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NEW ASPECTS IN THE FREE-RADICAL CHEMISTRY OF PYRIMIDINE NUCLEOBASES

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The contribution will cover three aspects:

i) It has been known for some time that OH radicals and H atoms react with the pyrimidines by adding to the C(5)–C(6) double bond, but only the u.v.-spectra of the sum of these radicals have been reported so far. It will be shown how to arrive at the individual spectra of the C(5) and the C(6) adduct radicals.

ii) α-Hydroxyalkyl radicals are known to inactivate biologically active DNA. In contrast to the electrophilic radicals H and OH they are nucleophilic and the hydroxymethyl radicals add exclusively at the C(6) position of 1,3-dimethyluracil ($k \approx 10^4\,dm^3\,mol^{-1}\,s^{-1}$). In the corresponding thymine derivative this reaction also occurs, but one third of the hydroxymethyl radicals abstract an H-atom from the C(5)-methyl group thereby forming an allylic radical. In the course of these reactions pyrimidines with an exocyclic double bond are formed. These products react much more rapidly with hydroxymethyl radicals than the starting material leading to highly hydroxymethylated material at very low doses.

iii) The direct effect of ionizing radiation which would produce a pyrimidine base radical cation can be mimicked by reacting the pyrimidine with SO_4^-, a very good electron acceptor. In water, the radical cation of 1,3-dimethyluracil is rapidly ($t_{\frac{1}{2}} < 2\,\mu s$) converted into the C(5) OH adduct radical. In the presence of peroxodisulphate a chain reaction sets in which leads to the *cis*-glycol.

The relevance of these findings to radiobiological aspects of nucleic acid research will be discussed.

KEY WORDS: Pyrimidines, pulse radiolysis, hydroxymethyl radical, sulphate radical.

INTRODUCTION

From radiation-biological studies there is increasing evidence that non-repaired DNA double-strand breaks have to be considered as a lethal lesion in cells, at least with respect to reproduction. Hence there is a great interest in unravelling the mechanisms behind DNA strand breakage (for a review see[1]). DNA strand breakage must result from sugar radicals as precursors, and for a while it seemed as if the reactions of nucleobase radicals might be of a lesser importance. However, new attention has been paid to the reactions of the nucleobase radicals when it became apparent that, in the nucleic acid poly(U), base radicals induce strand breakage[2] and release of unaltered bases.[3–5] It is now quite clear, that, in this system, base radicals are capable of attacking the sugar moiety forming radicals which then give rise to the observed damage: strand breakage and base release. It is the radiation chemistry of the pyrimidines (for an updating review see[6]) that we are concerned with, because this base radical-induced strand break formation is more apparent in poly(U) than in the corresponding systems containing purines (Bothe and Schulte-Frohlinde, unpublished results).

In the present paper some recent studies on the free-radical chemistry of pyrimidines will be reported. As initiators OH radicals, solvated electrons, and H radicals

have been used, but the studies have also been extended to $^{\cdot}CH_2OH$ and $SO_4^{\cdot-}$ radicals. The main substrates were 1,3-dimethyluracil and 1,3-dimethylthymine which have certain advantages over the free bases or the nucleosides. The methyl group at N(1) blocks this position and deprotonation reactions at N(1) which often occur in the free-radical chemistry of pyrimidines can no longer take place. Thus the methyl group mimicks the situation in nucleic acids. The methyl group at N(3) does not influence the free-radical chemistry but is most useful for analytical reasons, allowing quantitative product studies by GC after trimethylsilylation of those products containing hydroxyl groups.

UV-SPECTRA OF PYRIMIDINE RADICALS

When radicals, e.g. OH or H, add to the C(5)–C(6) double bond two types of radicals are formed. As has been shown by Fujita and Steenken,[7] the C(5) OH adduct radicals with the free spin at C(6) have reducing properties (readily reduce tetranitromethane) while the C(6) OH adduct radicals with the free spin at C(5) have oxidizing properties (oxidize N,N,N′,N′-tetramethylphenylene diamine). Similar studies have now been extended to the H-atom.[8] Both, OH and H, are electrophilic radicals and in uracil add preferentially to the C(5) position. In thymine and its derivatives there is also considerable attack at the C(6) position and to a smaller extent at the methyl group. While

