Allosteric inhibition of individual enzyme molecules trapped in lipid vesicles

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AUTHOR SUMMARY

It has been known for a while that protein molecules may possess multiple conformational substates, which they randomly sample over time. The implications of these substates for protein function; e.g., enzymatic activity are still unknown. In recent years, single-molecule experiments have investigated the dynamics of enzymatic reactions, and found that proteins may reside in a given substrate for long periods of time. Such experiments showed that catalytic rates not only vary from one enzyme molecule to another (a phenomenon termed static disorder) (1), but also that these rates can fluctuate over time, as a protein transforms between substates (dynamic disorder) (2).

From a structural point of view, it is quite difficult to understand the slow transformations of proteins between substates. One way to gain insight into these processes is to investigate different functions of a protein and find their interrelationships. For example, one could study how the binding of an effector molecule to an enzyme at a site other than its active site affects its catalytic rate (this is often termed allosteric regulation). In the current work, we studied individual enzyme molecules and probed not only their catalytic rates, but also their allosteric inhibition by product molecules.

In order to facilitate examination of single molecules of the enzyme horseradish peroxidase (HRP), we trapped these molecules within lipid vesicles, which were then tethered to a surface (Fig. P1). Once an individual HRP molecule was confined within a tethered lipid vesicle, we were able to utilize a fluorescence-based technique to examine product formation at the single-molecule level. We identified a fluorogenic substrate of HRP that becomes trapped within the vesicle after it reacts with the enzyme. This phenomenon allows the concentration of the product within the vesicle to increase to a high enough level that it inhibits further enzymatic activity; this is seen in the right box, which shows that the growth in the number of product molecules is gradually slowing down over time. Analysis of many single-molecule traces like this one allowed us to identify a surprising correlation between the initial velocity of each HRP enzyme molecule and the number of product molecules required for its full inhibition.

Fig. P1. Encapsulation of horseradish peroxidase (HRP) molecules within surface-tethered lipid vesicles facilitates their study at the single-molecule level. We identified a fluorogenic substrate of HRP that becomes trapped within the vesicle after it reacts with the enzyme. This phenomenon allows the concentration of the product within the vesicle to increase to a high enough level that it inhibits further enzymatic activity; this is seen in the right box, which shows that the growth in the number of product molecules is gradually slowing down over time. Analysis of many single-molecule traces like this one allowed us to identify a surprising correlation between the initial velocity of each HRP enzyme molecule and the number of product molecules required for its full inhibition.

Our experiments thus demonstrate allosteric modulation of an enzyme’s activity that is observed at the level of the individual molecule.

Analyses of hundreds of traces similar to the one in the right box of Fig. P1 allowed us to plot a histogram of the initial velocities of HRP molecules (taken from the initial slope of each trace) and the number of molecules required for full inhibition (the level reached after a long period of time). We found that both parameters were broadly distributed, which is just a manifestation of the static disorder alluded to in the first paragraph. However, what is both unique and remarkable in the current study is that the two parameters were significantly correlated with each other.

Our analysis shows that the correlation between the initial enzymatic velocity and the level of inhibition must be due to a similar degree of correlation between the catalytic rate of the enzyme and the rate of binding of the product at the inhibitory site. Thus, although each of these rate constants fluctuates significantly, and on a time scale much shorter than the typical length of an experimental trace (hundreds of seconds), their fluctuations seem to be linked together, and

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this is why we observe correlations between them in our experiments.

What do we learn from these results on the activity of enzymes? Disorder in the activity, observed on the single-molecule level, seems to be a universal phenomenon, and could therefore be simply an outcome of the complex structure and energy landscape of proteins. However, it seems that enzymes have evolved to counter this disorder, by introducing correlated motions at distant sites. Based on our finding, and borrowing from the pictorial terminology of Frauenfelder et al. (3), we can say that the dynamics at the allosteric site of an enzyme are “slaved” to the dynamics at the active site. Such correlated motions, which survive even very long time fluctuations, may help proteins regularize their functions. Characterization of the structural mechanisms underlying such intramolecular “slaving” deserves future attention, and may shed new light on an old question: why are enzymes so much larger than their active sites?