Substrate Catalysis Enhances Single-Enzyme Diffusion

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Self-propulsion of micro- and nanoscale objects can be achieved by harnessing the chemical free energy of the environment through substrate catalysis. For example, we and others have demonstrated that energy arising from catalytic reactions can drive the movement of asymmetric particles on the micrometer and submicrometer length scales by self-electrophoresis, self-diffusiophoresis, and bubble propulsion.[1] Autonomous motion of symmetric colloidal particles with enzymatic (catalytic) sites in the presence of a substrate has been proposed[2] but to date has not been demonstrated experimentally. In addition, catalysis-enhanced diffusion at the single-molecule scale has not been reported. Because of their great diversity, the use of enzymes as catalytic motors would vastly expand the available methods for powering nano- and micromotors. In this study, we provide the first single-molecule-scale measurements of catalysis-enhanced diffusion of the urease enzyme and calculate the force responsible for this enhancement using Brownian dynamics simulations.

Diffusion coefficients were measured by fluorescence correlation spectroscopy (FCS) using time-correlated single-photon counting (TCSPC) instrumentation developed previously.[3] Briefly, fluctuations in fluorescence intensity arising from diffusion of probe molecules were autocorrelated and fit by a multicomponent 3D diffusion model to determine the diffusion coefficient of individual species (e.g., free dye and tagged enzyme).[4,5] Fluorescent tagging of the enzyme was confirmed on the basis of the FCS autocorrelation curves by noting that the fast- and slow-diffusing components corresponded to free dye and fluorescently tagged urease, respectively. The diffusion coefficient of the free dye in buffer was 3.09×10^{-6} cm^2/s and exhibited no significant change in the presence of urea. Thus, the diffusion time of the free dye was fixed for all subsequent curve fits. The diffusion coefficient of urease in buffer was 3.18×10^{-7} cm^2/s, corresponding to a hydrodynamic radius of 6.9 nm, which is consistent with the hydrodynamic radius of 7 nm reported by Follmer et al.[6] The urease concentrations used in this study ranged from 10 to 100 μg/mL, well below the reported concentrations at which aggregation of urease occurs.[6]

Diffusion coefficients of urease at urea concentrations ranging from 0 to 1 M increased from 3.18×10^{-7} to 4.06×10^{-7} cm^2/s, respectively (Figure 1A), representing a catalysis-induced increase in diffusion coefficient of 28%. This increase in diffusion coefficient of urease saturated at ~0.1 M urea, which is similar to the concentration at which the urea hydrolysis reaction rate saturates. The viscosities of these different urea solutions as measured using a cone–plate viscometer were not significantly different than that of the buffer. The urea concentrations were kept below 1 M to avoid denaturation.[7] To test whether enhanced diffusion in the presence of urea was a result of catalysis, urease activity was inhibited by pretreatment of the enzyme with 1 mM pyrocatechol for 2 h.[8] In the presence of inhibitor only, the diffusion of urease
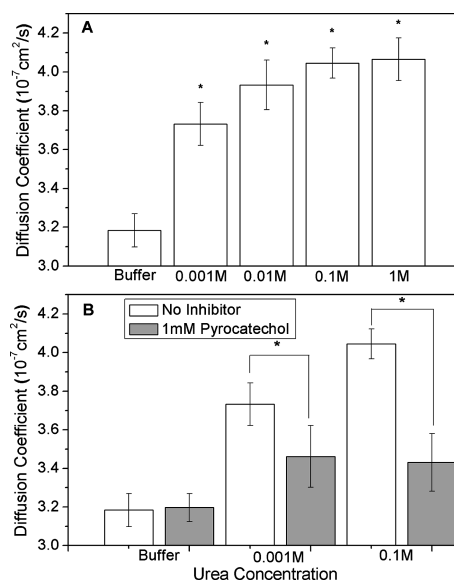
Figure 1. (A) The diffusion coefficient of urease increased with increasing substrate concentration. (B) The increase in urease diffusion coefficient was significantly attenuated by the urease inhibitor pyrocatechol. Error bars represent standard deviations. * indicates a significance value of $P < 0.05$.

in buffer was not significantly different from that of urease without inhibitor, suggesting that the inhibitor itself does not alter the hydrodynamic radius of the enzyme. Similarly, when urease was inhibited and exposed to either 0.001 or 0.1 M urea, the diffusion coefficients were significantly attenuated in comparison with matched controls (Figure 1B).

We hypothesized that the origin of enhanced diffusion could be the generation of charged reaction products, which results in an asymmetric electric field within angstroms of the enzyme, as has been observed for asymmetric particles.[1,9] The diffusion coefficient of NH_4^+ ions is higher than those of the anions (HCO_3^- and HPO_4^{2-}) generated by the hydrolysis of urea in phosphate buffer. As each urea molecule is hydrolyzed, these ions are released at the surface of the enzyme, and the faster diffusion of NH_4^+ creates a local electric field. This field could exert an electrophoretic force on the order of few piconewtons for a short interval of time until the ions diffuse away from the double layer. Since it is technically challenging to measure such short-time-scale forces, we estimated the impulsive force due to a single turnover using Brownian dynamics simulations.[5]