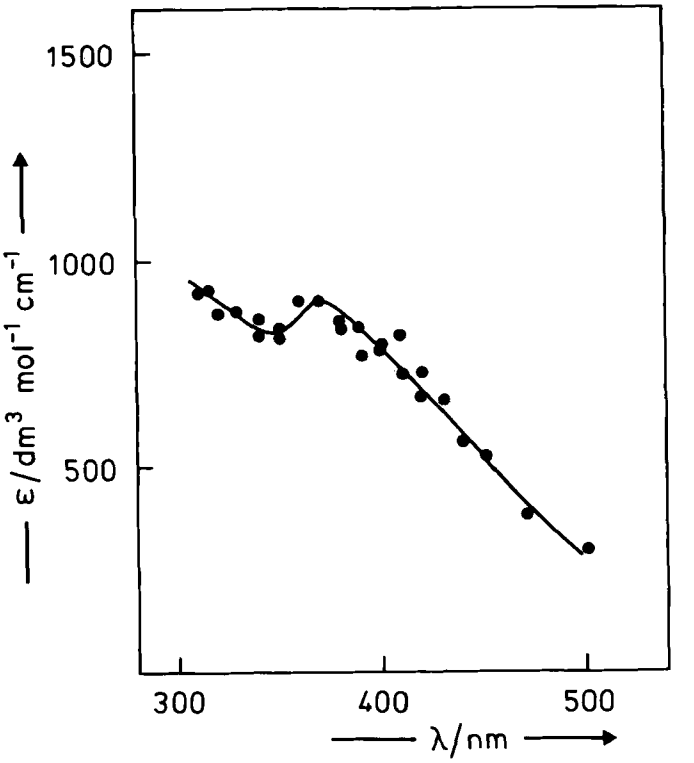
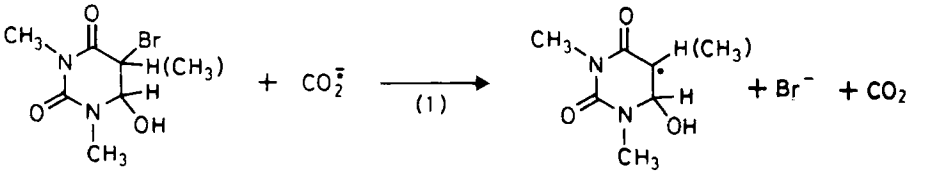


FIGURE 1 Absorption spectrum of the mixture of the OH adduct radicals formed by the reaction of OH radicals with 1,3-dimethylthymine.

the UV absorption spectrum in the uracil derivatives is strongly dominated by the C(5) OH adduct radical this is no longer so in the case of thymine derivatives. Until recently, only the spectrum of the sum of these three radicals was known. The C(6) OH adduct radical can be produced separately and quantitatively by making use of reaction 1.[9]



The knowledge of the contribution of the various radicals to the overall spectrum (Figure 1) allows subtraction of the C(6) OH adduct component (Figure 2). The remaining absorption is now mainly due to the C(5) OH adduct, but especially at longer wavelengths the allylic radical formed by H abstraction from the methyl group is noticeable. This radical is formed in high yields when $O^{\cdot-}$ (the basic form of the OH radical) is reacted with the thymine derivative. The spectrum, obtained by D.J. Deeble in our laboratory, is shown in Figure 3.

