the equilibrium constant for H^+ transfer between $H_2O^{\bullet+}$ and adjacent H_2O molecules results as $\geqslant 10^4\,M$, i.e., $pK_a(H_2O^{\bullet+}) \leqslant -4$. Comparison of this value with that (15.7) for water gives $\Delta pK_a(H_2O/H_2O^{\bullet+}) \leqslant -20$, i.e., the Brönsted acidity of H_2O increases on one-electron oxidation by $\geqslant 20$ orders of magnitude.



(14)

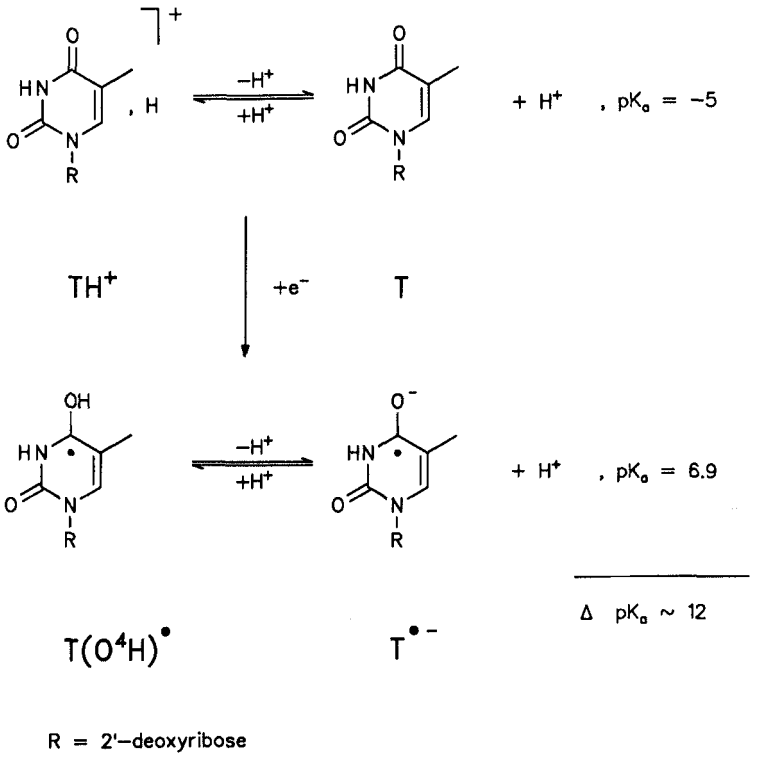
3.2. *Electron Addition (One-Electron Reduction)*

All the nucleic acid bases have a very high reactivity with the hydrated electron, e_{aq}^- (for a collection of the rate constants see[9,59]), a reaction in which the corresponding "electron adducts" are formed, cf. Eq. (15):

$$e_{aq}^- + \text{base} \rightarrow \underset{\text{electron adduct}}{\text{base}^{\bullet -}} \qquad (15)$$

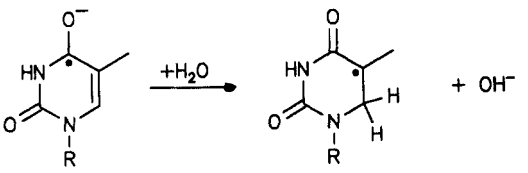
Due to the increase in electron density, the electron adducts are considerably stronger bases (proton acceptors) than their parental precursors. Examples for this phenomenon will be given in the following sections.

3.2.1. Thymidine (T). On the basis of electron spin resonance[60] and pulse radiolysis with optical[61–63] and conductance[63] detection, the electron adduct of T is negatively charged (i.e., it is a radical anion) at pH values above ~7. At lower pH values, it protonates rapidly and reversibly at O^4 to form the neutral radical $T(O^4H)^{\cdot}$ (Eq. 16).[63] The pK_a has been determined by time-resolved optical spectroscopy (pulse radiolysis) to be 6.9.[62] If this value is compared with that for the parent (≈ -5)[64] it is evident that electron addition to T increases its basicity by ~12 orders of magnitude:



(16)

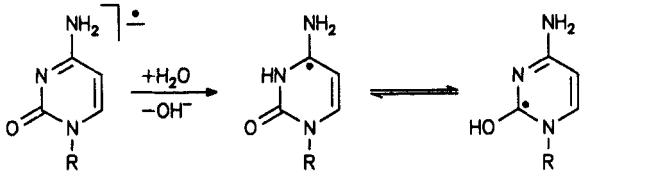
One of the consequences of the increased electron density of the T system is that protonation at *carbon* becomes possible. This *irreversible* reaction takes place at C6 leading to the (oxidizing) 5,6-dihydrothymidine-5-yl radical,[60,63] abbreviated as $T(C6H)^{\cdot}$ (Eq. (17)). The protonation on carbon is catalyzed by phosphate, the great efficiency of this reaction making it possible to observe the product radical by pulse radiolysis or *in-situ*-radiolysis ESR on the ~ms timescale.[60,63] The radical $T(C6H)^{\cdot}$, often abbreviated as $TH^{\cdot}$ in the ESR literature, has been observed also on irradiation of single crystals of T systems[65] and of DNA at temperatures ≥ 77 K,[66–68] where it is the most prominent, if not the only, "final" radical product from the chain of events that start with the capture of the electron ejected by the ionizing radiation.[1,2,20–22,39]



$T^{\bullet -}$ $T(C6H)^{\bullet}$ (17)

It is noteworthy that in single crystals of thymidine (and of 1-methylthymine) at 8 K the electron adduct is protonated at O^4 (like it is in aqueous solution at pH $\leqslant$ 7).[65,69]

3.2.2. Deoxycytidine (C). The radical anion $C^{\bullet -}$, formed by electron addition to C in neutral solution, is protonated by water[70] (Eq. (18)) in less than 4 ns,[71] as shown by conductance techniques. This protonation does *not* lead to a loss of reducing ability[70] (the neutral radical is able to one-electron-reduce even *very* weak oxidants such as *N*-methylpyridine cations).[62] From this it is concluded that $C^{\bullet -}$ gets protonated on a hetero-atom (O^2 or N3, see eq. (18)), and *not* on carbon, in which case the radical would *not* be a good reductant (as concluded from the behaviour[72] of the corresponding radical from uracil or thymine). In aqueous solution there is probably a tautomeric equilibrium between the N3 and the O^2-protonated electron adducts, analogous to the situation with the adenosine electron adduct (see section 3.2.3). These radicals are symbolized by $C(N3H)^{\bullet}$ and $C(O^2H)^{\bullet}$:

