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PHOTODIMERIZATION OF PYRIMIDINE NUCLEIC ACID DERIVATIVES IN AQUEOUS SOLUTION AND THE EFFECT OF OXYGEN

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Two well-known chemical changes induced in nucleic acids by UV are the dimerization and hydration of pyrimidine bases. Recently the dimerization in solutions of thymine, uracil (Lamola and Mittal, 1967) and orotic acid (Beukers and Berends, 1961; Sztumpf-Kulikowska, Shugar and Boag, 1967) has been studied with a view to clarifying the mechanisms involved in the photochemical reactions. Orotic acid when irradiated in aqueous solution appears to form dimers almost exclusively, the yield of which can be determined by absorbance measurements. The dimer yield is markedly reduced by the presence of oxygen, or of paramagnetic ions. By assuming that oxygen competes for an excited state involved in dimer formation, Sztumpf-Kulikowska et al., (1967) have estimated that the lifetime of the excited state is 2×10^{-6}s. In this paper, the effect of oxygen on dimer production in aqueous solutions of a variety of pyrimidine derivatives has been examined. By separating the photoproducts chromatographically, it has been shown that uracil dimerization, but not hydration, is affected by oxygen. From kinetic data, the lifetime of the uracil excited state which leads to dimerization is estimated to be 10^{-5}s.

METHODS

Samples were irradiated in 0.005 M CH_3COONH_4 (pH 4.4) using the UV monochromator and irradiation techniques described previously (Johns and Rauth, 1965; Johns, Pearson, Leblanc and Helleiner, 1964). Uracil-^{14}C was chromatographed on Whatman No. 4 paper in n-butanol:H_2O (86:14, v/v) pH 9.3 or iso-butyric acid:conc. $NH_4OH:H_2O$ (134:11:55, v/v/v) pH 4.5.

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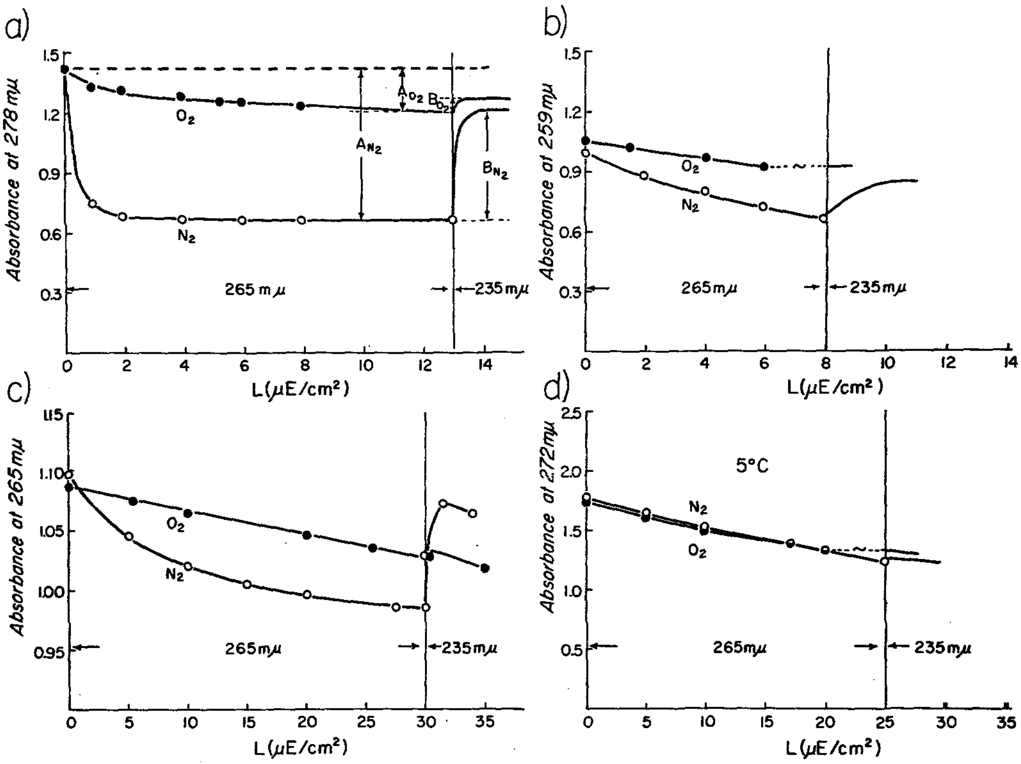

Fig. 1. Decrease in absorbance on irradiation at 265 mμ, followed by reversal at 235 mμ for oxygenated solutions and solutions deoxygenated by bubbling with N_2. Absorbances were measured with a 1 cm optical path at the peak absorption of each base, a) orotic acid, 1.9×10^{4}M, b) Uracil, 1.25×10^{-4}M, c) Thymine, 1.35×10^{-4}M and d) Cytosine, 1.3×10^{-4}M. Note the different vertical scales. The exposure is in μ Einsteins/cm^2.

