dynamics are predominantly governed by the homolytic dissociation energy of the $^{+\bullet}C-H$ bond, although other factors such as solvent reorganization should be taken into account to a minor extent.

Acknowledgment. This work has been jointly supported by the CNRS, the Office of Basic Energy Sciences of the US Department of Energy. P.H. thanks the NIST for allowing him to carry out the laser flash photolysis experiments and Dr. R. E. Huie for his help in using the laser system.

Registry No. MAH radical cation, 105784-79-0; BQCNH radical cation, 141063-18-5; BQAH radical cation, 141063-19-6; BNAH radical cation, 72533-28-9; NADH radical cation, 88764-56-1.

One-Electron-Reduction Potentials of Pyrimidine Bases, Nucleosides, and Nucleotides in Aqueous Solution. Consequences for DNA Redox Chemistry[1]

S. Steenken,* J. P. Telo,[2] H. M. Novais,[2] and L. P. Candeias

Contribution from the Max-Planck-Institut für Strahlenchemie, D-4330 Mülheim, Germany.
Received November 18, 1991

Abstract: The reduction potentials in aqueous solution of the pyrimidine bases, nucleosides, and nucleotides of uracil (U) and thymine (T) were determined using the technique of pulse radiolysis with time-resolved spectrophotometric detection. The electron adducts of U and T were found to undergo reversible electron exchange with a series of ring-substituted *N*-methylpyridinium cations with known reduction potential. From the concentrations of the pyrimidine electron adducts and the reduced *N*-methylpyridinium compounds at electron-transfer equilibrium, the thermodynamical equilibrium constants were obtained and from these the reduction potentials. The results show U and T and their nucleosides and nucleotides to have very similar reduction potentials, ~ -1.1 V/NHE at pH 8, i.e., the effect of methylation at C5, C6, or of substitution at N1 is small, ≤ 0.1 V. In the case of cytosine (C) the electron adduct is protonated (probably at N3), even up to pH 13. The protonated adduct (C(H)$^\bullet$) undergoes a reversible electron transfer with the *N*-methylpyridinium cations. This is accompanied in one direction by transfer of a proton but by that of a water molecule in the other direction. As a result of the protonation of the electron adduct, the effective ease of reduction of C in aqueous solution is similar to that of U and T. It is suggested that in DNA the tendency for $C^{\bullet-}$ to be protonated (by its complementary base G) is larger by ≥ 10 orders of magnitude than that for protonation of $T^{\bullet-}$ by its complementary base A. This results in C and not T being the most easily reduced base in DNA. A further consequence is that lack of neutralization by *intra*pair proton transfer of $T^{\bullet-}$ enables the irreversible *extra*-pair protonation on C6 of the radical anion to take place.

Introduction

The destructive, mutagenic, and carcinogenic effect of ionizing radiation on living matter is almost exclusively due to the changes induced in the DNA of the cell nucleus.[3] If is for this reason that there has been a continuing and, lately, increasing interest to unravel the chains of mechanism by which the oxidizing and the reducing species that result from ionization of a molecule lead to the biologically visible damage.[4] On a molecular or "chemical" level one of the questions that has attracted considerable attention is whether the lesions to the pyrimidine and purine bases, which are produced in a *statistical* way, lead to damage at *specific* sites. Evidence for such "damage migration" phenomena has originally come from ESR studies in matrices at low temperatures,[4] but the conclusions[4] have recently been supported by methods such as pulse radiolysis[5,6] or laser photolysis[7] on DNA (bases) in aqueous solution at room temperature. There have also been theoretical studies on the feasibility of charge or energy transduction between bases of different nature or along the DNA chain.[8] The ESR evidence available to data can be summarized[4] by stating that, at room temperature, the negative charge produced in the ionization is trapped by thymine and the positive one by guanine. This is equivalent to saying that thymine is the most electron-affinic and guanine the most easily oxidized base under "DNA conditions", i.e., as constituents of the polynucleotide chain of DNA. This result has been regarded as being in agreement with the electron affinities (EA) and the ionization potentials (IP) of the bases. Concerning the *ionization potentials*, experimentally determined values are available,[9] and these show the purines to be more easily oxidized than the pyrimidines, and, among the former, guanine to be the best electron donor of all the bases, and this is in agreement with the results[10] of MO calculations.

(1) A preliminary report on this topic was given at the International Radiation Biology Conference held at New Orleans, LA, April 1990.

(2) Instituto Superior Tecnico, P-1096 Lisboa, Portugal.

(3) For reviews see, e.g.: (a) *Effects of Ionizing Radiation on DNA*; Bertinchamps, A. J., Hüttermann, J., Köhnlein, W., Teoule, R., Eds.; Springer: Berlin, 1978. (b) *Mechanisms of DNA Damage and Repair*; Simic, M. G., Upton, A. C., Eds.; Plenum: New York, 1986. (c) von Sonntag, C. *The Chemical Basis of Radiation Biology*; Taylor and Francis: London, 1987.

(4) For reviews see, e.g.: (a) Reference 3a. (b) Bernhard, W. A. *Adv. Radiat. Biol.* **1981**, *9*, 199. (c) Hüttermann, J. *Ultramicroscopy* **1982**, *10*, 25. (d) Symons, M. C. R. *J. Chem. Soc., Faraday Trans. 1* **1987**, *83*, 1. (e) Close, D. M.; Nelson, W. H.; Sagstuen, E. In *Electronic Magnetic Resonance of the Solid State*; Weil, J. A., Ed.; Canadian Society of Chemistry: Ottawa, Canada, 1987; p 237. (f) Hüttermann, J. In *Radical Ionic Systems*; Lund, A., Shiotani, M., Eds.; Kluwer: Dordrecht, 1991; pp 435-62. (g) Bernhard, W. A. Initial Sites of One Electron Attachment in DNA. In *The Early Effects of Radiation on DNA*; Fielden, E. M., O'Neill, P., Eds.; Nato ARW Series, Springer: 1991; Vol. H **54**, pp 141-54.

(5) Sevilla, M. D.; Failor, R.; Clark, C.; Holroyd, R. A.; Pettei, M. *J. Phys. Chem.* **1976**, *80*, 353.

(6) Visscher, K. J.; Hom, M.; Loman, H.; Spoelder, H. J. W.; Verberne, J. B. *Radiat. Phys. Chem.* **1988**, *32*, 465.

(7) Candeias, L. P.; Jones, G. D. D.; O'Neill, P.; Steenken, S. *Int. J. Radiat. Biol.* **1992**, *61*, 15.

(8) Gueron, M.; Eisinger, J.; Shulman, R. G. *J. Chem. Phys.* **1967**, *47*, 4077. Voltz, R. *Radiat. Res. Rev.* **1968**, *1*, 301. Dee, D.; Baur, M. E. *J. Chem. Phys.* **1974**, *60*, 541.

(9) (a) Hush, N. S.; Cheung, A. S. *Chem. Phys. Lett.* **1975**, *34*, 11. (b) Peng, S.; Padva, A.; LeBreton, P. R. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 2966. (c) McGlynn, S. P.; Dougherty, D.; Mathers, T.; Abdulner, S. In *Excited States in Organic Chemistry and Biochemistry*; Pullman, B., Goldblum, N., Eds.; Reidel: Dordrecht, 1977; p 247.

0002-7863/92/1514-4701$03.00/0

However, concerning the *electron affinities*, experimentally determined values do *not* seem to exist, the published values[11] being derived from MO calculations. Although there is agreement that the purines are less electron-affinic than the pyrimidines, the calculations[11] do not permit an answer to the important question whether among the pyrimidines it is the cytosine or the thymine moiety that is the better electron acceptor.

In addition, the calculated values relate, of course, to the isolated molecule in vacuo, no account being taken of the effects of neighbors or solvent. However, these effects can obviously be of great influence in modifying the properties of electron acceptors. Of particular importance are protonation reactions of electron adducts by which the reduction potentials of the electron acceptors are changed. Such protonation reactions are possible not only in aqueous solution but also within double stranded DNA, where protonation of an electron adduct of a particular base is likely to occur by its "natural" proton donor, i.e., its complementary base, through the channel of the preexisting hydrogen bonds between the bases in the pair.[12]

It is for this reason that the use of gas phase electron affinities to predict the trapping site of electrons in a system such as DNA, where protonation is possible, is not likely to give correct results. It is therefore necessary to determine experimentally the "electron affinities under protonating conditions", i.e., the reduction potentials of the pyrimidine bases in aqueous solution, in order to obtain reliable values for their ease of reduction. Electrochemical measurements on nucleic acid bases have been performed previously;[13] however, the electrode reactions were not reversible in aqueous solution, so the measured potentials are not the thermodynamically defined numbers. However, by using "electrochemistry in homogeneous solution", i.e., molecules with known reduction potential to which electron transfer is reversible and by measuring the equilibrium constants for the electron exchange with the pyrimidine bases, their reduction potentials can in principle be determined. The results, if applied to DNA, should shed some light on the most likely trapping sites for the electron and should give an explanation for the recent ESR observations that not only in di- and oligonucleotides (containing cytosine and thymine),[14] but also in DNA itself[15] e^- is trapped more by cytosine than by thymine.

