Site of H Atom Attack on Uracil and Its Derivatives in Aqueous Solution

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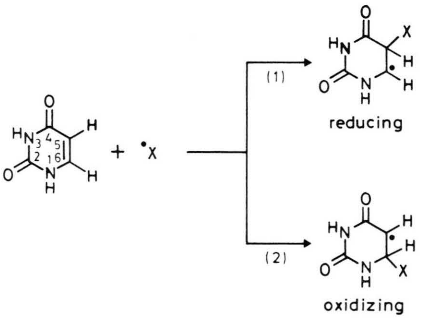
Z. Naturforsch. **40 c,** 292 – 294 (1985);
received November 19, 1984

Dedicated to Professor Dr. D. Schulte-Frohlinde
on the Occasion of His 60th Birthday

Pyrimidines, H Atom, Pulse Radiolysis, Electron Transfer

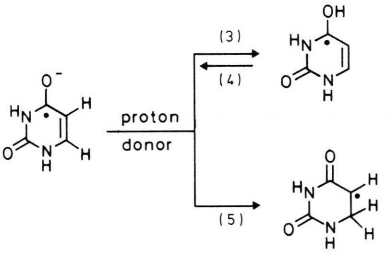
Hydrogen atoms from the radiolysis of water at pH 1.6 add to the 5,6-double bond of pyrimidines. The preferential site of attack is the C(5) position (values in brackets) in the case of 6-methyluracil (87%), 1,3-dimethyluracil (71%), uracil (69%) and poly(U) (60%). This reaction yields a radical of reducing properties which can be monitored by its reaction with tetranitromethane in a pulse radiolysis experiment. In thymine (37%), thymidine (32%) and 1,3-dimethylthymine (25%) H-addition no longer preferentially occurs at C(5), but addition is now mainly at C(6). Hydrogen abstraction from the methyl groups or the sugar moiety is negligible (≦ 5.5%). A comparison is made with literature values for the equivalent reactions of OH radicals.

The main radiation-induced damage to the living cell is considered to be due to lesions formed in its DNA. Most of the reactions that lead to such lesions are free radical reactions. Hence considerable effort has been put into unravelling the underlying reactions by studying low-molecular-weight model systems [1]. In the course of such studies a technique was developed by Fujita and Steenken [2] which enabled the position of OH attack on the C(5)−C(6) double bond of pyrimidines to be monitored by pulse radiolysis. As depicted for uracil a radical X˙ (e.g. ˙OH) may add at C(5), a reaction which results in a radical with the free spin at C(6) (reaction (1)).



Such radicals have reducing properties and are characterized by their rapid reaction with tetranitromethane (TNM). This reaction leads to the formation of the nitroform anion (NF⁻) which has a strong absorption at 350 nm ($\varepsilon = 13\,250\ \mathrm{dm^3\,mol^{-1}\,cm^{-1}}$). On the other hand, if a radical adds at C(6) (reaction (2)) a radical is created which has oxidizing properties and readily oxidizes N,N,N′,N′-tetramethylphenylenediamine (TMPD). In the studies carried out so far with this dual monitoring system reasonable material balances have been obtained [2 – 4].

In the course of our investigation on the protonation reactions of the electron adducts of uracil and its derivatives, it has been observed that the ultimate fate of the electron adducts is a protonation at C(6) (reaction (5)), changing the reducing electron adducts (and their oxygen-protonated forms, *cf.* reaction (3)) into an oxidizing radical [5].



Since this protonated electron adduct may be regarded as an H atom adduct, the question arose as to which pyrimidinic sites H atoms add. This is in itself a question of some importance, since it has been shown that in the DNA model poly(U), H atoms induce strand breaks [6] and base release [7] with high efficiency, although H atoms do not (≦ 5%) abstract hydrogen from the sugar moiety but (≧ 95%) add to the C(5)−C(6) double bond of the bases [7].

To study the reactions of H atoms in aqueous solution with the pyrimidines (2×10^{-3} M), solvated electrons (e^-_{aq}) from the radiolysis of water (reaction (6)) have been converted into H atoms in acid solutions (pH = 1.6, reaction (7)) while OH radicals were scavenged by *tert*-butanol (0.5 M, reaction (8)).

$$H_2O \xrightarrow[\text{radiation}]{\text{ionizing}} {}^{\cdot}OH,\ e^-_{aq},\ H^{\cdot},\ H^+,\ H_2O_2,\ H_2 \qquad (6)$$

$$e^-_{aq} + H^+ \rightarrow H^{\cdot} \qquad (7)$$

$$^{\cdot}OH + (CH_3)_3COH \rightarrow H_2O + {}^{\cdot}CH_2C(CH_3)_2OH. \quad (8)$$

