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Februar 1970 KFK 1170

Institut für Strahlenbiologie

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KARLSRUHE

Reprinted from

Biochimica et Biophysica Acta
Elsevier Publishing Company, Amsterdam - Printed in The Netherlands

BBA 96409

AN ELECTRON PARAMAGNETIC RESONANCE STUDY OF SHORT-LIVED FREE RADICALS FROM NUCLEIC ACID CONSTITUENTS AND RELATED COMPOUNDS IN AQUEOUS SOLUTION

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(Received October 3rd, 1969)

SUMMARY

Short-lived radicals produced by the reaction of $OH^{\cdot}$ and $NH_2^{\cdot}$ with a number of nucleosides and other compounds were identified by means of the EPR-flow technique. The particularities and differences in the EPR spectra of the nucleosides and the free bases were interpreted in terms of structural alterations caused by the attachment of the sugar. Investigations concerning the compounds 5-bromouracil and caffeine showed that C-6 and C-8, respectively, are the sites of attack.

INTRODUCTION

A large number of EPR investigations of irradiated dry nucleic acids as well as their constituent molecules have been published demonstrating that free radicals are formed in these compounds upon irradiation[1–4]. The EPR-flow technique makes possible the study of transient free radicals resulting as intermediates in reactions of organic compounds with chemically generated radicals like $OH^{\cdot}$ and $NH_2^{\cdot}$ (ref. 5). Since $OH^{\cdot}$ is one of the main products of water radiolysis such studies yield additional information concerning the action of ionizing radiation on aqueous solutions.

Free radicals produced by the reaction of $OH^{\cdot}$ and $NH_2^{\cdot}$ with pyrimidine bases have been identified in a recent publication[6]. It is the aim of the present investigation to extend these studies on pyrimidine nucleosides as well as on two other compounds of biological interest, caffeine and 5-bromouracil. Special reference is made to the structural peculiarities of the free radicals produced in solution.

MATERIALS AND METHODS

The radicals $OH^{\cdot}$ and $NH_2^{\cdot}$ were generated by reduction of H_2O_2 and NH_2OH, respectively, with $TiCl_3$ at pH 2. The two solutions containing H_2O_2 (NH_2OH) and $TiCl_3$ as well as the organic target molecules were mixed in a four jet mixing chamber used together with a Varian flat cell. The total flow rate was measured to be 5 ml/sec. Details of this technique have been described[5,7]. All reagents were commercially available (Merck and Schuchardt products) and used without further purification. EPR spectra were recorded with an AEG X-band spectrometer in conjunction with a proton resonance Gaussmeter for precise field measurements. The g factors were determined against the DPPH value of 2.0036.

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RESULTS

The pyrimidine nucleosides cytidine, thymidine and uridine were subjected both to $OH^{\cdot}$ and $NH_2^{\cdot}$ attack. While the spectrum of cytidine was practically identical

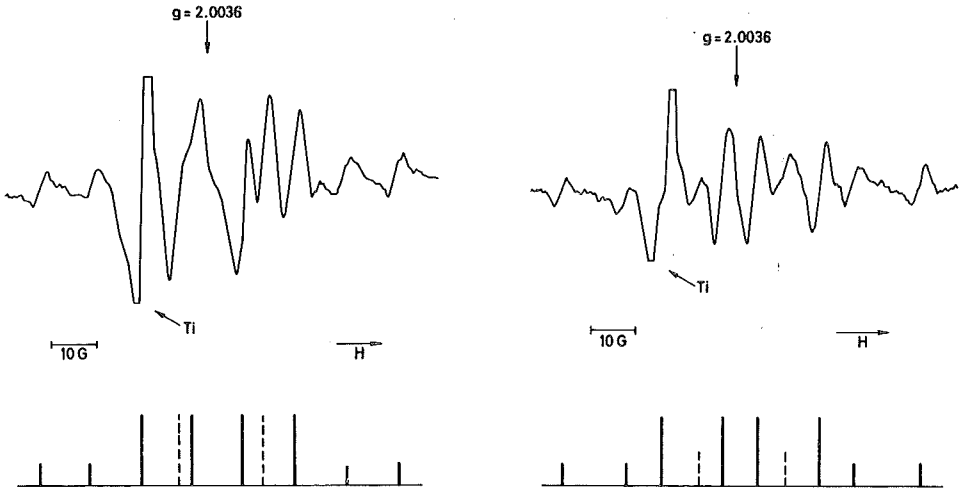


Fig. 1. EPR spectrum of thymidine upon reaction with $OH^{\cdot}$. Solid and dashed lines of the stick diagram belong to the C-6 and C-5 adducts, respectively.