The simulation parameters were as follows: The maximum reaction rate per catalytic site was 2.3×10^4 s^{-1}, as reported by Blakeley et al.[10] A 10 ns impulse time per reaction corresponded to the time it would take for the ions to diffuse one Debye length from the urease molecule, dissipating the electrophoretic force. The force per impulse was then adjusted in successive Brownian dynamics simulations until the diffusion coefficient increased from

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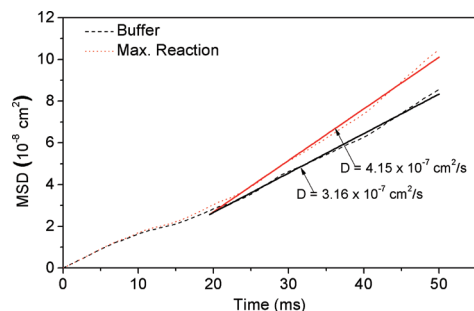
Figure 2. Mean square displacement (MSD) of urease with no reaction (black) and at the maximum reaction rate assuming a force of 12 pN per turnover (red) obtained from Brownian dynamics simulations. Solid lines are linear fits at longer time scales.

3.16×10^{-7} cm²/s (no reaction) to 4.15×10^{-7} cm²/s (maximum reaction rate), as observed in the experiments. To account for this change, self-electrophoresis would need to produce a force of 12 pN per turnover (Figure 2). This is close to the force (20 pN) calculated for an anion ($q = -e$) 2 nm from the center of an enzyme ($q = 25e$) in water ($\varepsilon = 78$). In addition, the electrophoretic force should increase with decreasing ionic strength of the medium, potentially further increasing the diffusion rate.

As an alternative explanation, we explored the possibility that local pH changes upon catalysis might enhance diffusion, as the hydrolysis of urea should result in an increase in the local pH. This hypothesis was based on a study in which bovine serum albumin diffusion depended strongly on pH, ionic strength, and protein concentration.[11] In that study, the diffusion coefficient of the protein increased with an increase in pH, with a minimum at the isoelectric point, where the surface charge on the protein is zero.[12] The increase in pH is associated with an increase in surface charge of the protein, resulting in stronger protein–protein and protein–counterion columbic interactions, which can cause an increase in the protein's diffusion coefficient.

To measure the pH in the vicinity of the enzyme, we tagged urease with SNARF-1, the fluorescence lifetime of which changes with the pH of the local medium. SNARF-1-based pH measurements reflect the relative fraction of the molecules in the protonated and deprotonated states, which exhibit different fluorescence lifetimes.[13] Fluorescence lifetime curves of SNARF-1 measured in solutions of various pH were fit with a double-exponential decay model[4] with decay time constants of 0.44 and 1.42 ns, corresponding to the protonated and deprotonated states, respectively.[14] This result is shown in the inset of Figure 3, where the relative fraction of protonated SNARF-1 molecules decreases and the amplitude-weighted fluorescence lifetime increases with an increase in pH. As shown in Figure 3, the pH in the vicinity of the enzyme increased modestly but significantly at urea concentrations of 0.01 M and above, which would result in a less than 5% increase in diffusion, far below the 28% increase measured using FCS.[5] No increase in local pH was observed when the enzyme was inhibited, suggesting that the pH changes were due to catalysis. Thus, although catalysis-induced changes in pH around single enzymes may be of biological and chemical significance (and their observation represents a novel measurement), the observed increase in pH does not explain the large enhancement of diffusion induced by catalysis. In addition, the local rise in the temperature due to urea hydrolysis was estimated to be in the microkelvin range, which is too small to explain the observed changes in the diffusion coefficient.[5] Thus, movement through a phoretic mechanism remains the most plausible explanation.
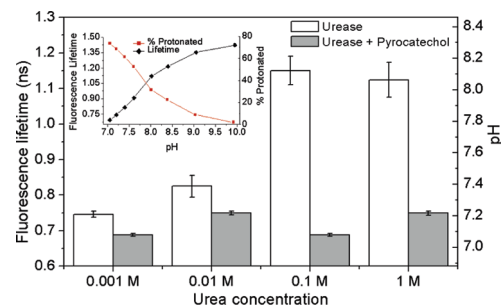
Figure 3. SNARF-1 fluorescence lifetimes were measured as a function of solution pH (inset). Catalysis of urea was accompanied by a significant increase in pH (relative to [urea] = 0.001 M). This increase was significantly attenuated by pyrocatechol for all urea concentrations tested. Error bars represent standard deviations.

In conclusion, we have reported that catalysis can alter the diffusive mobility of an enzyme in the presence of a substrate. We anticipate that in the presence of a substrate gradient, the enzyme molecules will exhibit collective directional motion (chemotaxis).[15] These observations lay the foundation for development of novel enzyme-driven nanomotors by asymmetric placement of the enzyme on micro- and nanoscale objects. Additionally, many molecular machines in living systems function as Brownian ratchets.[16] The movements of these enzyme-based biological machines may be greatly facilitated by catalysis-induced forces resulting from substrate turnover.

Supporting Information Available: Experimental methods, Brownian dynamics simulation methodology, and estimates of local temperature and electrolyte friction coefficient. This material is available free of charge via the Internet at http://pubs.acs.org.

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