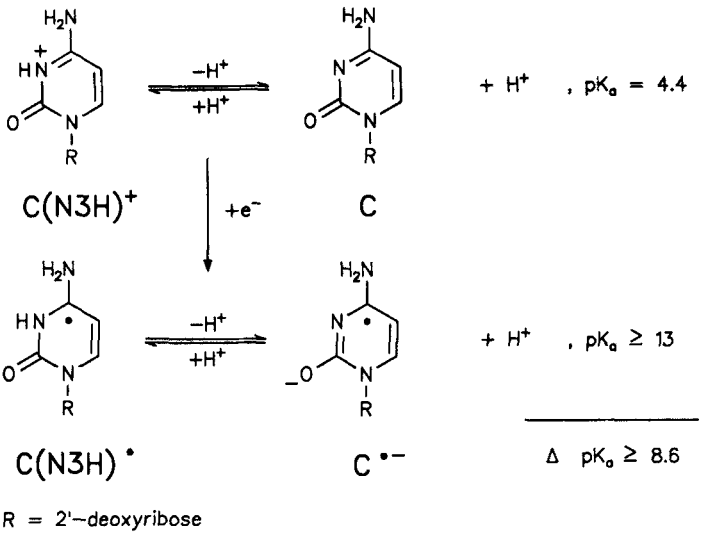


$C^{\bullet -}$ $C(N3H)^{\bullet}$ $C(O^2H)^{\bullet}$ (18)

R = 2'-deoxyribose(5'-phosphate)

For the sake of simplicity, it will in the following be assumed that the electron adduct is protonated at N3.

With conductance methods it was shown[70] that $C(N3H)^{\bullet}$ does not deprotonate up to pH 10.6. The optical absorption spectrum of the radical remains unaltered from pH 6 up to 13.[9,62] If it is assumed that the spectra of $C(N3H)^{\bullet}$ and $C^{\bullet -}$ are different (as they are for $T(O^4H)^{\bullet}/T^{\bullet -}$,[61–63] $U(racil)(O^4H)^{\bullet}/U^{\bullet -}$,[62] and $A(NH)^{\bullet}/A^{\bullet -}$,[8] this observation means that the pK_a of $C(N3H)^{\bullet}$ is larger than 13. Comparison with the pK_a for the parent base (4.4) gives $\Delta pK_a(C(N3H)^+/C(N3H)^{\bullet}) \geqslant 8.6$, i.e., the basicity of C is increased by electron addition by $\geqslant 8.6$ orders of magnitude.



(19)

As mentioned above, the absorption spectrum of C does not change between pH 6 and 13. It also remains qualitatively the same between 1 μs and 1 ms after its generation. This probably means that there is no change in the nature of the radical over this time period, thus also no rearrangement to give a radical that is protonated on *carbon*. It is interesting that also in the crystalline state the electron adduct of C shows *no* tendency to protonate on carbon.[73]

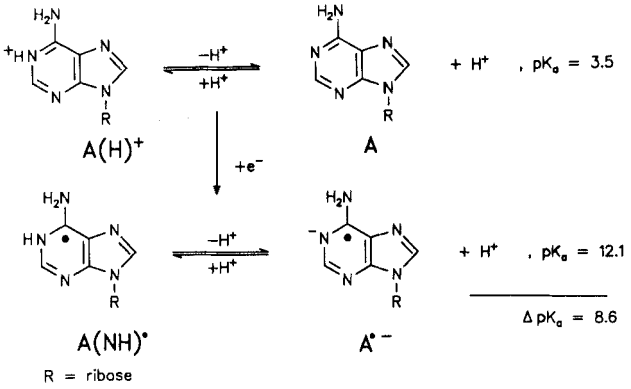
It has recently been unambiguously demonstrated that in low temperature matrices the electron adduct of C is protonated at one of the hetero atoms of the molecue,[74–77] like it is in single crystals[73] and in aqueous solutions.[70] As pointed out previously,[9] the reason for this pronounced tendency to be protonated is obviously the very strong Brönsted basicity of the electron adduct. In this context a comparison with thymidine is appropriate: The pK_a of T is -5,[64] that of C is 4.4, i.e., C is a better base than T by 9.4 orders of magnitude. The basicity of T increases by 11.9 orders of magnitude on one-electron addition ($pK_a(T(O^4H)^\cdot = 6.9$, see section 3.2.1). If this factor applies also to the C system, the pK_a of $C(N3H)^\cdot$ results as $(4.4 + 11.9 =) 16.3$,* a number which is in line with the experimentally determined lower limit of 13 (see above). Such high pK_a values for hetero-atom acids mean that protonation on *carbon* of the (hypothetical) radical anion is relatively unlikely. *The radical is protected from being protonated on carbon by protonation on a hetero-atom.*

Particularly interesting is the situation of the electron adduct in DNA. Here the strong proton affinity of $C^{\cdot -}$ is *supported* by the relatively high proton donicity (acidity) of the complementary base, guanine.[9,10] The consequences of this will be discussed in section 3.4.2.2.

3.2.3. Adenosine (A). The adenosine electron adduct, $A^{\cdot -}$, has been shown by conductance to be rapidly protonated by water.[42,78,79] The initial protonation is on the

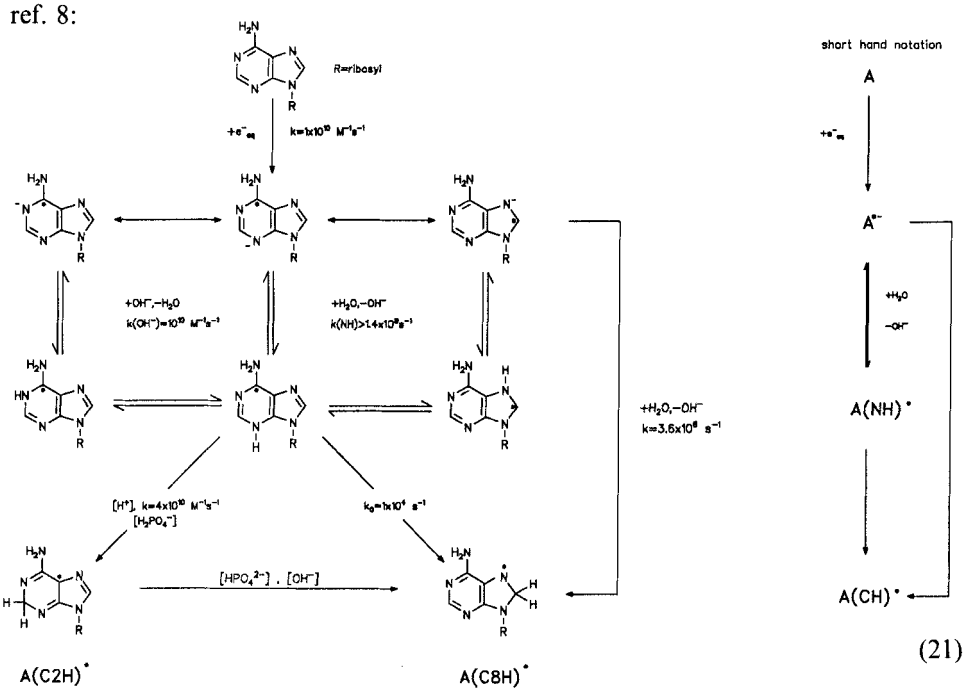
*The same number is obtained by considering the basicity difference between T and C (9.4) and the pK_a of $T(O^4H)^\cdot$ (6.9).

nitrogens and is reversible with a pK_a of the N-protonated neutral radical A(NH)$^{\bullet}$ (this radical probably exists in aqueous solution as a mixture of the N1, N3, and N7 protonated isomers, see scheme 21) of 12.1.[8,9] Compared with the pK_a of the protonated parent (3.5), this means an increase in basicity by 8.6 orders of magnitude ($\Delta pK_a(A(NH)^+/A(NH)^{\bullet} = 8.6$).