Experimental Section

The pyridines and pyrimidines were obtained from Aldrich/Bader, Boehringer, Merck, P + L Biochemicals, and Sigma, and they were used as received. The argon-saturated aqueous solutions ($[O_2] \leq 1$ μM, water purified with a Millipore-Milli-Q system) typically contained 0.2 M *tert*-butyl alcohol to scavenge $OH^•$, (0.2 to) 2 mM pyrimidine, 1–10 mM phosphate (except for solutions of the nucleotides), and 10–500 μM of the redox standard. Five different redox standards were used: (a) terephthaldialdehyde (=TEA; it was recrystallized from 2-propanol), (b) 1-methyl-4-carboxypyridinium (MCP^+) chloride, (c) 1,4-dimethylpyridinium (DMP^+) iodide, synthesized by methylation of 4-picoline with methyl iodide, (d) 1-methyl-4-(4-cyanostyryl)pyridinium ($MCSP^+$) iodide, prepared as described,[16] and (e) *N*-methylnicotineamide (MNA^+) iodide. In the case of d), in addition to the "usual" cut-off filters at 280, 320, or 360 nm, a filter with λ(cut-off) = 435 nm was used to minimize photochemical transformation of the standard by the analyzing light.

The aqueous solutions were irradiated with 100–400 ns pulses from a 3 MeV van de Graaff accelerator using doses such that 0.5–2 μM radicals were produced. The temperature of the solutions was kept at 20 °C to within 0.1° using cells that are an integral part of a heat exchanger.[17] The optical signals were digitized with a Tektronix 7612 transient recorder interfaced with a DEC LSI $11/73^+$ computer which controlled the experiment and was also used for on-line preanalysis of the data. Day-to-day dosimetry was performed with N_2O saturated 10 mM KSCN solutions for which $G(OH) = 6.0$ and $\epsilon((SCN)_2^{•-})$ at 480 nm = 7600 M^{-1} cm^{-1}. In addition, in most cases special dosimetries were performed using solutions that contained only the redox standard or the pyrimidine under representative reducing conditions. Final data analysis was performed with a Microvax connector with the LSI via Ethernet.

Results and Discussion

1. Acid–Base Properties of the Electron Adducts. Since the Brönsted basicities of the electron adducts of the naturally occurring pyrimidines have been measured only for a few systems, a systematic study of the protonation states of pyrimidine electron adducts in the pH range 5–10 was carried out. The electron adducts were produced by pulse-irradiating 0.5 mM pyrimidine derivative (P) solutions that contained 0.1–0.2 M *tert*-butyl alcohol to scavenge the OH radicals (eq 2) generated in the water radiolysis (eq 1) and 5–10 mM phosphate to speed up the proton exchange reactions. In eq 1, the numbers in brackets indicate the radiation chemical yields, expressed as number of species produced per 100 eV of absorbed radiation. Since the radical

$$H_2O \rightarrow OH^• (2.8),\ e^-_{aq} (2.7),\ H^• (0.55) \quad (1)$$

$$OH^• + (CH_3)_3COH \rightarrow H_2O + (CH_3)_2C(OH)CH_2^• \quad (2)$$

$$H^• + P \rightarrow H\text{-}P^• \quad (3)$$

$$e^-_{aq} + P \rightarrow P^{•-} \quad (4)$$

formed by H-abstraction from *tert*-butyl alcohol (eq 2) does not absorb at $\lambda > 200$ nm, the optical signals observed above this wavelength must be due to the reactions of e^-_{aq} and $H^•$ with the pyrimidines (eqs 3 and 4).

In an experiment with cytidine, the $H^•$ atoms were scavenged, together with the $OH^•$ radicals, by 65 mM 2-propanol, eq 5.

$$OH^•(H^•) + (CH_3)_2CHOH \rightarrow H_2O(H_2) + (CH_3)_2C^•OH \quad (5)$$

Under these conditions, the optical absorptions observed after the pulse at 300–350 nm were very similar to those measured in the presence of the $H^•$ atoms (i.e., with *tert*-butyl alcohol as $OH^•$ scavenger), which indicates that the optical signals are predominantly due to the products of reaction of e^-_{aq} (eq 4) and not of $H^•$ (eq 3).[18] In agreement with this is the fact that the transients could be removed by typical e^-_{aq} scavengers such as $ClCH_2CH_2OH$ or CH_2Cl_2, which essentially do not react with $H^•$.

The absorptions observed on reaction of e^-_{aq} with the pyrimidines increased in intensity in going from ~350 to ~300 nm, with a sharp drop of optical density at lower wavelengths. This decrease is due to the onset of the strong absorption band of the parent pyrimidines, which are depleted by the formation of radicals. If corrected for this depletion, the λ_{max} of the electron adducts is at $\lambda < 300$ nm.

It was found that with all pyrimidines, except the cytidines, the absorption at all wavelengths between 300 and 350 nm decreased on going from pH ~ 10 to pH ~ 5. The pH-dependent changes of the absorptions are explained in terms of a protonation/deprotonation equilibrium.[19] In the case of uracil and thymine derivatives, it has been established by ESR in aqueous solution that electron addition occurs at O^4 to give oxyallyl type radicals.[20] The electron adducts are rapidly protonated on O^4 (see eq 6b),[21] a reaction also seen in single crystals at ≈4 K,[22]

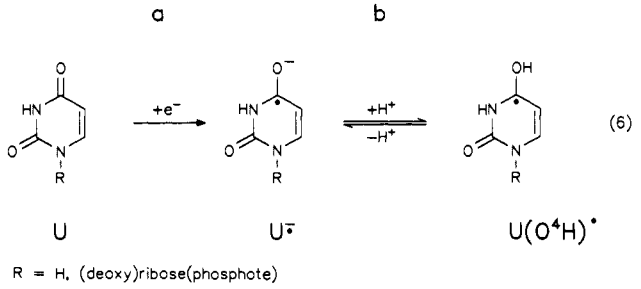
(10) See refs 9 and 11.
(11) (a) Pullman, B.; Pullman, A. *Quantum Biochemistry*; Wiley-Interscience: London, 1963. (b) Berthod, H.; Giessner-Prettre, C.; Pullman, A. *Theor. Chim. Acta* **1966**, *5*, 53. (c) Bodor, N.; Dewar, M. J. S.; Harget, A. J. *J. Am. Chem. Soc.* **1970**, *92*, 2929. (d) Younkin, J. M.; Smith, L. J.; Compton, R. N. *Theor. Chim. Acta* **1976**, *41*, 157. Compton, R. N.; Yoshioka, Y.; Jordan, K. D. *Theort. Chim. Acta* **1980**, *54*, 259. (e) Rakvin, B.; Herak, J. N. *Radiat. Phys. Chem.* **1983**, *22*, 1043.
(12) (a) Steenken, S. *Chem. Rev.* **1989**, *89*, 503. (b) Steenken, S. *Free Radical Res. Commun.* **1989**, *6*, 117. (c) Steenken, S. *Free Radical Res. Commun.*, in press.
(13) Janik, B.; Elving, P. J. *Chem. Rev.* **1968**, *68*, 25. Elving, P. J.; Pace, S. J.; O'Reilly, J. E. *J. Am. Chem. Soc.* **1973**, *95*, 647. Webb, J. W.; Janik, B.; Elving, P. J. *J. Am. Chem. Soc.* **1973**, *95*, 8495; Santhanam, K. S. U.; Elving, P. J. *J. Am. Chem. Soc.* **1974**, *96*, 1653. Cummings, T. E.; Elving, P. J. *J. Electroanal. Chem.* **1978**, *94*, 123.
(14) Bernhard, W. A. *J. Phys. Chem.* **1989**, *93*, 2187.
(15) Sevilla, M. D.; Becker, D.; Yan, M.; Summerfield, S. R. *J. Phys. Chem.* **1991**, *95*, 3409.
(16) Phillips, A. P. *J. Org. Chem.* **1949**, *14*, 302. Horwitz, L. *J. Am. Chem. Soc.* **1955**, *77*, 1687. Williams, J. L. R.; Adel, R. E.; Carlson, J. M.; Reynolds, G. A.; Borden, D. G.; Ford, J. A. *J. Org. Chem.* **1963**, *28*, 387. Güsten, H.; Schulte-Frohlinde, D. *Z. Naturforsch.* **1979**, *34B*, 1556.
(17) Jagannadham, V.; Steenken, S. *J. Am. Chem. Soc.* **1984**, *106*, 6542.
(18) The H-atom probably reacts by addition at the C5–C6 double bond, see: Das, S.; Deeble, D. J.; von Sonntag, C. *Z. Naturforsch.* **1985**, *40C*, 292.
(19) See, also: Hayon, E. *J. Chem. Phys.* **1969**, *51*, 4881, and ref 21.
(20) Novais, H. M.; Steenken, S. *J. Am. Chem. Soc.* **1986**, *108*, 1.

