SCIENCE CHINA
Chemistry

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https://doi.org/10.1007/s11426-023-1569-2

Single-molecule conductance measurements reveal a new catalytic mechanism of formate dehydrogenase

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Received March 12, 2023; accepted March 15, 2023; published online March 21, 2023

Citation: Zhong S, Yang Z, Guo X. Single-molecule conductance measurements reveal a new catalytic mechanism of formate dehydrogenase. *Sci China Chem*, 2023, 66, https://doi.org/10.1007/s11426-023-1569-2

Elucidating the functional mechanism of biological enzyme functions is always an important topic in biochemistry because of its great importance for understanding life activities and developing potential drugs. However, ensemble methods commonly used in the analysis of enzyme kinetics can only provide average information on a large number of molecules, mixing the information on other components that are irrelevant to the enzyme catalytic mechanism and thus covering up individual differences and possible transient intermediates [1]. The single-molecule technology, which detects only a single molecule, has unique advantages in acquiring enzyme catalytic mechanisms and has been applied to probe enzyme catalytic mechanisms in the last century [2]. It can (i) eliminate the interference of irrelevant components, (ii) avoid the shielding of the averaging information on the single-molecule behavior and (iii) observe real-time electrical or optical signal variations in the enzyme catalysis process with extremely superhigh temporal resolution (~17 μs). Furthermore, relying on real-time monitoring for a single molecule, the dynamic conversion relationships among different conformations can be directly visualized to provide the direct experimental evidence for the analysis of the reaction mechanism.

The coenzyme-dependent oxidoreductase formate dehydrogenase (FDH) was early considered to be the key enzyme in the coenzyme regeneration system in redox biosynthesis. In 1951, the Nobel Prize winner, Hugo Theorell, proposed the widely accepted Theorell–Chance catalytic mechanism that coenzyme-dependent oxidoreductases had transitions between active and inactive states [3]. This mechanism explained the dynamic process of enzymes and was crucial to understand the enzyme behavior. However, direct experimental evidence for this state transition is lacking. In addition, some studies have observed that the catalytic rate was significantly higher than the nicotinamide adenine dinucleotide (NADH) dissociation rate [4], suggesting a possible new catalytic mechanism. They offer an opportunity to further investigate the mechanism of FDH using the single-molecule technology.

In a recent article published in *Nature Catalysis* [5], Wenjing Hong, Baishan Fang, Binju Wang, and coworkers from Xiamen University used a single-molecule electrical technology to monitor the catalytic cycle of FDH in real time, and creatively proposed a new catalytic mechanism for FDH. They built a scanning tunneling microscope break junction (STM-BJ) (Figure 1a) and tested the conductance of single complex FDH–nicotinamide adenine dinucleotide (NAD^+), FDH–NADH, FDH and FDH–HCO_2^-, respectively. After that, the dynamic conversion process among five conductance states (T1, T2, T3, T4, and T5) in the FDH catalytic cycle was monitored in real time. The states corresponding to T1, T2, and T5 were confirmed and two new

