Enhancing Enzyme-Catalyzed Polymerization

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Abstract

This review discusses enzyme-catalyzed polymerization and proposes several ideas for further research and experiments. An experimental method is proposed for determining growing polymer chain dynamics as it interacts with the active site of an enzyme. Additionally, further investigation is proposed to explore the possible stabilization effects of high pressure on enzymes used in enzymatic polymerization at high temperature, as well as the use of enzymes from extremophiles for polymerization.

Introduction

The transformative progress made by Chemistry and Materials Science in the past several centuries was made possible by humanity's ability to rationally accumulate capital. We use ever more expensive and complex equipment to produce raw materials in unnaturally high concentration and purity, to create completely unearthly reaction conditions, and to control them precisely.

Evolution makes up in patience and trial and error what it lacks in ingenuity, resources, and planning. Confined mostly to dirt, water, air, sunlight– humble, earthly reaction conditions – and only trace concentrations of all but a few elements, biology has produced a dazzling array of complex macromolecular machinery, orders of magnitude more sophisticated and adjustable at the nano/micro scale than most man-made chemicals.

Although we do not yet completely understand the nuances and working principles of biological macromolecules, our knowledge improves continuously. It seems that there is an enormous opportunity to augment conventional industrial chemistry with machinery and insights borrowed from the enormous warehouse of specialized and efficient chemistry that biology offers.

This review will discuss several ideas for enhancing the understanding and practical implementation of enzymatically catalyzed polymerizations.

Enzymes

Enzymes are a class of ubiquitous protein macromolecule catalysts that make life happen at the speed which we are accustomed to. This is not trivial– enzymes often increase reaction rates to nearly the limits of diffusion speed. Most reactions crucial to biological metabolism would not proceed at all near ambient temperatures if not for the catalysis performed by enzymes. Since the finding of amylase in 1833 (ichiro Shoda et al., 2016)

enzymes have been a key research topic in biology and chemistry. Their amazing efficiency in catalyzing countless complex reactions at mild conditions, as shown by Wolfenden and coworkers (Edwards et al., 2011) (Lad et al., 2003)(Radzicka and Wolfenden, 1995), is both fascinating and desirable for many practical applications. Enzymes are already used as industrial catalysts in the manufacturing and processing of food, medicine, textiles, and the possibility of human-designed enzymes (Kries et al., 2013) further increases the scope of potential applications.

How do enzymes work?

The physical mechanisms behind enzyme catalysis are not settled despite 100 years of structural data from X-ray crystallography studies and the last several decades of simulations. There are many reviews that discuss the issue, including several confidently titled "How do enzymes work?" (Garcia-Viloca, 2004)(Blow, 2000)(Kraut, 1988), but there is no clear consensus. E. Fischer first proposed the "lock and key" explanation in 1984 (Fischer, 1894), now called molecular recognition, which refers to the formation of an enzyme-substrate complex due to supramolecular interaction. The substrate is somehow more active in the complex and thus catalysis is achieved. More detailed theories emerged over the years as experimental/spectroscopic data accumulated and computational models became available. Linus Pauling hypothesized that enzymes deform their substrates to increase the energy of the substrate and bring it energetically closer to transition state (GSD- ground state destabilization) or somehow stabilize transition states (TSS-transition state stabilization) (PAULING, 1946). Given the number and variety of unnatural substrates that enzymes have been shown to activate, it seems that there is something more complex and dynamic going on than is suggested by the "lock and key" analogy. Researchers have more recently been attempting a more quantitative and detailed look at enzyme catalysis mechanisms including the effect of multiple enzyme conformations (perhaps the enzyme binds or accepts/transports the substrate in one conformation and then changes shape to another stable conformation, thus pushing the reaction forward).

Some recent work of particular interest by Stephen D. Fried and Steven G. Boxer (BOXER et al., 2018) indicates that enzyme active sites may operate by subjecting the substrate to a high localized electric field. To investigate this possibility, they took advantage of the "Stark Effect," first demonstrated by Johannes Stark (STARK, 1913) in 1913, who found that applying an external electric field split the spectral lines of hydrogen. In larger organic molecules, an external E-field induces vibrational energy level changes in dipole groups such as Carbonyls. They started by measuring the vibrational frequency shifts experienced by 19-nortestosterone's (a Ketosteroid Isomerase inhibitor) carbonyl dipole when placed in a known electric field to determine the substrate's response as applied E-field varies. Then, taking this data for known external E-fields and comparing it to the measured vibrational shift the 19-nortestosterone's carbonyl experiences when inside Ketosteroid Isomerase's active site, the authors were able to demonstrate that the active site has a high local electric field (144 +/- 6 MV/cm), which according to free energy models accounts for 72% of the enzyme's total rate acceleration.

