Redox Potentials of Free Radicals. II. Pyrimidine Bases

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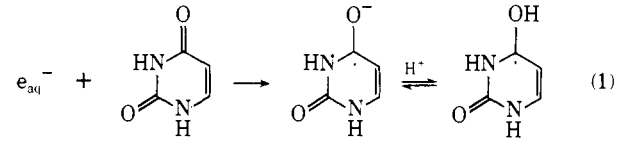
Abstract: The technique of pulse radiolysis and kinetic absorption spectrophotometry was used to determine the redox potentials (E° [1]) of various pyrimidine radicals (·RH) in aqueous solutions. The method used to derive the potentials of these free radicals is based on the electron transfer properties of these radicals to a range of acceptor molecules A, whose redox potentials are known, $\cdot RH + A \rightleftharpoons R + \cdot A^- + H^+$. The intermediates produced from the reaction of OH radicals with thymine, uracil, 1-methyluracil, 1,3-dimethyluracil, cytosine, dihydrothymine, and dihydrouracil were studied. These give characteristic E° [1] values of $\sim+0.13$ V at pH 7.0. In alkaline solutions, beyond the pK_a[1] of these pyrimidines, the "enol form," of these intermediates have significantly lower (more negative) potentials, making these radicals stronger reducing agents. The intermediates produced from dihydropyrimidines at pH 7.0, however, have E° [1] = ~-0.20 V. The electron adducts to these pyrimidines produce ketyl-type radicals, and these have been found to have very low oxidation potentials, E° [1] = -1.3 V, making these radicals powerful reducing agents. The implications of these results are discussed. The reaction rate constants of some of these electron transfer processes have been measured and are found to be $\leqslant 5 \times 10^{-9}$ M^{-1} sec^{-1} for acceptors whose redox potentials are more positive than those of the donor radicals.

The chemical behavior of the intermediates produced from the reaction of hydroxyl radicals and solvated electrons with the pyrimidine bases of DNA is considered to be of biochemical and radiobiological importance. A more recent interest in these reactions is related to the electron transfer properties of these intermediates with respect to their role in the mechanism of radiation sensitization (see ref 2–5 and references cited therein), as applied to the radiation therapy of tumors.

The primary site(s) for addition of hydrated electrons, e_{aq}^-, to uracil, thymine, and other pyrimidines has been postulated[6] to be the C_2 and C_4 carbonyl groups, *e.g.*



(1)

$pK_a = 7.3$

and the ionization constants of these ketyl radicals are $\sim$7.0 for many pyrimidines.[6] Delocalization of the unpaired electron throughout the ring probably takes place. SCF–MO calculations[7] have supported these assignments.

The reaction of OH radicals with pyrimidines has been interpreted on the basis of addition across the 5,6 carbon–carbon double bond (see ref 5 and references cited therein). The observed[5] changes in the transient optical absorption spectra of these intermediates with pH were shown[5] to be due to the tautomerization of the pyrimidine molecules, and not to the acid–base properties of the OH-radical adducts. However, no decision could be reached on the extent of OH-radical addition at the C_5 and/or C_6 positions.

A new method[8,9] has been developed in this laboratory for the determination of the redox potentials of free radicals in aqueous solutions. It is based on the electron transfer properties of free radicals (·RH) to a range of acceptors (A) whose redox potentials are known.

$$\cdot RH + A \rightleftharpoons \cdot A^- + R + H^+ \qquad (2)$$

This method (see more below) has now been used to determine the redox potentials of the radicals produced from the reaction of OH radicals and e_{aq}^- with various pyrimidines in aqueous solutions.

Experimental Section

The free radicals studied were produced using the technique of pulse radiolysis, and the electron transfer processes occurring in the presence of various acceptors were followed by kinetic absorption spectrophotometry.

The experimental details of the pulse radiolysis setup used have been described.[10,11] The radicals formed from the reaction with OH radicals were produced in N_2O-saturated solutions ($\sim 2.5 \times 10^{-2}$ M) in order to convert $\geq$90% of the e_{aq}^- into OH radicals.

$$e_{aq}^- + N_2O \longrightarrow N_2 + OH + OH^- \qquad (3)$$

The electron adducts to the pyrimidines were produced in the presence of $\sim$1.0 M *tert*-butyl alcohol to scavenge[10] the OH radicals. The radicals produced from the reaction of OH with *t*-BuOH were shown not to interfere with the reactions studied.

