Enhanced Enzyme Diffusion
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Introduction

With fluorescence correlation spectroscopy, the diffusivity of enzymes is observed (1) to increase in a substrate-dependent manner during catalysis. When excited to laser heating, green fluorescent proteins have been shown to blink in response (2) to local temperature and pH changes. However, a similar effect has not been attributed to the heat exchanged in an enzyme-catalyzed reaction even though enzymes, like catalase, release enough heat to unfold a proteins.

In this regard, single-molecule fluorescence correlation spectroscopy suggests catalase enhanced diffusion is caused by the enzyme undergoing the chemoacoustic effect depending on the heat released during the chemical reaction. The chemoacoustic effect is thought to generate an asymmetric pressure wave produced at the catalase substrate interface that displaces the catalase relative to the H2O2 substrate as illustrated in Figure 1.

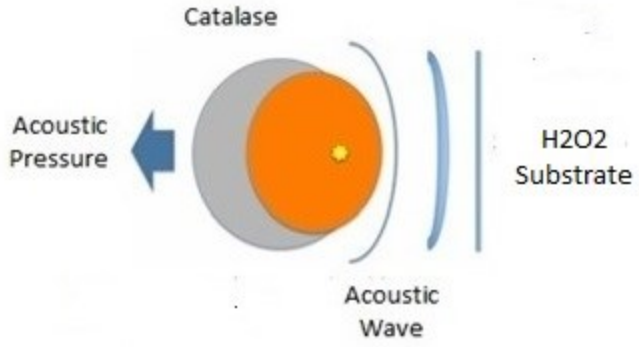

Figure 1. Chemoacoustic Effect

Alternatively, other mechanisms (2,3) that might account for enhanced catalase diffusion include global temperature increase of the solution, charged product induced electrophoresis, and pH changes around the catalase immediately following catalysis. Indeed, the diffusion coefficient of catalase, which mediates the conversion of H2O2 into water and oxygen, also increases in a substrate-dependent manner. Similarly, global or local temperature changes of the solution may explain the enhanced catalase diffusion phenomenon. More recently, the catalase diffusion coefficient increase arises from chemotactic behaviour in which the catalase preferentially diffuses towards higher H2O2 gradients. Indeed, experiments (1) show that when enzymes catalyze reactions, the heat released in the process is thought responsible for accelerating the proteins as observed by fluorescence spectroscopy.

Proposal

In this regard, the diffusion of enzymes in their substrate is proposed to be the electrostatic charging of enzymes by simple QED to explain enhancement. Simple QED relies on real photons (4) and differs from the virtual photons in Feynman's QED. Simple QED based on QM was developed for nanoscale heat transfer and is applicable to nanoscale globular proteins. QM stands for quantum mechanics. Taking catalase as a representative enzyme, the heat Q of the chemical reaction places interior atoms under high EM confinement that by the Planck law of QM denies the catalase the heat capacity to allow conservation of Q heat to proceed by an increase in temperature. Instead, the heat of the reaction is conserved by creating EM radiation E, typically beyond the UV as illustrated in Figure 2.