Table I. Rate and Equilibrium Constants at 20 °C for Electron Transfer between Pyrimidines P and Redox Standards S

pyrimidine (P)	pK_a(PH•)	S,[a] E^1, V/NHE, λ(obs), nm	k_f,[b] M^{-1} s^{-1}	k_r,[c] M^{-1} s^{-1}	$K_{Kin} = k_f/k_r$[d]	K[e]	E(P) − E(S),[f] V/NHE	E(P),[g] V/NHE	$f(e^-)$,[h]
uracil	7.3[i]	MCP^+, −0.94, 395	4.0×10^9	6.4×10^6	1200	623 ± 48	−0.165	−1.10	0.98
pH 8.2									
uridine	7.0	TEA, −0.80, 395, 625	2.5×10^9						
pH 8.2–8.7		$MCSP^+$, −0.87, 485	4.8×10^9	4.1×10^6	1200	1165 ± 176	−0.181	−1.05	1.00
		MCP^+, −0.94, 395	2.3×10^9	2.1×10^6		1119 ± 218	−0.180	−1.06	0.95
		DMP^+, −1.15, 365	4.3×10^9			~24	~−0.082	~−1.2	
		MNA^+, −1.01, 420	4.2×10^9	3.0×10^6	1300	770	−0.170	−1.18	
deoxyuridine		TEA	2.8×10^9						0.92
pH 8.3–8.7		$MCSP^+$	5.1×10^9	4.2×10^6		1218 ± 103	−0.183	−1.05	1.03
		MCP^+	2.6×10^9	1.9×10^6		1319 ± 249	−0.185	−1.12	0.94
uridine-3′-phosphate	7.1	MCP^+	2.0×10^9	3.9×10^6	180	509 ± 270	−0.160	−1.10	1.00
pH 8.4									
uridine-5′-phosphate	7.9	TEA	2.3×10^9						0.96
pH 8.8		MCP^+	2.3×10^9	3.9×10^6		590 ± 91	−0.164	−1.10	0.98
			2.3×10^9[k]	6.7×10^6[k]	570[k]	345[k] ± 23	−0.150[k]	−1.09[k]	0.95[k]
uridine-3′,5′-diphosphate	8.5	MCP^+	2.3×10^9	1.3×10^7		170 ± 20	−0.13	−1.07	0.97
pH 9.2									
thymine	7.2[i]	MCP^+	3.1×10^9	6.9×10^6	1700	448 ± 30	−0.157	−1.10	1.00
pH 8.6									
thymidine	6.9	TEA	3.1×10^9						
pH 8.4–8.7		MCP^+	2.8×10^9	7.0×10^6	1800	402 ± 13	−0.154	−1.09	1.00
		DMP^+	3.9×10^9[l]			~8[l]	~−0.053[l]	~−1.20[l]	
			$<5 \times 10^8$[m]						
		MNA^+	3.9×10^9	8.0×10^6	490	632	−0.165	−1.18	
thymidine-3′-phosphate	7.1	MCP^+	2.2×10^9	4.6×10^6	200	479 ± 73	−0.159	1.10	1.00
pH 8.7									
thymidine-5′-phosphate	8.3	MCP^+	2.4×10^9	5.5×10^6		435 ± 29	−0.156	−1.10	0.97
pH 9.2			2.2×10^9[n]	6.9×10^6[n]	1000[n]	317[n] ± 46	−0.148[n]	−1.09[n]	0.94[n]
6-methyl-uracil		MCP^+	3.2×10^9	5.6×10^6	1000	569 ± 27	−0.163	−1.10	0.98
pH 8.6									
orotic acid	7.0	TEA	2.1×10^9						1.02
pH 8.6		MCP^+	2.6×10^9	5.5×10^6	473	469 ± 60	−0.158	−1.10	1.00
		MNA^+	3.6×10^9	1.3×10^8	28	289	−0.145	−1.16	
isoorotic acid		TEA	2.1×10^9						0.99
pH 8.6		MCP^+	3.4×10^9	1.3×10^7	350	270 ± 67	−0.144	−1.08	1.00
cytosine		MCP^+	2.8×10^9	7.1×10^6	1100	397 ± 43	−0.154	−1.09	0.98
pH 8.8									
cytidine	>13	MCP^+	2.0×10^9	5.9×10^6		340 ± 43	−0.150	−1.09	1.00
pH 8.5–8.8									
		$MCSP^+$	2.8×10^9	6.1×10^6	540	456 ± 55	−0.157	−1.03	1.00
		DMP^+				6.0 ± 3.2	−0.046	−1.20	
		MNA^+	2.6×10^9	1.2×10^7	217	222 ± 22	−0.139	−1.15	
								−1.16[p]	
								−1.14[q]	
cytidine-5′-phosphate		TEA	2.0×10^9						
		MCP^+	1.6×10^9	3.0×10^6	1800	534 ± 129	−0.161	−1.10	0.97
pH 8.5–8.7									

[a] Key: TEA = terephthaldialdehyde, MCP^+ = 1-methyl-4-carboxypyridinium, DMP^+ = 1,4-dimethylpyridinium, $MCSP^+$ = 1-methyl-4-(4-cyanostyryl)pyridinium, MNA^+ = 1-methylnicotineamide. [b] Determined from k_{obsd} vs [S] plots containing ≥5 points. [c] Calculated from eq 11 using K from column 7. [d] Obtained from $k_{obsd}/[S]$ vs $[P]/[S]$ plots. [e] Obtained from the optical densities at equilibrium. [f] Calculated from K (column 7) using the Nernst equation. [g] Error limits ±50 mV. [h] f = fraction of electrons scavenged by S at [S]:[P] ≥ 2. The error limits correspond to ±0.03. [i] See: Hayon, E. J. *Chem. Phys.* **1969**, *51*, 4881. [k] At pH 8.2. [l] At pH 8–8.5. [m] At pH 5–6. [n] At pH 8.7. [o] Due to overlap of spectra OD buildup due to DMP• is compensated by decay due to C(H)•. [p] At pH 5.5. [q] At pH 10.4.

and this causes the decrease in absorption at 300–400 nm, understandable on the basis of the overall decrease in electron density on protonation.



By monitoring the OD changes of the electron adducts as a function of pH (in most cases at 320 nm, where the signal quality was optimal), the pK_a values of the protonated electron adducts of the pyrimidines P, P(H)• (see eq 6b), were obtained from the inflection points of the sigmoidal curves. These values are listed in column 2 of Table I.

It is evident that the basicities of the radical anions of uracil, thymine, (deoxy)uridine, and thymidine are very similar (pK_a(P(H)•) = 7.1 ± 0.2), which means that substitution of H at C5 by methyl or at N1 by (deoxy)ribose has only a very small influence on the acid–base properties of the electron adducts. The same is true for the substituent at N1, (deoxy)ribose-3′-phosphate, but *not* for the corresponding 5′-phosphate: A phosphate group at 5′ raises the pK_a by 0.9–1.3 units. When there are *two* phosphate groups as with uridine-3′,5′-phosphate, the acidity of the protonated electron adduct (pK_a = 8.5) is slightly further weakened. The difference between the effect of the phosphate group at C3′ and C5′ can be explained in terms of H-bond formation between O^4–H and one of the oxygens of the phosphate group at C-5′. Such an H-bond would weaken the acidity of

(21) Deeble, D. J.; Das, S.; von Sonntag, C. *J. Phys. Chem.* **1985**, *89*, 5784.
(22) Sagstuen, E.; Hole, E. O.; Nelson, W. H.; Close, D. M. *J. Phys. Chem.* **1989**, *93*, 5974. Hole, E. O.; Sagstuen, E.; Nelson, W. H.; Close, D. M. *J. Phys. Chem.* **1991**, *95*, 1494.

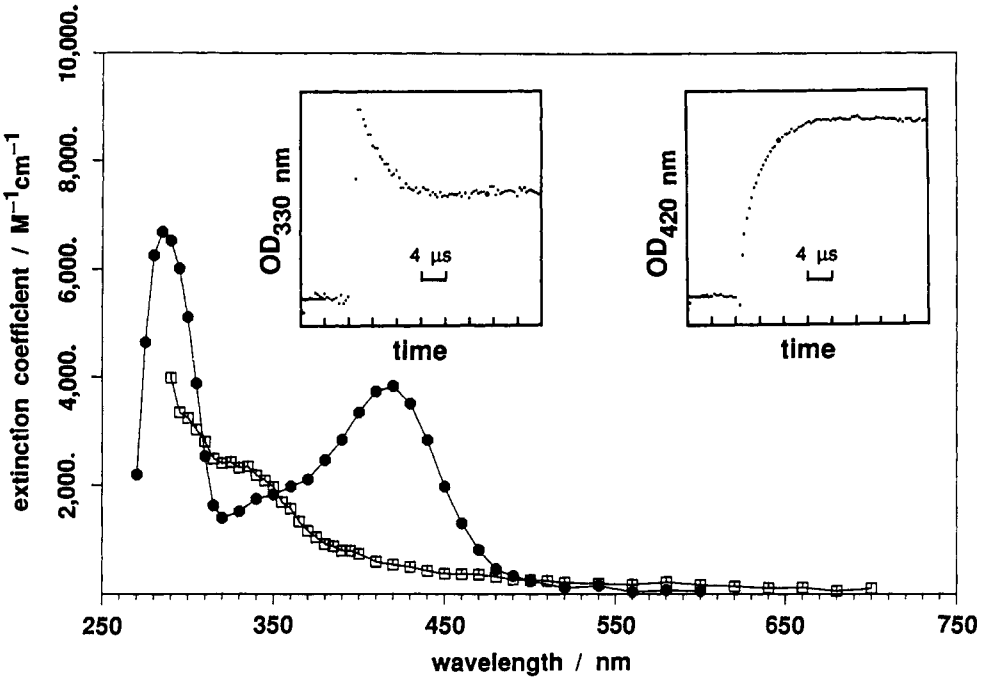


Figure 1. Absorption spectra of $T^{\bullet-}$ (squares) recorded using a 1 mM solution of thymidine, 4 μs after the pulse, and of MNA$^{\bullet}$ (circles) recorded with a 0.5 mM solution of *N*-methylnicotineamide iodide, 20 μs after the pulse. Insets: changes of OD on irradiation of a solution containing 2 mM thymidine and 0.05 mM *N*-methylnicotineamide iodide (all Ar-saturated aqueous solutions containing 0.2 M *tert*-butyl alcohol, pH = 8.5–8.7 and T = 20 °C).