The details of the method used to determine the redox potential of free radicals have been fully described.[9] Briefly, this method depends on following the formation and the amount of the $\cdot A^-$ radical, reaction 2, produced by electron transfer from the donor radical. Table I lists the acceptors used, their redox potential values (taken from ref 12), the wavelengths monitored, and the pK_a of the $\cdot A^-$ radicals. The percentage efficiency of the electron transfer processes was determined based on the determination of the 100% efficiency ("blank") for formation of the $\cdot A^-$ or $\cdot A^- H^+$ radicals. This was done with every acceptor, just prior to carrying out the experiment, by monitoring the absorbance of $\cdot A^-$ produced *via* reaction 4, in the presence of 1.0 M *t*-BuOH. These "blanks"

$$e_{aq}^- + A \longrightarrow \cdot A^- \qquad (4)$$

(1) Radiation Chemistry Section, C.S.M.C.R.I., Bhavnagar, India.
(2) E. Hayon and M. Simic, *Radiat. Res.*, **50**, 464 (1972).
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(4) M. Simic and E. Hayon, *Biochem. Biophys. Res. Commun.*, **50**, 364 (1972).
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(9) P. S. Rao and E. Hayon, *J. Amer. Chem. Soc.*, **96**, 1287 (1974).
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(12) (a) "Handbook of Biochemistry," Chemical Rubber Publishing Co., 1970, p J-33; W. M. Clark, "Oxidation Reduction Potentials of Organic Systems," Williams and Wilkins, Baltimore, Md., 1960; (b) L. Meites, "Polarographic Techniques," Interscience, New York, N. Y., 1967; I. M. Kolthoff and J. J. Lingane, "Polarography," Interscience, New York, N. Y., 1958.

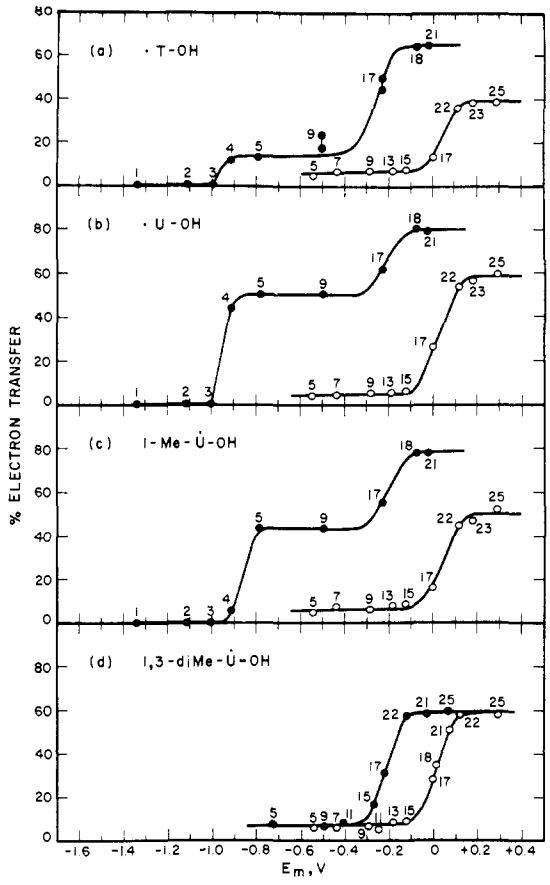


Figure 1. Dependence of the efficiency (expressed in percentage) of electron transfer to various acceptors A from the OH radicals adducts of some pyrimidines in aqueous solutions upon the redox potential of A: (a) thymine, (b) uracil, (c) 1-methyluracil, and (d) 1,3-dimethyluracil. Experiments carried out using ~1 mM concentrations in the presence of N_2O (1 atm). Open symbols at pH 7.0 and dark symbols at pH 10.8. Total dose ~0.15–1.2 krads/pulse. See Table I for a listing of acceptors used.

were obtained under identical experimental conditions of concentration, dose, wavelength, monochromator slits, and pH. Based on the Nernst equation, it was shown[9] for eq 2 that at 50% conversion of the initially produced $\cdot R^-$ radicals, and for $[A]/[\cdot R^-] \sim 15$

$$E_{\cdot R^-} = E_A^\circ + 0.085 \quad \text{(A)}$$

Under equilibrium conditions and at 50% conversion, eq B can be derived when studying electron adducts (*i.e.*, when R in eq 2 is also the starting substrate)

$$-E_{\cdot R^-} = E_A^\circ + \frac{0.059}{n} + \log \frac{2[A] - [\cdot R^-]}{2[R] - [\cdot R^-]} \quad \text{(B)}$$

The redox potential notation used is as follows: $E^\circ{}^1$ is the value at pH 7.0 and ~25°, and E_m is the value at the stated pH of the experiment. The redox potentials of free radicals are presented as *reduction* potentials (see ref 9).

