DECYL NITRITE: AN ALDEHYDE ANALOG IN THE BACTERIAL BIOLUMINESCENCE REACTION

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Decyl nitrite was found to be an effective substitute for aliphatic aldehydes in the light producing reactions of bacterial luciferase. Two other aldehyde analogs, decyl formate and decyl formamide, were inactive. A kinetic isotope effect of 1.7 was found for 1-^{2}H-dodecanal in comparison to dodecanal. These results support a Baeyer-Villiger type mechanism for bioluminescence proposed previously. Alkyl nitrites may be useful in the study of other enzymic reactions involving aldehyde substrates.

Bacterial luciferase catalyzes the simultaneous oxidation of reduced flavin mononucleotide ($FMNH_2$) and a long chain aliphatic aldehyde by molecular oxygen.[1] The products are the corresponding carboxylic acid[2-4] and an excited state of FMN (presumably enzyme bound) which emits a photon.[5,6] The reaction appears to have the following stoichiometry:

$$FMNH_2 + O_2 + RCHO \xrightarrow{\text{luciferase}} FMN^* + H_2O + RCO_2H$$

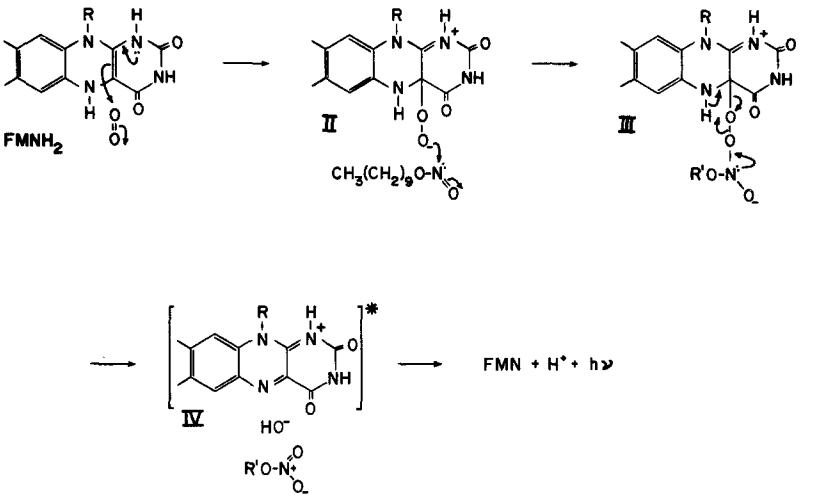
$$FMN^* \longrightarrow FMN + h\nu$$

The time required for a single catalytic cycle is of the order of tens of seconds, while the substrate $FMNH_2$ is autoxidized in less than one second. Since luminescence in an in vitro assay thus lasts much longer than substrate, the enzyme molecules function only once. The rate constant for the decay of luminescence is therefore a direct measure of the lifetime of an enzyme intermediate.

A detailed mechanism has recently been proposed for this reaction.[8,9] We have now found that long chain alkyl nitrites, R-O-N=O, also lead to light production in the luciferase reaction.[10] Alkyl nitrites, which are electronic analogs of aldehydes, can be directly substituted for aldehydes in the previously proposed mechanism, as shown in Scheme I.

Decyl nitrite, prepared by reaction of decanol with sodium nitrite and sulfuric acid[11] was purified by distillation (bp 90°/3mm) followed by

Scheme I



preparative gas-liquid chromatography. Ultraviolet,[12] infrared and nuclear magnetic resonance spectra were in agreement with the assigned structure. At 19° decyl nitrite ($2x10^{-5}$M), O_2, $FMNH_2$ and a sample of pure luciferase from Photobacterium fischeri strain MAV[13] gave 7.0% of the initial light intensity and 22% of the quantum yield compared with an identical dodecanal assay. The first order rate constant for the decay of luminescence was 0.0091 sec^{-1} compared to 0.026 sec^{-1} with dodecanal. The control reaction with only luciferase, $FMNH_2$ and O_2 gave 0.7% of the initial light intensity and 0.4% of the quantum yield compared to dodecanal, with a rate constant of 0.048 sec^{-1}.

Decyl nitrite also stimulates light production in vivo. Mutants of MAV which are dim until a solution of aldehyde is added to the medium (aldehyde mutants)[14,15] also became bright with decyl nitrite. Although the intensity of the light produced with the nitrite was only 10% of that with a similar quantity of aldehyde (10^{-5}M in a culture at OD 1.0), far more light was ultimately produced, since with aldehydes the bacteria turn dim again within a few minutes while with the nitrite they remained at a constant level of brightness for well over a half hour. These results indicate that decyl nitrite is a good substrate for bacterial luciferase both in vitro and in vivo.

It has been reported previously[16] that there is no deuterium isotope effect in the luciferase reaction with 1-deuteroaldehydes. We have synthesized 1-^{2}H-dodecanal,[17] purified it by distillation and preparative glc, and found an isotopic purity of about 98% at the 1-position by nmr. The reaction of pure MAV luciferase, $FMNH_2$, O_2 and the 1-^{2}H-dodecanal compared to that with dodecanal gave a $k_H/k_D = 1.7$ for the first order decay of luminescence, a lower initial light intensity and the same quantum yield. In a previous publication[8] the k_H/k_D was assumed to be 1 based on the earlier report,[16] and consequently the rate determining step in the reaction was taken to be the conversion of intermediate II to III. However, the finding here of a substantial isotope effect makes it more likely that the rate determining step lies in the conversion of III to IV, a step closely analogous to a Baeyer-Villiger oxidation. Our $k_H/k_D = 1.7$ is consistent with the k_H/k_D for the Baeyer-Villiger oxidation of aldehydes which was recently reported to vary from 1.4 to 3.0 depending on the conditions.[18] It is possible that the previous failure to find a deuterium isotope effect[16] can be attributed to the use of a different enzyme (derived from strain ATCC 7744 rather than strain MAV) which may have a different rate determining step. Also, the previously used enzyme was much less pure than the gel-electrophoretically pure enzyme used in this study, and decanal, rather than dodecanal, was used. If the conversion of III to IV is the rate determining step, then the reaction of II with aldehydes (or, presumably, nitrites) to give III must be reversible in order for the mechanism to be consistent with the earlier finding that the later addition of one aldehyde can change the kinetics of a reaction in progress with another aldehyde.[16] Indeed, additions of anions to carbonyl groups are usually freely reversible.

Two other aldehyde analogs, decyl formate and decyl formamide, acted as competitive inhibitors of light production with aldehydes. A mixture of sodium nitrite and decanol, the possible hydrolysis products of decyl nitrite, did not lead to light production. According to our mechanism, this was

expected since the peroxyanion II should not be able to attack the anion nitrite due to electrostatic repulsion.

In sum, the following new findings support our postulated mechanism: a kinetic deuterium isotope effect with a value similar to that for Baeyer-Villiger oxidations of aldehydes; light production with decyl nitrite both *in vitro* and *in vivo* with aldehyde mutants with a mechanism presumably analogous to that with aldehydes; and lack of light production with sodium nitrite and decanol. We hope that alkyl nitrites will be useful in the study of other enzymic reactions involving aldehydes.

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