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ON THE MECHANISM OF BACTERIAL LUCIFERASE. 4a,5-DIHYDRO-FLAVINS AS MODEL COMPOUNDS FOR REACTION INTERMEDIATES.

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Introduction

The monooxygenation of long-chain aldehydes to form the corresponding carboxylic acids catalyzed by the FMN-dependent bacterial luciferase produces light as one of the reaction products (1):

$$FMNH_2 + O_2 + RCHO \rightarrow FMN + H_2O + RCOOH + h\nu$$

The reaction producing excited states involves a 4a,5-dihydroflavin intermediate (3). The breakdown of a 4a-peroxyhemiacetal intermediate is the key step and has been formulated as an intramolecular CIEEL (chemically initiated electron exchange luminescence) mechanism (2,5,6). Based on the assumption that charge or electron transfer from an activator to the peroxide is connected with the rate-limiting step, a linear free energy relationship (LFER) should be found between the one-electron oxidation potential of the electron-donating activator (the reduced 4a,5-dihydroflavin) and the rate of light emission decay. This relationship was tested by us some years ago using 8-substituted FMN derivatives as cofactors of the luciferase. Indeed we observed the expected dependence on the redox potentials of such FMN analogues in a linear free energy relationship (4). For this correlation the $2e^-$-redox potentials of the Fl_{ox}/1,5-Fl_{red} couples were used since the one-electron oxidation potentials of 4a,5-dihydroflavins were not available; however, we reasoned that the two sets of potentials would exhibit the same dependence on substitution. To verify experimentally this fundamental premise, we have synthesized several 4a,5-dihydroflavins with varying residues at the 8-position and measured their one-electron oxidation potentials by cyclic voltammetry (for detailed experimental conditions see (7)).

Results and Discussion

One-electron oxidation of the 4a,5-dihydroflavin model compounds is reversible under the conditions used. The one-electron oxidation potentials were tested on a Hammett-relationship concerning substituent effects according to the

Figure 1a

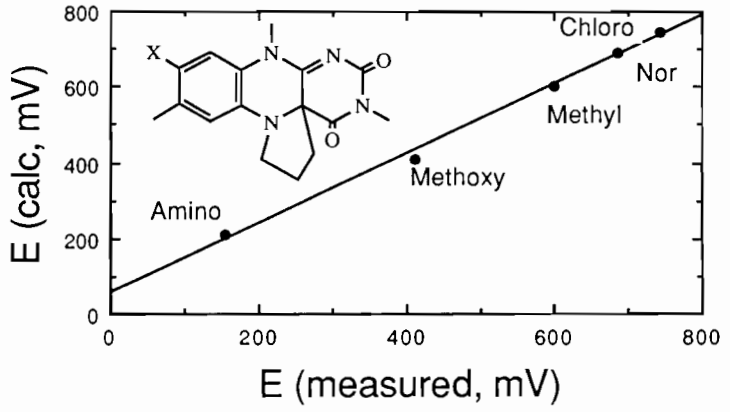
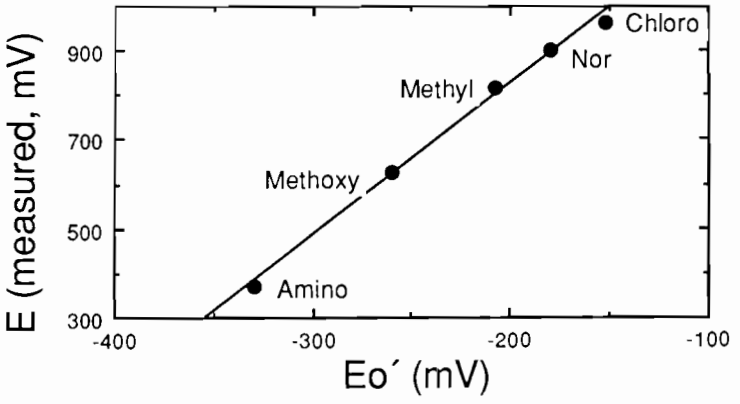


Figure 1b



method described by Swain et.al.(8). Using optimized resonance and field constants an excellent correlation was obtained

between calculated and measured one-electron oxidation potentials (figure 1a). Comparing the effect of substitution at the 8-position on the *one-electron oxidation potentials* of 4a,5-dihydroflavins and on the *2e⁻-redox potentials* of FMN derivatives we have found the very good correlation shown in figure 1b. This confirms that the assumptions mentioned in the introduction are correct. A plot of the log of the rate of light emission decay (k) in the luciferase reaction against the one-electron oxidation potentials yields a value for α being about one third (≈ 0.3[1]) of the value found in a correlation with the $2e^-$-redox potentials of FMN derivatives (figure 2). Altogether we are now confident that the electron/charge donating properties of the reduced flavin moiety of the luciferase-bound 4a,5-dihydroflavin intermediate determines the rate and effectiveness of excited state production. The dependence shown in figure 2 is consistent with an intramolecular CIEEL mechanism in which partial charge transfer in the transition state can be envisaged as well(10).

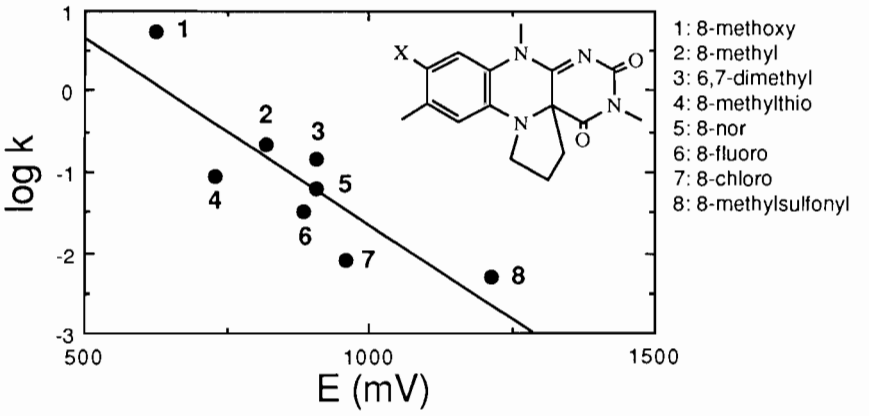


Figure 2 Correlation of one-electron oxidation potentials with the rate of light emission decay in an assay with V.harveyi luciferase. Rate constants were taken from (4). One-electron oxidation potentials were measured by cyclic voltammetry or calculated by the method of Swain et.al (8).

[1]For discussion of typical α-values of ≈ 0.3 refer to (9)

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