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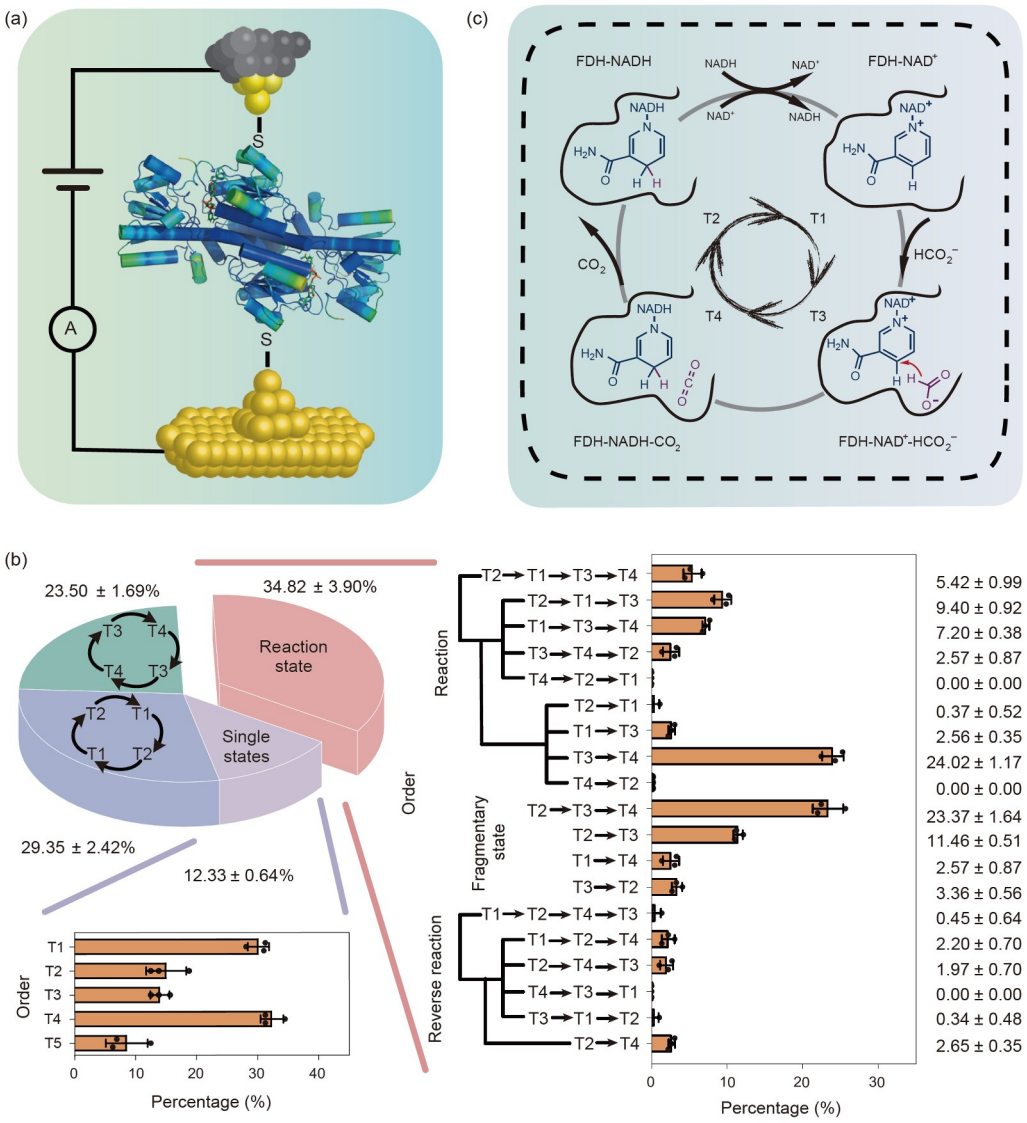

Figure 1 (a) Schematic diagram of a STM-BJ for measuring single-molecule FDH conductance. (b) Statistical analysis of the conductance change trajectory, revealing a T2→T1→T3→T4 catalytic process. (c) The new catalytic cycle mechanism of FDH. Reproduced from Ref. [5] with permission of Springer Nature (color online).

states (T3 and T4) were discovered according to the conductance in comparison with control experiments. By statistically analyzing the data, they found that the order of T2→T1→T3→T4 dominates the catalytic process that is different from the traditional Theorell–Chance mechanism and does not undergo the coenzyme dissociation state T5 (Figure 1b), suggesting the existence of a new catalytic mechanism. To further investigate this mechanism, various computational simulation methods including molecular dynamics (MD), quantum mechanics/molecular mechanics (QM/MM)–MD, and molecular mechanics/generalized Born surface area (MM/GBSA) were employed to simulate key steps in the catalysis process. They identified the structures of T3 and T4, proved that the π–π stacking effect can stabilize the combination of T3 with another molecule of oxidative coenzyme I, and proposed a mechanism by which the reduced coenzyme I in FDH could change to be oxidized coenzyme I through the *in-situ* transfer of negative hydrogen ions. Finally, a new catalytic cycle mechanism of FAD was established (Figure 1c).

In general, for the first time, they dynamically observed the catalytic reaction process of FDH at the single-molecule level, and combined statistical analysis and theoretical calculation techniques to propose a new catalytic cycle mechanism for FDH. This is a breakthrough innovation that challenges the traditional Theorell–Chance mechanism, which might revolutionize the traditional biological detection technology and provide a deeper understanding of

the enzymatic reaction mechanisms at the single-molecule level. These results build a useful single-molecule enzymatic detection platform, which provided endless opportunities for subsequent studies on single-molecule enzyme dynamics.

Conflict of interest These authors declare no conflict of interest.

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