Modeling single-molecule enzyme kinetics based on Planck's radiation formula and the principle of enthalpy-entropy compensation.

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The prosthetic group of cholesterol oxidase (COx), FAD, is fluorescent when oxidized and non-fluorescent when reduced, making it possible to monitor the oxidation-reduction catalytic cycle of a single molecule of COx fluorometrically. H. Lu et al [*Science* **282**:1877-1882 (1998)] report that the on-times, the times that COx wait before being reduced, are distributed asymmetrically over a wide range from 10 to 2,300 ms, depending on the concentration of cholesterol. We find that the on-time distributions of Lu eta al fit the equation,

$$P(w) = (a/w^5) \exp(-b/w) + c(w) \quad \ldots\ldots\ldots\ldots\ldots (1)$$

where P(w) is the probability of the occurrence of waiting time, w, a and b are constants, and c(w) is a correction term that depends on w.

The first term of the right-hand-side of Eq. (1) is of the same form as Planck's radiation law that predicts the probability, P_lambda, of radiation of a black body as a function of wavelength, lambda, at a given temperature, suggesting the following isomorphism between black-body radiation and enzymic catalysis: i) P_lambda = P(w), ii) lambda = w, iii) E = Delta H*, iv) Ultraviolet catastrophe = 'Catalytic catastrophe', and v) Energy quantization (photons) = 'Conformational energy quantization' (conformons), where E is the energy of radiation, Delta H* is the activation enthalpy of the enzyme-substrate complex, and conformons are the mechanical energy stored in sequence-specific sites in biopolymers driving all non-random molecular motions in the cell [S. Ji, BioSystems **54**: 107-130 9(2000)]. Based on Equalities i) through iii), which are supported by firm experimental data, it has been postulated (a) that the Lu et al's waiting time distributions embody, in analogy to the ultraviolet catastrophe, the "catalytic catastrophe" defined as the precipitous drop in the probability of catalysis when the activation enthalpy decreases below a critical threshold, and (b) that, just as energy quantization removed the ultraviolet catastrophe, the 'catalytic catastrophe' can be removed by quantizing the conformational free energy. Quantized energies in physics are referred to as photons. The 'quantized conformational energy' postulated to underlie catalysis was previously referred to as conformons [S. Ji, Ann. N. Y. Acad. Sci. **227**:211-226, 419-437 (1974)].

The c(w) term in Eq. (1) can be accounted for in terms of entropy-dependent regulatory mechanisms of catalytic rates which are thought to involve the exchange of the local conformational disorder (Delta s*) at its active site with local conformational order (- Delta s*) imported from elsewhere in the enzyme when the enzyme is activated, leading to little or no net change in the over-all activation entropy of the enzyme-substrate complex, Delta S . This is analogous to the garage door whose gravitational potential energy is converted into the mechanical potential energy of its spring and vice versa during its operation cycle of opening and closing (the "garage-door principle of enzymic catalysis" (GDPEC)). It is suggested that GDPEC can be viewed as the 'entropic version' of the enthalpy-entropy compensation principle, Delta G = Delta H – T Delta S, since it can represent GDPEC when Delta H = 0, and Delta S = Delta s' + Delta s'', where s' and s'' are the oppositely changing (or mutually compensating) entropies of its active site and an allosteric site mechanically coupled to it.