Fig. 2. EPR spectrum of thymine upon reaction with $OH^{\cdot}$. Solid and dashed sticks belong to the C-6 and C-5 adducts, respectively.

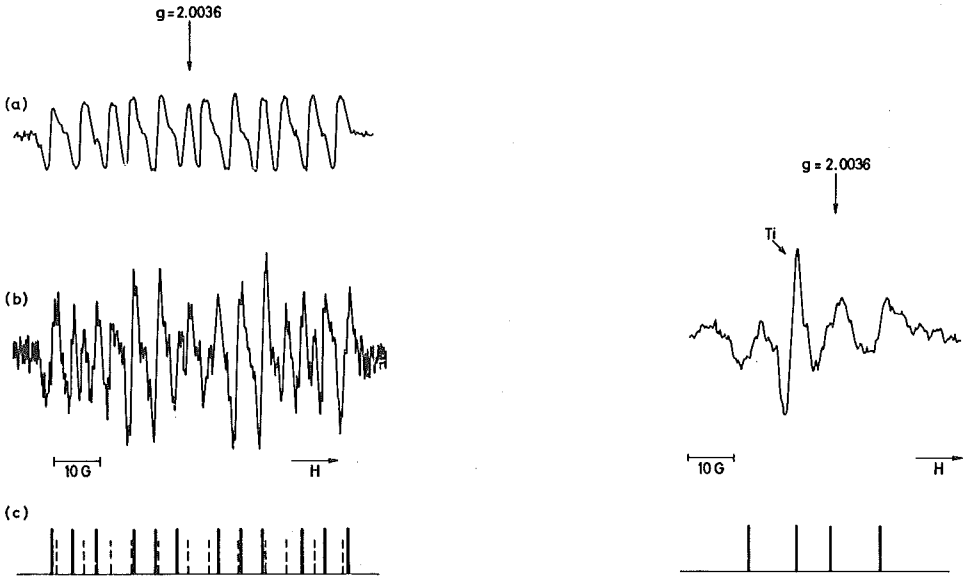


Fig. 3. (a) EPR spectrum of uracil upon reaction with $NH_2^{\cdot}$ (ref. 6). (b) EPR spectrum of uridine upon reaction with $NH_2^{\cdot}$. (c) Analysis of the uridine signal; solid lines: C-6 adduct; dashed lines: C-5 adduct.

Fig. 4. EPR spectrum of 5-bromouracil upon reaction with $OH^{\cdot}$.

with that of cytosine[6], considerable alterations in the spectra of thymidine and uridine as compared with the free base spectra were observed. Fig. 1 shows the spectrum of thymidine after reaction with OH·. For comparison the thymine spectrum is shown in Fig. 2. The signal of uracil after NH_2· attack is reproduced in Fig. 3a. When this signal is compared with Fig. 3b representing the signal of NH_2·-attacked uridine,

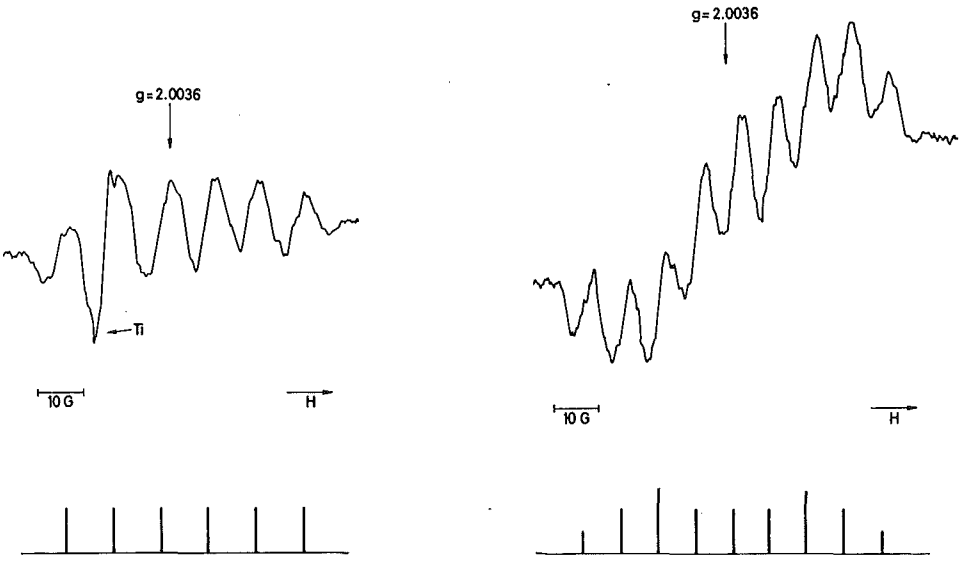


Fig. 5. EPR spectrum of the caffeine–OH· adduct.
Fig. 6. EPR spectrum of the caffeine–NH_2· adduct.