(20)

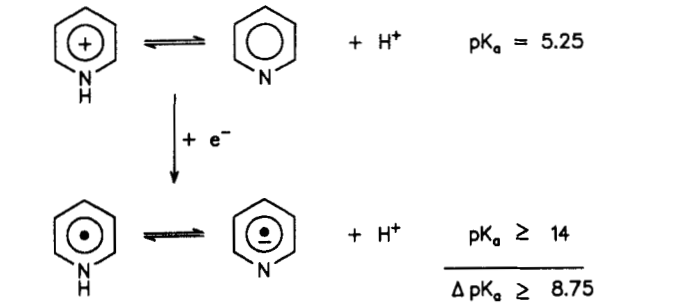
$A^{\bullet -}$ also reacts rapidly by protonation on *carbon* ($k = 3.6 \times 10^6 s^{-1}$), and even the less electron-rich A(NH)$^{\bullet}$ still protonates on carbon with $k = 1 \times 10^4 s^{-1}$. This rate can be considerably accelerated by phosphate.[8] Protonation occurs at C2 and C8,[8] giving the same radicals (A(C2H)$^{\bullet}$ and A(C8H)$^{\bullet}$, respectively) as those[1,2,20,22,37,38,80–82] observed in single crystals. Of the two adducts at carbon, A(C8H)$^{\bullet}$ is the thermodynamically more stable one, in the solid state[80–82] as well as in aqueous solution.[8] The reversible and *irr*eversible protonations of $A^{\bullet -}$ (which will be discussed in more detail in section 3.5.2.2) are summarized in scheme 21, which is taken from ref. 8:



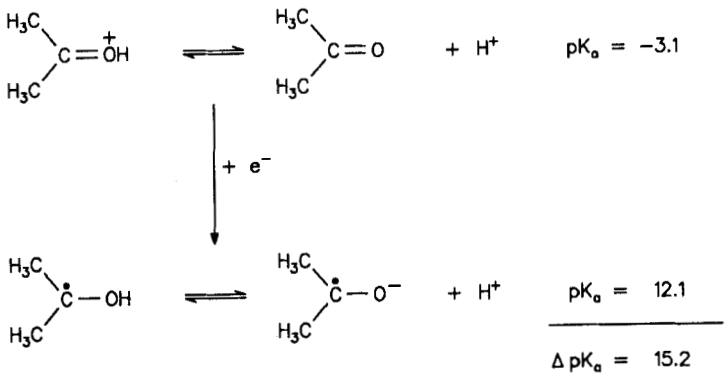
(21)

3.2.4. Deoxyguanosine (G). The radical anion of guanosine had so far not been fully studied in aqueous solution. However, pulse radiolysis experiments with optical and conductance detection indicate that the behavior of the radical anion is similar to that of adenosine, i.e., there is a rapid protonation by water on heteroatom (probably at O^6) followed by a transformation ($k = 1.2 \times 10^6\,s^{-1}$) leading to protonation on carbon, probably at C8.[48] This conclusion is based on the very weak reducing properties of the resulting radical(s). In acid solution, the C-protonated electron adduct undergoes a further protonation to give a radical cation $G(H_2)^{\cdot +}$, whose pK_a is 5.6.[48] Protonation of $G^{\cdot -}$ on O^6 has also been observed to take place in single crystals, and the production of the "H-adduct" to C8 of the guanine moiety has been interpreted[22] as due to protonation of the radical anion at C8.

3.2.5. Other compounds. Analogous to the case of one-electron *oxidation*, the changes in the acid/base properties that occur on one-electron *reduction* are not restricted to nucleic acid bases. The two examples given below, one involving an aromatic, the other an aliphatic system, demonstrate this point:



(22)



(23)

3.3. Reduction Potentials of T and C

The reduction potential of a molecule is a quantitative measure of its ease of reduction. In the ideal case, reduction potentials are measured by electrochemical methods. However, with many molecules and particularly in aqueous solution the electrode reactions are often *irr*eversible which prevents the determination of thermodynamically defined potentials. The method of measuring electron transfer equilibria in

homogeneous solution circumvents these problems. The reduction potential of T was determined by measuring by pulse radiolysis the equilibrium constants for the reversible electron exchange with substituted *N*-methylpyridinum cations such as *N*-methylnicotineamide.[62]

Similar experiments were performed with cytidine instead of thimidine. It was found that the electron exchange between the electron adduct of (deoxy)cytidine (which is protonated at pH < 13, see section 3.2.2) and, e.g., *N*-methylnicotineamide is reversible at pH 6–8 and that the tendency of (deoxy)cytidine to be reduced in aqueous solution is comparable to that of thymidine whose reduction potential at pH ~ 8 is -1.1 V/NHE.[62] On the basis of the considerably higher electron density of the cytosine system compared to that of thymidine, as judged by the much larger Brönsted basicity of (deoxy)cytidine ($pK_a(CH^+) = 4.4$) relative to that of thymidine ($pK_a(TH^+) = -5$), or by the lower ionization potential of cytosine (IP = 8.9 eV) as compared to thymine (IP = 9.4 eV),[83–86] their similar ease of reduction may be surprising. However, the ease of *reduction* of C is considerably enhanced by the ease of *protonation* of the electron adduct. Since $C^{\bullet -}$ is a much stronger base than $T^{\bullet -}$ ($pK_a(T(O^4H)^\bullet) = 6.9$[62] and $pK_a(C(N3H)^\bullet) \geqslant 13$) it is evident that the free energy of protonation is larger in the case of C. The driving force for protonation contributes to the free energy of electron addition, i.e., the driving force for electron addition is larger if that for protonation of the electron adduct is larger. The consequences of the different acid/base properties of the nucleic acid bases and/or their one-electron oxidized or reduced forms for the redox chemistry of DNA will be discussed in sections 3.4 and 3.5.

3.4. Proton-Transfer Between the Bases of a Pair ("Intra-Pair" Proton Transfer)

As has been pointed out previously,[9,10] in DNA the stage is set in a perfect way for proton transfer to occur, i.e., along the preset channels of the hydrogen bonds between the bases. In principle, these proton transfers could be very fast, involving just one vibrational period. The adjustment of the system to changes in proton affinity due to ionization (electron loss) or electron gain can thus take place essentially instantaneously. An aspect of this concept is that, in order to understand the redox chemistry of a particular base in DNA, the properties and the behavior of its partner have to be considered as well.