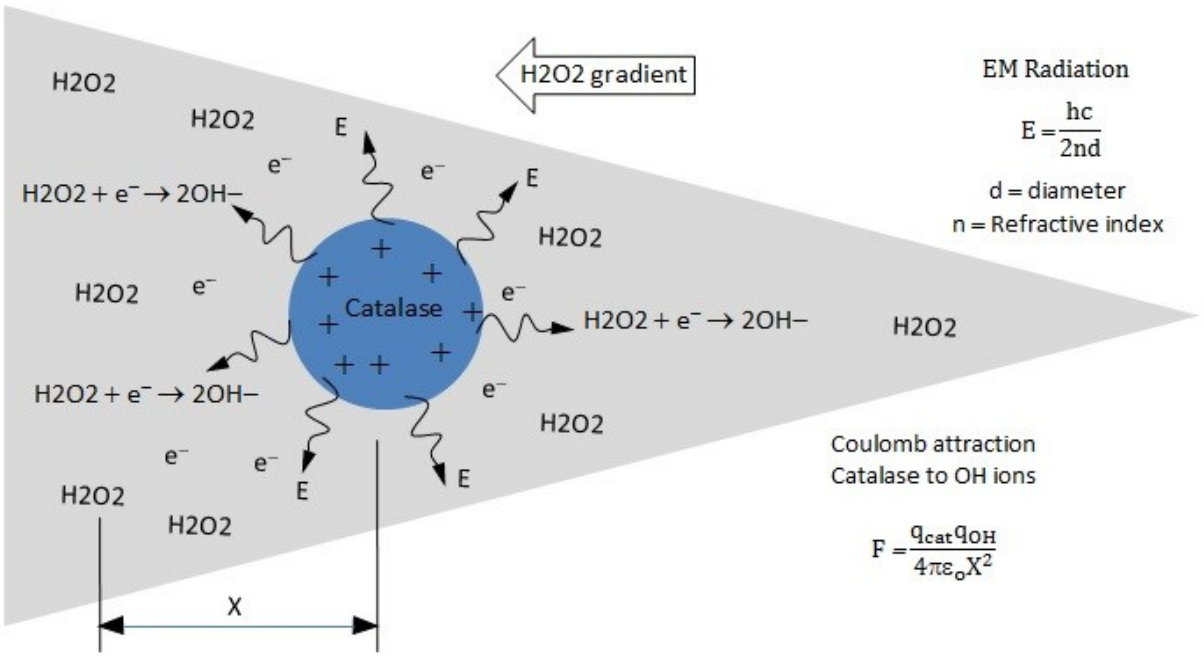

Figure 2. Simple QED in enhanced diffusion of catalase

In application to enhanced diffusion, simple QED induces EM radiation E beyond the UV that removes electrons e^- from the otherwise neutral pH ~ 7 catalase to produce a positive charged + catalase protein. The Planck energy E of simple QED corresponds to a EM wave standing across the diameter d of the catalase protein, E = hc/2nd, where h is Planck's constant, c the speed of light, and n the refractive index of the protein. For catalase, d ~ 10 nm and for n ~ 1.5, E ~ 40 eV which is in the EUV and sufficient to ionize the protein atoms as shown in Figure 3.

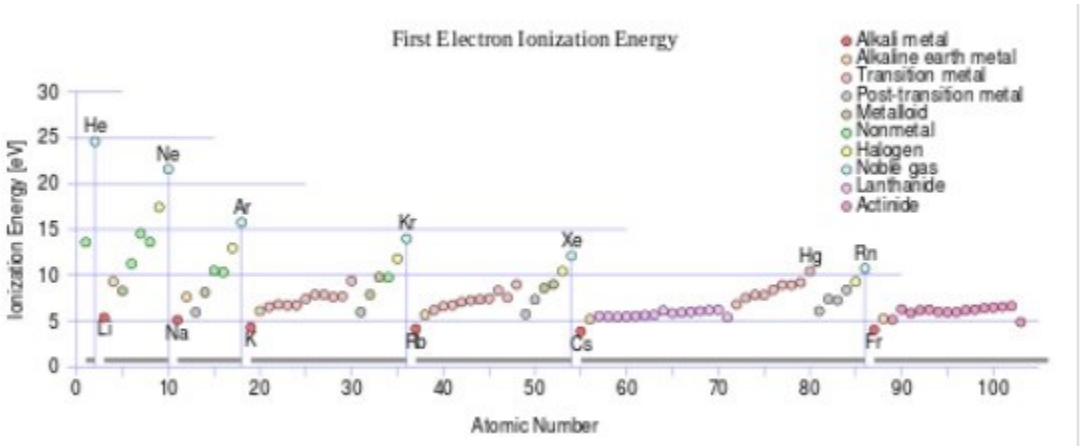

Figure 3. Ionization Potentials

But 40 eV > most elemental ionizations. Nevertheless, lower quantum states of molecules and surface plasmons having E < 40 eV are excited by fluorescence.

