Some Observations on the Effects of Light and Solvent Polarity on the Kinetics of Tetrahydrobiopterin Autoxidation

By **John A. Blair** and **Anthony J. Pearson,*** Department of Chemistry, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET

Autoxidation of tetrahydrobiopterin is accelerated by light, the effect being more pronounced at low oxygen pressures, and is retarded by reduction in solvent polarity.

ISOLATION and storage of reduced pterins is normally carried out with daylight excluded, but no quantitative information is available concerning its influence on their lability, particularly towards autoxidation. In a previous publication[1] we reported that autoxidation rates of tetrahydrobiopterin in aqueous solution are only slightly increased by the admission of light, using a gas phase of 100% oxygen.

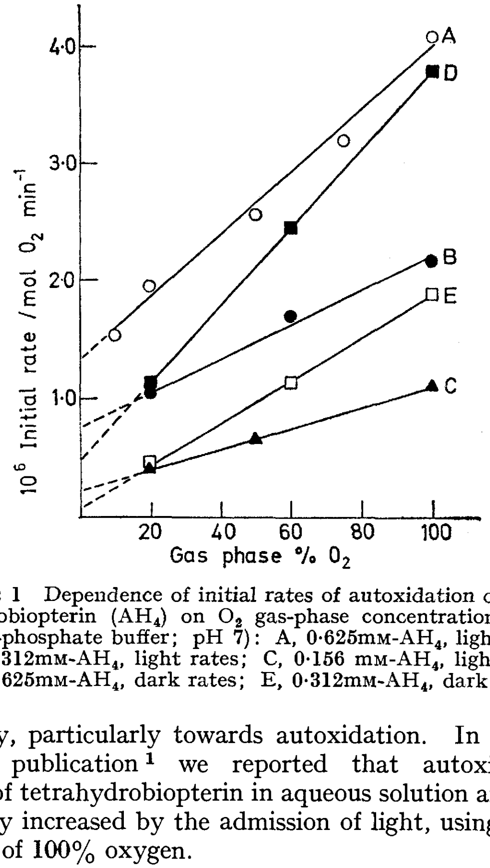

FIGURE 1 Dependence of initial rates of autoxidation of tetrahydrobiopterin (AH_4) on O_2 gas-phase concentrations (25°; 0·1M-phosphate buffer; pH 7): A, 0·625mM-AH_4, light rates; B, 0·312mM-AH_4, light rates; C, 0·156 mM-AH_4, light rates; D, 0·625mM-AH_4, dark rates; E, 0·312mM-AH_4, dark rates

We now describe the interesting observation that more pronounced acceleration is produced at low gas-phase concentrations of oxygen (Figure 1). There is an apparent combination of first- and zero-order dependence on oxygen, and the zero order : first order ratio is increased in the presence of laboratory illumination. The small residual effect with dark rates is probably due to incomplete exclusion of radiation, and not trace impurities as earlier suggested.[1] In contrast, the reaction remains first order in tetrahydrobiopterin under both sets of conditions (Figure 2).

The observation of a comparatively large zero-order contribution for oxidations under air appears to validate the neglect of thermal oxygen initiation in the overall rate equation, and supports the treatment of kinetic data made[1] to show that the chain carrier is $HO_2\cdot$ rather than organic peroxyl radicals.

Spectrophotometric determinations of initial specific rates for oxidation under air show that the reaction is considerably slower in ethanol–water (8 : 1) (0·051% oxidation min^{-1}) than in water (0·825% oxidation min^{-1}). Since oxygen is more soluble in the former,[2] the result is not due to changes in its concentration. Furthermore, it is unlikely that the tetrahydrobiopterin molecule is more protonated in ethanol, though protonation is known to retard oxidation.[1,3] This result indicates that autoxidation involves electron-transfer steps, since such an ionic-type process would be facilitated by polar solvents.†

The observed photochemical effect is possibly due to electron transfer from excited tetrahydrobiopterin, $\lambda_{max.}$ 290—300 nm, to oxygen or chain carrier during initiation and/or propagation, though the complete kinetic scheme has not yet been solved. A simplified mechanism, neglecting photochemical effects during propagation [step (7)], is outlined in the Scheme, and application of the steady state approximation leads to the rate equation