In the following, the information given in sections 3.1 and 3.2 on the acid/base properties of the one-electron oxidized/reduced (2′-deoxy)nucleosides* is combined with the aim of obtaining a *quantitative* picture of redox-induced proton transfer, hopefully reflecting the situation in DNA.

It is, of course, clear that the pK_a-values refer only to aqueous solution where proton transfer is always to or from a water molecule or from a hydronium ion, i.e., where *water* is the proton exchange partner and, in addition, where all the species involved in the proton exchange are hydrated, the hydration free energies influencing the position of the equilibria. The environment is obviously different in the case in DNA, and the applicability of pK_a-values to the situation in DNA may therefore be

*It is assumed and there is experimental evidence[62] that the redox-induced changes in acid/base properties of the nucleo*tides* are not very different from those of the nucleo*sides*.

questioned. However, it is likely that the solvation energies of the *radical* species involved in the proton transfer reactions (such as in Eqs. (24–30)) are approximately the same as those in the corresponding reactions of the parent (*non*-radical) species (Eqs. (24–30)). If this is the case, these energies cancel which means that the ΔpK_a-values *do* have a good chance of reflecting the proton-transfer situation in environments other than aqueous phase.

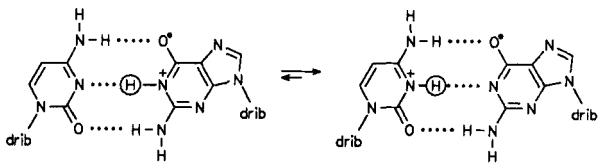
Another aspect is the fact that in H_2O, the pK values relate to (de)protonation from the *average* of the various tautomeric forms present, whereas in DNA there is possibly only one tautomeric form (of the radical) present. Thus, the *collective* aqueous-phase pK values may not correctly represent the *site-specific* proton transfers in the base pairs. Therefore, the use of (gas phase) *proton affinities* may, in principle, be more appropriate for estimating the position of proton transfer equilibria in DNA. However, experimentally determined values for the nucleobase *radicals* (i.e., the one-electron oxidized or reduced forms) are not available. On the other hand, the protonation state of bases in base pairs of which one member was one-electron oxidized or reduced, has recently been calculated, using MO methods.[87] The (qualitative) results of these calculations are in very good agreement with those using the pK_a approach (via supra et infra), lending support to both methods.

3.4.1. Oxidation of bases in the base pairs.

3.4.1.1. The G–C pair. Equation (24) describes what probably happens after an electron is removed from a guanine moiety. Deoxyguanosine is a weak acid (pK_a = 9.4). The radical cation, however, is a much stronger acid (pK_a = 3.9). If G is ionized, the equilibrium positions of one or of all of the three protons involved in the hydrogen bonds with the cytosine moiety (C) will therefore be shifted toward the cytosine with the result of an overall transfer of positive charge to that base, whereas the unpaired spin will of course remain on the oxidized base. Proton transfer thus leads to a separation of charge from spin: The spin residing on the damaged base and the charge on the complementary base in the other strand. For clarity the proton *moving* across the strands is encircled, in the following equations.

The equilibrium for protonation of C or for deprotonation from $G^{\bullet+}$ can be easily calculated by combining the corresponding reactions, as follows:

$$
\begin{array}{lcll}
G^{\bullet+} & \rightleftharpoons & G(-H)^{\bullet} + H^{+} & K = 10^{-3.9} \\
C + H^{+} & \rightleftharpoons & C(H)^{+} & K = 10^{4.3} \\
\hline
G^{\bullet+} + C & \rightleftharpoons & G(-H)^{\bullet} + C(H)^{+} & K = 10^{0.4}
\end{array}
\qquad (24)
$$



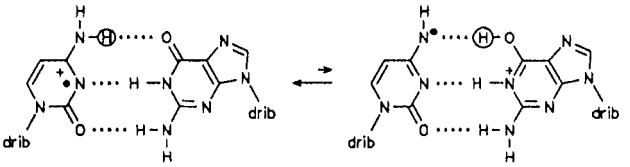
The result is that in DNA the radical cation of the G moiety should deprotonate by H^+ transfer to C. However, the equilibrium constant for this process is not very large,

which means that G will also have some radical cation nature. The radical G(−H)$^{\bullet}$ has in fact been observed on oxidation of double-stranded DNA in aqueous solution at room temperature. In agreement with the above scheme (see also ref. 5), it was found that G$^{\bullet+}$ deprotonates at N1,[88] i.e., that it is present as G(N1 − H)$^{\bullet}$.

If ionization occurs at the cytosine end of the G–C base pair (Eq. (25)), the ionization-induced driving force for deprotonation is considerably larger than in the case of ionization of G (since $\Delta pK_a(C/C^{\bullet+}) \leqslant -10$ compared to $\Delta pK_a(G/G^{\bullet+}) = -5.6$). On the other hand, G is a weaker *base* than C, so the tendency of G to *accept* a proton from C$^{\bullet+}$ is less than in the reverse case (Eq. (24)). These opposing properties result in a situation where the proton remains essentially at the C moiety. In other words, the radical cation produced by ionization of C *remains a radical cation*, since it does not lose its charge by proton transfer to its base partner. This is thus different from the situation resulting from ionization of G, where the equilibrium between G$^{\bullet+}$ and G(−H)$^{\bullet}$, formed by proton transfer to C, is in favor of the neutral G(−H)$^{\bullet}$.

C$^{\bullet+}$	⇌	C(−H)$^{\bullet}$ + H^{+}	$K \sim 10^{-4}$
G + H^{+}	⇌	G(H)$^{+}$	$K = 10^{2}$
C$^{\bullet+}$ + G	⇌	C(−H)$^{\bullet}$ + G(H)$^{+}$	$K \sim 10^{-2}$

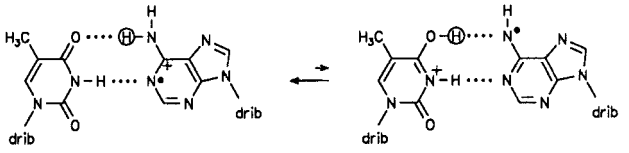
(25)



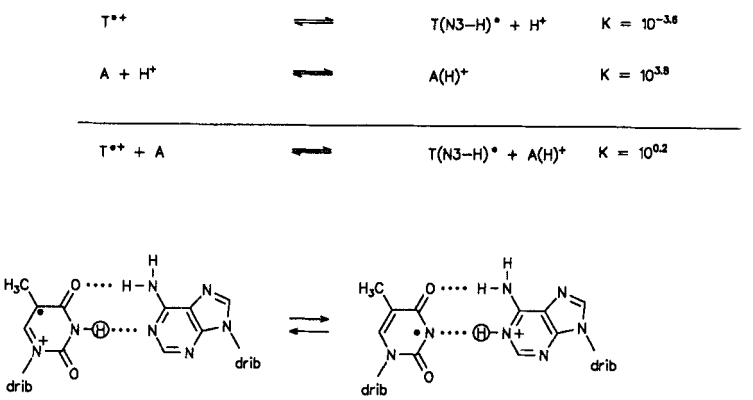
3.4.1.2. The A–T pair. In the case of the A–T base pair, ionization of A leads to the strongly acidic species A$^{\bullet+}$, so one would expect this species to deprotonate. However, this is essentially prevented by the extremely low basicity of T($pK_a(T(H)^{+}) = -5$). Therefore, analogous to the case of C$^{\bullet+}$, it is concluded that the radical cation of A remains a cationic species.

