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The Quantum Yield of Luciferase is Dependent on Atp and Enzyme Concentrations

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THE QUANTUM YIELD OF LUCIFERASE IS DEPENDENT ON ATP AND ENZYME CONCENTRATIONS

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Abstract The Quantum Yield of firefly luciferase relative to its substrate luciferin is a function of enzyme and ATP concentrations. The implications for molecular electronic applications are discussed.

INTRODUCTION

Luciferase, the light-producing enzyme from the firefly *Photinus pyralis* is an excellent candidate for use as a molecular electronics biocomputing element. Its substrates are adenosine-5'-triphosphate (ATP), the heterocyclic co-factor luciferin and oxygen. Its products are carbon dioxide, pyrophosphate, adenosine-5'-monophosphate (AMP), oxyluciferin and light [1,2]. This can be exploited to produce a molecular switch with an 'ON' light emitting state and an 'OFF' dark state. In principle the smallest possible unit in such a device would be a single luciferase enzyme molecule. Such an application would require that each enzyme turnover produced one photon.

This paper describes the measurement of the number of photons produced per luciferin molecule consumed, i.e. the quantum yield of luciferase relative to luciferin, Q. A previous measurement at a single concentration of ATP and luciferase[3] gave a value of 0.88. These results show that Q is dependent upon the concentration of reactants. The effect can be explained in terms of an equilibrium between fully active enzyme dimers and partially active enzyme dimers. Previous evidence of this effect has been proposed on the basis of luciferase specific activity[4]. This has important implications for any molecular electronics applications as well as for any proposed reaction mechanisms.

MATERIALS AND METHODS

Purified luciferase was obtained from Sigma Chemical Co. Purified luciferin was obtained from Boehringer-Mannheim. All other materials were obtained from Aldrich Chemical Co. All reactions were performed in buffer containing 25 mM glycyl glycine, 5 mM magnesium chloride, 10 mM EDTA, 50 mM ß-mercaptoethanol, pH 7.8. Bioluminescence was measured by a photon-counting photomultiplier. Absolute efficiency of the luminometer was obtained with reference to a photodiode and a commercial power meter.

The reaction was initiated by addition of ATP to luciferase + luciferin. In all cases reaction conditions were such that [ATP] > [luciferase] > [luciferin]. Light emission decayed with first order kinetics. Data was recorded until it reached < 10% of the original value. A log plot was used to estimate the total area beneath the bioluminescence curve, and hence the total number of photons released. Separate control experiments showed that the reaction ceased due to exhaustion of luciferin, rather than non-specific hydrolysis of substrates or enzyme deactivation.

The quantum yield of luciferase with respect to luciferin was calculated from the relation:

$$N_p = \int_0^\infty A_0 . \exp\left(\frac{-t}{\tau}\right)$$

$$= A_0 . \tau$$

$$Q = \frac{N_p}{N_L}$$

where : N_p = Total photons emitted

N_L = Total number of luciferin molecules added

A_0 = Initial Bioluminescence

t = Time

τ = Time Constant of Bioluminescence

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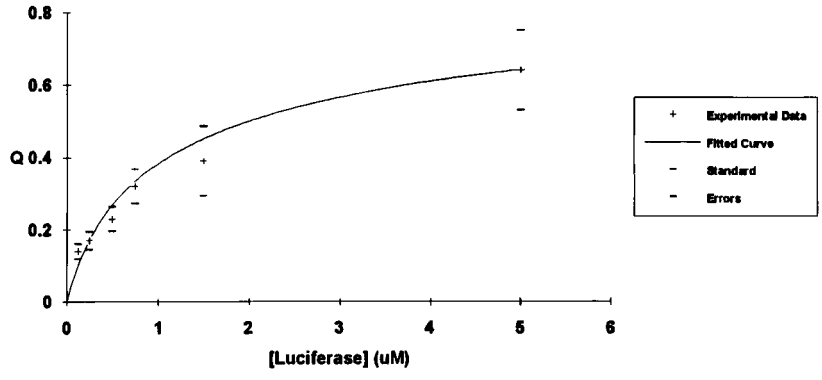
RESULTS

The dependence of Q on luciferase concentration is shown in Figure 1.

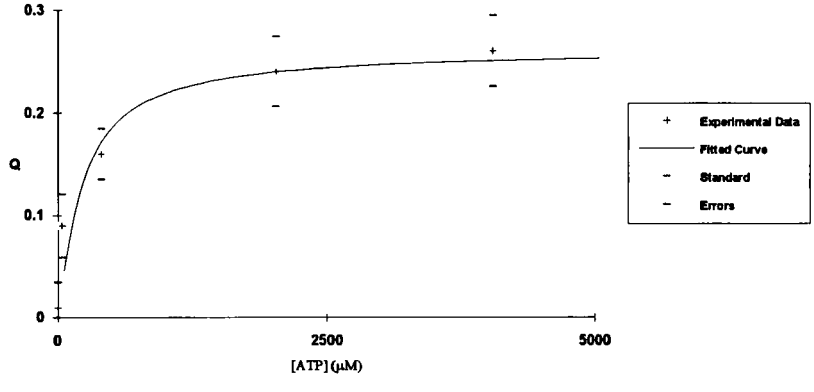
Figure 1



The initial luciferin concentration was 5.0 nM. The ATP concentration was 400 μM. As luciferin concentration increased from 0.13 μM to 5.0 μM the observed Q increased from 0.14 to 0.66. The relationship was non-linear.

The dependence of Q on ATP concentration is shown in Figure 2. The initial luciferin concentration was 5.0 nM. The luciferase concentration was 250 nM. As the ATP concentration increased from 0.4 μM to 4.0 mM the value of Q increased from 0.01 to 0.26. The relationship was non-linear.