O^4–H. With the 5′-phosphate the base and the phosphate group are on the same side of the (deoxy)ribose ring and can bend over to almost touch each other.[23] With the 3′-derivative, however, the phosphate group is on the opposite side of the ring, such that interaction between the base and the phosphate is not possible.

The electron adduct of cytidine has been shown[24] by conductance measurements to be protonated at pH 6–10.6. Protonation occurs by water acting as an acid, and this process (eq 8) is complete in ≤20 ns.[6,25] We have measured the absorption

$$C + e^- \rightarrow C^{\bullet-} \quad (7)$$

$$C^{\bullet-} + H_2O \rightarrow C(H)^{\bullet} + OH^- \quad (8)$$

spectrum of the electron adduct of cytidine and found it not to change in the pH range 6–13. Under the assumption that the absorption spectrum of the neutral (protonated) electron adduct is different from that of its conjugate base (as it is in the case of U and T, see above) the invariance of the absorption spectrum with pH means that the pK_a value of the protonated electron adduct is >13. This means that upon electron addition the Brönsted basicity of the molecule is increased by >9 orders of magnitude (from a comparison with pK_a(cytidineH$^+$) = 4.4). A similar increase in proton accepting power is observed in the uridine or thymidine system, e.g., pK_a (thymidineH$^+$) = −5,[26] pK_a (thymidineH$^{\bullet}$) = 6.9 (see Table I), thus $\Delta pK_a \sim 12$. Large increases in *basicities* upon electron *addition* are well-known,[27] e.g., pK_a(pyridineH$^+$) = 5.25, pK_a(pyridineH$^{\bullet}$) ≥ 14,[28] or pK_a-((CH$_3$)$_2$C=OH$^+$) = −3.06,[29] pK_a((CH$_3$)$_2$C$^{\bullet}$OH) = 12,[30] i.e., ΔpK_a = 15. Reciprocal to this are the increases in *acidities* upon electron *removal*,[12,31] e.g., pK_a(deoxyguanosine) = 9.5, pK_a(deoxyguanosine$^{\bullet+}$) = 3.9,[31] i.e., ΔpK_a = 5.6, or pK_a(phenol) = 10, pK_a(phenol$^{\bullet+}$) = −2,[32] i.e., the acidity increases by 12 orders of magnitude in this case.[33]

2. Electron Exchange with 1-Methylpyridinium Cations. Electron adducts of uracil, thymine, and cytosine and their nucleosides and nucleotides were produced by reacting e^-_{aq} with 0.5–4 mM solutions at pH 8–9 as described in section 1. With the rate constants for reaction with e^-_{aq} of $\approx 10^{10}$ M^{-1} s^{-1}, the formation of the electron adducts is complete in ≤200 ns, which means that reactions that occur at a longer time scale cannot be due to reaction of e^-_{aq}. At radical concentrations of ≈1 μM, the decay of the electron adducts was essentially by second order and their lifetime was ≥1 ms. To these solutions were than added 0.01–0.5 mM substituted 1-methylpyridinium ions ("standard" S^+) such as 1-methylnicotineamide (MNA$^+$) or 1,4-dimethylpyridinium (DMP$^+$), whereby the concentration of the standard was typically ≤10% of that of the pyrimidine. As a result of the addition of the standard, the absorptions due to the pyrimidine radicals $P^{\bullet-}$ (with the uracil or thymine system) or P(H)$^{\bullet}$ (with the cytosines) were replaced (see Figure 1) by those (for the λ_{max} values, see Table I) of the semireduced standard, $S^{\bullet}$, with rates (on the μs to ms time scale) roughly proportional to the concentrations of the pyridinium cations S^+. This shows that the decay of the (protonated) electron adducts is due to transfer of an electron to the pyridinium cations. This transfer turned out to be reversible, as documented by the fact that the *yields* of electron transfer depended on the concentrations of pyridinium cation *and* on that of the pyrimidine, higher concentrations of the latter leading to a decrease in the yield of semireduced pyridinium cation, higher concentrations of the former to an increase. The dependence of the concentrations of the electron adducts of the pyrimidines ($P^{\bullet-}$) and of the semireduced pyridinium cations ($S^{\bullet}$) on the concentrations of the parent compounds is given by the equilibrium constant K for the electron exchange, which for the case of the uracils and thymines can be formulated as

$$P^{\bullet-} + S^+ \underset{k_r}{\overset{k_f}{\rightleftharpoons}} P + S^{\bullet}$$

$$K = k_f/k_r = [P]\,[S^{\bullet}]/[P^{\bullet-}]\,[S^+] \quad (9)$$

(23) For NMR data on this molecule, see: Schweizer, M. P.; Broom, A. D.; Tso, P. O. P.; Hollis, D. P. *J. Am. Chem. Soc.* **1968**, *90*, 1042.
(24) Hissung, A.; von Sonntag, C. *Int. J. Radiat. Biol.* **1979**, *35*, 449.
(25) Visscher, K. J.; de Haas, M. P.; Loman, H.; Vojnovic, B.; Warman, J. M. *Int. J. Radiat. Biol.* **1987**, *52*, 745.
(26) Garcia, B.; Palacios, J. C. *Ber. Bunsenges. Phys. Chem.* **1988**, *92*, 696.
(27) For a recent review, see: ref 12c.
(28) Fessenden, R. W.; Neta, P. *Chem. Phys. Lett.* **1973**, *18*, 14.
(29) Bagno, A.; Lucchini, V.; Scorrano, G. *Bull. Soc. Chim. Fr.* **1987**, 563.
(30) Asmus, K.-D.; Henglein, A.; Wigger, A.; Beck, G. *Ber. Bunsenges. Phys. Chem.* **1966**, *70*, 756. Laroff, G. P.; Fessenden, R. W. *J. Phys. Chem.* **1973**, *77*, 1283.
(31) Candeias, L. P.; Steenken, S. *J. Am. Chem. Soc.* **1989**, *111*, 1094.
(32) Dixon, W. T.; Murphy, D. *J. Chem. Soc., Faraday Trans. 2* **1976**, *72*, 1221.
(33) For a recent review on Brönsted acidities and basicities of *radicals*, see: Dohrmann, J. K. *Landolt-Börnstein, Zahlenwerte und Funktionen aus Wissenschaft und Technik* **1985**, *13e*, 5.

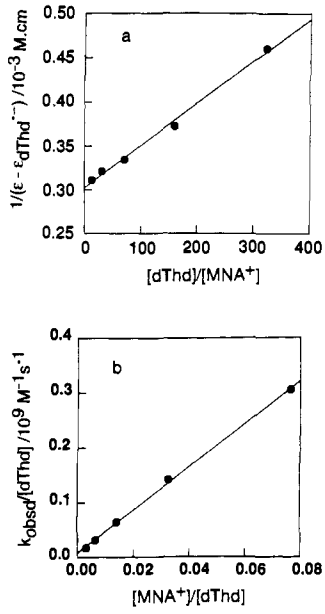


Figure 2. Graphical determination of the equilibrium constant for the electron transfer between the thymidine electron adduct (=dThyd$^{\bullet-}$) and MNA$^+$ (at 20 °C and pH = 8.5) from (a) the optical densities at equilibrium and (b) the rates of establishment of equilibrium.

In order to obtain reliable values for K, the optical densities due to S$^\bullet$ and P$^{\bullet-}$ were measured using at least five solutions that differed from one another by up to a factor of 40 with respect to the [P]:[S$^+$] ratio, while the sum [P] + [S$^+$] was kept constant. The optical densities at λ_{max} (S$^\bullet$ or P$^{\bullet-}$) were converted into concentrations as previously described.[34] Alternatively, a graphical method to obtain K was applied. For this purpose, eq 9 is rearranged to give eq 10

$$1/(\epsilon - \epsilon(P^{\bullet-})) = a/K[P]/[S^+] + a \qquad (10)$$

where ϵ is the effective extinction coefficient of the solution after establishment of electron transfer equilibrium and $a \equiv 1/[\epsilon(S^\bullet) - \epsilon(P^{\bullet-})]$. Therefore, if $1/[\epsilon - \epsilon(P^{\bullet-})]$ is plotted versus [P]/[S$^+$], K is obtained as the ratio of intercept and slope, see Figure 2a.

Column 7 of Table I contains the equilibrium constants thus obtained, together with the standard deviations which are on the average between 10 and 20%. In many cases, between two and four different redox standards with different redox potentials were employed to determine the equilibrium amount of electron transfer from the electron adducts of the pyrimidines, again using ≥5 different ratios of concentrations of pyrimidine and standard. In a few cases, it was also possible to get an estimate of the equilibrium constant from an analysis[34,35b] of the kinetics of approach to equilibrium, see Figure 2b for an example. The values thus obtained are listed in column 6 of Table I. They are considered less reliable (due to combination of errors from slope and intercept) than those calculated from the optical densities at equilibrium. However, from a qualitative point of view the kinetically derived values are valuable since they demonstrate directly the existence of reversible electron transfer.

