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A KINETIC STUDY OF THE AUTOXIDATION OF TETRAHYDROBIOPTERIN

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The kinetics of autoxidation of tetrahydrobiopterin (1) in aqueous solution have been studied manometrically at 25°C using the initial rates method. The reaction has a 1:1 stoichiometry and is first order in (1) and oxygen (rate constant = 1.0 ± 0.1 l $mol^{-1}sec^{-1}$). Retardation by phenols and the absence of any effect of light suggests a free-radical chain reaction. A linear dependence of rate on per cent ionisation of the 3,4-amide group (pK = 10.5, estimated spectrophotometrically) is found on varying the solution pH. (Fig. 1).

Hydrogen peroxide has been reported in solutions of autoxidised tetrahydropterins[1,2], and e.s.r. studies[3,4,5] have shown the presence of free-radicals during oxidation.

This data is consistent with the mechanism shown, involving formation of transient hydroperoxides having the structure shown (IV) (atom 4a is the only carbon atom having unpaired electron density in radical (III)). Rapid loss of H_2O_2 and subsequent rearrangement of the quinonoid from (V) gives the observed first product, 7,8-dihydrobiopterin (VI) indicated by T.L.C. and characterised spectroscopically: U.V. : λ_{max}(nm.) pH 1, 267, 350; pH 7, 240, 280, 330; pH 13, 280, 330, cf. ref. 6. N.m.r : 2 - proton singlet τ 4.9, characteristic of 7,8-dihydropterins, CH_3 group at τ8.7, doublet J = 8 Hz, two CH groups at τ5.5 and τ6.1). This is then oxidised more slowly to give the final product, biopterin (VII)[1,7]. Present work in this laboratory indicates the same mechanism for other tetrahydropterins.

Tetrahydropterins are cofactors in enzymatic hydroxylation[6] of phenylalanine to tyrosine, a process in which hydroperoxides may have a significant role.

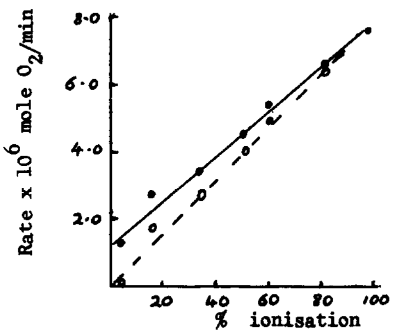
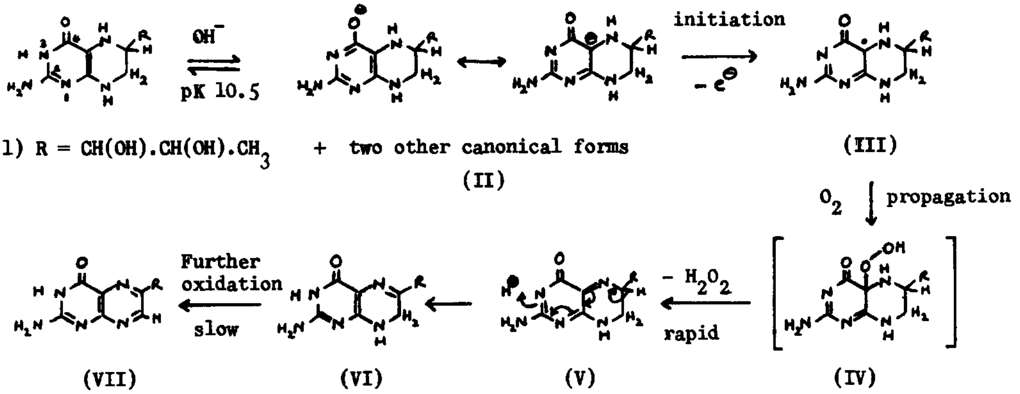


Fig 1

— (a) 0.3 mM tetrahydrobiopterin solutions under pure O_2. pH varied using NaOH solutions of different concentrations. Intercept due to slow reaction of non-ionised compound.

- - - (b) Rates corrected for reaction of residual non-ionised compound.

Mechanism:



(1) R = $CH(OH).CH(OH).CH_3$ + two other canonical forms (II)

References:

1. R. Stocks-Wilson, Ph.D. Thesis, (1971) University of Aston in Birmingham.
2. H.I.X. Mager and W. Berends, Rec. Trav. Chem. 84, 1329 (1965).
3. P. Doyle, Unpublished data, University of Aston in Birmingham.
4. A. Bobst, Helv. Chim. Acta., 50, 2222 (1967).
5. A. Ehrenberg, P. Hemmerich, F. Müller, T. Okada and M. Viscontini, Helv. Chim. Acta., 50, 411 (1967).
6. R.L. Blakley, "The Biochemistry of Folic Acid and Related Pteridines" North-Holland (1969).
7. H. Rembold, H. Metzger and W. Gutensohn, Biochim. Biophys. Acta, 230, 117 (1971).