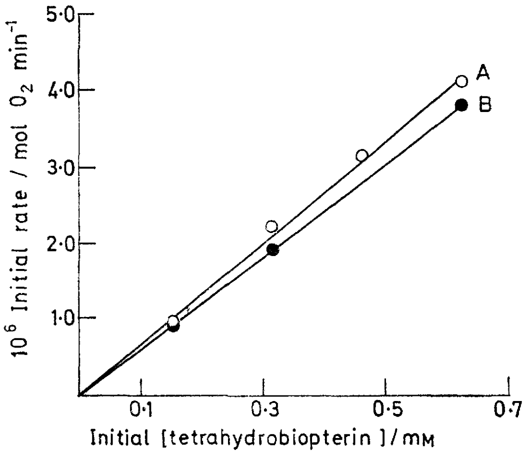

FIGURE 2 Effect of light exclusion on tetrahydrobiopterin dependence (1·00%) O_2): A, light rates; B, dark rates

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† However, we have observed that the related compound, 1,3,6,7,8-pentamethyl-5,6,7,8-tetrahydropteridine-2,4-dione, which is soluble in non-polar media, is autoxidised *ca.* 100 times more rapidly in water than in moist pyridine, in support of the above observations (unpublished data).

[1] J. A. Blair and A. J. Pearson, *J.C.S. Perkin II*, 1974, 80; A. J. Pearson, *Chem. and Ind.*, 1974, 233.

[2] W. F. Linke and A. Seidell, ' Solubilities of Inorganic and Metal Organic Compounds., Amer. Chem. Soc., 1965, 4th edn., vol 2.

[3] M. C. Archer, K. G. Scrimgeour, and D. J. Vonderschmitt, *Canad. J. Biochem.*, 1972, **50**, 1174.

$$\mathrm{AH_4} \xrightarrow{h\nu} \mathrm{AH_4^*} \qquad \text{rate} = kI\,[\mathrm{AH_4}] \tag{1}$$

$$\mathrm{AH_4^*} \xrightarrow{k_\mathrm{d}} \mathrm{AH_4} \tag{2}$$

$$\mathrm{AH_4^*} + \mathrm{O_2} \xrightarrow{k_\mathrm{q}} \mathrm{AH_4} + \mathrm{O_2} \qquad \text{quenching} \tag{3}$$

$$\mathrm{AH_4^*} + \mathrm{O_2} \xrightarrow{k_1} \mathrm{AH_3\cdot} + \mathrm{O_2{}^{\bullet -}} + \mathrm{H^+} \tag{4}$$

$$\mathrm{AH_4} + \mathrm{O_2} \xrightarrow{k_2} \mathrm{AH_3\cdot} + \mathrm{O_2{}^{\bullet -}} + \mathrm{H^+} \tag{5}$$

$$\mathrm{AH_3\cdot} + \mathrm{O_2} \xrightarrow{k_3} \mathrm{AH_2} + \mathrm{O_2{}^{\bullet -}} + \mathrm{H^+} \tag{6}$$

$$\mathrm{AH_4} + \mathrm{HO_2\cdot} \xrightarrow{k_4} \mathrm{AH_3\cdot} + \mathrm{HO_2{}^-} + \mathrm{H^+} \tag{7}$$

$$\mathrm{AH_3\cdot} + \mathrm{HO_2\cdot} \xrightarrow{k_5} \mathrm{AH_2} + \mathrm{HO_2{}^-} + \mathrm{H^+} \tag{8}$$

SCHEME I = Incident radiation intensity, $\mathrm{AH_4}$ = tetrahydrobiopterin, $\mathrm{AH_4^*}$ = excited tetrahydrobipterin, $\mathrm{AH_3\cdot}$ = the derived semiquinone free radical, and $\mathrm{AH_2}$ = quinonoid dihydrobipterin (see ref. 1)

(9). This predicts a complex oxygen dependence which becomes first order in the absence of light ($I = 0$).

$$\text{Rate} = \frac{-\mathrm{d}[\mathrm{O_2}]}{\mathrm{d}t} = \left\{\frac{kk_1I}{k_\mathrm{d} + (k_\mathrm{q} + k_2)[\mathrm{O_2}]} + k_2 + \left(\frac{k_3}{k_5}\right)^{\frac{1}{2}}\left[\frac{kk_1k_4I}{k_\mathrm{d} + (k_\mathrm{q} + k_1)[\mathrm{O_2}]} + k_2k_4\right]^{\frac{1}{2}}\right\}[\mathrm{AH_4}][\mathrm{O_2}] \tag{9}$$

EXPERIMENTAL

Tetrahydrobiopterin was a gift from Roche Products Ltd.

Kinetics.—Oxygen uptake rates were determined as described previously.[1] Spectrophotometric measurements were made with a Perkin-Elmer 137 u.v. spectrophotometer, using solutions in equilibrium with air, by following the changes in absorbance at 330 nm ($\lambda_{\text{max.}}$ for 7,8-dihydrobiopterin, the product under these conditions) at ambient temperature.

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