The last column of Table I contains data on the yields of electron transfer from the pyrimidine electron adducts to the standards under conditions that electron transfer to the standards is quantitative, which is true when the ratio [S$^+$]:[P] ≥ 0.2. These yields of electron transfer are close to 1.0, i.e., electron transfer is complete under these conditions. An oxidant with a somewhat higher reduction potential than the pyridinium cations was also used. Terephthaldialdehyde, TEA, which has a potential of −0.80 V/NHE at pH 7–8,[36] oxidizes essentially irreversibly the pyrimidine electron adducts.

Attempts were also made to one-electron reduce the pyrimidines with aliphatic radicals known to be powerful electron donors. Examples are $CO_2^{\bullet-}$ (E^1_7 = −1.85 V/NHE),[37] $(CH_3)_2CO^{\bullet-}$ (E^1_{13} = −2.5 V/NHE),[35] and the radical anion of the cyclic disulfide $\overline{SCH_2HC(OH)CH(OH)CH_2S}$ (RSSR), whose reduction potential may be estimated to be −1.6 V/NHE.[38] The disulfide radical anion RSSR$^{\bullet-}$ was produced by one-electron *oxidation* by OH of dithiothreitol which gives a thiyl radical which at pH > 10 cyclizes to the disulfide radical anion, recognizable by the absorption band with λ(max) = 390 nm.[39] In the absence of oxidants, the decay of RSSR$^{\bullet-}$ is by second order kinetics, with a half-life ≥1 ms. This decay was not accelerated by 1 mM cytidine. The rate constant for the reaction of RSSR$^{\bullet-}$ + cytidine is thus $k/M^{-1}\,s^{-1} \leq 10^3/10^{-3} = 10^6$. The radicals $CO_2^{\bullet-}$ and $(CH_3)_2CO^{\bullet-}$ have a similarly low reactivity, as judged by the absence of formation within ≤1 ms of the cytidine electron adduct on producing the aliphatic radicals in the presence of 1 mM of the nucleoside. The reason for the lack of reactivity cannot be a lack of thermodynamic driving power for electron transfer, which in the case of $(CH_3)_2CO^{\bullet-}$/cytidine corresponds to 1.4 V = 32 kcal/mol, and to still 0.5 V if RSSR$^{\bullet-}$ is the reductant. The conclusion is then that the activation barrier for electron transfer is high for these aliphatic reductants, but obviously not for the *less* strongly reducing aromatic radicals S$^\bullet$ of the pyridinyl type as described earlier in this section.

3. One-Electron Reduction Potentials.[40] The equilibrium constants K_n for the electron exchange at pH n between the pyrimidines P and the pyridinium cations (standard S$^+$, see eq 9) are related to (reflect the) the difference in reduction potential between the two types of electron acceptor as described by the Nernst equation

$$E^1_n(P) - E^1_n(S) = -(RT/F) \ln K_n \qquad (11)$$

The absolute value for one-electron reduction of P at pH n, $E^1_n(P)$,[41] can thus be obtained from K_n if $E^1_n(S^+)$ is known. The standard most frequently used (see Table I) was 1-methylnicotineamide (MNA$^+$), whose E^1_7 has been accurately determined[42] to be −1.01 V/NHE. This was also used as a standard against "secondary" standards such as 1,4-dimethylpyridinium (DMP$^+$; this is the cation with the lowest reduction potential, as expected on the basis of the electron-donating methyl group in the 4-position) or 1-methyl-4-carboxypyridinium (MCP$^+$). The potentials of the standards MCSP$^+$ and terephthaldialdehyde (TEA) are based on electron exchange equilibration with 2,2′-cyclobutylbispyridinium dication (E^1_7 = −0.60 V/NHE).[43] The values measured for the standards (listed in column 3 of Table I) are estimated to be accurate to ±20 mV, and the best values for the pyrimidines are those obtained using the most "negative" standards MNA$^+$ and DMP$^+$.

Using the reduction potentials of the standards and the K values presented in Table I, the reduction potentials at pH 8–9 of the

(34) Meisel, D.; Czapski, G. *J. Phys. Chem.* **1975**, *79*, 1503. Ilan, Y. A.; Czapski, G.; Meisel, D. *Biochim. Biophys. Acta* **1976**, *430*, 209. Wardman, P.; Clarke, E. D. *J. Chem. Soc., Faraday Trans. 1* **1976**, *72*, 1377. Steenken, S.; Neta, P. *J. Phys. Chem.* **1979**, *83*, 1134; **1982**, *86*, 3661.

(35) (a) Steenken, S. In *Landolt-Börnstein, Zahlenwerte und Funktionen aus Wissenschaft und Technik* **1985**, *13e*, 147. (b) Wardman, P. *J. Phys. Chem. Ref. Data* **1989**, *18*, 1637.

(36) Bolte, G.; Steenken, S. unpublished material.

(37) Koppenol, W. H.; Rush, J. D. *J. Phys. Chem.* **1987**, *91*, 4429. Surdhar, P. S.; Mezyk, S. P.; Armstrong, D. A. *J. Phys. Chem.* **1989**, *93*, 3360.

(38) Surdhar, P. S.; Armstrong, D. A. *J. Phys. Chem.* **1986**, *90*, 5915; **1987**, *91*, 6532.

(39) Akhlaq, M. S.; von Sonntag, C. *Z. Naturforsch.* **1987**, *42C*, 134.

(40) For a collection of one-electron reduction potentials of organic molecules and radicals, see: ref 35.

(41) Following pulse radiolysis nomenclature (see ref 34), the superscript 1 to the potential E denotes that a *one*-electron reduction is involved, and the subscript n indicates the pH of the aqueous solution in which the potential is measured. E.g., E^1_7 is the potential of a one-electron reduction at pH 7.

(42) Anderson, R. F.; Patel, K. B. *J. Chem. Soc., Faraday Trans. 1* **1984**, *80*, 2693. Jensen, M. A.; Elving, P. J. *Biochim. Biophys. Acta* **1984**, *764*, 310. See, also: ref 35 for reviews.

(43) Measured by cyclic voltammetry in an aqueous solution containing 1 mM substrate and 1 mM phosphate.

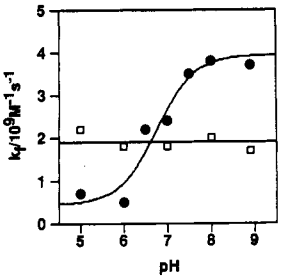
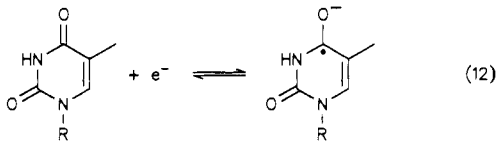


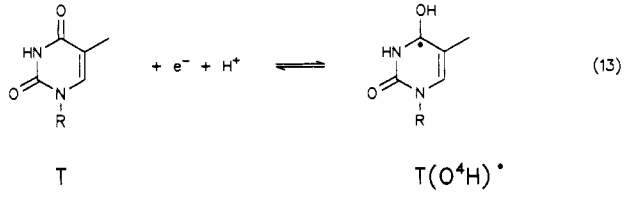
Figure 3. pH dependence of the rate of reduction of DMP^+ by the electron adduct of thymidine (circles) and of the rate of reduction of MCP^+ by the electron adduct of cytidine (squares). The sigmoidal curve is from a computer fit of the data based on $pK_a(T(O^4H)^\bullet) = 6.9$.

pyrimidines were calculated with eq 11, and the results are shown in column 9 of Table I. It is obvious that the E^1 values are essentially independent of the redox standard used, evidence that the data reflect the existence of true electron transfer equilibria.

a. Uracil and Thymine Derivatives. Concerning the uracil (U) and thymine (T) systems, electron addition at pH 8–9 produces the radical anions (see section 1 and Table I), so the electrode reaction defining the potential is, e.g., in the case of T



Since the electron adducts are bases, they are protonatable and this reaction (see eq 6b) changes the reduction potential of the system which is now defined by eq 13

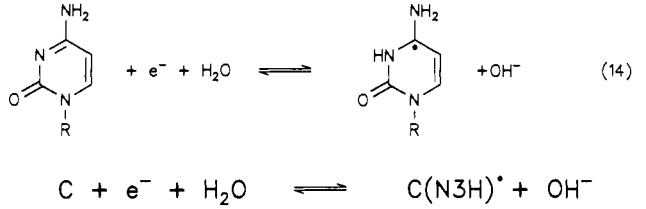


Equation 13 contains an electron and a proton transfer equilibrium. The thermodynamic treatment[35b,44] of this combined electron/proton transfer process leads to the prediction that, on lowering the pH, the potential remains the same until the pK_a of the protonated electron adduct, $T(O^4H)^\bullet$ ($\equiv T(H)^\bullet$), is reached ($pK_a = 6.9$ in the case of thymidine, see Table I). Below the pK_a the potential increases by 59 mV per pH unit.[35b,44] This increase results from the driving force for protonation of the electron adduct (eq 13 forward), which grows with increasing $[H^+]$.

