Enzymically Generated Triplet Acetone

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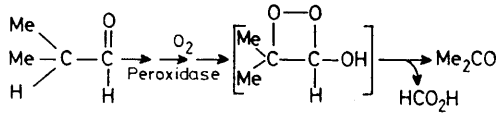
Summary The oxidation of isobutyraldehyde in the horseradish peroxidase–O_2 system produces triplet acetone in high yield, as shown by efficiently sensitized 9,10-dibromoanthracene-2-sulphonate emission and by the occurrence of the expected photoproducts, *i.e.* isopropyl alcohol and tetramethylglycol.

THE oxidation of isobutyraldehyde in the peroxidase–O_2 system generates the products expected from the cleavage of an intermediate dioxetan, *i.e.*, acetone and formic acid[1] (Scheme). We suspect therefore that acetone is generated in the triplet state.[2] This has been confirmed both by sensitized chemiluminescence and by identification of the expected photoproducts,[3] *i.e.*, isopropyl alcohol and tetramethylglycol.

The system used consisted of $4{\cdot}2 \times 10^{-2}$M isobutyraldehyde, $2{\cdot}5 \times 10^{-6}$M horseradish peroxidase (Sigma, type VI), and 0·034 M ethanol (for solubilizing the aldehyde) in 0·1 M

phosphate buffer, pH 7·0, at 25 °C. Emission was detected with a liquid scintillation counter; it increased as oxygen was depleted. In the presence of 9,10-dibromoanthracene-2-sulphonate a dramatic enhancement (200 fold at infinite sensitizer concentration) was observed as a result of the long range triplet–singlet energy transfer; thus the non-halogenated sensitizer, unable to overcome the spin-forbidden nature of the transfer,[4] was inefficient.

In dimethyl sulphoxide–potassium t-butoxide (no enzyme), chemiluminescence was observed with conventional equipment; its spectrum matched the fluorescence spectrum of acetone. Obviously in the aprotic solvent, the emitter is singlet acetone which was isolated from the spent reaction mixture as the 2,4-dinitrophenylhydrazone. Formic acid was also properly identified.[5]



SCHEME

Product formation in the enzymic system was analysed by g.l.c. after leaving the solution undisturbed for 96 h in the dark. The yields based on isobutyraldehyde which had reacted (50%) were: acetone, 86 ± 10%; tetramethylglycol, 0·5—4%; and isopropyl alcohol, 6—7%. This indicates that acetone must be formed in an excited state, the chemiexcitation quantum yield being no less than, and probably well above, 0·1. Rigorous control experiments confirmed that neither formic acid nor ethanol could have reduced ground state acetone.

It is possible that isopropyl alcohol reacts with triplet acetone to form tetramethylglycol.[6] However, the very occurrence of photoproducts and the dramatic enhancement in emission with 9,10-dibromoanthracene-2-sulphonate implies that triplet acetone must be considerably protected from oxygen quenching by the enzyme. This indicates that the enzyme is the hydrogen donor. The enzyme radical would then abstract a hydrogen atom from an exogenous donor (presumably ethyl alcohol or formic acid) thus regenerating the enzyme. The participation of ethyl alcohol is suggested by the occurrence of a g.l.c. peak corresponding to acetaldehyde.

We expect to obtain a high chemiexcitation quantum yield from other closely related systems under study in this laboratory.[7] Whether an oxidation similar to that reported herein occurs with the luciferin[8] of the bioluminescent worm *Diplocardia longa* remains to be ascertained.

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