It was found that the activity of various mutant strains of Ketosteroid Isomerase depended strongly on the magnitude of the electric field. This result cannot completely be generalized to all enzymes- Ketosteroid Isomerase is a particularly good test case because it has no conformational changes during catalysis and needs to stabilize a highly polarized dipole in the transition state. However, electrostatic catalysis is pervasive in enzymology because most chemical reactions in biology involve charge rearrangements. The authors go on to demonstrate that this effect is present in many enzymes and those with the highest catalytic activity may be the ones that rely, like Ketosteroid Isomerase, exclusively on E-field stabilization of dipoles (conformational rearrangements other enzymes perform take time).

How does nature adapt enzymes to ambient conditions?

Another incredible facet of enzyme science is the analysis of the changes made to enzymes by evolution to make them operate optimally in extreme environments, such as deep-sea thermal vents. It is not a trivial matter that life at the bottom of the ocean, in hot springs, and in other extreme environments is able to utilize homologues of the proteins and macromolecules that are used by organisms living in mild conditions. Understanding the adaptations that allow this to happen should provide interesting insights for adjustments that may be made to enzymes or to reaction conditions in order to increase enzyme performance and resilience in industrial use cases. A classic example worth mentioning here is the use of thermostable TAQ polymerase in PCR (Saiki et al., 1988).

Pressure + Temperature effects on enzymes

There are several aspects of enzyme structure affected by pressure. Firstly, there are hydrophobic regions in enzyme structures that can become hydrated if the water pressure is high enough to force water molecules into the less dense hydrophobic regions(Huang et al., 2016). Secondly, many enzymes vacillate between multiple conformations that may all somehow be involved in the catalysis process or the transport of substrate and products to and from active site respectively (Ichiye, 2016).

High pressure can increase the free energy of conformations that have higher effective volume and in some cases crush/denature the enzyme entirely. Low temperatures can also make some conformations thermodynamically inaccessible/unlikely, thus preventing the activity of the enzyme. High temperatures, on the other hand, can make the enzyme too free to change shape and thus either decrease its activity or even denature it permanently.

Evolution tuning the rigidity of enzymes to accommodate different Pressure-Temperature combinations

It has been found that extremophiles which thrive in harsh conditions have evolved homologues of standard enzymes with altered stiffness. "Flexibility matching" has been found by comparing homologous proteins from psychrophile (low-temperature), thermophile (high-temperature) and mesophile organisms (Bae and Phillips, 2004). The effective flexibility of the proteins from the extremophiles (modeled at their natural temperatures and pressures) matches the flexibility of homologous proteins in mesophiles at standard temperatures and pressures. Structural analysis of homologous proteins from thermophiles and mesophiles shows that there are actually two types of adaptations that are used to tune the flexibility of the protein structure. Thermophiles need to increase rigidity in order to keep the shape of the enzyme at higher thermal energies. There are actually two distinct adaptations reported for achieving greater rigidity in the enzyme structure. Thermophilic archaea generally have excess hydrogen bonding while thermophilic bacteria generally have several more salt-bridges (Berezovsky and Shakhnovich, 2005). This is considered consistent with prevailing evolutionary theory, as Archaea are thought to have evolved first at high temperatures and then adapted to lower ones (by losing H-bonds) and bacteria did the opposite (evolved first at low temperature and then adapted to high). It may be easier to lose Hydrogen bonding to become more flexible (Archaea) or gain several salt bridges (bacteria) to become more rigid.

There are other variables besides the structure of the enzyme itself, such as dissolved piezolytes produced by extremophiles(Huang et al., 2016), but if we take the simple model of flexibility matching, there is an interesting implication. It seems that to a first approximation pressure and temperature may have somewhat

opposite effects. High temperature makes enzymes more flexible (capable of overcoming structural rigidity to take less energetically favorable conformations) while high pressure tends to make some conformations less favorable by greatly increasing the importance of volume– thus effectively making the enzyme less flexible. Empirically, it has been found that high pressures can increase the optimal and maximum growth temperatures of various bacteria. For example, the highest growth temperature on record for M. kandleri strain 116 is 122°C, achieved at 40 MPa(Takai et al., 2008).

Perhaps, at least in some cases, activity loss of industrially useful enzymes at high temperature could be prevented by applying high pressure to the reaction vessel/environment. This would be an interesting result because high pressure and temperature should be favorable for polymerizations that result in a more condensed product compared to the monomer substrate. Additionally, it seems possible that this idea can be generalized to other industrial processes using enzymes today.