Since the H2O2 substrate having an acidic pH < 5 is naturally charged positive, diffusion of charged catalase at pH ~ 7 is not electrostatically enhanced. But in the presence of a H2O2 substrate concentration gradient, catalase is found (2) to diffuse toward areas of higher substrate concentration.

What this suggests is Coulomb attraction controls enzyme diffusion, the proposal: first requiring simple QED to create EM radiation from heat Q released by the chemical reaction to remove electrons and charge the catalase enzyme positive, and second the delocalized electrons dissociate H2O2 to $2OH^-$ hydroxyl radicals. The Coulomb attraction is noted in Figure 2. In the H2O2 gradient, more OH^- radical dissociation occurs in the higher H2O2 concentration region, thereby allowing electrostatic attraction to enhance enzyme diffusion in the direction of higher H2O2 concentration.

Analysis The analysis presented in support of the proposed simple QED explanation of enzyme diffusion is based on the experimental data (2) and Supporting Information for catalase shown in Figure 4.

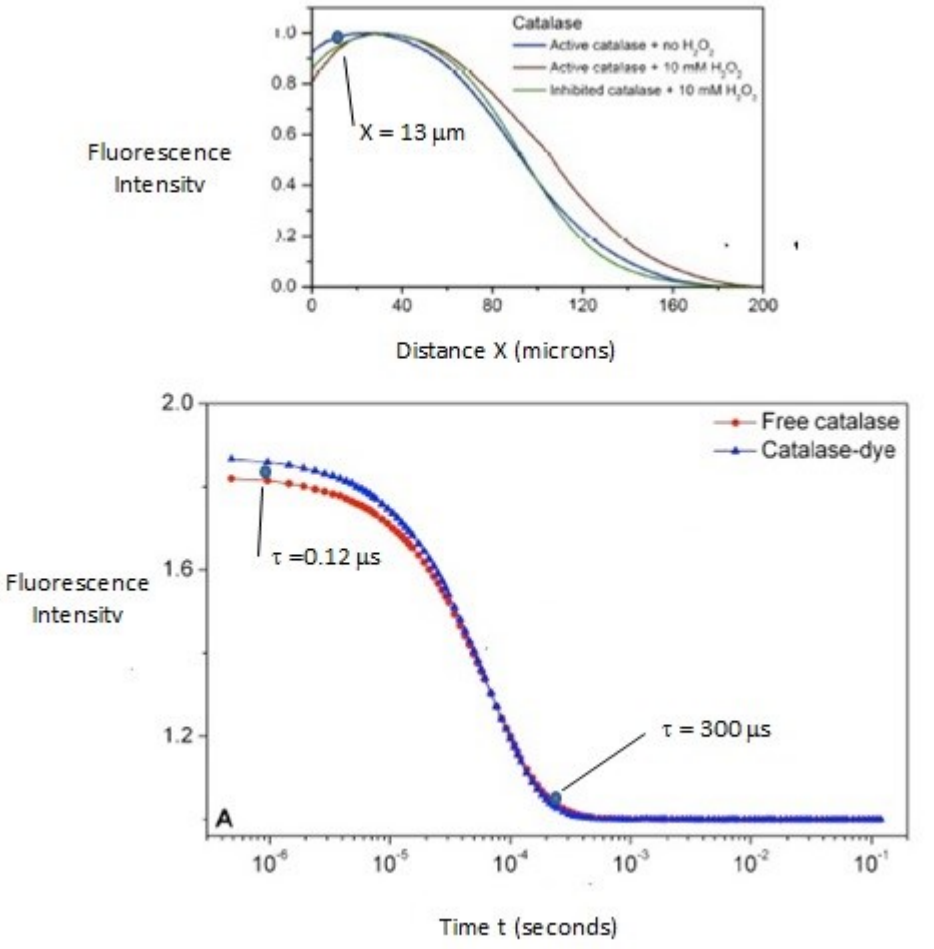

Figure 4. Catalase FCS Response

To initiate enzymatic diffusion, H2O2 upon reacting with the catalytic site liberates (2) heat Q by,