An attempt was made to measure the reduction potential of thymidine in a pH range below the pK for protonation of its electron adduct, i.e., to determine the potential for reaction 13. Unfortunately, it was not possible to establish electron transfer *equilibrium* between the protonated electron adduct and the standards. However, it was found that the *rate* of reduction of the standard 1,4-dimethylpyridinium (DMP^+) decreased with decreasing pH in a sigmoidal way with an inflection point at pH 7, the same as the pK value for protonation of the electron adduct of thymidine (see Figure 3). This indicates that the protonated electron adduct of thymidine is a weaker reductant than its conjugate base, the radical anion. The rate constant measured at pH 5–6 for reduction of DMP^+ by the neutral radical is $\approx 5 \times 10^8\ M^{-1}\ s^{-1}$. The fact that this number cannot be given with greater precision is due to distortion of the buildup kinetics of $DMP^\bullet$ by its bimolecular decay.

Concerning the data collected for the uracils and thymines (Table I), the single most important result is that the reduction potentials at pH 8–9 (i.e., those for formation of the radical *anions* from the neutral precursors, eq 12) are essentially the same for all the systems, bases, nucleosides, and nucleotides, i.e., $E^1_{8-9} = -1.11 \pm 0.05$ V. The effect on E^1_{8-9} of methylation at C5 or C6 or replacement of H at N1 by (deoxy)ribose or (deoxy)ribose-3′ or 5′-phosphate is thus within the experimental accuracy of ±50 mV. Even an ionized carboxyl group at C5 or C6 does not have a strong influence on the reduction potential, a situation similar to that observed with respect to the pK_a values.

b. Cytosine and Derivatives. In contrast to the pH dependence of the rate of reduction by the thymidine electron adduct are the results for cytidine: as shown in Figure 3, the rate of reduction of the standard 1-methyl-4-carboxypyridinium (MCP^+) is *in*dependent of pH between 5 and 9, which reflects the fact[24] that the protonation state of the electron adduct of C remains the same in this pH range. In this region (and up to pH 13; see section 1) the electron adduct of cytidine is not a radical anion but a neutral species,[24,25] formed by rapid (≤20 ns)[6,25] protonation of the electron adduct by water, eq 14.



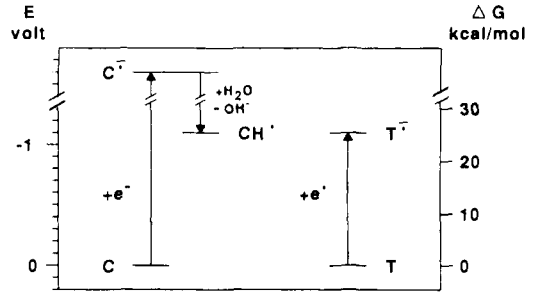
It was found that $C(N3H)^\bullet$ ($\equiv C(H)^\bullet$) undergoes reversible electron transfer with pyridinium cations, eq 15

$$C(H)^\bullet + S^+ \underset{k_r}{\overset{k_f}{\rightleftharpoons}} C + S^\bullet + H^+ \qquad (15)$$

for which the equilibrium constant is defined as $K = [C][S^\bullet][H^+]/[C(H)^\bullet][S^+]$. The K values were determined for a number of cytosine derivatives and (pyridinium) standards S^+ and from these the reduction potentials E were calculated. The values (average = −(1.12 ± 0.05) V/NHE, see Table I) are similar to those found for the uracil and thymine derivatives. At first sight, this is a surprising result, since one expects the cytosines (which are more electron-rich, based on their lower IPs[9]) to be *less* easily reduced than the uracils and thymines. The reason for the higher (than expected) reduction potentials is the protonation of the electron adduct (eq 14). Protonation of the electron adduct provides to the overall reduction an extra driving force, with the consequence that the reduction potential becomes more positive than it would be in the absence of protonation. This results in the reduction potentials of the cytosines not being smaller than those of the uracils and thymines. A related aspect of the higher electron density is the fact that cytosine ($pK = 4.5$) is a better proton acceptor than thymine ($pK = -5$)[26] by 9.5 orders of magnitude. A similar difference in proton accepting power is exhibited by the electron adducts, $pK_a(T(H)^\bullet) = 6.9$, $pK_a(C(H)^\bullet) \geq 13$. The stronger tendency of the cytosine system to protonate thus compensates its probably lower "intrinsic" (gas phase) tendency to pick up an electron. Equation 16 is a graphical representation of the effect of protonation on the reduction potential of cytidine at pH 8.5, which is compared with thymidine, taking the free energies of the free bases as equal to zero.

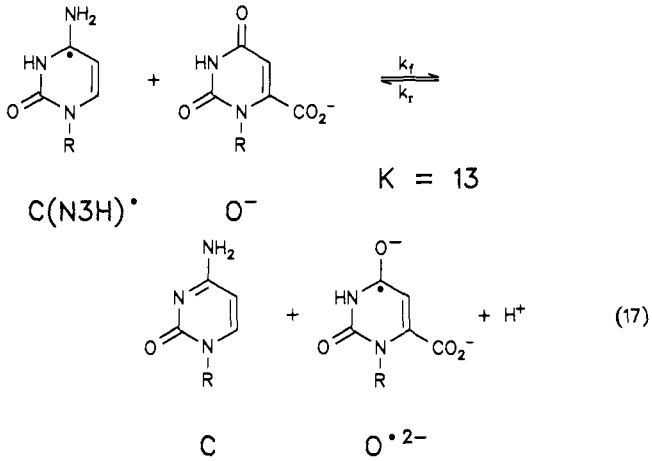
c. Electron Exchange between Pyrimidine Bases. In order to check the consistency of the reduction potentials determined using the pyridinium cations as standards, experiments involving electron transfer *between two pyrimidine* systems were carried out. A serious difficulty in interpreting such experiments is due to the

(44) Michaelis, L. *Biochem. Z.* **1932**, *250*, 564. *Chem. Rev.* **1935**, *16*, 243. Michaelis, L.; Schubert, M. P. *Chem. Rev.* **1938**, *22*, 437.



(16)

fact that the absorption spectra of all pyrimidine radicals are very similar, and therefore the electron transfer causes only a very small change in optical density at any wavelength. However, exceptions to this are the radicals of orotic and isoorotic acid, whose spectra are visibly different from those of other pyrimidine radicals, so the electron exchange between these and the other members of the pyrimidine family can be observed, see Figure 4 and eq 17.[45] This means that orotic and isoorotic acid can be used as redox standards for uracil, thymine, and cytosine derivatives. The equilibrium constants for eq 17 and analogous reactions and the corresponding ΔE values for the electron transfer are presented in Table II. Within the limits of experimental error, the values are in agreement with the reduction potential data obtained by use of the pyridinium cations as presented in Table I.



(17)

4. Relevance to the Radiation Chemistry of DNA. The finding that the reduction potential in aqueous solution at pH ~ 8.5 of the cytosine moiety is equal to that of thymine is difficult to reconcile with the "classical" conclusions drawn[4a-d,46] from electron spin resonance of irradiated DNA that it is the thymine system which selectively traps the electrons formed in the ionizing events. However, it has recently been convincingly demonstrated that in DNA at low temperatures (≤77 K) the electron is predominantly trapped at *cytosine* and not at thymine.[4g,14,15] This can easily be explained by the larger electron affinity of cytosine as compared to thymine *under protonating conditions*, not only in aqueous solution but even more so in DNA.

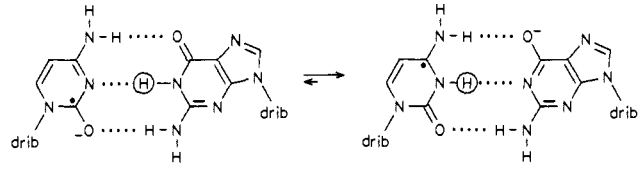
In contrast to the situation in aqueous solution, in DNA, due to the pairing of the bases, the *likelihood of protonation does depend not only on the basicity of the electron adduct but also on the acidity of its complementary base.* For instance, if in the G–C pair the electron is picked up by C, the chances of $C^{\bullet-}$ to be protonated by G are quite good, since G is a relatively stong acid (pK_a (deoxyguanosine) = 9.5)[47] and $C^{\bullet-}$ is a strong base (pK_a ($C(H)^\bullet$) ≥ 13), i.e., ΔpK_a ($G/C(H)^\bullet$) ≤ −3.5. If these pK values, which, of course, refer to aqueous solution, describe as well the situation in DNA, the proton transfer equilibrium can be predicted.[48] E.g., in the $C^{\bullet-}$–G pair, $C^{\bullet-}$ will be quantitatively protonated

$$C^{\bullet-} + H^+ \rightleftharpoons C(H)^\bullet \qquad K \geq 10^{13} \tag{18}$$

$$G \rightleftharpoons G(-H)^- + H^+ \qquad K = 10^{-9.5} \tag{19}$$

$$C^{\bullet-} + G \rightleftharpoons C(H)^\bullet + G(-H)^- \qquad K \geq 10^{3.5} \tag{20}$$

Consequently, the N1 proton of guanine will be pulled over to the cytosine N3, as shown below.