A$^{\bullet+}$	⇌	A(−H)$^{\bullet}$ + H^{+}	$K \geq 10^{-1}$
T + H^{+}	⇌	T(H)$^{+}$	$K = 10^{-5}$
A$^{\bullet+}$ + A	⇌	A(−H)$^{\bullet}$ + T(H)$^{+}$	$K \geq 10^{-6}$

(26)



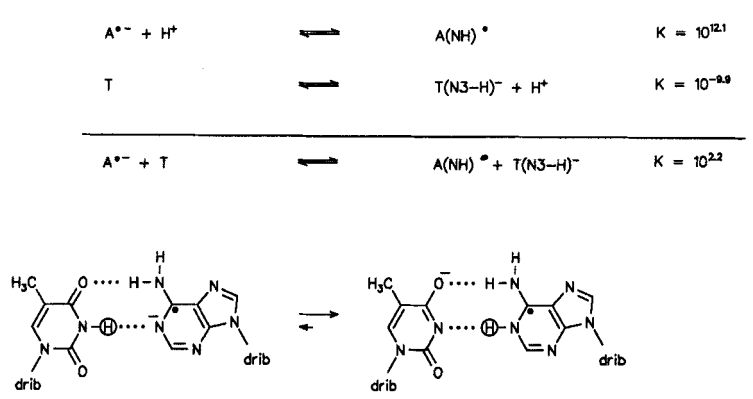
The situation is different in the A–T pair if it is T which is ionized. Combining $pK_a(T^{\bullet +}) = 3.6$ with $pK_a(A(H)^+) = 3.8$ yields the value of $1.6 (\equiv 10^{0.2})$ for the proton transfer equilibrium from $T^{\bullet +}$ to A. It can therefore be concluded that the reactivity of one-electron oxidized T in DNA should be characterized by its *two* protonation states, $T^{\bullet +}$ and $T(N3 - H)^{\bullet}$, being approximately equally important.



(27)

3.4.2. Reduction of bases in the base pairs.
The rate constants for reaction (in aqueous solution) of e_{aq}^- with purines and pyrimidines are essentially the same.[9,59] On this basis it may be assumed that the chances of an electron reacting with a purine-pyrimidine base pair to end up on either are equal.

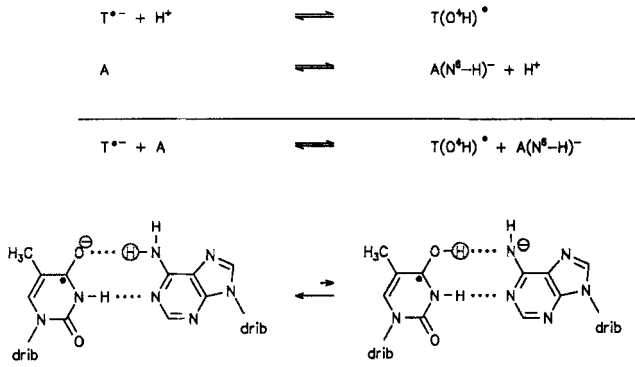
3.4.2.1. The A–T pair. If the electron is picked up by adenine, the Brönsted basicity of this molecule increases by 8.6 orders of magnitude, as reflected by the pK_a for deprotonation from the protonated radical anion of 12.1 (see section 3.2.3). Thymine, the base partner of adenine, is a weak acid. Thus, it should be able to protonate strong bases. Combining $pK_a(A(NH)^{\bullet}) = 12.1$ with the pK_a for deprotonation of neutral thymidine ($= 9.6$) shows that the equilibrium for proton transfer between T and $A^{\bullet -}$ is such that $A^{\bullet -}$ is quantitatively protonated:



(28)

This means that the chances for one-electron reduced adenine to remain a radical *anion* are low. Thermodynamically, the system is more stable if a proton is transferred to $A^{\bullet -}$, i.e., if the negative charge is on the thymine moiety (with the unpaired spin, of course, still on the adenine). It is evident that also in the case of *reduction*-induced proton transfer all proton transfer reactions that occur in a base pair lead to a separation of charge from spin such that charge and spin end up in opposite strands of the double helix.

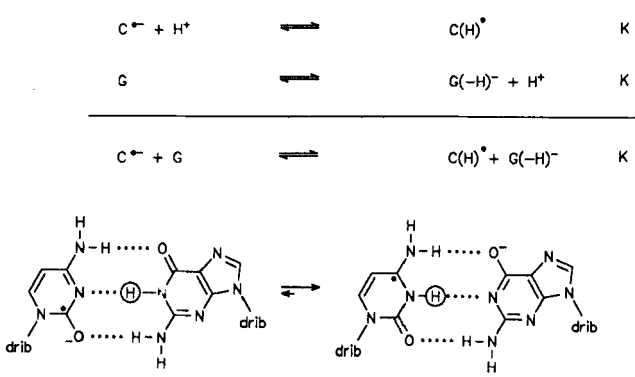
If the electron is scavenged not by A but by T, the resulting radical anion remains an anion, in spite of the fact that the Brönsted basicity of $T^{\bullet -}$ is $\geqslant 12$ orders of magnitude higher than that of T. The reason is that the base partner, A, is an extremely weak acid ($pK_a(A) \geqslant 14$). The following equations describe the situation:



(29)

With the negative charge on T not neutralized by proton transfer from A, other protonating agents such as water can react with $T^{\bullet -}$. In DNA, it is the C5/C6 double bond of T which is exposed to the water molecules in the hydration shell of the helix. The consequences of this are discussed in section 3.5.

3.4.2.2. The G–C pair. If the electron is scavenged by C, the Brönsted basicity of this molecule is further increased, as reported in section 3.2.2. Nature has coupled this powerful *base* with the strongest *acid* among the nucleic acid bases, namely G. Proton transfer from G to $C^{\bullet -}$ is therefore expected to be highly favored, and this is the case as seen from the following equations:



(30)

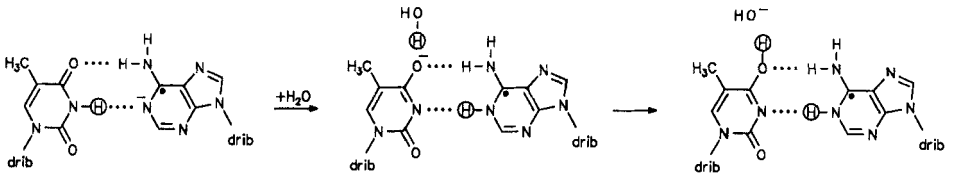
The conclusion is thus that, in contrast to the pyrimidine base T, in DNA the cytosine radical anion is *protonated* (by its complementary base, G) on a hetero atom, probably on N3, as shown above. One of the consequences is that the radical is thereby protected with respect to undergoing irreversible protonation on carbon (see section 3.5.2).

