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Michaelis-Menten is dead, long live Michaelis-Menten!

Nils G Walter

Modern single-molecule tools, when applied to enzymes, challenge fundamental concepts of catalysis by uncovering mechanistic pathways, intermediates and heterogeneities hidden in the ensemble average. It is thus reassuring that the Michaelis-Menten formalism, a pillar of enzymology, is upheld, if reinterpreted, even when visualizing single turnover events with a microscope focus.

The seminal 1913 discovery of Leonor Michaelis and Maud Menten[1] arguably represents the beginning of enzyme kinetics as a systematic field and remains a pillar of enzymology. Thousands of enzymes have been characterized using the Michaelis-Menten formalism, which describes the rate of multiple enzymatic turnovers as a function of substrate concentration (**Fig. 1**). Close to a hundred years of successful, largely undisputed use does not imply, however, that modern insight cannot add exciting new twists. In this issue of *Nature Chemical Biology*, Sunney Xie and co-workers at Harvard[2] have employed creative single-molecule microscopy to ask the simple question of whether the Michaelis-Menten formalism is upheld even when visualizing catalysis one substrate molecule at a time. The answer is yes, but the microscopic interpretation changes in light of enzymatic heterogeneity.

Michaelis and Menten showed that invertase (now known as β-fructofuranosidase), a yeast enzyme central to sugar metabolism that catalyzes the hydrolysis of sucrose into an optically distinct mixture of glucose and fructose ('invert sugar'), has a characteristic hyperbolic dependence on substrate concentration. Two regimes can be distinguished under conditions where substrate is in excess over enzyme (multiple-turnover conditions) (**Fig. 1**). At limiting (low) substrate concentration, the measured rate constant increases linearly with substrate concentration, indicating that reversible substrate binding (k_{on}, k_{off}) is mostly rate limiting. At saturating (high) substrate concentration, the measured rate constant is independent of substrate concentration and the catalytic turnover (k_{cat}) becomes fully rate limiting.

Applying single-molecule microscopy to β-galactosidase, a bacterial enzyme essential for sugar utilization and a modern enzymological work horse, the Harvard group has now taken a closer look at these two regimes (**Fig. 1**). A derivative of the enzyme's lactose substrate yields brief fluorescent bursts upon hydrolytic turnover before the fluorescent product diffuses out of a laser focus. A succession of turnovers by a single immobilized enzyme thus yields a meteor shower of fluorescence bursts. Kinetic information on multiple turnovers by a single enzyme is derived from a large number of waiting times between two successive fluorescence bursts. The authors find that the average waiting time of a single enzyme molecule plotted against the inverse of the substrate concentration recapitulates the linear Lineweaver-Burke relationship observed in an ensemble measurement. This demonstrates that the Michaelis-Menten equation holds even at the single-molecule level. The average waiting time from a large number of single enzyme molecules then is related to the macroscopic turnover rate constant.

At low substrate concentration, Xie and co-workers find that a single time constant characterizes the waiting times between substrate turnovers of a single enzyme. This implies that the limiting rate constants under these conditions, k_{on} and k_{off}, are uniform over long periods of time (**Fig. 1**). By contrast, at high substrate concentration the waiting times between substrate turnovers show an asymmetric probability distribution. This implies that the limiting rate constant under these conditions—the catalytic turnover rate constant, k_{cat}—varies over time for an individual enzyme (**Fig. 1**). Although the molecular basis for such catalytic heterogeneity is unclear, Xie and co-workers propose that conformational isomers of the enzyme are the cause. The broad distribution of k_{cat} values (referred to as χ^2 in the context of a single-molecule observation) (**Fig. 1**) suggests that large numbers of such conformers with highly variable catalytic powers exist for a single enzyme, and interconvert only slowly (as compared to the catalytic turnover rate). Such slow interconversion is also referred to as a 'memory effect', in the sense that each enzyme molecule has a memory of its conformational state and retains it for some time longer than the turnover time; such single enzymes are described as showing dynamic disorder, indicating that they display various conformational states that are not static but slowly interconvert. From the autocorrelation function of the fluorescence bursts observed for single enzymes at high substrate concentration (that is, the correlation of the fluorescence time trace against a time-shifted version of itself), Xie and co-workers were able to extract time constants for these conformational isomerizations, which themselves show a broad distribution ranging from milliseconds to tens of seconds. The good agreement between this distribution and the known range of timescales of conformational fluctuations in proteins[3] further supports the notion that catalytic heterogeneity is caused by (dynamic) conformational heterogeneity.

What consequences does all this heterogeneity at the single-enzyme level have for the Michaelis-Menten formalism? The good news is that the Michaelis-Menten equation as a phenomenological description still holds. Yet our interpretation of the extracted k_{cat} rate constant has to be significantly revised. More specifically, the k_{cat} (or, formally, χ^2) value derived at saturating substrate concentration turns out to be the weighted harmonic mean of the different catalytic turnover rate constants represented in the single enzyme over time. Consequently, the Michaelis constant

Nils G. Walter is in the Department of Chemistry at the University of Michigan, 930 North University Avenue, Ann Arbor, Michigan 48109-1055, USA.
e-mail: nwalter@umich.edu

$K_M = (k_{cat} + k_{off})/k_{on}$ (or the equivalent, C_M, for a single molecule, where χ^2 substitutes for k_{cat}) also acquires an ensemble-averaged meaning.