Lowest possible doses were used to minimize radical–radical reactions and enhance the electron transfer process, reaction 2.

The chemicals used were the highest research grade available commercially and were obtained from Cyclochemicals, Calbiochem, J. T. Baker, Eastman Chemicals, Aldrich, K & K Laboratories, and Baker and Adamson. The solutions were prepared just previous to use and were buffered using perchloric acid, KOH, and 1 mM phosphate and tetraborate buffers. Special care was taken[9] to minimize exposure of these solutions to light.

Results and Discussion

The various pyrimidine free radicals examined below have all been previously studied[5,6] by pulse radiolysis of their aqueous solutions. The transient species formed absorb at wavelengths below those used to monitor the formation of the $\cdot A^-$ and $\cdot A^- - H^+$ radicals from the acceptors used. In those few cases where the intermediates do absorb at the wavelengths monitored, their extinction coefficients are ≤ 0.1 that of the acceptor radicals or of the dyes. The electron transfer from $\cdot RH$ to certain acceptors (particularly dyes) was followed by monitoring the disappearance or "bleaching" of the dye at the appropriate wavelength.

In all cases, the concentrations of the acceptors used were 20–50 μM.

OH Radical Adducts. Thymine. On pulse radiolysis of 1 mM aqueous solutions of thymine, 1 atm of N_2O, at pH 7.0 in the presence of 20–50 μM concentrations of acceptors having a $E^\circ{}^1 \leq -0.125$ V, no electron transfer was observed from the OH radical adduct to thymine. Using acceptors with a higher redox potential, electron transfer was observed; see Figure 1a. A "titration" type of dependence upon the $E^\circ{}^1$ values of A can be seen, from which the redox potential of the radical transferring an electron can be determined from the midpoint of the titration curve; $E^\circ{}^1 = +0.13$ V (see Table II).

It can be noted from Figure 1a that only ~40% of the radicals produced from the reaction of OH with thymine were found to transfer at pH 7.0. This is interpreted to mean that (a) at least two or more radicals are produced from the reaction with OH and (b) the other radicals(s) has a higher redox potential. Three main reactions with thymine are likely to occur: OH addition at the C_5 position, OH addition at the C_6 position, and OH abstraction from the C_5–CH_3 group to form the C_5–$CH_2\cdot$ radical. No evidence exists at present for OH abstraction from N_1–H and N_3–H groups to form >N· radicals.

At pH 10.8, the per cent transfer *vs.* $E^\circ{}^1$ curve

Table I. List of Acceptors Used

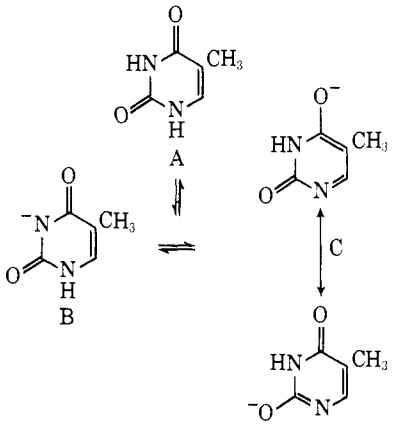
No.	Acceptor	$E^\circ{}^1$, V[a]	λ (nm[b]) monitored	pK_a (radical)
1.	Acetophenone	−1.290	300, 445	9.9
2.	Benzophenone	−1.000	545, 615	9.25
3.	3-Benzoylpyridine	−0.750	530, 605	9.2
4.	Fluorescein	−0.572	500*	
5.	Rhodamine B	−0.542	520*	
6.	Eosin Y	−0.500	520*	
7.	Methyl viologen	−0.446	385	
8.	Crystal violet	−0.357	525*	
9.	Safranine T	−0.289	520*	
10.	Phenosafranine	−0.254	520*	
11.	9,10-Anthraquinone-2-sulfonate	−0.250	400	3.25
12.	Riboflavine	−0.208	560	8.3
13.	9,10-Anthraquinone-2,6-disulfonate	−0.184	385, 400	3.2
14.	2-Hydroxy-1,4-naphthoquinone	−0.139	390	4.7
15.	Indigodisulfonate	−0.125	610*	
16.	Indigotetrasulfonate	−0.046	610*	
17.	Menaquinone	+0.002	370, 400	4.5
18.	Methylene Blue	+0.011	580*	
19.	Thionine (pH 8)	+0.031	600*	
20.	1,5-Naphthoquinone	+0.050	370, 390	4.1
21.	Indophenol (pH 9)	+0.089	610*	
22.	1,4-Naphthoquinone-2-sulfonate	+0.018	380, 400	4.3
23.	2,5-Dimethyl-*p*-benzoquinone	+0.176	440	4.6
24.	2,6-Dichloroindophenol	+0.217	600*	4.0
25.	*p*-Benzoquinone	+0.293	415, 430	3.6

[a] Values at pH 7.0, 25° from ref 12. [b] Asterisks indicate that "disappearance" or "bleaching" was used to monitor the oxidation of the donor radicals.