TABLE I

PARAMETERS OF THE EPR SPECTRA OF THE OH· AND NH_2· ADDUCTS

Compound	*g factor*	*OH· radical Hyperfine splitting (Gauss)*	*Site of attack*
Thymidine	2.0029	a(H) = 18.0	C-5, *cf.* Fig. 1
	2.0032	a(Met) = 23.6, a(H) = 10.8	C-6, *cf.* Fig. 1
Cytidine	2.0027	$a(H_I) = a(H_{II}) = 18.3$	C-5*
Uridine	2.0029	$a(H_I) = 21.2$, $a(H_{II}) = 18.1$	C-5*
Bromouracil	2.0066	a(Br) = 10 (7)	C-6. *cf.* Fig. 4
Caffeine	2.0028	a(H) = 28.5, a(N) = 9.5	C-8, *cf.* Fig. 5

Compound	*g factor*	*NH_2· radical Hyperfine splitting (Gauss)*	*Site of attack*
Thymidine	2.0027	a(H) = 16.7, a(N) = 5.05	C-5*
	2.0031	a(Met) = 23.1, a(H) = 16.8, a(N) = 7.2	C-6*
Cytidine	2.0028	$a(H_I) = 11.8$, $a(H_{II}) = 18.6$, a(N) = 11.8	C-5*
Uridine	2.0029	$a(H_I) = 18.0$, $a(H_{II}) = 34.2$, a(N) = 6.4	C-5, *cf.* Fig. 3
	2.0029	$a(H_I) = 18.0$, $a(H_{II}) = 37.0$, a(N) = 4.5	C-6, *cf.* Fig. 3
Caffeine	2.0032	$a(H) = 31.0$, $a(N_I) = a(N_{II}) = 7.7$	C-8, *cf.* Fig. 6

* The same parameters as measured for the free base[6].

striking differences become obvious. Although we failed to detect reproducible signals from the purine bases adenine, guanine and xanthine, well-resolved spectra were obtained from caffeine (1,3,7-trimethylpurine) which are shown in Figs. 4 and 5. The reaction of OH· with 5-bromouracil leads to the production of a weak signal which appears to be a quartet (Fig. 6). The strong low-field signals in Figs. 1, 2, 4 and 6 are attributed to a titanium complex which was recently described[8]. Hyperfine splittings and g factors of the radicals under investigation are summarized in Table I.

DISCUSSION

Nucleosides

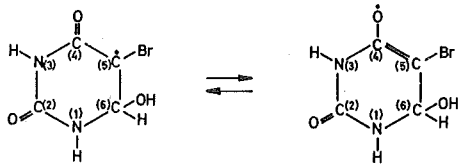
Comparing the characteristics of the EPR signals of the nucleosides with those of the corresponding bases, the principal observation made is that no sugar radicals can be detected although the isolated sugars give large signals when attacked with OH· (ref. 9). The failure to detect sugar signals in pyrimidine nucleosides parallels, however, the situation of γ-irradiated dry nucleosides where, in general, the base signal is by far the largest spectral component as was, for example, demonstrated in the case of thymidine[10]. The analysis of the spectra obtained with thymidine and thymine after OH· attack (Figs. 1 and 2) confirms that the only significant difference between the signals is due to an alteration in the hyperfine splitting constants. As may be seen from Figs. 1 and 2, the splitting of the methyl protons of the C-6 adduct (solid sticks in Figs. 1 and 2) is approximately the same in thymine and thymidine. However, the splitting caused by the β-proton at C-6 is very much smaller in the case of thymidine, namely 10.8 G as compared with 15.1 G for thymine[6]. The hyperfine interaction of the α-proton in the C-5 adduct (dashed sticks in Figs. 1 and 2) was measured to be 18.0 G (Table I) thus being approximately equal to the 18.7 G reported for thymine[6]. The observation that the β-proton splittings of the C-6 adduct are different in thymine and thymidine is also made with γ-irradiated or hydrogen-bombarded dry thymidine and thymine. In that case there are hydrogen additions at C-6 giving rise to the hyperfine interaction with the two protons at this position. While these protons were found to be equivalent in γ-irradiated thymidine[10], different splitting constants are required to explain the signal characteristics of hydrogen-bombarded thymine[11]. To account for this observation it must be assumed that in thymine the two substituents at C-6 form different torsional angles with the π-orbital axis at C-5.