RESULTS AND DISCUSSION

Effect of oxygen: Fig. 1 shows the loss of absorbance with UV exposure at 265 mμ, for oxygenated and deoxygenated solutions. The presence of dimers, as indicated by the recovery of absorbance upon reirradiation at 235 mμ, was observed for orotic acid, thymine and uracil in the absence of oxygen. Dimerization was inhibited markedly by the presence of oxygen in the solution. Cytosine showed neither dimer formation, nor a significant effect of O_2.

Experiments similar to those illustrated in Fig. 1 were performed on a variety of nucleic acid derivatives. These results are given in Table 1. Generally, three types of behaviour were observed. Group A compounds

TABLE I

EFFECTS OF OXYGEN ON DIMER FORMATION IN NUCLEIC ACID DERIVATIVES

GROUP	COMPOUNDS	EXPOSURE AT 265mu L($\mu E/cm^2$)	PERCENT LOSS OF ABSORBANCE AT ABSORPTION PEAK		PERCENT RECOVERY OF ABSORBANCE ON REIRRADIATION AT 235mμ	
			N_2	O_2	N_2	O_2
A	O_2 EFFECT ON DIMER YIELD					
	THYMINE-t	30	10	6	9	1
	THYMIDINE-T	12	12	3	9	0
	THYMIDYLIC ACID Tp5[1]	11	11	2	6	0
	1 METHYL THYMINE	27	6	-	1	-
	5 HYDROXYMETHYL URACIL	14	30	11	22	1
	URACIL-u	20	69	64	29	2
	URIDINE-U	2	54	48	2	0
	Up5[1]	6	95	95	2	0
	Up2[1] (3[1])	2	64	61	2	0
	dUp5[1]	2	60	58	3	1
	OROTIC ACID	10	54	16	39	5
	5 METHYL OROTIC ACID	25	2	--	0.5	-
B	NO O_2 EFFECT ON DIMER YIELD					
	1-3 DIMETHYL URACIL	12	92	92	2	1.5
	UpU	1	50	50	9.5	7.5
	TpT	3	60	56	45	44
C	NO DIMERS					
	c,C, Cp3[1],dCp5[1], 5 HYDROXYMETHYL CYTOSINE (ALL AT 5°C), 6 HYDROXY URACIL 5 HYDROXY URACIL, 5 BROMOURIDINE, 5 IODO-DEOXYURIDINE, ADENINE, GUANINE.					

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exhibited dimer formation in deoxygenated solutions, and dimerization was efficiently inhibited by the presence of O_2. Compounds in group B also formed dimers, but here O_2 had no inhibiting effect. This group includes the dinucleoside phosphates, such as UpU, in which the proximity of the bases favours dimerization over inhibition by O_2. In the compounds of group C, which includes cytosine derivatives, no dimerization was observed under these irradiation conditions.

The nature of the reactions of thymine and uracil was further studied using ^{14}C-labelled bases and chromatography to separate individual products. Thymine was converted into two products which were chromatographically similar to dimers A and B of Weinblum and Johns (1966). Uracil yielded a hydrate and several dimers. Fig. 2a shows the total dimer production in the absence of O_2,

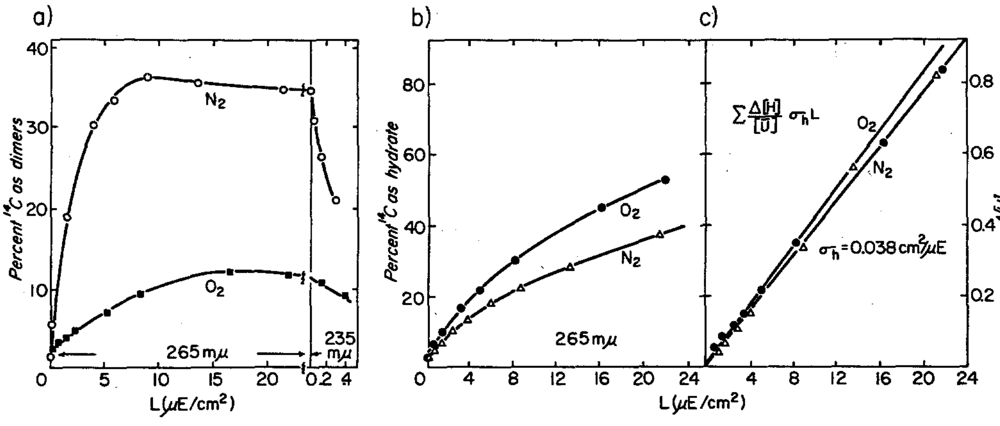

Fig. 2. Formation of a) dimers and b) hydrate in ^{14}C-uracil measured chromatographically. The amount of each photoproduct is shown as a percent of the total activity. In (c), the data in b) are replotted as $\Sigma\Delta[H]/[\overline{U}]$ where $[\overline{U}]$ represents the average unchanged [U] during an increment of exposure. The cross section for hydration, σ_h is equal to the slope of the lines (Brown et al., 1965).