Table II. Redox Potentials of Free Radicals Derived from Pyrimidine Bases in Aqueous Solution

Substrate	pK_a	Radical form	pH	E_m, V[a]	$E^{\circ 1}$, V[a,b]
Thymine	9.9, >13.0	.T–OH	7.0	+0.04	+0.13
			10.8	−0.28[c]	+0.04
			10.8	~−0.96	~−0.65
Uracil	9.5, 13.0	.U–OH	7.0	+0.03	+0.12
			10.8	−0.21	+0.11
			10.8	−0.95	−0.64
1-Methyluracil	9.8	1-Me-Ü–OH	7.0	+0.04	+0.13
			10.8	−0.20	+0.06
			10.8	−0.86	−0.55
1,3-Dimethyluracil		1,3-diMe-Ü–OH	7.0	+0.02	+0.11
			10.8	−0.21	+0.11
Cytosine	4.6, 12.2	·C–OH	7.0	−0.20	−0.12
			3.2	+0.20	+0.06
Dihydrothymine		·TH	7.0	+0.26	−0.18
Dihydrouracil		·UH	7.0	−0.29	−0.21
Uracil	7.3[d]	·U^-–H^+	5.4	−1.15	−1.32
		·U^-	8.5	−1.45	−1.53
Thymine	7.2[d]	·T^-–H^+	5.4	−1.15	−1.32
		·T^-	8.5	−1.45	−1.53
Cytosine		·C^-	9.2	−1.47	−1.55

[a] Values to ±0.03 V. [b] $E^{\circ 1}$ represents the redox potential values of the freer adicals at pH 7.0, corrected according to eq A and B. [c] Value approximate only. [d] pK_a (radical) values of electron adducts.



changes and two steps can now be seen, Figure 1a. These changes are not due[5] to the ionization of the OH radical adduct to thymine but to tautomeric changes of the molecule itself. At this pH, thymine (pK_a^1 = 9.9) undergoes tautomeric changes which have been suggested[13] to be a 1.1:1 mixture of the mono-anions B and C. The radicals produced on reaction of OH with forms B and C can certainly be expected to be different from those produced from A.

At pH 10.8, ~15% of the OH radicals produce a transient species with a $E^{\circ 1}$ ~ −0.65 V, ~50% a species with $E^{\circ 1}$ ~ +0.04 V, and the remaining 35% of the OH radicals react at a different site(s). It cannot be categorically stated whether form B or form C gives rise to the radicals produced.

Uracil. At pH 7.0, ~60% of the OH radicals react with uracil to form a radical whose electron transfer properties to acceptors can be observed, Figure 1b. This intermediate has a potential $E^{\circ 1}$ = +0.12 V, fairly close to that produced from thymine at pH 7.0 (see Table II).

The results obtained at pH 10.8 are significantly different, Figure 1b. About 50% of the OH radicals produce an intermediate with a $E^{\circ 1}$ = −0.64 V, *i.e.*, a relatively strong reducing agent, ~30% of the OH radicals give rise to a second intermediate with $E^{\circ 1}$ = +0.11 V, and the remaining ~20% of the OH radicals give a third (or more) species having a relatively high redox potential.