It has in fact been found experimentally, using ESR, that in DNA at low-temperature (4–77 K) the electron adduct of C is *protonated*,[74–76,89,90] probably at N3.[76,77] Furthermore, the abundance of this radical is much larger than that of the electron adduct of T, i.e., the electron prefers to localize at C rather than at T.[89,90] On the basis of the acidity/basicity concepts developed above (Eqs. 24–30), this preference for C is easy to understand: In the G–C pair protonation of the electron adduct of C is thermodynamically highly favored, which is much in contrast to the situation in the A–T pair if the electron is scavenged by T. If it is assumed that the electron affinity of C is less than that of T,* this is an example which shows that the thermodynamics of proton transfer can qualitatively change the energetics of electron transfer.

The case that the electron is scavenged by G also has to be considered. Without knowing the pK_a-values of the protonated electron adduct it is, however, not possible to make quantitative predictions as to the position of the proton(s) between the members of the G–C pair. However, since C is a poor acid it is unlikely that a proton will be transferred to $G^{\bullet -}$. This gives external protonating agents (H_2O) a good chance to protonate $G^{\bullet -}$ (at a carbon; see section 3.5.2).

3.5. *"Extra-Pair" Proton Transfer (Proton Transfer To and From Water Molecules)*

In DNA hydrogen bonds do not only exist between the partners of a base pair, but also between the bases and adjacent water molecules in the minor and major groove.[11–18] Therefore, the interactions with these water molecules of the base radicals formed by oxidation or reduction (termed *extra-pair* processes) have to be taken into account as well. The extra-pair processes may be divided into two categories: Those between the water molecules and the *hetero*-atoms of the bases (these processes are reversible), and those between water and the *carbon*-atoms of the base radicals (these are very likely *ir*reversible). In the following are given two examples for the reversible type of extra-pair proton transfer: Due to the close contact between the bases and water molecules in the hydration shell of DNA,[11–18] it is possible that base ions such as, e.g., $T(N3 - H)^-$ (formed by proton transfer to $A^{\bullet -}$, see Eq. (28) are rapidly protonated by water molecules, leading to generation of the OH^- ion, Eq. (31):

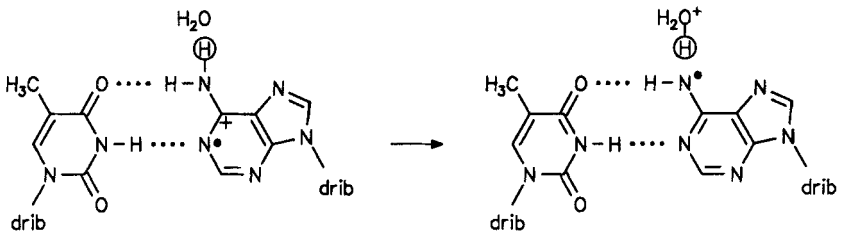


(31)

The negative charge introduced by the electron picked up by A is thus seen to end up in the hydration shell. Looking from $A^{\bullet -}$, its partner T just serves as a relay in the

*Experimentally determined electron affinity values for the nucleic acid bases do not seem to be available.

proton transfer from H_2O. Analogous reactions are conceivable for all cases where proton transfer takes place between the strands (Eqs. (24, 27, 28, 30)). It is, of course, also possible that the radical ions react with water in their own strand, i.e., without the involvement of their base partner. Deprotonation of the radical cation of adenine in its pair is an example:

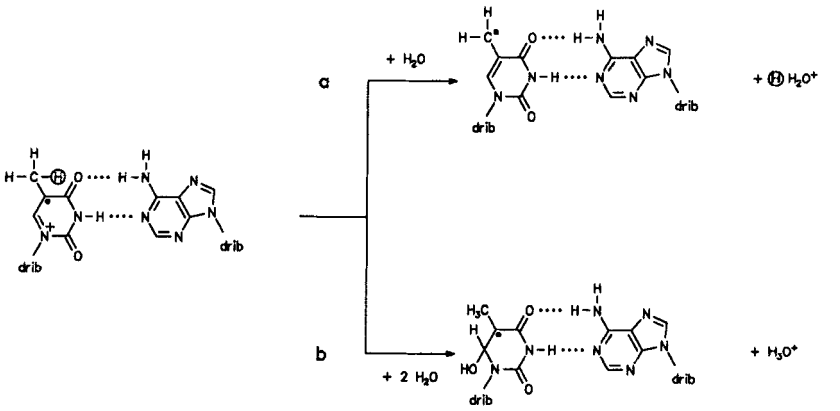


(32)

All these reactions lead to neutral radicals *in* the strands and the corresponding charge (as H^+ or $OH)^-$ *outside*, i.e., in the hydration shell, where neutralization can take place.

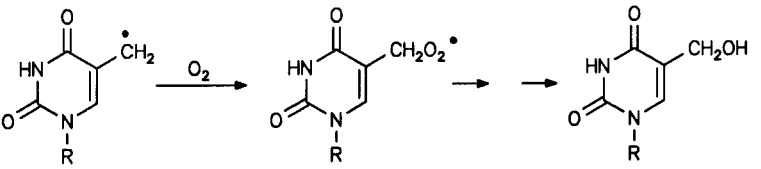
The second, and possibly more important type of extra-pair proton transfer, involves protonation on and deprotonation from *carbon*. These processes are very likely to be *ir*reversible and therefore prone to lead to irrepairable damage. In the following, these reactions will be described, again basing the considerations on what is known from homogenous aqueous solution. It is obvious that the conclusions drawn can only be of approximative nature, since many of the reactions that occur in DNA are likely to be dependent on the orientation in space[19] (conformations) of the potential reaction partners.

3.5.1. Involvement of carbon. Oxidation *of the bases.* As pointed out in section 3.4.1.2, in DNA the radical cation of thymine is unlikely to fully transfer the N3-proton to its complementary base adenine. One-electron-oxidized thymine will therefore retain its radical cation nature, i.e., its electrophilicity that expresses itself in sidechain-deprotonation (deprotonation from the methyl group at C5) to yield an allyl-type radical (Eq. (12a))[91,92] and in addition of a (nucleophilic) water molecule at C6 followed by deprotonation to give the 6-hydroxy-5,6-dihydrothymin-5-yl radical (Eq. (12b)). The two types of reaction, i.e., elimination, and electrophilic addition, which are characteristic of radical cation behavior in nucleophilic environments,[93] are therefore likely to take place also in DNA:



(33)

Concerning the deprotonation shown in Eq. (33a) the occurrence in DNA of this process can be concluded from the presence of 5-(hydroxymethyl)uracil after γ-irradiation.[94] This compound, which has been identified as an indicator of oxidative stress,[95] results from the allyl radical after its reaction with oxygen,[96,97] i.e.,