Another interesting phenomenon is observed in the EPR spectrum of NH_2·-attacked uridine. In Fig. 3a the signal of uracil after reaction with NH_2· which has been attributed to the C-5 adduct[6] is reproduced. The analysis of the spectrum of uridine (Fig. 3b) shows that it contains the spectrum of Fig. 3a on which a twelve-line pattern is superimposed, obviously, closely resembling that of uracil. However, different coupling constants are measured for this spectrum. This is indicated by the solid sticks in Fig. 3c, the dashed ones representing the superimposed signal of uracil. One is thus faced with the fact that two spectra belonging to the same radical type are present in uridine after NH_2· attack. To account for the uridine spectrum we suggest that the C-5 adduct (dashed sticks in Fig. 3c) as well as the C-6 adduct (solid sticks) contributes to the total signal, which means that the selectivity of the NH_2·

attack observed with uracil is removed in uridine. A similar observation can be made in the case of OH·-attacked thymidine and thymine (Figs. 1 and 2) where the relative intensities of the C-5 and C-6 adduct spectra are different. The reason for this phenomenon, most probably, lies in changes of the charge distribution and in the modifications of the base geometry caused by the attachment of sugar[12]. Furthermore, the observation that the hyperfine splitting constants may be different in the base and the nucleoside (Figs. 1 and 2) due to altered torsional angles is consistent with such an assumption.

5-Bromouracil

If thymine in deoxyribonucleic acid is replaced by 5-bromouracil, a considerable sensitization of microorganisms to ionizing radiations is observed[13,14]. For this reason an investigation of the reaction of this compound with OH· appeared to be a promising attempt at a better understanding of the sensitizing mechanism. However, as shown in Fig. 4, only a very weak EPR signal is obtained upon reaction with OH·. It consists of 4 lines of nearly equal intensity centered at $g = 2.0066$. This high g factor clearly reflects the neighborhood of the heavy bromine nucleus. The quartet splitting has to be attributed to the interaction of the electron spin with the bromine nucleus of nuclear spin 3/2, rather than to the coupling of two different protons. As may easily be seen, such a spectrum cannot be produced by the hydrogens available in 5-bromouracil without the occurrence of additional nitrogen hyperfine splitting. Thus a C-6 adduct of OH· is proposed to fit the EPR data. However, the g factor is not as high as should be expected if the unpaired electron would be confined to the C-5 position. Therefore a strong delocalization of the spin on the oxygen attached to C-4 is suggested (Radicals 1 and 2) which explains qualitatively the magnitude of the g factor as well as the relatively small bromine interaction as compared with observations in the solid state[15]. Evidence for a similar delocalization has been presented for



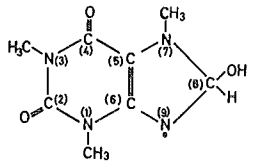
(1) (2)

the C-6 adduct of thymine which in that case, however, causes an increase of the g factor[6]. The reasons for the nonequidistance of the bromine lines at present are not fully understood, although indications for an analogous situation in irradiated single crystals of 5-bromouracil have been obtained recently[15]. An isotope effect can be excluded because the naturally occurring isotopes ^{79}Br and ^{81}Br have the same nuclear spin and approximately equal nuclear moments. The fact that the same adduct is generated both in 5-bromouracil and in thymine seems to rule out the possibility that the sensitizing action of this compound is due to the so-called "indirect radiation effect".

Caffeine

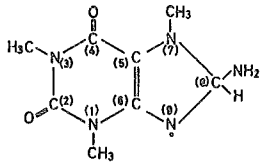
The reaction of caffeine with OH· leads to the production of a radical spectrum which consists of 6 equal intense lines indicating interaction of the unpaired spin with a nitrogen and a hydrogen nucleus. This spectrum is assigned to a C-8 adduct

in which the unpaired spin is centered at N-9 (Radical 3). Further evidence that C-8 is the site of attack comes from the reaction of caffeine with NH_2· which gives rise to a nine-line pattern with line intensities of 1:2:3:2:2:2:3:2:1. Again a C-8 adduct accounts for this spectrum, the unpaired electron at N-9 interacting with the



(3)

nitrogen and the hydrogen nucleus at C-8 as well as with the central atom N-9 (Radical 4). A comparison of the splitting constants of the OH· and NH_2· adduct



(4)

(Table I) shows that the splittings of N-9 and of the proton change, however, in opposite directions indicating differences in structure and spin distribution between the two adducts. It may be concluded from these results that in solution, too, C-8 is the site of radical attack in purines, as has already been confirmed for the dry state by means of single crystal studies[3,16].

ACKNOWLEDGMENT

Work on caffeine has been initiated by one of us (Cl. N.) together with Dr. Y. Rosenthal at the Weizmann Institute of Science, Rehovot (Israel) to whom we are grateful.

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