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Enzymes in Coherent Motion

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Abstract

A single-molecule approach reveals coherence in long trajectories of enzyme conformational dynamics.

Subtle conformational changes often play a crucial role in enzyme functions, and enzyme conformations are highly dynamic. The use of only a static structural characterization from an ensemble-averaged measurement at equilibrium is therefore often inadequate for predicting dynamic conformations and understanding correlated enzyme functions. Single-molecule approaches (1), which investigate individual molecules under specific physiological conditions, are a powerful tool for characterizing and analyzing complex enzymatic reaction dynamics and correlated conformational dynamics. On page 319 of this issue, Choi *et al.* (2) report a novel approach to probing single-molecule conformational dynamics that reveals the conformational motions of the enzyme's active site during enzymatic reaction turnovers.

The authors studied T4 lysozyme, an enzyme that catalyzes the hydrolysis of the glycosidic bonds in the polysaccharide found in bacterial cell walls (3). During the enzymatic reaction, an active substrate-enzyme complex forms, followed by chemical transformation and product release (see the figure, panel A). Choi *et al.* attached the T4 lysozyme molecule to a single-walled carbon nanotube (SWNT) device (see the figure, panel B). In their single-molecule enzymatic reaction assay, enzyme molecular motions create changes in electrostatic potentials that can be converted and amplified into dynamically changing electron fluxes. Using this approach, the authors were able to record hinge-bending motions of the T4 lysozyme active site under enzymatic reactions with high time resolution and signal clarity.

The authors detected two different electronic signals caused by the lysozyme hinge-bending motions: Slow signal oscillations result from the transduction of catalytic turnover events at ~15 Hz, and fast signal oscillations correspond to nonproductive binding events at ~330 Hz. These results are in good agreement with earlier results from single-molecule fluorescence resonance energy transfer (smFRET) spectroscopy under enzymatic reactions (see the figure, panel C) (4–6). This is encouraging, as the conclusions are reached from very different technical approaches that probe electronic and photon signals, respectively.

More intriguingly, both this work (2) and previous single-molecule spectroscopic analyses (5, 6) suggest that the hinge-bending motions in T4 lysozyme show intermittent coherent conformational dynamics. The reported enzyme dynamics imply that active-site conformational motion times tend to bunch in a narrow time window (5–7). The dynamics reported for enzymes (2, 5–10) are likely common in the conformational dynamics of other proteins, especially those that perform conformation-gated functions. Substrate-enzyme binding and substrate-enzyme complex formation serve as a driving force and negative-entropy source for the coherent dynamics at the single-molecule level. Accordingly, the

conformational motions are driven by the local environment, such as electrostatic field, and hydrophobicity at the active site of the enzyme (2, 4–7).

The results reported by Choi *et al.* show that SWNT electronic detection is complementary to smFRET measurements. For example, with their SWNT-based approach, Choi *et al.* were able to overcome signal bleaching (11), a typical barrier for single-molecule spectroscopic measurements. This enabled them to record trajectories of hundreds of hinge-bending open-close motion events under enzymatic reaction conditions. The same approach could be used to explore the dynamics of other molecules with charged functionalities.

In the future, it will be critical to probe the conformational change dynamics at a time scale of nanoseconds or even picoseconds, because many important protein conformational motions occur at the nanosecond time scale. (The reported results are in the time scale of minutes to microseconds.) Furthermore, a suite of single-molecule experiments will be needed to probe different aspects of the complex reaction dynamics and associated conformational dynamics of enzymes. In recent years, such a combined approach has yielded detailed mechanistic information about enzymatic conformational dynamics, including structures of the intermediate enzyme-substrate complex and the associated energy landscape.

A particularly promising technique uses correlated mechanical force to perturb enzyme conformational dynamics and reactivity, thereby allowing enzyme activities to be manipulated in order to explore new enzyme properties and obtain fundamental understanding of enzymatic dynamics (12–15). The next generation of single-molecule approaches will be capable not only of probing complex single-molecule protein dynamics but also of manipulating protein conformational dynamics and controlling protein activities.

References and Notes

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1. . Single-molecule enzyme studies

Active-site conformational motions are essential for the catalytic function of T4 lysozyme (**A**). Through open-close motions, the enzyme adjusts its physical and chemical flexibility toward an active state (ES^*), which consists of the binding complex of an enzyme (E) and a substrate (S) for a specific catalytic reaction. Choi *et al.* now report SWNT electronic measurements of single-molecule T4 lysozyme conformational dynamics (**B**), which allow longer trajectories to be recorded than with FRET spectroscopic studies (**C**).