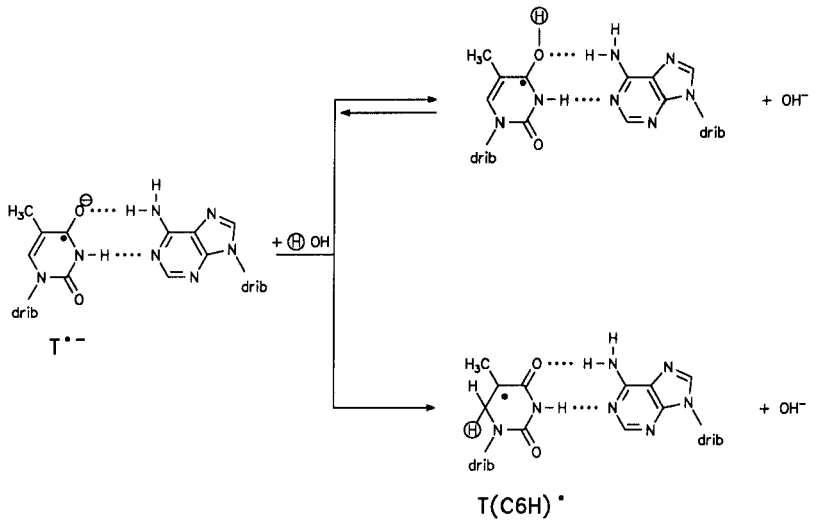


(34)

With the base radical cation from A and G deprotonation from carbon or hydration (hydroxylation) reactions (analogous to Eq. (33b)) do *not* appear to be important.

3.5.2. Involvement of carbon. Reduction *of the bases.*

3.5.2.1. Thymidine. Thymine also plays a special role with respect to irreversible reactions involving radical *anions*. In DNA at >77 K the reductive equivalents resulting from γ-irradiation show up as the 5,6-dihydrothymine-5-yl radical[20–22] T(C6H)$^{\bullet}$, which originates from the electron adduct of the thymine moiety by protonation by water at C6 (see Eq. (17)). In section 3.4.2.1 it was shown that in the A–T$^{\bullet-}$ pair in DNA the negative charge of T$^{\bullet-}$ is probably not neutralized by proton transfer from A. This gives external protonating agents such as water or phosphate a chance to protonate T$^{\bullet-}$ on carbon (the C5–C6 double bond is exposed to the exterior where there are ≈ 6 water molecules per base pair). Protonations on carbon are considerably faster with radical anions than with their (heteroatom protonated) conjugate acids.[8,60,98,99] On this basis, in the hypothetical case that T$^{\bullet-}$ was protonated (on O^4) by its complementary base A, (irreversible) protonation on C6 would be much *less* likely. Protonation on a heteroatom would thus protect the base with respect to protonation on carbon.



(35)

3.5.2.2. Adenosine. Protonations on carbon are also very important in the case of the electron adduct of the adenine moiety, $A^{\cdot -}$. As shown in section 3.2.3, in an initial step, this radical protonates rapidly on one of the nitrogens ($k \geqslant 1.4 \times 10^8 s^{-1}$)[78,79] to give the neutral radical A(NH)$^{\cdot}$. At pH 7, A(NH)$^{\cdot}$ transforms in a slow reaction ($k = 10^4 s^{-1}$) into its C8-protonated isomer, A(C8H)$^{\cdot}$. By converting A(NH)$^{\cdot}$ back into its conjugate base $A^{\cdot -}$ by reaction with OH^-, the rate of A(C8H)$^{\cdot}$ formation is considerably increased. $A^{\cdot -}$ protonates on C8 with the rate constant $3.6 \times 10^6 s^{-1}$, i.e., considerably faster than its (less electron-rich) conjugate acid, (A(NH)$^{\cdot}$.[8]

In the presence of H^+, A(NH)$^{\cdot}$ is transformed into another C-protonated isomer, that produced by addition to C2, A(C2H)$^{\cdot}$. A(C2H)$^{\cdot}$ is less thermodynamically stable than A(C8H)$^{\cdot}$, as concluded from the fact that A(C2H)$^{\cdot}$ can be converted into A(C8H)$^{\cdot}$ by catalysts such as phosphate or acetate.[8] As pointed out in section 3.2.3, A(NH)$^{\cdot}$ and A(C8H)$^{\cdot}$ have also been identified in the crystalline state upon irradiation with γ-rays. Also here, A(C8H)$^{\cdot}$ is the thermodynamically most stable isomer. The formation of the same species under such different conditions (single crystal/aqueous solution) demonstrates (a) the strong intrinsic driving force for these protonation reactions, and (b) the relevance of the data obtained from single crystal studies (using essentially ESR/ENDOR for analysis)[20–22] for understanding the radical chemistry of the DNA bases.

If one compares the uncatalyzed rate of protonation on carbon of adenosine ($k = 1 \times 10^4 s^{-1}$)[8] or of dAMP($k = 3.4 \times 10^4 s^{-1}$)[8] in aqueous solution with that of thymidine ($k \leqslant 10^3 s^{-1}$)[62] or thymidylic acid (TMP; $k = 1 \times 10^3 s^{-1}$),[100] it is evident that in the aqueous phase the tendency for protonation on carbon is more pronounced with the adenine than with the thymine system. An obvious question is thus whether in DNA the situation should be analogous. In order to judge this, it is useful to compare the driving force for protonation of the radical anions *in the base pair*, i.e., A–$T^{\cdot -}$ and $A^{\cdot -}$ – T. As pointed out in section 3.4.2.1, the thermodynamically preferred forms should be A–$T^{\cdot -}$ and A(NH)$^{\cdot}$–T(–H)$^-$. Thus, the tendency for protonation on carbon of the radical *anion*, $T^{\cdot -}$, has to be compared with that of the *neutral* radical, A(NH)$^{\cdot}$. On the basis of the behavior of the electron adducts of the separate nucleos(t)ides in aqueous solution, A(NH)$^{\cdot}$ protonates on C8 much more rapidly than does $T^{\cdot -}$ on C6. It is thus reasonable to assume that the same happens in DNA, i.e., that A(C8H)$^{\cdot}$ should be formed in DNA.