Why is such a new, 'microscopic' interpretation of the Michaelis-Menten formalism important? First, the underlying single-enzyme behavior may well be a universal phenomenon, as conformational and catalytic heterogeneity has been observed for diverse enzymes, including cholesterol oxidase[4], lipase[5], λ-exonuclease[6] and the hairpin ribozyme[7]. Second, this behavior is likely to be biologically relevant, as heterogeneity becomes more obvious the less ensemble averaging occurs (an effect that led to the discovery of heterogeneity in single-molecule experiments in the first place). In small volumes with few enzyme molecules of one kind, such as the cytosol of a living cell, this heterogeneity may therefore have a significant role in determining biological function, especially if an enzyme at the tip of a catalytic cascade is affected. To test this idea, *in vivo* studies of catalysis by single enzymes are greatly needed. Third, this single-enzyme behavior focuses our attention on the molecular basis of the conformational differences underlying the catalytic heterogeneity. For example, the enzyme studied by Xie and co-workers, β-galactosidase, is actually a homotetramer with four well-separated and independent active sites[8], all of which must contribute to the observed heterogeneity. This raises the intriguing question of whether and how the four active sites are coordinated in their heterogeneity. The discovery of catalytic signatures of conformational heterogeneity even in as well-characterized a formalism as that of Michaelis and Menten lays the foundation for ultimately tracking down its molecular origin. Perhaps such an accomplishment will be easier for ribozymes, where the conformational isomers are considerably longer lived[7,9], plausibly as a result of the higher thermodynamic stability of secondary structure in RNA as compared to protein.

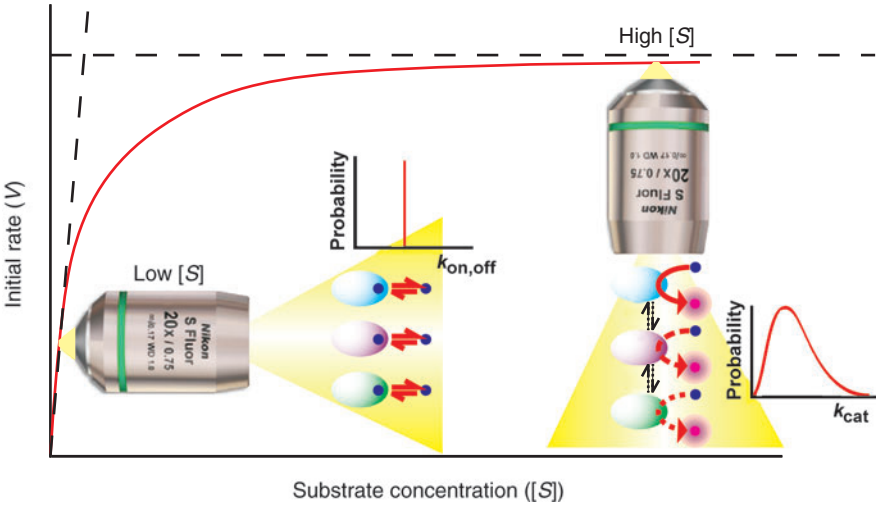

Figure 1 Focusing in on the hyperbolic Michaelis-Menten relationship between enzymatic rate and substrate concentration under multiple-turnover conditions[2]. Applying single-molecule microscopy to β-galactosidase, Xie and co-workers have taken a close look at the low- and high–substrate-concentration regimes. At low substrate concentration, the waiting times between substrate turnovers are characterized by a single time constant, which reflects uniform substrate-binding and -dissociation rate constants, k_{on} and k_{off}, respectively. By contrast, the waiting times in the high-substrate-concentration regime show an asymmetric probability distribution. This finding implies that the catalytic turnover rate constant k_{cat} varies over time in an individual enzyme as a result of slowly interconverting conformational isomers.

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Sweet insights into learning and memory

Gerald W Hart

Studies have linked the Fucα(1-2)Gal modification of neuronal glycoproteins to cognitive processes such as learning and memory. The identification of synapsins Ia and Ib as the main Fucα(1-2)Gal-bearing neuronal proteins not only suggests previously unknown molecular mechanisms of neuronal plasticity but also indicates the existence of previously unknown glycosylation pathways in neurons.

Neuronal plasticity, which is key to learning and memory, is the remarkable ability of neurons or neuronal networks to dynamically alter their biochemical, physiological or morphological properties in response to intrinsic or extrinsic input[1]. Over the past decade, several groups have used glycan-specific antibodies and specific fucosylation inhibitors, in combination with behavioral studies in animals, to suggest that the increased modification of brain proteins by sugars terminating in Fucα(1-2)Gal residues correlates with improved learning and memory and with increased synapse formation[2,3]. However, almost nothing is known about the nature of the modified proteins or about the possible molecular mechanisms by which such sugar modifications could influence neuronal communication. In the latest issue of the *Proceedings of the National Academy of Sciences (USA)*, Murrey, Hsieh-Wilson *et al.*[4] use synaptic vesicle purification, immunoblots and gel electrophoresis, combined with

Gerald W. Hart is in the Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205-2185, USA.
e-mail: gwhart@jhmi.edu