Figure 2



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DISCUSSION

To explain changes in quantum yield it is postulated that the monomeric form of the enzyme binds both ATP and luciferin but that this path leads to products without the emission of light, whereas the dimer binds two ATP and luciferin molecules and produces light.

$$D{:}(ATP)_2 \xleftrightarrow{K_1} 2ATP + D \quad \text{.....1}$$

$$D \xleftrightarrow{K_2} M \quad \text{.....2}$$

$$M{:}(ATP) \xleftrightarrow{K_3} M + ATP \quad \text{.....3}$$

$$D{:}(ATP)_2 + L + O_2 \rightarrow D{:}(AMP)_2{:}OL + CO_2 + h\nu + 2PP_i \quad \text{.....4}$$

$$M{:}(ATP) + L + O_2 \rightarrow M{:}(AMP){:}OL + CO_2 + PP_i \quad \text{.....5}$$

M	= luciferase monomer
D	= luciferase dimer
$D{:}(ATP)_2$	= luciferase dimer with bound ATP
M:(ATP)	= luciferase monomer with bound ATP
L	= luciferin
OL	= oxyluciferin
K_1, K_2, K_3	= equilibrium dissociation constants.

The equilibria of equations 1, 2 and 3 are established rapidly compared to the overall rate of reaction. Since both luciferase and ATP are in excess relative to luciferin, the position of the equilibria will not be affected by the turnover of the enzyme or the luciferin concentration. Under these conditions Q is related directly to the fraction of luciferase in the dimeric state, assuming monomer and dimer have identical reaction rates:

$Q = D/E_0$ where E_0 = total luciferase concentration

As enzyme concentration is increased, the fraction of enzyme in the dimeric state will increase, which in turn leads to an increased quantum yield. Similarly, as the ATP concentration is increased, so the amount of $dimer{:}(ATP)_2$ complex relative to monomer:(ATP) complex will increase since the two molecules of ATP bind to dimer while only one binds to monomer.

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These assumptions and equations 1-3 predict that the expected quantum yield is related to the luciferase concentration, the ATP concentration and the three equilibrium constants K_1, K_2 and K_3 by the expression:

$$Q.[E_0]+\frac{(K_3+[ATP])}{K_3}.\sqrt{\frac{K_1.K_2.[E_0].Q}{2(K_1+[ATP]^2)}}+[E_0]=0 \qquad .....6$$

Figs 1 and 2 show the experimental data and predictions from equation 6 using values for $K_1 = 1540 \pm 3245$ μM^2, $K_2 = 23.9 \pm 0.01$ μM and $K_3 = 197.4 \pm 0.01$ μM. These three parameters were varied to obtain the best fit to both sets of data. The values given correspond to a $\chi^2 = 0.89$. K_1 has a large error because K_1 is small relative to $[ATP]^2$, so changing K_1 and K_2 values had little effect on the goodness of fit providing $K_1.K_2$ is a constant. If K_1 is assumed to be negligible relative to $[ATP]^2$, the best fit values obtained were $K_1.K_2 = 1151.2 \pm 0.2$ μM^3, $K_3 = 26.4 \pm 0.1$ μM, $\chi^2 = 1.08$.

It is conceivable that the monomer has a low but finite light-emitting activity. However from Fig. 1. the quantum efficiency of the monomer cannot be greater than 0.08 while the quantum yield of the dimer cannot be less than 0.80 (maximum observed quantum yield). Since the model already gives a $\chi^2 < 1$, inclusion of further variables is not justified.

Previous evidence for activity of only dimeric luciferase comes from the observation that at low enzyme concentrations, specific activity is non-linear with enzyme concentration in the range 0.18 to 18 $\mu g/ml$[4]. The same authors show that at 0.025 mM ATP the apparent monomer-dimer equilibrium constant was 15 nM, reducing to 5 nM with 1.28 mM ATP. This was interpreted as a conformational change caused by ATP binding, but may also be explained in terms of an ATP induced perturbation of the monomer-dimer equilibrium.

The proposed reaction mechanism may be explained by a de-excitation pathway in the monomeric form of luciferase which is prohibited in the dimer. One possibility is that solvent is allowed free access to the monomer active site, while in the dimer, the active site is protected from solvent molecules. Thus non-radiative de-excitation to solvent would be possible in the monomer but not the dimer. This suggests that the active sites of luciferase dimer are located at the monomer-monomer interface.

A second possibility is that only dimer can provide the reaction pathway leading to the formation of dioxetanone. It is believed[5,6] that dioxetanone loses CO_2 by thermal excitation to create the excited state of oxyluciferin responsible for luminescence.

It is interesting to speculate that the enzyme is likely to be exclusively dimeric in the firefly since the enzyme is membrane associated *in vivo*. It seems unlikely that the firefly

might use modulation of the monomer-dimer equilibrium in order to control its 'flashing' behaviour since this would represent energetically sub-optimal behaviour.

For the purposes of exploiting this reaction as an analytical tool or its use as an amplifier or memory device it can be concluded that quantum yield is very high only under conditions shown in Figure 1. The proposed reaction scheme can account quantitatively for the dependence of Q on the concentrations of ATP and enzyme.

It is clear that in any application using luciferase as a component of a molecular electronics device this effect will limit the minimum possible unit size. If a device is to use single molecules as individual array elements it will be necessary to cross-link or otherwise position monomers into permanent dimers to ensure complete activity. Alternatively it may be possible to manipulate the monomer-dimer equilibrium in order to switch individual luciferase molecules between 'ON' and 'OFF' states, providing the basis for a molecular switch.

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