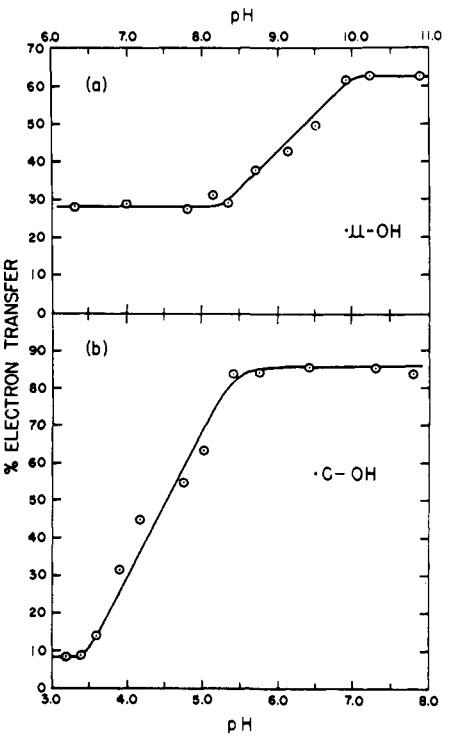


Figure 2. Dependence upon pH of the efficiency (expressed in percentage) of electron transfer from the OH radical adduct to (a) 1 m*M* uracil using 50 μM menaquinone ($E^{\circ 1}$ = +0.002 V) as the acceptor, 1 atm of N_2O, and (b) 1 m*M* cytosine using 20 μM indigodisulfonate ($E^{\circ 1}$ = −0.125 V) as the acceptor, 1 atm of N_2O. Total dose for (a) 1.2 and for (b) 0.15 krads/pulse.

The dependence upon pH of the efficiency of electron transfer to menaquinone is shown in Figure 2a. From this curve, a pK_a ~ 9.2 ± 0.2 is obtained, close to the value pK_a^1 = 9.5 for uracil. This result confirms the earlier[5] observation and conclusion that the changes with pH are due to tautomerization of the molecule.

On studying the intermediates produced from reaction of OH with 1-methyluracil at pH 7.0, 50% formation of

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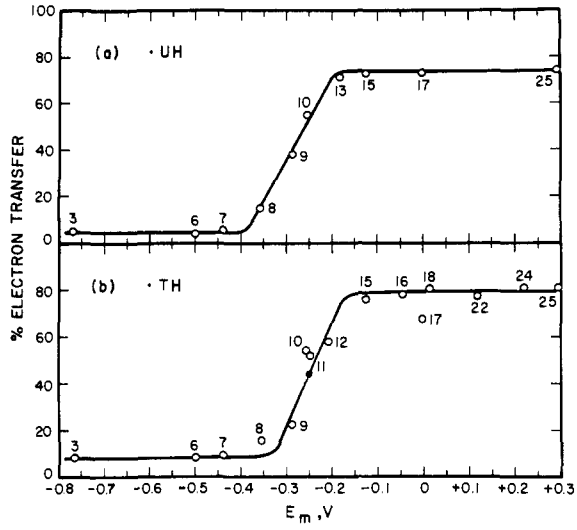

Figure 3. Dependence of the efficiency (expressed in percentage) electron transfer to various acceptors A from the free radicals produced from the reaction of OH radicals with (a) dihydrouracil and (b) dihydrothymine, upon the redox potential of A. Solutions of 2 mM concentration were used in the presence of N_2O (1 atm) at pH 7.0. Total dose ~0.15–1.2 krads/pulse. See Table I for a listing of acceptors used.

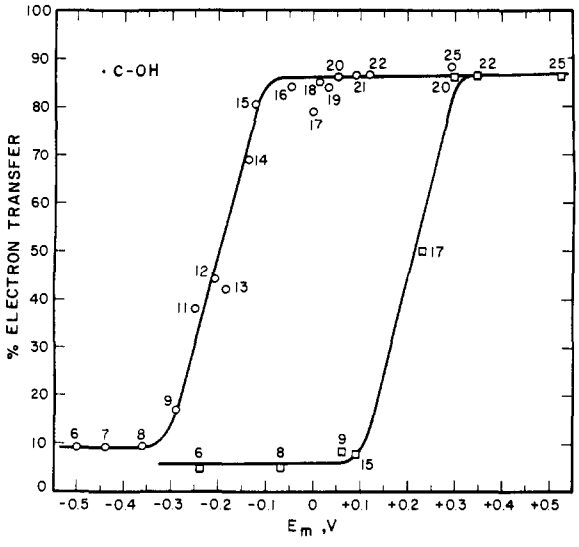

Figure 4. Dependence upon the redox potential of A of the efficiency of electron transfer from the OH radical adducts to cytosine (1 mM, 1 atm of N_2O) to various acceptors A at pH 7.0 (○) and 3.2 (□). Total dose ~0.12–1.20 krads/pulse. See Table I for a listing of acceptors used.

a radical with a $E^{\circ\,1} = +0.13$ V is produced, Figure 1c. For uracil, ~60% of the OH radicals produced a similar intermediate. It is suggested that this decrease may be due to H atom abstraction by OH radicals from the N_1–CH_3 group. It has already been shown that nitrogen in amides[14] and peptides[15] activates a methyl group attached to it. At pH 10.8, the intermediates produced from 1-methyluracil ($pK_a = 9.8$) appear to be similar to those produced from uracil at this pH.