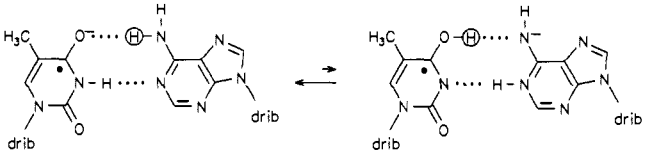
(21)

In contrast, with the AT pair, assuming that the electron resides with T, the situation is reversed, since $T^{\bullet-}$ is only a weak base (pK_a ($T(H)^\bullet$) = 6.9) and A a very weak acid (pK_a (deoxyadenosine) ≥ 13.75),[49] i.e., ΔpK_a ($A/T(H)^\bullet$) ≥ 6.85. This means that the thymine radical anion will *not* be protonated by its complementary base, adenine, cf. eqs 22–24.

$$T^{\bullet-} + H^+ \rightleftharpoons T(O^4H)^\bullet \qquad K = 10^{6.9} \tag{22}$$

$$A \rightleftharpoons A(N^6\text{–}H)^- + H^+ \qquad K \leq 10^{-13.75} \tag{23}$$

$$T^{\bullet-} + A \rightleftharpoons T(O^4H)^\bullet + A(N^6\text{–}H)^- \qquad K \leq 10^{-6.85} \tag{24}$$



(25)

If the A–T(H)• pair is compared with the G–C(H)• pair, the $\Delta\Delta pK_a$ results as ≥(6.85 + 3.6) = 10.45, i.e., the tendency for $C^{\bullet-}$ to be protonated (by G) is larger by ≥10 orders of magnitude than that for $T^{\bullet-}$ (by A). This $\Delta\Delta pK_a$ corresponds to a differential driving force for protonation of $C^{\bullet-}$ compared to $T^{\bullet-}$ in their base pairs of ≥0.59 V or ≥13.6 kcal mol^{-1}.

A second way of looking at the difference in "protonation pressure" on $C^{\bullet-}$ is by comparing the situation in water with that in DNA, i.e., the following equilibria.

$$C^{\bullet-} + H_2O \rightleftharpoons C(H)^\bullet + OH^- \qquad K_{aq} = [C(H)^\bullet][OH^-]/[C^{\bullet-}][H_2O] \tag{26}$$

$$C^{\bullet-} - G \rightleftharpoons C(H)^\bullet - G(-H)^- \qquad K_{DNA} = [C(H)^\bullet][G(-H)^-]/[C^{\bullet-}][G] \tag{27}$$

(45) Electron transfer from electron adducts of pyrimidines to orotic acid has been seen before: Adams, G. E.; Greenstock, C. L.; van Hemmen, J. J.; Willson, R. L. *Radiat. Res.* **1972**, *49*, 85. However, it was not recognized that the reactions are reversible.

(46) Gräslund, A.; Ehrenberg, A.; Rupprecht, A.; Ström, G.; Crespi, H. *Int. J. Radiat. Biol.* **1975**, *28*, 313. For reviews, see: Hüttermann, J.; Voit, K.; Oloff, H.; Köhnlein, W.; Gräslund, A.; Rupprecht, A. *Faraday Discuss. Chem. Soc.* **1984**, *78*, 135. Hüttermann, J.; Voit, K. In *Electron Magnetic Resonance of the Solid State*; Weil, J. A., Ed.; Canadian Society of Chemistry: Ottawa, Canada, 1987; p 267, and refs 4a–d.

(47) In the subsequent discussion, the pK_a values of the bases are taken as those of the corresponding 2′-deoxynucleosides (from Tso, P. Bases, Nucleosides, and Nucleotides. In *Basic Principles in Nucleic Acid Chemistry*; Tso, P., Ed.; Academic: New York; 1974; p 453.

(48) It is obvious that in DNA the species involved in the proton transfer between the bases in the pair are not hydrated. In contrast, the pK_a values, which refer to aqueous solution, contain the free energies of hydration of all the proton exchange partners. If the free energy changes due to the hydration changes resulting from (de)protonation of the *radical* (such as in eq 18) are the same as those of a *parent* base (such as in eq 19), their contributions to the overall proton transfer equilibrium (such as in eq 20) *cancel*. Under this condition the ΔpK_a values quantitatively reflect the proton transfer equilibria also under nonaqueous conditions.

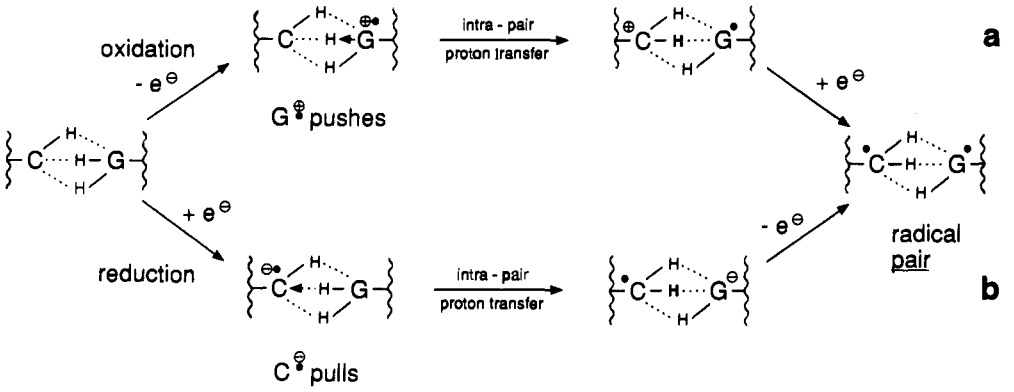
(49) Hissung, A.; von Sonntag, C.; Veltwisch, D.; Asmus, K.-D. *Int. J. Radiat. Biol.* **1981**, *39*, 63.

Table II. Data on Intermolecular Electron Transfer between Individual Pyrimidines[a]

P_1	P_2	pH	λ(obs), nm	k, M^{-1} s^{-1}	K^b	$[E(P_1) - E(P_2)]$, V
cytidine (CH•)	orotic acid (O)	8.5	315	$k_f = (9.1 \pm 0.2) \times 10^8$	13	0.066
				$k_r = (3 \pm 2) \times 10^7$		
cytidine (CH•)	isoorotic acid (iso-O)	8.5	315		0.46	−0.020
thymidine (T•⁻)	orotic acid (O)	8.5	315	$k_f = 6.9 \times 10^8$	89	0.115

[a] $[P_1]$:$[P_2]$ was varied between 0.1 and 10. $[P_1] + [P_2] = 1$ mM, 20 °C. [b] K is defined for electron transfer from e^- adduct of P_2 to P_1.

Scheme I. Sensitization of Complementary Base by Proton Transfer



Taking the ratio of the two equilibrium constants and inserting the dissociation constant of G, $K_a(G)$, and the ion product of water, K_W, one obtains

$$K_{DNA}/K_{aq} = K_a(G)/K_W = 10^{-9.5}/10^{-14} = 10^{4.5} \quad (28)$$

This means that the tendency for $C^{\bullet-}$ to be protonated by G in DNA is larger by the factor $10^{4.5}$ as compared to that in water.

On the basis of these considerations it is reasonable to assume that $C^{\bullet-}$ will be protonated in DNA. It is obvious that the reduction potential for C increases with increasing driving force for protonation of $C^{\bullet-}$, and, as a result of the strong tendency for $C^{\bullet-}$ to be protonated "under DNA conditions", i.e., by G, the reduction potential of C could become more positive than that of T, i.e., C is more easily reducible than T. This difference in potential between C and T in their base pairs can be calculated from the potentials in aqueous solution at pH 8 (−1.1 V for both C and T) and the difference in pK_a between G (9.5) and A (≥13). The ΔpK_a (≥3.5) corresponds to ≥0.21 V, by which C is more "electron-affinic under DNA conditions" than is T.

It is likely that the guanine anion, formed by proton abstraction by $C^{\bullet-}$, is eventually neutralized by reaction with a water molecule, probably one residing in the minor groove. However, as long as G is *de*protonated, it is the perfect trap for positive holes, due to its considerably increased ease[50] of one-electron oxidation, compared to neutral G or any of the other bases. This means that, in a generalized way, pick-up of an *electron* by a particular base leads to an increased probability of a *positive hole* in the complementary strand being trapped at the base exactly opposite to the reduced base, see Scheme I (part b).

This situation is reciprocal to what happens when a base is *oxidized* rather than reduced: oxidation leads to an enormous increase in the *acidity* of the base, resulting in the transfer of a proton to the complementary base,[12,31] whose electron affinity is thereby considerably enhanced. That base has therefore an improved change of scavenging an electron contained in its strand,[51] Scheme I (part a). The common feature of these interstrand proton transfers is that damage in one base, whether of oxidative or of reductive nature, attracts damage of the opposite type to deposit in the complementary base on the other strand. The result in each case is a (uncharged) radical *pair* located on a base *pair*. As pointed out by Bernhard,[51] it is possible that the double-stranded radical pair leads to a double strand break. Also possible is interstrand electron transfer (followed by or concerted with proton transfer between the strands), a reaction that constitutes repair.[52] The driving force for this process can be calculated from the reduction potential of C (−1.1 V, see Table I) and the oxidation potential of G (≥1.17 V)[53] to be ≥2.3 eV or ≥53 kcal/mol. From a Marcus theory point of view, this large number is likely to correspond to the "inverted region" of the electron transfer rate-free energy relation and this implies a considerable lifetime of the radical pair.

