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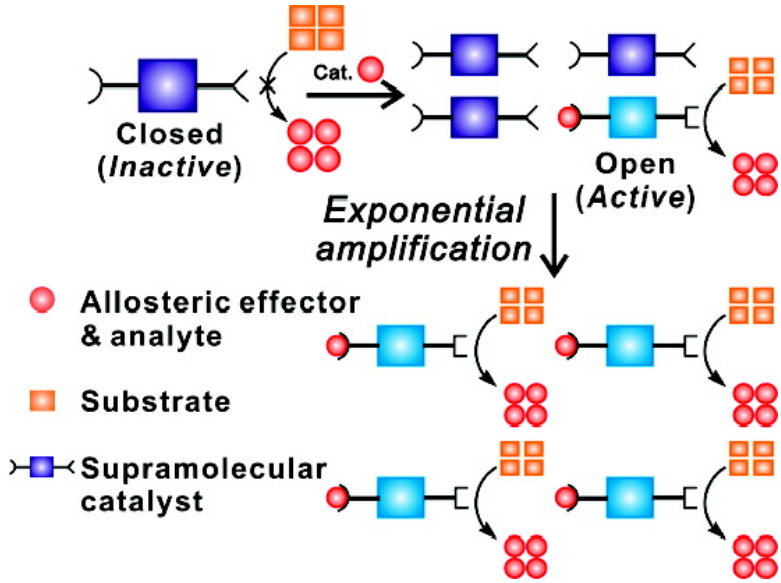

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PCR-like Cascade Reactions in the Context of an Allosteric Enzyme Mimic

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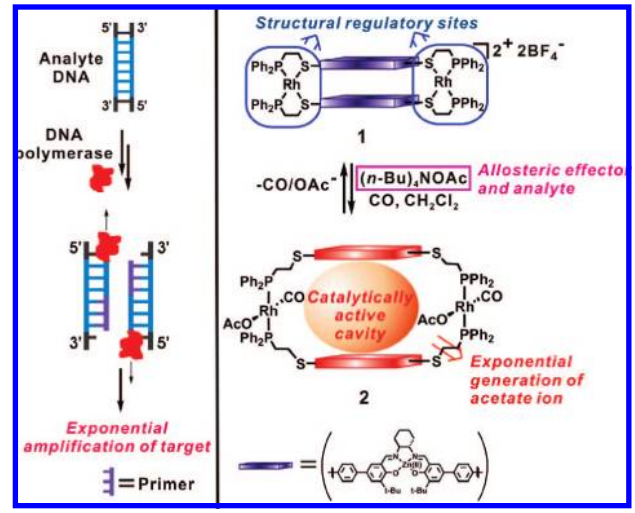
The polymerase chain reaction (PCR) is utilized in the biochemistry and molecular biology communities for exponentially amplifying DNA by making copies of a specific region of a nucleic acid target. When coupled with diagnostic probes, this technique allows one to detect a small collection of molecules under very dilute conditions.[1] PCR-based methods are the backbone of the modern biodiagnostics industry and allow one to identify genetic markers for disease with unprecedented sensitivity and very high reliability.[2] A limitation of PCR is that it only works with nucleic acid targets, and there are no known analogues of PCR for other target molecule candidates. However, if one could create a way to amplify recognition events in the case of non-nucleic acid targets, one could open up the ability to create ultrasensitive detection systems for a much wider class of analytes, including ones that are important both inside and outside the field of biology.

Our group has developed a variety of supramolecular allosteric enzyme mimics assembled via the weak-link approach (WLA) for the detection of small molecule and elemental anion analytes.[3] The WLA, which builds upon the growing number of methods for preparing metal containing macrocyclic complexes,[4] allows one to synthesize structures through coordination chemistry with hemilabile ligands and transition metal based regulatory sites that can be modulated through ligand displacement reactions.[5] With these systems, a small molecule reacts at the allosteric regulatory site, which induces a conformational change and subsequently turns on a catalytic reaction that generates a surrogate signaling molecule (typically one that is fluorescent).[6] These systems are not mimics of PCR but rather resemble Enzyme Linked ImmunoSorbent Assays (ELISAs) where a target sandwiching event creates a catalyst target complex that generates a chemiluminescent or fluorescent signal. ELISA is a signal amplification approach rather than a target amplification approach, and its sensitivity is often intimately linked to the binding constant of the recognition element. Therefore, with signal amplification one cannot achieve the sensitivity associated with a target amplification approach like PCR, which is constantly driving the equilibrium reaction between the recognition complex and target by generating more of the target. These observations pose the following question: can coordination chemists design allosteric structures that mimic the properties of PCR? In particular, can allosteric enzymes be designed that recognize a small molecule through metal ligand binding at a regulatory site, which subsequently turns on a catalytic reaction that generates more of the molecule that was initially recognized? Herein, we report the first example of a coordination chemistry based catalytic system that mimics some of the properties of PCR in the context of a small molecule, rather than a nucleic acid, recognition event and exhibits the cascade amplification curves one typically associates with a PCR-like reaction.