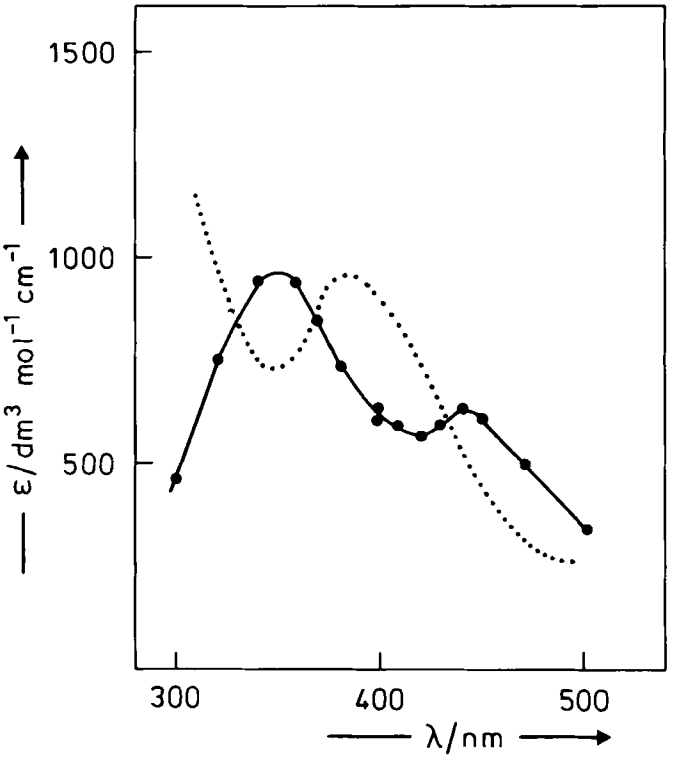


FIGURE 2 Absorption spectrum of the C(6) OH adduct radical of 1,3-dimethylthymine obtained by reacting $CO_2^{\cdot-}$ with 5-bromo-5,6-dihydro-1,3-dimethylthymine. The dotted line is the calculated spectrum of the corresponding C(5) OH adduct radical.

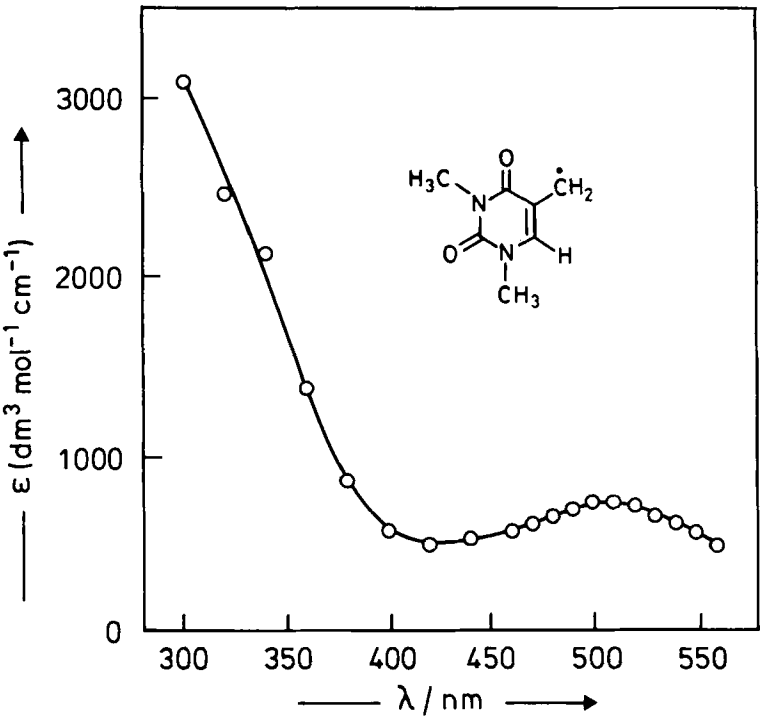


FIGURE 3 Absorption spectrum of the allylic radical of 1,3-dimethylthymine formed by reacting O^- with 1,3-dimethylthymine.

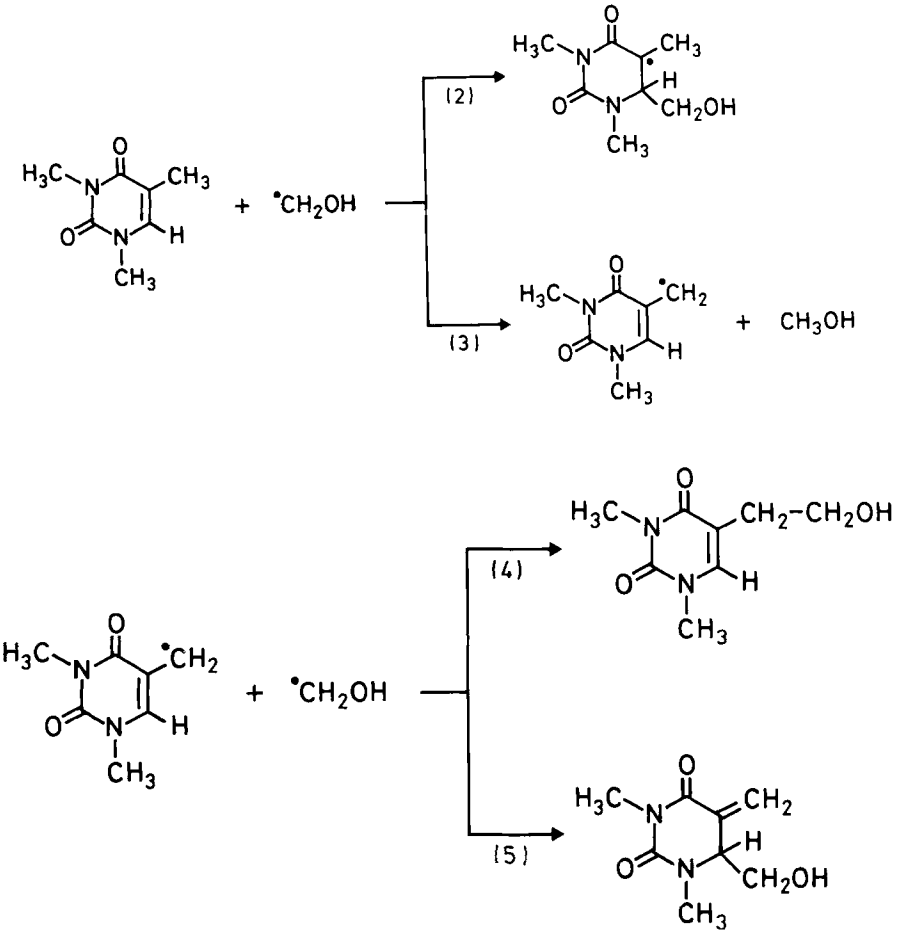
H-abstraction from the methyl group by the H-atom can be neglected.[8] In this case we therefore deal only with the C(5) and the C(6) adduct radicals. It is also possible to arrive at the separate spectra of these two H adduct spectra. The C(6) H adduct radical is formed quantitatively by the buffer-catalysed conversion of the electron adduct[10–12] while (in favourable cases) the C(5) adduct is formed in the reaction of the OH radical with the dihydropyrimidine. The H-adduct spectra do not differ as drastically from one another as the OH adduct spectra (cf.[11]).

