Journal of

The Chemical Society,

Chemical Communications

NUMBER 8/1976 21 APRIL

Oxidation of Dialkyl Sulphides by Bacterial Luciferase

By Frank McCapra*† and Russell Hart

(*School of Molecular Sciences, University of Sussex, Brighton* BN1 9QJ)

Summary Long chain dialkyl sulphides are oxidised, without the emission of light, by one of the intermediates responsible for the light reaction in *Photobacterium phosphoreum;* this is the first example of an alternative substrate for the reaction, indicating something of the size of the active site and the nature of the intermediate.

Luminescent bacteria generate light according to the Scheme.[1] Recent advances in understanding the mechanism of light emission include the identification of fatty acid as product,[2,3] with a quantum yield of 0·10—0·17 and the isolation of the intermediate (II) by low temperature chromatography.[4] Although two suggestions for the structure of the excited molecule produced in the reaction have been made,[2,5] no direct evidence for either is available nor do the structures point to a mechanism in agreement with current knowledge.

In order to reconstruct the molecular events which lead to the production of light, one must know the structure of the intermediate (II) and its characteristic reactions. Alternative substrates may reveal part of the chemistry or assist in trapping the intermediate. For example, it has been reported[6] that alkyl nitrites may be used instead of aldehyde, with light emission. If this is so the implications for the mechanism are considerable. However, we have synthesised and carefully purified n-decyl nitrite, and find no trace of light emission, using the enzyme from *P. phosphoreum* under a variety of conditions. No reaction with any of the intermediates was observable. There is however a series of compounds, the long chain dialkyl sulphides, which do react, strongly indicating that the intermediate (II) is an active peroxide, with properties perhaps analogous to those of a peroxy-acid. The product of the reaction is

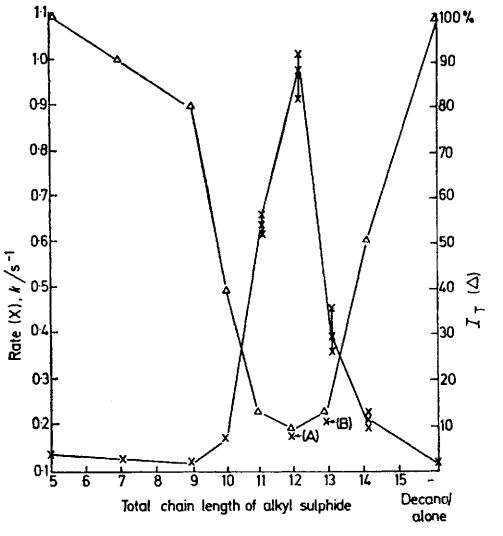

Figure Rate of decay of light emission (×) and total yield of quanta emitted (I_T,Δ) plotted against length of chain, including the sulphur atom, of the alkyl methyl sulphide. The concentration of the aldehyde and sulphide were approximately equal (5×10^{-4}M) and the reaction conditions were those developed by Hastings and Gibson.[9] (A), Nonyl ethyl sulphide; (B), decyl ethyl sulphide.

† Present address: Chemistry Department, University of British Columbia, 2075 Wesbrook Place, Vancouver, B.C., Canada V6T 1W5.

the corresponding sulphoxide. The enzyme has a very limited turnover under the conditions used and a radioactive dilution method was employed to show that the expected sulphoxide was formed. Radioactive [*Me*-^{14}C]-decyl sulphide (purified by t.l.c. followed by distillation) and

Luciferase + $FMNH_2$ ⇌ Intermediate (I) (E. $FMNH_2$)

E. $FMNH_2$ + O_2 → Intermediate (II)

Intermediate (II) + RCHO → Luciferase + FMN + RCO_2H + light (490 nm)

SCHEME FMN = riboflavin mononucleotide.

NADH were added to a luciferase preparation containing some associated FMN reductase.[2] Pure methyl decyl sulphoxide was added after 2 h and extracted in the usual way. Although the sulphoxide could well be optically active the quantity of sulphide involved precluded examination of this point. No difficulty was experienced in obtaining constant activity in successive recrystallisations. Table 1 gives the results, including those of appropriate control experiments. Although the blank reactions show significant incorporation of radioactivity, there is reasonable evidence for formation of sulphoxide. It is possible that the sulphide causes the dissociation of the presumed flavin peroxide–enzyme complex in addition to reduction, but we have no evidence for this.

TABLE 1. Oxidation of methyl decyl sulphide.