In an effort to design a system that mimics the properties of PCR, we initially focused on probing the coordination chemistry of the previously synthesized tetranuclear ELISA-mimic complex

Scheme 1. Small Molecule Regulated Target Amplification with an Allosteric Enzyme Mimic



1 (Scheme 1).[7] This structure can be opened in the presence of Cl^- to generate a cavity capable of catalyzing an acyl transfer reaction between pyridyl carbinol and acetic anhydride.[6] The byproduct of that reaction is acetic acid. When coupled to a pH indicator this provides a convenient way of reading out the signal amplification event associated with the initial Cl^- recognition event. We hypothesized that one could realize PCR-like behavior if the complex exhibited significant reactivity with acetate instead of Cl^-, and the reaction was carried out under basic conditions where the product generated would be acetate ion rather than acetic acid. Significantly, when complex **1** is reacted with 2 equiv of $(n\text{-Bu})_4$NOAc at room temperature under a CO atmosphere, it is quantitatively converted to the open macrocycle **2**, which possesses a *trans* acetate and CO ligand at each Rh(I) site.[8]

The open macrocycle **2** was characterized by ^{1}H and ^{31}P{^{1}H} NMR spectroscopies, FT-IR spectroscopy, and electrospray ionization mass spectrometry (ESI-MS).[9] All data are consistent with the proposed structural formulation (see Supporting Information), and the reaction is completely reversible. Indeed, upon removal of solvent and CO by vacuum, complex **1** quantitatively reforms. From these experiments, we confirmed that the acetate anion can act as an allosteric switch to chemically toggle the macrocycle from a closed and presumably inactive to an open and catalytically active state.

The catalytic activity of the Zn(II)-salen based supramolecular compound **1** was studied in the context of the acyl transfer reaction involving acetic anhydride and pyridyl carbinol as the substrates (Scheme 2). In the absence of acetate, there is almost no catalytic activity. However, if 1 mL of 0.04 mM (433 nmol) $(n\text{-Bu})_4$NOAc is added to a 10 mL solution containing 4.44 μmol (0.4 mM) of inactive macrocyclic complex **1**, CO (1 atm), 13.5 μmol of the

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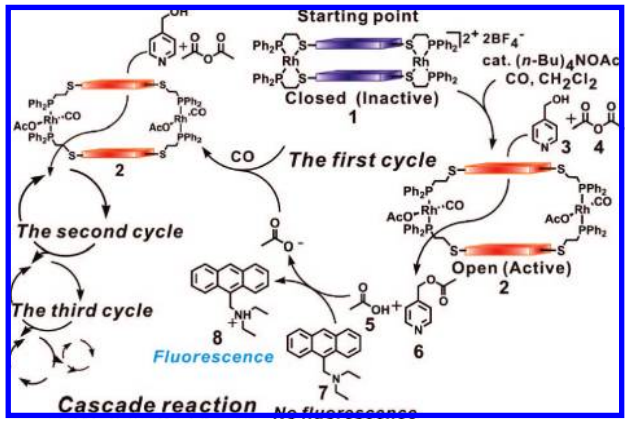
Scheme 2. Proposed Cascade Mechanism for the PCR-Mimic in the Context of Acetate Detection



substrates (pyridyl carbinol **3** and acetic anhydride **4**, 1.22 mM), and 13.5 μmol of the fluorophore (9-(*N*,*N*-diethylaminomethyl)anthracene **7**, 1.22 mM),[10] the mixture begins to catalyze the acyl transfer reaction. Once a small amount of $(n\text{-Bu})_4$NOAc reacts with inactive complex **1** at its two rhodium centers that serve as structural regulatory sites, the complex is converted into open cavity complex **2**, which then catalyzes the reaction between **3** and **4**. Bimetallic acyl transfer reactions involving Zn are well-known.[3b,6,11] In the early stages of the reaction, only a minor amount of the catalyst is activated (in the form of **2**, ~5% based upon stoichiometry), but as the reaction proceeds, more acetate is generated, which leads to the formation of more **2** and progressively faster catalysis. The pH-sensitive fluorescent dye **7** allows one to follow the amount of acetate generated during the catalytic cycles.[12] This type of cascade reaction results in an exponential increase of the amount of products **5** and **6**.

The formation kinetics of the product, 4-acetoxymethylpyridine **6**, were studied by gas chromatography (GC) as a function of starting acetate concentration (Figure 1; see Supporting Information for experimental procedure of catalysis). Significantly, one sees the sigmoidal product generation curves, which are characteristic of PCR-like processes and reminiscent of those observed by Rebek et al. in the study of autocatalytic and self-replicating systems with synthetic macromolecules, such as cylindrical dimeric capsules and adenine-imide conjugate molecules.[13] Slow induction followed by rapid exponential amplification, linear growth, and then eventual saturation (after ~80% of the substrate has been consumed) is observed, regardless of initial analyte concentration. As with PCR, the time at which the exponential step turns on correlates with analyte concentration.

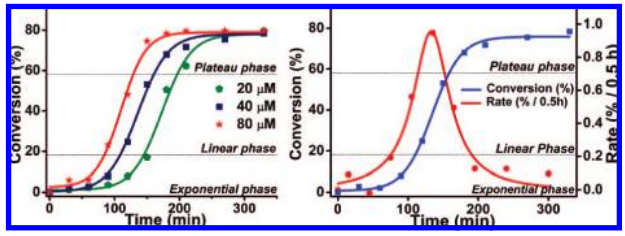


Figure 1. Conversion (%) vs time (min), starting with various concentrations of analyte (left). Conversion (■) and rate (●) vs time in the case of 40 μM of analyte (right). Product formation was monitored by GC (product **6**) and fluorescence (acetate ion by pH indicator; see Supporting Information).

In conclusion, this work presents the first demonstration of a PCR-type cascade reaction involving a supramolecular allosteric catalyst. Indeed, the supramolecular catalyst is activated by recognition of a small molecule, which leads to the catalytic generation of more analyte creating a cascade that resembles the amplification processes in PCR. In principle, the approach can be extended to other analytes as long as one can envision catalytic reactions that generate an analyte of interest. Efforts in this direction are underway.

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Supporting Information Available: Experimental procedures and spectral data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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