$$Q = nk_{cat}\frac{\Delta H}{N_{avag}}$$

where, n is number of catalytic sites per enzyme molecule, n = 4 per catalase, k_{cat} the catalase turnover, $k_{cat} = 2.12 \times 10^5$/ s, ΔH the enthalpy change in the reaction ΔH = -100 kJ/ mol, and N_{avag} is Avogadro's number, $N_{avag} = 6.023 \times 10^{23}$ /mol giving the heat Q = 1.41 x10^{-13} J/s.

But how does the heat enhance enzyme diffusion?

Currently, the heat Q of the chemical reaction is thought conserved (1) by the chemoacoustic effect generating an asymmetric pressure wave to displace the catalase. But (2) suggests the temperature change in water surroundings is insignificant suggesting the chemoacoustic effect is likely negligible in enhanced enzyme diffusion.

In this paper, QM precludes conservation of the heat Q by an increase in catalase temperature, and instead, simple QED photons beyond the UV are created inside the catalase that remove electrons to charge the catalase positive. Hence, the positive charged catalase is electrostatically attracted to the momentary electron cloud giving the impression of enhanced diffusion.

The number N_p of E = 40 eV simple QED photons is, $N_p = Q / E = 1.41 \times 10^{-13} / (40\ e) = 22{,}000$/s, where e = 1.6 x 10^{-19} J/eV. But the number N_{atom} of atoms in catalase is, $N_{atom} = 4\pi(R/\Delta)^3/3$ where R is the catalase radius and Δ the cubical dimension of atomic volume. For R ~ 5 nm and Δ ~ 0.25 nm, N_{atom} ~ 33,500. Hence, simple QED radiation at 40 eV ionizes all catalase atoms in about 1.5 s.

The delocalized electrons move outward and attach to H2O2 molecules, thereby producing OH^- radicals. But beyond photo dissociation of neutral H2O2, the anion state of H2O2 involving O^- leads (4) to the formation of OH^-. Regardless, the number of negative charged delocalized species is equal to the number of positive charged catalase atoms, and therefore the distance X between the catalase and the negative charged electron cloud is estimated by Coulombs law,

$$X = \frac{N_{atom} e}{\sqrt{4\pi\varepsilon\varepsilon_o F}}$$

where, ε_o is the permeability, $\varepsilon_o = 8.854 \times 10^{-12}$ C^2/Nt-m^2, and in water ε = 78. From (2), the typical force F ~ 20 x10^{-12} Nt gives X ~ 13 μm which is only a fraction of the full response. Nevertheless, the catalase moves X ~ 13 μm to recombine with the delocalized electron cloud to once again allow the heat Q to repeat the process and increase the X position, i.e., the force F is reduced and X is incremented, say by 10 μm to X = 23 μm. In effect, the catalase in small increments 'swims' up the H2O2 gradient in the direction of higher H2O2 concentration. The initial X increment at F = 20 x10^{-12} Nt and X = 13 μm is noted in Figure 4.

If the force F = 20 x10^{-12} Nt is constant until reaching X = 200 μm, the acceleration a of the catalase molecule, a = F/m, where $m = 4\pi\rho R^3/3$. For ρ = 1400 kg/m^3, m = 7.3 x 10^{-22} kg giving a = 2.7 x 10^{10} m/s^2. Hence,

$$\tau = \sqrt{\frac{2X}{a}}$$

Figure 4 notes τ = 1.2 x 10^{-7} s ~ 0.12 μs, but the experimental FCS data shows X = 200 μm requires τ > 300 μs. To reach X = 200 μm requires F ~ 3 x 10^{-18} Nt << 20 x10^{-12} Nt which is not be correct.