Reaction	Total activity of the sulphide added (counts s^{-1})	Wt. of sulphoxide added (mg)	Final activity of sulphoxide (counts s^{-1} mg^{-1})	Conversion (%)
Normal reaction[a]	$3{\cdot}001 \times 10^4$	113·92	23·95	9·1
H_2O_2 added,[b] no NADH	$1{\cdot}07 \times 10^4$	78·13	3·414	2·5
Normal reaction	$8{\cdot}23 \times 10^3$	70·91	9·205	7·9
H_2O_2 added, no NADH	$8{\cdot}23 \times 10^3$	74·96	2·382	2·2
Normal reaction	$2{\cdot}45 \times 10^3$	69·76	3·261	9·2
No FMN	$2{\cdot}45 \times 10^3$	62·70	0·922	2·4
Sulphide only[c]	$2{\cdot}45 \times 10^3$	65·74	0·534	1·4

[a] Reaction conditions for this radioactive assay have been described previously.[2,9] The sulphide was substituted for decanal. [b] Hydrogen peroxide was added (30 equiv. based on FMN present) to simulate effect of $FMNH_2$ autoxidation. [c] [*Me*-^{14}C]Decyl sulphide was suspened in buffer using Triton-X as detergent, and the reaction worked up as before in order to take into account possible traces of sulphoxide produced during synthesis of the sulphide.

The effect of the alkyl sulphides, when used as competitive substrates (Figure) depends markedly on the chain length, and even on the position of the sulphur atom in the chain. These compounds are unique in both increasing the rate of light emission and depressing the quantum yield when used competitively. We have examined a large variety of long chain compounds including sulphoxides, sulphones, alkanes, alkanethiols, alkenes, alkyl isocyanates, α-ketoacids, alkynyl aldehydes, and other would-be electrophilic inhibitors. These are either without effect, or react in a general way with the enzymic sulphydryl groups, or as Hastings showed,[1] lower the rate slightly leaving the total light yield unchanged.

From these observations we conclude that the intermediate (II) is an active peroxide for which the sulphide is a specific reductant. The sulphides have no effect on the enzyme–flavin complex in the absence of oxygen. So far we have been unable to intercept the intermediate (IIa), the result of the combination of aldehyde and (II), which should also be a peroxide. Further support for the direct and specific reaction with the intermediate (II) was obtained by isolating (II) by chromatography at 0 °C. Table 2 shows the result of the competition between aldehyde and sulphide for the intermediate.

TABLE 2

Substrate	I_0	I_T	k/s^{-1}
Decanal	100	100	$4{\cdot}5 \times 10^{-2}$
Decanal + decyl methyl sulphide	49·7	8·2	0·24
(1) Decyl methyl sulphide	0	0	—
(2) Decanal[a] (30 s later)	0	0	—

[a] The intermediate (II) is stable for many hours at this temperature (0–5 °C).

It is known that peroxides with distinctive activating features are preferentially reduced by diphenyl sulphide,[7] and we feel that the intermediate (II) may well have the properties of a carbonyl oxide.[8] There is evidence that the compound formed by the reduction of the peroxide is enzyme bound and an inhibitor of the light reaction, although so far we have only been able to identify FMN as a product. Since luciferase behaves as a flavin-catalysed hydroxylase our present work may have more general application.

We thank the S.R.C. for financial support and Professor J. W. Hastings for advising us of the chromatographic procedure developed in his laboratory for isolating the intermediate (II).

(*Received*, 3*rd October* 1975; *Com.* 1134.)

[1] J. W. Hastings, Q. H. Gibson, J. Friedland, and J. Spudich, in 'Bioluminescence in Progress', eds. F. H. Johnson and Y. Haneda, Princeton University Press, 1966, p. 151.
[2] F. McCapra and D. W. Hysert, *Biochem. Biophys. Res. Comm.*, 1973, **52**, 298.
[3] O. Shimomura, F. H. Johnson, and Y. Kohama, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 2086; D. K. Dunn, G. A. Michaliszyn, I. G. Bogacki, and E. A. Meighen, *Biochemistry*, 1973, **12**, 4911; A. Vigny and A. M. Michelson, *Biochimie*, 1974, **56**, 171.
[4] J. W. Hastings, C. Balny, C. LePeuch, and P. Douzou, *Proc. Nat. Acad. Sci. U.S.A.*, 1973, **70**, 3468.
[5] M. Eley, J. Lee, J. M. Lhoste, C. Y. Lee, M. J. Cormier, and P. Hemmerich, *Biochemistry*, 1970, **9**, 2902.
[6] D. Bentley, A. Eberhard, and R. Solsky, *Biochem. Biophys. Res. Comm.*, 1974, **56**, 865 (Dr. Eberhard has indicated that he has some difficulty in repeating these results).
[7] H. H. Wasserman and I. Saito, *J. Amer. Chem. Soc.*, 1975, **97**, 906; B. S. Campbell, D. B. Denney, D. Z. Denney, and L. S. Shih, *ibid.*, p. 3850.
[8] G. A. Hamilton in 'Progress in Biorganic Chemistry,' eds. E. T. Kaiser and T. J. Kezdy, Wiley–Interscience, New York, 1971, Vol. 1, p. 83.
[9] J. W. Hastings and Q. H. Gibson, *J. Biol. Chem.*, 1963, **238**, 2537.