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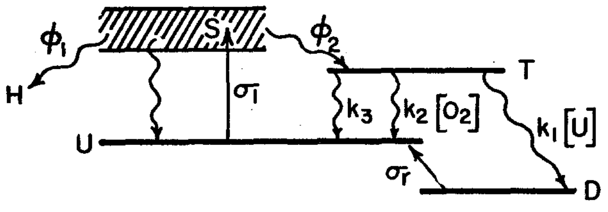
and the inhibition of dimers due to O_2. More hydrate was formed in O_2 than in N_2 saturated solutions (Fig. 2b). However, since there were fewer dimers in O_2, more uracil was available for hydration. In fact, the actual cross section, σ_h, is unaffected by O_2, as shown in Fig. 2c, in which $\Sigma\frac{\Delta[H]}{[\overline{U}]}$ is plotted against L (Brown, Freeman and Johns, 1965). The cross section for hydration, σ_h, as measured from the slope of the lines, is approximately 0.038 cm^2/μE whether O_2 is present or not. The differential effect of O_2 on dimer and hydrate formation supports the conclusion of Lamola and Mittal (1967) that dimer and hydrate production involve different excited states of uracil.

Proposed reaction scheme: The observed photochemical reactions of uracil in aqueous solution are consistent with the following simplified scheme:



In this scheme, ϕ_1 is the efficiency of production of hydrate, H, from excited state(s), S, (probably singlet(s)), and ϕ_2 is the efficiency for formation of an excited state, T, (probably a triplet) from S. Dimer, D, is formed from T by reaction with uracil with rate constant $k_1(M^{-1}\ s^{-1})$, in competition with quenching by O_2 with rate constant $k_2(M^{-1}\ s^{-1})$ and the spontaneous de-excitation of T with rate constant $k_3(s^{-1})$. The cross sections for H and T formation are then $\sigma_h = \sigma_1 \cdot \phi_1$ and $\sigma_T = \sigma_1 \cdot \phi_2$, respectively. This scheme is similar to the one proposed for orotic acid by Sztumpf-Kulikowska et al., (1967), but reactions have been added to account for the hydration of uracil.

Some of the parameters in the scheme can be obtained from kinetic data. The initial rate of dimer formation, $\left(\frac{d[D]}{d\ L}\right)_o$, at known concentrations of U and O_2 is given by

$$\left(\frac{d[D]}{d\ L}\right)_o = \sigma_T \cdot [U] \cdot \left\{\frac{k_1\ [U]}{k_1\ [U] + k_2\ [O_2] + k_3}\right\} = \sigma_T \cdot [U] \cdot \underline{f}$$

where $\sigma_T \cdot [U]$ is the rate of production of the state T and $\underline{f}$ is the fraction of this excited state which leads to dimers. $\left(\frac{d[D]}{d\ L}\right)_o$ has been measured as a function of $[O_2]$ (Fig. 2a), and also at different U concentrations; by using the above equation, the following values have been obtained:

$k_2/k_1 = 1.7$, $k_3/k_1 = 1.2 \times 10^{-4}$ M, $\sigma_T = 0.11\ cm^2/\mu E$ at 265 mμ.

The total absorption cross section at 265 mμ calculated from the extinction coefficient is 17 $cm^2/\mu mole$. If this is σ_1, as suggested by the scheme, then $\phi_1 = 0.002$ and $\phi_2 = 0.006$. By increasing the efficiency of forming state T from S it would be possible to increase the dimer yield. Lamola and Mittal (1967) have reported a higher quantum yield for absorbance loss of uracil irradiated in acetonitrile. We have also observed this effect but the products are not the same as those described here.

Porter (1954) and Kasche and Lindquist (1964) obtained a rate constant of $10^9\ M^{-1}\ s^{-1}$ for quenching of triplet states by O_2. Using this value for k_2, the lifetime of T is estimated to be 10^{-5}s. The fact that oxygen and other paramagnetic molecules are efficient triplet state quenchers, together with the lifetime of 10^{-5} s provides some, though not conclusive evidence that dimer formation occurs via a triplet state.

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