Dimethyluracil cannot undergo keto–enol tautomerism, and no changes with pH in the transient optical

(14) E. Hayon, T. Ibata, N. N. Lichtin, and M. Simic, *J. Amer. Chem. Soc.*, **92**, 3898 (1970).

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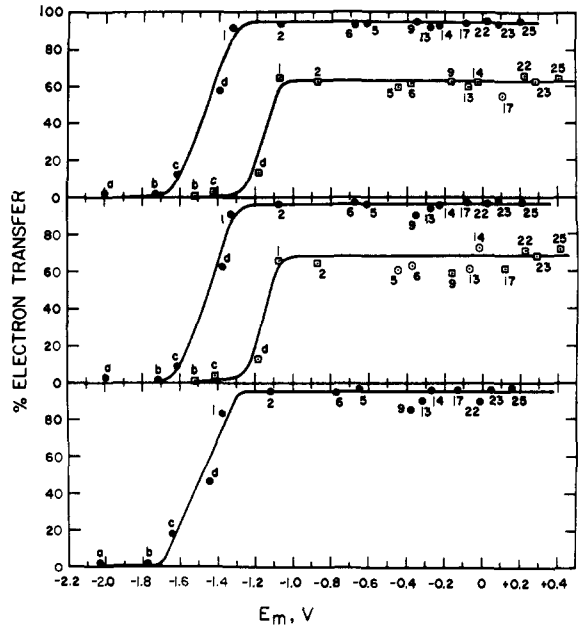

Figure 5. Dependence upon the redox potential of various acceptors A of the efficiency of electron transfer from the e_{aq}^- adducts to some pyrimidines in aqueous solutions: (a) 1.0 mM uracil at pH 5.4 (⊡) and 8.5 (●); (b) 1.0 mM thymine at pH 5.4 (⊡) and 8.5 (●); and (c) 1.0 mM cytosine at pH 9.2. Experiments carried out in 1.0 M *tert*-butyl alcohol and argon (1 atm). Total dose ~0.15–1.20 krads/pulse. See Table I for a listing of acceptors used. In addition, the following acceptors were used: (a) benzonitrile (−1.90 V), (b) crotonic acid (−1.625 V), (c) benzamide (−1.525 V), and (d) 4,4′-dihydroxybenzophenone (−1.28 V).

absorption spectrum of the OH radical adducts were observed[5] by pulse radiolysis. On irradiation at pH 7.0 in presence of various acceptors, only ~60% of the OH radicals give rise to the intermediate with $E^{\circ\,1} = +0.11$ V. At pH 10.8, again only 60% of the OH radicals are accountable for, as expected. The remaining OH radicals probably abstract an H atom from the N-methyl groups. The shift in the curves, Figure 1d, is due to the shift of 0.059 V per pH unit. This intermediate also has a $E^{\circ\,1} = +0.11$ V (see Table II).

Dihydropyrimidines. Hydroxyl radicals primarily abstract an H atom from dihydropyrimidines. The transient optical absorption spectra of the resulting radicals are somewhat similar to those produced by OH radical *addition* to the corresponding pyrimidines (see ref 5 and other references cited therein).

Dihydrouracil (UH_2) and dihydrothymine (TH_2) both give a similar redox potential value for the ·UH and ·TH radicals; see Figure 3 and Table II. The

$$OH + UH_2 \text{ (or } TH_2) \longrightarrow \cdot UH \text{ (or } \cdot TH) + H_2O \quad (5)$$

$E^{\circ\,1}$ value of ~−0.18 to −0.21 V for these radicals are, however, substantially lower than those of the OH radical adducts to the corresponding pyrimidines (Table II). It follows, therefore, that the ·UH and ·TH radicals are stronger reducing agents than the ·U–OH and ·T–OH radicals. These observations were previously reported[2–5] and now can be understood based on the determined potentials of these radicals.

It is interesting to note that OH radicals can abstract an H atom from both the C_5 and C_6 positions in these dihydropyrimidines and that 80% of the OH radicals can be acccounted for (see Figure 3).