THE REACTIONS OF PYRIMIDINES WITH HYDROXYMETHYL RADICALS

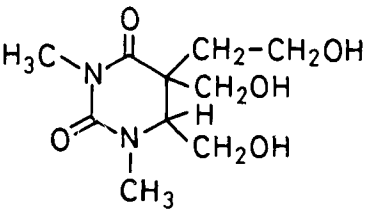
It has been shown by Nabben *et al*[13] that α-hydroxyalkyl radicals are capable of inactivating biologically active DNA. Furthermore, Bothe, Herak and Schulte-Frohlinde (unpublished results) observed that the hydroxymethyl radical induces strand breaks in poly(U). Both processes occur with lower efficiencies compared to that of the OH radical and H-atom. It therefore appeared desirable to have a closer look at the reactions of such radicals with the pyrimidine component.

The radicals H and OH react with pyrimidines with rate constant which are close to the diffusion-controlled limit. The hydroxymethyl radical (generated by the reaction of OH with methanol) reacts much more slowly ($k \approx 10^4\,dm^3\,mol^{-1}\,s^{-1}$).[14] This

radical is nucleophilic and in an investigation of the 1,3-dimethyluracil system it has been shown that it adds exclusively to the C(6) position. Very interesting results have been obtained with 1,3-dimethylthymine: 2/3 of the hydroxymethyl radicals also add to the C(6) position but 1/3 abstracts an H-atom from the methyl group (reactions 2 and 3).[14] In subsequent reactions pyrimidines with an exocyclic double bond are formed (e.g. reaction 5).



Such products cannot be isolated because they are much more efficient radical scavengers than the pyrimidines themselves, and they only reach extremely low steady state concentrations. Hence their products, e.g. triply hydroxymethylated compounds, such as **1**, appear without any noticeable induction period, i.e. they behave as if they were real primary products. Thus the system is very complex and this is reflected in the large number of (seemingly primary) products (for a more detailed description the reader is referred to Ref.[14]).



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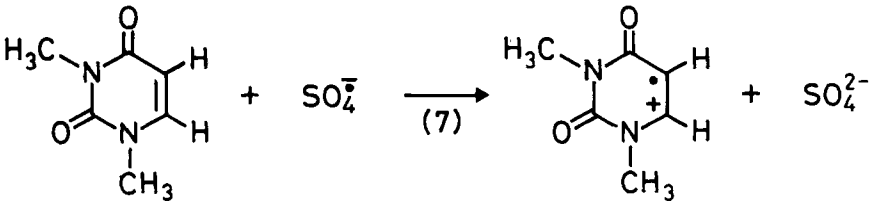
THE PYRIMIDINE RADICAL CATION