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Under acid conditions TMPD cannot be used to monitor oxidizing radicals, consequently only the yield of reducing radicals has been measured with TNM. The two major radicals expected (*cf.* Ref. [2]) are the C(5) and the C(6) H-adducts. The C(5) H atom adduct is known to be a reducing radical [4], whilest the C(6) H-adduct radical has no strongly reducing properties. It could be envisaged that H atoms might also add to O-(4) yielding the same radical as formed in reaction (3). This possibility has, however, been excluded on the grounds of both spectral and redox properties [5, 8]. The presence of *t*-butanol radicals does not effect the TNM monitoring system, since these radicals neither react with TNM nor with pyrimidines [7]. Using the yield of H atoms determined under similar conditions [7] and correcting for the small fraction ($G = 0.17$) of electrons reacting with the pyrimidine ($k\,(e^-_{aq} + H^+) = 2.5 \times 10^{10}\ \text{M}^{-1}\,\text{s}^{-1}$, $k\,(e^-_{aq} + \text{pyrimidine}) = 1.5 \times 10^{10}\ \text{M}^{-1}\,\text{s}^{-1}$ [9]) as well as for the fraction of H atoms scavenged by *t*-butanol ($k\,(H^\cdot + \text{pyrimidine}) = 2 \times 10^8\ \text{M}^{-1}\,\text{s}^{-1}$, $k\,(H^\cdot + t\text{-BuOH}) = 1 \times 10^5\ \text{M}^{-1}\,\text{s}^{-1}$, both measured in competition with $DCOO^-$ [10, 11]) a value of 3.3 (molecules/100 eV) is obtained for the *G* value of H + pyrimidine. In the presence of TNM ($0.3 - 1.0 \times 10^{-4}$ M) the C(5) H-adduct radicals are oxidized ($k \geqq 3 \times 10^9\ \text{M}^{-1}\,\text{s}^{-1}$) in times ($t_{1/2} < 8\ \mu\text{s}$) short compared to the bimolecular decay of the radicals ($2k \approx 2 \times 10^9\ \text{M}^{-1}\,\text{s}^{-1}$, therefore after a typical dose of 3 Gy the radical concentration would approximately equal 2×10^{-6} M and $t_{1/2} > 200\ \mu\text{s}$). Hence $G(NF^-)$ at about 15 µs (Table I) is a good reflection of the yield of the C(5) H-adduct radicals. Their yields, given as a percentage of the total number of H atoms reacting with the pyrimidines, are compiled in Table II. At longer times with an approximate halflife of 0.5 ms there is a further small increase in the absorbance at 350 nm. Since the calculated $G(NF^-)$ for this process (< 10% of the fast component) shows a marked dependence on TNM concentration and on dose rate, increasing with increasing TNM concentration and decreasing dose rate, it would seem not to be associated with the C(5) H-adduct oxidation nor with a shortlived TNM-radical-adduct (*cf.* [12]). Therefore, this slow formation of NF^- has not been taken into account in the evaluation of the data.

Table I. Radiation-chemical yields (G values) of nitroform anion (NF^-) and hydrogen for some pyrimidines (2 mM) irradiated in Ar-saturated aqueous solution at pH 1.6 in the presence of 0.5 M *t*-butanol. Tetranitromethane ($0.3 - 1.0 \times 10^{-4}$ M) was present in the NF^- determinations.

Substrate	$G(NF^-)$	$G(H_2)$
6-Methyluracil	2.9	0.46
1,3-Dimethyluracil	2.3_5	0.46
Uracil	2.3	0.46; 0.5 [7]
Poly(U)	2.1	0.5 [7]
Thymine	1.2	0.59
Thymidine	1.1	0.67
1,3-Dimethylthymine	0.8	0.52

Table II. Percentages of radicals formed by H and OH attack on pyrimidines.

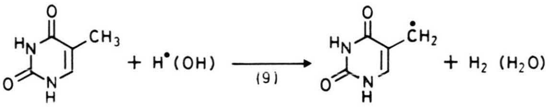
Substrate	C(5)-adduct		C(6)-adduct		H-abstraction	
	$H^\cdot$	$^\cdot OH$ [b]	$H^\cdot$	$^\cdot OH$	$H^\cdot$	$^\cdot OH$
6-Methyluracil	87	87 [2]	13	7 [2]	nil	nil
1,3-Dimethyluracil	71	74 [13]	29	19 [13]	nil	nil
Uracil	69	83 [2]	31	14 [2]	nil	nil
Poly(U)	60		40		nil [c]	
Thymine	37	61 [2]	59.5	28 [2]	3.5	9 [2]
Thymidine	32 [a]	–	62.5 [a]	–	5.5	–
1,3-Dimethylthymine	25	59	73	–	2	–

[a] Assuming 3.5% H-abstraction at methyl and 2% at the sugar moiety the latter radicals reacting with tetranitromethane.

[b] Calculated from original data for the total yields of reducing radicals formed in N_2O saturated solutions, and allowing for the small contribution by reducing H-atom adducts.

[c] Within experimental error (*cf.* Ref. [7]).

A comparison with the corresponding reactivity of OH radicals (Table II) reveals that the selectivity of these two radicals in their site of attack is similar, although addition at C(6) is somewhat more favoured in the case of the H atom. Concomitantly a methyl substituent at C(5) which diverts addition towards C(6) does so more pronouncedly for H atom addition.



The yield of the H atoms which react with the methyl group has been measured by determining the H_2 yield (*cf.* reaction (9)). For example, in acid solutions (pH 1.5) containing thymine (5×10^{-3} M) $G(H_2)$ has been found to be 0.59. From this value the socalled molecular yield (*cf.* reaction (6)) of $G(H_2) = 0.46$ (*cf.* uracil) has to be substracted yielding $G(H_2) = 0.13$ for H atoms that have reacted with the methyl group. The error in determining these low H_2 yields is ± 0.04. Obviously this leads to a considerable uncertainty in the values for H abstraction. Nevertheless the data clearly show that less than 4% of the H atoms abstract from the methyl group. This value is somewhat smaller than the corresponding value for OH abstraction (Table II).

It has been mentioned above, that in poly(U), base radicals produce strand breaks (for kinetic studies see Ref. [14, 15]) and base release. The efficiencies of these processes are higher for the OH radical than for the H atom and negligible for the solvated electron [6, 7]. It has now been shown that the fraction of C(5) radical-adducts is larger for OH than H attack and that no C(5) H-adducts are formed when the electron adduct is converted to a carbon-centred H-adduct radical [5, 8]. Thus one might speculate that base-radical-mediated strand breakage and base release are processes involving the C(5) radical-adducts. Scavenging experiments with TNM lead to the same conclusion [7].

Acknowledgment

We would like to express our gratitude to Ms. D. Schulz for skillful technical assistance.

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