Table III. Reaction Rate Constants k of Some Electron Transfer Processes in Aqueous Solution

Donor radical	Acceptor, A	k_2, $\times 10^{-9}$ M^{-1} sec^{-1} [a]	Ref
Thymine, .T–OH	Menaquinone	3.9 (7.0), 5.2 (11.0)	5
		5.2 (10.8)	This work
	Methylene Blue	4.0 (7.0)	This work
	p-Benzoquinone	3.8 (7.0)	This work
Uracil, ·U–OH[b]	Menaquinone	4.2 (7.0), 4.1 (11.0)	5
		4.0 (7.0)	This work
	Methylene Blue	4.7 (10.8)	This work
	p-Benzoquinone	4.0 (7.0)	This work
1,3-Dimethyluracil, 1,3-diMe-U–OH	Menaquinone	2.7 (7.0), 2.3 (10.8)	This work
	Methylene Blue	3.0 (7.0)	This work
	p-Benzoquinone	3.2 (7.0)	This work
Cytosine, ·C–OH	Anthraquinone-2,6-disulfonate	2.2 (7.0)	This work
	Menaquinone	3.0 (2.9), 4.9 (7.0)	5
		2.7 (7.0)	This work
	p-Benzoquinone	2.9 (3.2)	5
		5.0 (6.9)	4
Dihydrothymine, ·TH	Menaquinone	7.0 (7.0)	5
		5.1 (7.0)	This work
	p-Benzoquinone	4.5 (5.5)	4
		5.4 (7.0)	This work
Dihydrouracil, ·UH	Menaquinone	4.8 (7.0)	This work
	p-Benzoquinone	4.5 (7.0)	This work
Electron adducts			
Thymine	Menaquinone	4.0 (7.0)	17
		4.6 (5.4), 4.1 (9.4)	This work
	p-Benzoquinone	4.8 (5.4)	This work
		3.8 (12.0)	17
Uracil	Menaquinone	2.9 (5.4)	This work
		3.6 (7.0)	17
	p-Benzoquinone	2.8 (5.4), 3.0 (8.5)	This work
		3.0 (12.0)	17
1,3-Dimethyluracil	Menaquinone	4.1 (5.4)	This work
	p-Benzoquinone	2.9 (5.4)	This work
Cytosine	Menaquinone	4.0 (7.0)	17
		4.0 (9.2)	This work
	p-Benzoquinone	4.2 (9.2)	This work
		3.4 (12.0)	17

[a] Values in parentheses are the pH at which rates were determined. [b] The donor radical produced from OH reaction with 1-methyluracil gave identical rates with these acceptors.

Cytosine. Cytosine has $pK_a^1 \sim 4.6$ and $pK_a^2 \sim 12.2$. The site of proton dissociation of pK_a^1 has been shown[16] to be the N_3 position. The intermediates produced on reaction of OH radicals with cytosine at pH 3.1 and 6.5 are different and have been shown[5] to be dependent on the pK_a^1 of cytosine; *i.e.*, the radical does not exhibit a pK_a.

The redox potentials of these two intermediates were determined at pH 3.2 and 7.0; see Figure 4. These radicals have lower redox potentials than those produced from uracil and thymine (Table II), making them stronger electron donors.

The pH dependence on the efficiency of electron transfer to indigodisulfonate ($E^{\circ\,1} = -0.125$ V) from the radical produced by reaction of OH with cytosine is shown in Figure 2b. The characteristic titration curve is obtained, from which a $pK = 4.5 \pm 0.2$ can be derived. This value is closely similar to the pK_a^1 of cytosine and confirms the earlier[5] conclusion that this radical does not have a dissociation constant in this pH region.

Electron Adducts. The primary sites of addition of e_{aq}^- to pyrimidines are at the carbonyl groups,[6] *e.g.*, reaction 1. These ketyl radicals have ionization constants of $\sim 7.0 \pm 0.3$.

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The redox potentials of the ketyl radicals of uracil and thymine were determined at pH 5.4 and 8.5. At the higher pH, the ground state uracil and thymine molecules are still in the keto form (pK_a^1 are 9.5 and 9.9, respectively). At pH 5.4, ~60–70% of the ketyl radicals transfer an electron to a wide range of acceptors (see Figure 5), and these radicals have a $E^{\circ\,1} \sim -1.3$ V. The fate of the remaining 30–40% of the electron adducts is not known. Two possible explanations can be offered: (a) the electron adduct at the second carbonyl group has a much higher oxidation potential or (b) a strong delocalization of the unpaired electron occurs, with a long residence time at the 5,6 carbon–carbon double bond position.

At pH 8.5, the uracil and thymine radical anions transfer ~100% to acceptors having redox potentials more positive than −1.2 V. These results indicate that the $E^{\circ\,1}$ of these ketyl-radical anions are very low indeed; see Table II. The fact that they transfer 100% would seem to indicate that the explanation (a) above is probably not the reason why only ~60–70% of the radicals formed at pH 5.4 transfer an electron to acceptors.