There is also the possibility that the thermodynamically less stable, but (in aqueous solution) kinetically favored A(C2H)$^{\cdot}$ isomer is formed (by protonation of C2 by N3 – H of thymine, see scheme X in ref 9). In this connection it is interesting that neither A(C2H)$^{\cdot}$ nor A(C8H)$^{\cdot}$ have so far been detected in irradiated DNA.[20–22] There are essentially two explanations for this failure: (a) The radicals A(C2H)$^{\cdot}$ or A(C8H)$^{\cdot}$ are "ESR-silent", i.e., they cannot be detected due to their very broad lines and/or microwave saturation behavior. (b) The radical A(NH)$^{\cdot}$ (or possibly A(C2H)$^{\cdot}$, which is a much weaker reductant that A(NH)$^{\cdot}$, see ref. 8), could transfer an *electron*[101–103] on to the base above or below in its strand before protonation takes place at a carbon. The electron would finally be trapped at a thymine site. Obviously, this would require electron transfer to be considerably faster than proton transfer. From a theoretical point of view,[104] in DNA electron transfer rates (in the direction of the helix axis, intra-strand electron transfer) could in fact be very high ($\approx 10^{14} s^{-1}$), due to the almost perfect stacking of the bases. On the other hand, also rates of proton transfer could be up to this value since transfer of a proton in a hydrogen-bonded structure such as that in a base pair could occur in one vibrational cycle. However, these high rates refer

TABLE I
Charge and reactivity status of the base radicals *in the base pairs*

	Electron removal		Electron addition	
Base involved	Charge of the base in the base pair	Probability of irrev. reaction	Charge of the base in the base pair	Probability of irrev. reaction
A	positive	low	neutral	high
G	positive $\rightleftharpoons$ neutral	low	neutral?	High
C	positive	low?	neutral	low?
T	positive $\rightleftharpoons$ neutral	high	negative	high

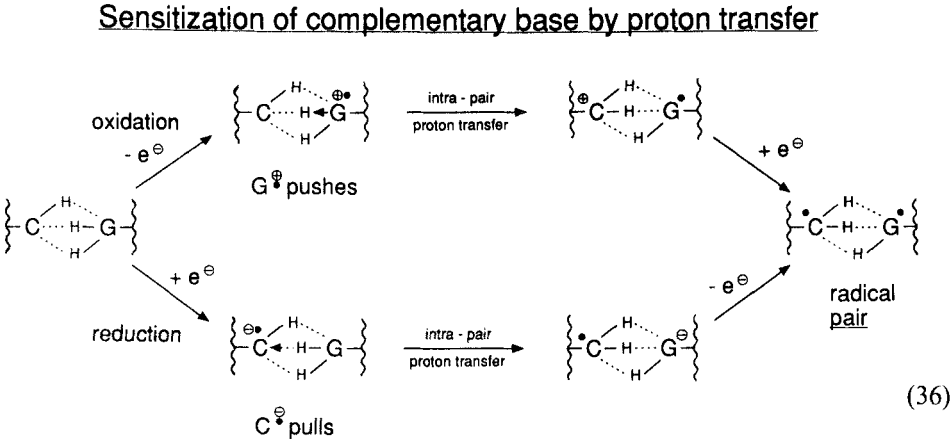
only to proton transfer between heteroatoms (as in *intra*-pair proton transfer), whereas proton transfer to and from carbons (which is of the *extra*-pair type) is always much slower.[26–30] Therefore, electron transfer, even from (heteroatom)-*protonated* electron adducts, probably can compete with their protonation on *carbon*. On the other hand, in DNA electrons do not appear to be able to migrate over large distances; on the basis of results from scavenger experiments, the journey of the electron rarely exceeds 5–50 nucleotide units.[3,105,106]

To summarize the situation regarding the likelihood of the individual bases in the base pairs to undergo *ir*reversible proton transfer reactions with water: Thymine is outstanding in so far as having a high reactivity when it is oxidized as well as when it is reduced. Adenine has a tendency to react irreversibly only upon one-electron reduction. With cytosine the situation is not yet clear. However, the evidence available at the moment is in favor of reversible reactions, while with guanine only the reversible path is observed upon *oxidation*. In the case of one-electron *reduction* of guanine, there is strong evidence for protonation on carbon of the electron adduct.[48] The charge and reactivity status of the base radicals in the base pairs, as based on the presently available information, is presented in Table 1.

3.6. G–C: A "Special Pair"?

In section 3.4.2.2 it was pointed out that if in the G–C pair the electron is picked up by C the resulting radical anion $C^{\cdot -}$ will be protonated by its complementary base G. Although the resulting guanine anion, $G(-H)^-$, produced by proton abstration by $C^{\cdot -}$, has a good chance of being protonated by an adjacent water molecule, as long as it remains an anion it should be the ideal trap for positive holes. Thus pick up of an electron by C in one strand leads to an increased probability of a positive hole to be trapped at G in the complementary strand, i.e., at the base exactly opposite to the reduced base. This situation is reciprocal to that when a base is *oxidized*. The increase in acidity on one-electron oxidation results in transfer of a proton to the complementary base (see Eq. (24)), whose electron affinity is thereby considerably enhanced. That base has therefore an increased chance of trapping an electron contained in its strand.[107] The common feature of these inter-strand proton transfers is that damage inflicted on one base, whether of oxidative or of reductive type, attracts damage of the opposite nature to deposit in the complementary base on the other strand. The common result is an (uncharged) *radical pair* on a *base pair*. Bernhard has pointed out that a double-stranded radical pair may well lead to a double-strand break.[107] The G–C pair thus seems to have unique abilities to scavenge electrons as well as holes.

Reductive damage at C increases the power of G to pick up positive holes, and oxidation of G makes its partner C an even better trap for electrons. The situation is summarized in scheme 36:



(36)

A comment may be made concerning the likelihood of (electron transfer) reaction between the two radicals in the pair, G(−H)$^{\cdot}$ and C(N3H)$^{\cdot}$. As long as the two molecules remain in the same plane as defined by the hydrogen bonds between them, there is not much overlap of the semi-occupied molecular orbitals and thus only a limited chance for reaction of the two radicals in the base pair.* On this basis, the lifetime of the radical pair with respect to spin–spin interaction could be quite long. At any rate, a reaction involving electron transfer (followed by or concerted with proton transfer) from C(N3H)$^{\cdot}$ to G(−H)$^{\cdot}$, would result in complete *repair* of the G–C pair.

3.7. Summary and Conclusions

It has been demonstrated that protonations and deprotonations result from one-electron reduction or oxidation, respectively, of the DNA bases. These proton transfer processes (by which the charge of the molecule is altered) not only change the nature and thereby the reactivity of the base radicals, but they also have a strong effect on electron transfer processes via their "modulation" of the free energies of reaction. An example of this is the base cytosine whose "electron affinity under protonating conditions" is *higher*, whereas the gas phase electron affinity is probably *lower* than that of the other bases. In addition to the reversible proton transfer processes (between the hetero-atoms) there are the irreversible ones (involving carbon atoms).

*For a discussion of the electron transfer in DNA by π–π interaction along the stacked bases (intra-strand electron transfer) see ref. 104. Estimates of rates of transfer *between* the strands (inter-strand electron transfer) do not seem to have been made. For general information on the dependence of electron transfer rates on various parameters such as distance see ref. 108.

The former are more likely to occur in the G–C base pair, whereas the A–T pair is more prone to engage in the latter. Since the reversible proton transfers are rapid they determine the *initial* sites of electron and positive hole deposition (as determined at $\approx$4–10 K), whereas in the irreversible processes, although they are slow, the *final* sites of damage (as measured at $\geqslant$77 K) are fixed. Future work will have to focus on the detailed mechanisms of "damage migration" from the initial to the final sites.

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