Enzymes as in-vitro catalysts for polymer production

There is an opportunity to use isolated enzymes to displace toxic metal catalysts and harsh process conditions for a wide range of chemical transformation (Drauz and Waldmann, 1995). An extensive review produced by Shin-ichiro Shoda et al (ichiro Shoda et al., 2016) reports a laundry list of in vitro polymerizations achieved using three classes of enzymes–oxidoreductases, transferases, and hydrolases– with both natural and unnatural substrates. Not only can enzymes potentially make polymer production cheaper and greener, but they can also provide regio, stereo and chiro selectivity. It is also possible to functionalize polymers in more controlled ways using enzymes instead of traditional catalysts.

However, in practice, using enzymes to effectively catalyze polymerizations poses challenges. Enzymes tend to get damaged or denatured and lose activity (or at mild conditions reactions slow as polymers grow and become more viscous) and they are hard to separate for reuse from the product polymer once the polymer is produced. It may make sense to attempt re-engineering the enzymes themselves for better performance or attempting to adjust reaction conditions or introduce co-factors of some kind, but the leading approach is to immobilize or protect the enzymes in various media to make them usable in continuous processes or removable from bulk processes. This has the effect of both improving enzyme recovery and longevity. There is, however, often a tradeoff however and depending on how aggressively the enzyme immobilized it can lose activity. Also, various process obstacles are encountered, notably the clogging of immobilization substrate with polymer, leading to pressure buildup in continuous flow reactor setups (Kundu et al., 2011) .

Exploring enzyme-catalyzed polymerization molecular dynamics

The basic chemical pathways for polymerization are known for many enzyme-monomer pairs, however, no studies have been found in the course of this literature search of the molecular dynamics that occur as the polymer grows via enzyme catalysis. Characterizing the molecular dynamics of particular monomer/solvent/enzyme combinations could be particularly helpful in optimizing immobilization structures using hydrophilic/hydrophobic copolymers such as those demonstrated by Gitsov et al (Scheibel and Gitsov, 2018).

This method is particularly interesting because the activity of the complexed/immobilized enzyme is actually as much as 1245% higher than the free enzyme. Typically enzyme activity drops due to distortions and other side effects of immobilization. In the case of ABA block copolymer complexes, the polymers can be engineers to interact favorably with the substrate and the resulting polymer, thus increasing the concentration of substrate in the vicinity of the enzyme and increasing the amount of time the product polymer chain can spend near the enzyme (thus growing longer), even as it may not be soluble in the bulk water solution. With a better understanding of the molecular dynamics of chain propagation dynamics, supporting copolymers can be designed to have appropriate porosity and size.

There are several possible mechanisms by which enzyme-catalyzed polymerization can progress:

1. Only the propagating chain end is inside the active site. The bulk chain is outside active site. Chain end drifts away from the active site and propagation stops until chain end is in an active site again.
2. Some part of the polymer chain stays in the active site for an appreciable time as the chain end grows. The chain end and eventually the entire polymer drift out of the active site, ending propagation until chain end returns to an active site (same enzyme or neighboring).
3. The entire polymer stays in or around the active site until it drifts away and chain propagation stops until chain end is in an active site again.

Proposed experimental design for elucidating molecular dynamics of enzymatic polymerization:

- Choose a monomer that is capable of producing a branched/crosslinked polymer via side chains that are also catalyzed by the enzyme used to perform main chain propagation. Perhaps doing a branched polymer that has side chains that can also be catalyzed by the same active site.
- Perform enzymatic polymerization
- Isolate resulting polymer
- Characterize and the degree of branching/crosslinking

A low degree of branching would imply that the enzymatic polymerization progresses according to mechanism (1) above, whereas a high degree of branching/crosslinking would imply that the reaction progresses according to mechanism (3).

Conclusion and proposed experiments

Enzyme-catalyzed polymerization may be made more industrially practical by exploring the fundamental molecular dynamics of the polymerization process and by pushing the boundaries of reaction conditions and their effects of enzyme performance. Understanding the molecular dynamics of chain propagation would be useful in designing immobilization substrates and choosing solvents and reaction conditions. We think the experimental direction proposed here for exploring molecular dynamics using crosslinking polymerization experiments could lead to some useful results in this direction. If these experiments are conducted over a range of enzymes, solvents and immobilization substrates the results could guide process optimization. Additionally, there seems to be an excellent opportunity to experiment with varying pressure and temperature to achieve better enzymatic polymerization results. High pressure has always been a crucial tool for industrial chemists, it would be exciting to see what possibilities it could open in the area of enzyme catalysis. Polymerizations are a good candidate for testing the concept of pressure assisted enzyme stabilization because many polymerizations result in a volume decrease. This means that all else being equal, reaction rates and yields should increase at high pressure. Ideally, high temperature and pressure enzymatic polymerization tests would be conducted both with standard enzymes and their deep-sea homologues using a variety of reaction conditions, including immobilization substrates. These experiments could produce industrially relevant results and lead to a better understanding of the effects of pressure and temperature on enzymes.

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