It is important to point out that the electron adducts to 1,3-dimethyluracil (results not presented) also transfer ~70% at pH 5.4 and ~100% at pH 8.5. The redox potentials of these radicals are also very low. Since this molecule cannot undergo keto–enol tautom-

erism, one can conclude that the N–H groups in these pyrimidines are *not* involved in the electron transfer reactions of these electron adducts. Hence, electron transfer probably occurs from the Ċ(OH) and Ċ(O^-) ketyl groups of these pyrimidines.

The electron adduct to cytosine (pK_a^1 = 4.6 and 12.2) at pH 9.2 is found to transfer 100% to acceptors and to have a very low redox potential; see Figure 5c and Table II.

Rates of Electron Transfer Reactions. In the process of determining the efficiency of electron transfer from the various radicals from pyrimidine bases to a number of acceptors, the formation kinetics of $\cdot A^-$ (or $\cdot A^-$–H^+) radicals, reaction 2, can be monitored. These k_2 rates are pseudo-first-order dependent on the concentration of A. Table III presents a selection of the rates[4,5,17] of electron transfer to a few acceptors A. These rates are the experimentally observed values and are not corrected for the back reaction. In most cases the correction is quite small.

It can be noted that (a) the intermediates produced from the reaction with OH radicals have k_2 values of $\sim$2–5 $\times$ 10^9 M^{-1} sec^{-1}, for acceptors having relatively high redox potentials, *i.e.*, $E_A^{\circ\,1} > E_{RH}^{\circ\,1}$. These rates are close to being diffusion controlled. These rates are also slightly higher for the basic forms of the donor radicals compared to the acid forms, and (b) the electron adducts to pyrimidines also have k_2 values of $\sim$2–5 $\times$ 10^9 M^{-1} sec^{-1}. It is somewhat surprising that these electron transfer rates are not higher than those from the OH adducts to pyrimidines.

Conclusions

It is clear that a knowledge of the redox potentials of the free radicals produced from pyrimidines in aqueous solution can be of great value in understanding and interpreting the various observations previously reported in the literature. The $\cdot$TH and $\cdot$UH radicals (produced by H atom abstraction from dihydropyrimidines) are stronger reducing agents than the $\cdot$T–OH and $\cdot$U–OH radicals (produced by OH addition to the pyrimidines). The very low redox potentials of the electron adducts to pyrimidines make these intermediates powerful reducing agents. It suggests and supports postulated mechanisms of electronic conductivity through DNA and biopolymers.

Thermochemical Isotope Effects. Chloroform/Chloroform-*d* and Acetone/Acetone-d_6 in Selected Solvents

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Abstract: Isotope effects on the heats of solution of the isotopic pairs chloroform/chloroform-*d* and acetone/acetone-d_6 have been measured in a variety of solvents at 25°. While both positive and negative isotope effects have been observed for the heats of solution of these solutes, the transfer of the deuterated compound from cyclohexane (at infinite dilution) to other solvents is in every case more exothermic than the transfer of the parent compound.

Thermochemical isotope effects have been studied in a number of ways. The most common type of investigation has dealt with the properties of a pure compound, such as melting point, boiling point, vapor pressure, heat of vaporization, etc. There is a growing body of data on the change in the thermochemical properties of a solute (at high dilution) caused by isotopic substitution on the solvent. Virtually all of this work has involved H_2O and D_2O as solvents. In a few cases, the effect of substitution of deuterium for hydrogen on one or both components of a binary system has been studied with respect to the integral mixing properties. Benjamin and Benson[2] compared the heats of mixing of the water–methanol and heavy water–methanol-*d* systems. Rabinovich and Nikolaev[3] measured the vapor pressures of the acetone–chloroform and acetone–chloroform-*d* systems to obtain the isotope effect on the excess free energy of mixing, and Morcom and Travers[4] studied heats of mixing in these systems to obtain the isotope effect on the heat of mixing. These investigations have led to the general conclusion that substitution of a deuterium atom for hydrogen increases the strength and/or the degree of hydrogen bonding.

Several workers have investigated the effects of isotopic substitution on equilibrium constants for formation of hydrogen bonds. Singh and Rao[5] observed very large isotope effects for hydrogen bonds of phenol and phenol-*d* with various bases in carbon tetrachloride, reporting both positive and negative isotope effects. Pluorde[6] investigated a similar series of bases and in every case found the ratio K_D/K_H to be greater than unity. Creswell and Allred[7] studied the hydrogen bonds of fluoroform and fluoroform-*d* with tetrahydrofuran in cyclohexane and found K_D/K_H = 1.04 at 25°, with the complex formation 130 cal/mol more exothermic for the deuterated compound.

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