The calculations show the force F cannot reach 200 μm in one step. Increments $X < 20$ μm are possible, but require many repetitive steps of ionization and recombination of the catalase molecule. It is unlikely a single catalase ionization of all 33,500 atoms producing the electron cloud that attracts the positive charged catalase can occur more than once. Indeed, the inactivation (5) of catalase occurring near pH 4.5 suggests the pathway for catalyst inactivation is by protonation. Hence, simple QED that induces catalytic heat to create charged atoms may be a valid explanation of FCS data only for a single catalase ionization.

Is it possible that simple QED could explain FCS measurements by another mechanism?

Discussion

FCS experiments generally show substrates enhance diffusion if the enzyme is catalytically active. However, many factors (6) contribute to the FCS signal that can give the impression of an increase in diffusion: the dissociation of enzyme oligomers upon interaction with the substrate, surface binding of the enzyme to glass during the experiment, conformational changes upon binding, and quenching of the fluorophore. Of these factors, simple QED offers an alternative to the dissociation of the catalase complex. Low catalase concentrations are typical for FCS measurements, but are close to the dissociation constants. So, the fluorophore significantly smaller than the parent enzyme diffuses much faster.

Simple QED requires a source of heat from enzyme catalysis. However, FCS measurements (5) of aldolase that is endothermic and does not produce catalytic, yet still shows enhanced diffusion. Later, aldolase was found (7) not to have enhanced diffusion ruling out simple QED as an explanation of enhanced diffusion. Because of this, aldolase was proposed (7) caused by a photo physical artifact other than simple QED. But FCS requires laser excitation to produce the fluorescence which is a source of heat that by simple QED may excite the enzyme complex, e.g., see optical emissions (4) by heating nanoparticles. Data is not available to support the argument that laser heating in FCS to initiate the fluorescence is the cause of enhanced enzyme diffusion.

Because of the artefacts in FCS, NMR has been proposed (8) as an alternative to the measurement of enhanced diffusion. NMR showed the active enzyme aldolase did not show enhanced diffusion even in the presence of its inhibitor pyrophosphate. To explain why FCS measurements differed from NMR, the dissociation of aldolase under substrate conversion was shown to occur because the low concentrations used in FCS lead to enzyme dissociation. Moreover, the aldolase sample required purification (8) of unbound fluorophores prior to the NMR measurements.

A recent DLS (7) study also finds no diffusion enhancement of aldolase. DLS strands for dynamic light scattering. DLS differs from FCS in that it relies on the measurement of scattered light and not absorbed light. The detected light in DLS does not originate from a fluorophore attached to the molecule of interest, but is light scattered by the molecule itself. While DLS spectra are typically acquired at nano molar concentrations, in FCS only a few fluorescently labeled molecules occupy the excitation volume. What this means is the molecules in FCS absorb more laser energy and are likely to dissociate by simple QED than in DLS where laser energy is scattered and not absorbed. Enzyme dissociation by simple QED is therefore a valid explanation of enhanced diffusion.

Conclusions

FCS measurements of enhanced catalase diffusion are caused by dissociation of the fluorophore from the catalase complex upon ionization from catalytic heat conserved by simple QED. Low concentrations of catalase used in FCS leading to enzyme dissociation are not necessary. The removal of electrons by simple QED produces a momentary negative charged cloud that electrostatically induces the dissociated fluorophore to diffuse faster than the larger catalase complex giving the false impression of enhanced catalase diffusion.

NMR measurements avoid the fluorophore dissociation in FCS even if the enzyme is not catalytically active. In aldolase, NMR does not show enhanced diffusion as laser heating is not required. DLS does not show enhanced aldolase diffusion because the laser radiation is scattered and not absorbed to produce ionization by simple QED.

Experimental verification in FCS enhanced enzyme diffusion is suggested to clarify simple QED is the mechanism of fluorophore dissociation.

References

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