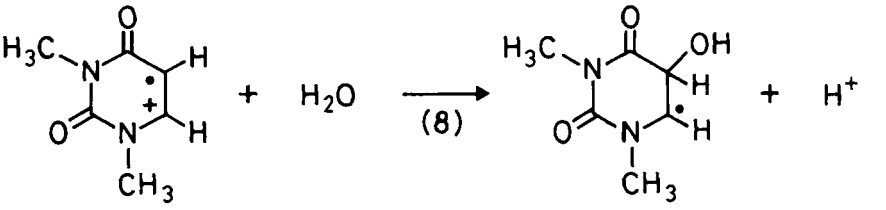
In radiation biology one distinguishes between the indirect effect (the reactions of the water radicals with a target molecule, e.g. DNA) and the direct effect. In the latter case the ionizing radiation is absorbed by the target molecule itself. In the nucleic acids the ionization of the nucleobases yields radical cations. To mimic the direct effect one can make use of biphotonic electron ejection with the help of a laser (cf.[15]) or by generating the radical cation by oxidation of the nucleobase with $SO_4^{\cdot-}$ (reaction 7). The $SO_4^{\cdot-}$ can be produced radiolytically (reaction 6).

$$S_2O_8^{2-} + e_{aq}^- \longrightarrow SO_4^{\cdot-} + SO_4^{2-} \qquad (6)$$



In the case of uracil the radical cation loses the proton at N(1).[16,17] In the nucleic acids this reaction cannot occur because this proton is substituted by the sugar moiety. Quantum mechanical calculations have shown that the electron density at N(1) is drastically reduced upon going from uracil to the uracil cation, but the N(3) position is not affected at all.[18] In accordance with this calculation it has been found in a pulse radiolysis experiment that the 1-methyluracil radical cation does not deprotonate at N(3) in neutral solutions, and further studies could be done equally well with the analytically more amenable 1,3-dimethyl derivative. Using pulse radiolysis with conductivity detection it has been shown that the $SO_4^{\cdot-}$ adduct, if formed, must have decomposed within $\leqslant 2\,\mu s$ thereby yielding a very short-lived radical cation which upon reaction with water produces a reducing radical ($\geqslant 90\%$ as monitored by the tetranitromethane method, confirming unpublished results by Lemaire, Bothe and Schulte-Frohlinde).[18] The UV spectrum also agrees with the above reported one. Hence we conclude that in this reaction water must have reacted at the C(5) position (reaction 8).

This is an interesting reaction in so far as the quantum mechanical calculations clearly show that the positive charge resides predominantly at C(6). The preferential reaction at C(5) appears to be due to a kinetically controlled reaction. In a sulfuric



acid glass the same reaction has been carried out and it is the C(6) OH adduct radical which is observed by ESR.[19] Thus it appears, that the latter is the thermodynamically more stable species (cf. also[20]).

In the radiolysis of 1,3-dimethyluracil in the presence of peroxodisulphate there is only one major product: the *cis*-glycol. It is formed in a rather complex chain reaction, the details of which will be published elsewhere.[18]

In the case of 1,3-dimethylthymine the $SO_4^{\cdot-}$ adduct is sufficiently long lived (several μs) to be monitored by pulse radiolysis (Deeble, Schuchmann, Steenken and von Sonntag, unpublished results). This system has not yet been fully elucidated, but it is quite obvious that there is no chain reaction that would lead to a single predominating product. Some additional preliminary experiments with uridine show that there must be some spin transfer to the sugar moiety or (less likely) primary attack at the sugar moiety.

RELEVANCE TO NUCLEIC ACIDS

It has been shown that the hydroxymethyl radicals react very slowly with the pyrimidines and it is conceivable that some of the low efficiency in inducing loss of biological activity (in DNA) and strand breakage (in poly(U)) is due to the slowness of the reaction. Strand breakage might in part also be caused by the hydroxymethyl radicals through direct H abstraction from the sugar moiety, a reaction for which evidence from model systems has been gained recently.[21] The comparative ease by which the thymine allyl radical is formed by the hydroxymethyl radical poses the question whether or not other organic radicals, e.g. those from nucleo-proteins would also attack the thymine moiety in a similar way, opening up a new route of the indirect effect. The studies with $SO_4^{\cdot-}$ are interesting insofar as they show that nucleobase radical cations (i.e. the "direct effect") can give rise to exactly the same product (the C(5) OH adduct radical) as does the reaction of the OH radical (i.e. the indirect effect).

It is hoped that a continuation of such model studies will help to improve our understanding of the free-radical chemistry of the nucleic acids and eventually lead to a better interpretation of the molecular processes involved in radiation-induced cell killing and mutagenesis.

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