To summarize, the C–G pair seems to have unique properties with respect to scavenging one-electron reducing as well as oxi-

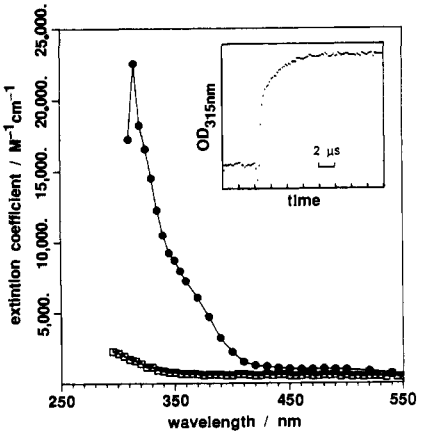
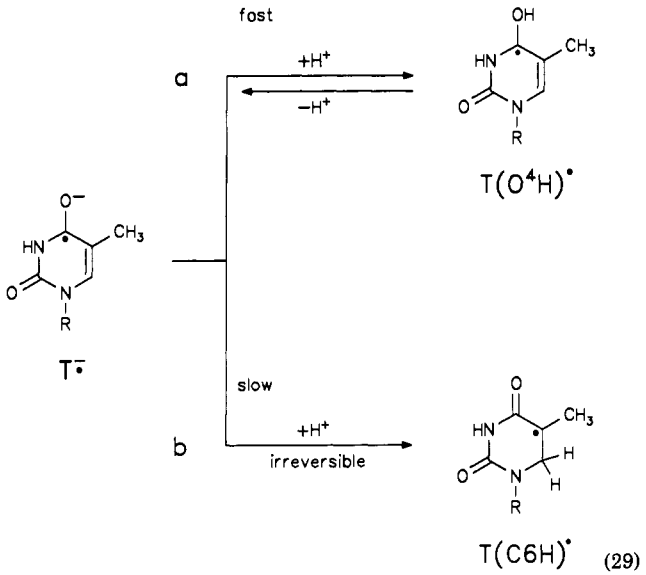


Figure 4. Absorption spectra of the radicals from electron addition to orotic acid (circles), recorded with a 1 mM solution of orotic acid, 25 µs after the pulse, and to cytidine (squares), recorded with a 1 mM solution of cytidine, 20 µs after the pulse. Inset: change of OD on irradiation of a solution 0.5 mM of orotic acid and 0.5 mM of cytidine (all Ar-saturated aqueous solutions containing 0.2 M *tert*-butyl alcohols, pH = 8.5 and T = 20 °C).

(50) Jovanovic, S. V.; Simic, M. G. *J. Phys. Chem.* **1986**, *90*, 974.
(51) Rackovsky, S.; Bernhard, W. A. *J. Phys. Chem.* **1989**, *93*, 5009.
(52) This repair reaction may be one of the reasons why in DNA the radiation chemical yield of radicals (see: Barnes, J.; Bernhard, W. A.; Mercer, K. R. *Radiat. Res.* **1991**, *126*, 104) is less than expected.
(53) This value is derived from ref 50 as an improved number. However, for the reference compound tryptophan at pH 13 the value 0.75 V/NHE (from Merenyi, G.; Lind, J.; Shen, X. *J. Phys. Chem.* **1988**, *92*, 134) is used.

dizing equivalents. Reductive damage at C leads to an enhanced probability of deposition of oxidative damage at G, and oxidation of G sensitizes C to become an even better trap for electrons.[51]

A final comment may be made on the observation[4a-e] that in DNA irradiated at or warmed up to room temperature the negative charge created on ionization ultimately ends up as the "C6-protonated thymine radical anion", the 5,6-dihydrothymine-5-yl radical T(H)•, or more precisely, T(C6H)•. This



(29)

is the result of the irreversibility[20,21] of this protonation on carbon. In the case of cytidine, there is so far no evidence for an analogous reaction in aqueous solution.[12c,54] Since the N3 (or the tautomeric O^2) protonated electron adduct of cytidine is able to donate an electron in aqueous solution,[24,55] even to weak electron acceptors such as 1,4-DMP$^+$ (see Table I) or to orotic acid[45] (see Table II), it is conceivable that it is able in DNA to transfer an e^- to thymidine, where it will be finally trapped by protonation of C6 (eq 29b).[56] In other words, although in DNA an electron has a better chance of being at first trapped at C, due to the strong driving force for protonation of the electron adduct, the *final* site of deposition, the "burial site", will be T, where it is trapped, after transfer along the helix axis, by the irreversible protonation of carbon. This explains the firmly established[3,4,15] formation of T(H)• in DNA irradiated at or warmed up to room temperature.

Acknowledgment. The financial support from a travel grant (Nato 0748/85) is gratefully acknowledged. J. P. Telo and L. P. Candeias thank the Deutscher Akademischer Austauschdienst (DAAD) for stipends.

Registry No. Uracil, 66-22-8; uridine, 58-96-8; deoxyuridine, 951-78-0; uridine-3′-phosphate, 84-53-7; uridine-5′-phosphate, 58-97-9; uridine-3′,5′-diphosphate, 2922-95-4; thymine, 65-71-4; thymidine, 50-89-5; thymidine-3′-phosphate, 2642-43-5; thymidine-5′-phosphate, 365-07-1; 6-methyluracil, 626-48-2; orotic acid, 65-86-1; isoorotic acid, 23945-44-0; cytosine, 71-30-7; cytidine, 65-46-3; cytidine-5′-phosphate, 63-37-6.

(54) However, on irradiation of cytosine in the solid state at 300 K, H-addition to C5 and C6 has been observed (Flossmann, W.; Westhof, E.; Müller, A. *Int. J. Radiat. Biol.* **1976**, *30*, 301).

(55) Greenstock, C. L.; Dunlop, I. *Radiat. Res.* **1973**, *56*, 428.

(56) In DNA, the C5–C6 double bond of T is exposed to the outside of the helix. In this region, water molecules (~6 per base pair, located mainly in the minor groove) are available to protonate C6. This reaction requires the presence of negative charge at C6. It is likely that protonation of O^4, which leads to a decrease of charge density also at C6, would decrease the rate of C6 protonation considerably, see ref 20), and thereby protect T. However, due to the low acidity of adenine, $T^{\bullet-}$ remains an anion and can thus be irreversibly protonated by water at C6.

Nickel(II) Ion–Support Interactions as a Function of Preparation Method of Silica-Supported Nickel Materials

Olivier Clause,*,† Maggy Kermarec,‡ Laurent Bonneviot,‡,§ Françoise Villain,‡,∥ and Michel Che‡

Contribution from the Laboratoire de Réactivité de Surface et Structure, URA 1106 CNRS, Université Pierre et Marie Curie, 4 place Jussieu, 75252 Paris Cedex 05, France, and Institut Français du Pétrole, BP 311, 92506 Rueil-Malmaison Cedex, France. *Received May 8, 1991*

Abstract: The adsorption on silica of various amminenickel(II) complexes has been investigated as a function of the preparation procedure, i.e., the composition of the impregnating solution and the washing and drying steps. Quite different adsorption modes can be distinguished by EXAFS, XANES, and infrared spectroscopies depending on the pH of the impregnating solution: $[Ni(NH_3)_6]^{2+}$ weakly adsorbs (electrostatic adsorption) while $[Ni(H_2O)_{6-n}(NH_3)_n]^{2+}$ ($n < 6$) complexes strongly interact with the carrier, giving rise to the formation of layered nickel silicate structures. A classification of the bonding of cations on supports, the latter acting as dispersing agents, macroanions, or chemical reagents, is proposed. The EXAFS technique is shown to be a powerful tool for the determination of the ion–support interaction during the first steps of the preparation of silica-supported nickel materials.

Studies focusing on ion adsorption on clays, inorganic oxides, and colloids have received much attention in a variety of domains, such as catalysis,[1-4] clay chemistry,[5] electrochemistry at the oxide/electrolyte interface,[6-11] and materials science.[12-14]

The present paper concerns silica-supported nickel materials prepared by deposition from aqueous solutions. Nickel supported

* To whom correspondence should be addressed.
† Institut Français du Pétrole.
‡ Université Pierre et Marie Curie.
§ Present address: Département de Chimie, Université Laval, G1V1E6, Québec, Canada.
∥ Present address: LURE, Université Paris-Sud, 91405 Orsay, France.

(1) See, for instance: Hegedus, L. L.; Avis, R.; Bell, A. T.; Boudart, M.; Chen, N. Y.; Gates, B. T.; Haag, W. O.; Somorjai, G. A.; Wei, J. *Catalyst Design, Progress and Perspectives*; Wiley: New York, 1987.

(2) Che, M.; Bennett, C. O. *Adv. Catal.* **1989**, *36*, 55–172.

(3) Che, M.; Bonneviot, L. *Successful Design of Catalysts*; Elsevier: Amsterdam, 1988; pp 147–158.

(4) Marcilly, C.; Franck, J. P. *Rev. Inst. Fr. Pet.* **1984**, *3*, 337–364.

(5) See, for instance: Barrer, R. M. *Zeolites and Clay Minerals as Sorbents and Molecular Sieves*; Academic Press: London, 1978.

0002-7863/92/1514-4709$03.00/0