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Development of an Enzyme Mimic Using Self-Selection

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Abstract: The development of a serine protease model using a self-selection protocol is described. The developed system mimics the regeneration step of an enzyme involved in covalent enzyme catalysis. A transition-state analogue of a transesterification reaction is used to self-select functional groups able to accelerate ester cleavage. It is shown that the insertion of a tertiary amine substituent flanking the reaction center reinforces transition-state stabilization by directing the reactive center towards the self-selected functionality. In addition, the tertiary amine activates a bland (solvent) nucleophile for attack on an ester bond similar to what occurs in a serine protease. A quantitative correspondence is observed between the amplification factors and catalytic activity, illustrating the potential of the dynamic covalent capture strategy to precisely detect and quantify weak noncovalent interactions.

Keywords: dynamic combinatorial chemistry · enzyme catalysis · supramolecular chemistry · thermodynamic control · transition states

1. Introduction

Catalyst discovery is a challenging task because of the subtle energy differences that determine the efficiency and selectivity of a catalyst.[1] The search for new catalysts is facilitated by combinatorial methodologies, which permit a high-throughput synthesis and screening of catalyst libraries.[2–8] In recent years, supramolecular approaches towards catalyst discovery are rapidly emerging, mainly driven by the advantage of self-assembly as a tool to generate structurally diverse catalyst libraries with minimal synthetic effort.[9–11] An alternative approach relies on the use of self-selection protocols for the spontaneous identification of a catalyst from a dynamic combinatorial library of potential candidates.[12] This relies on the shift in the thermodynamic equilibrium towards the optimal catalyst upon exposure of the library to a transition-state analogue (TSA) of the reaction under scrutiny.[13–17] The attractiveness of this approach is twofold. First, it has the potential of identifying catalysts from a large virtual library, which eliminates the need of actually synthesizing and screening all of them. Second, self-selection is by definition the result of *all* energetic interactions that occur between catalyst and transition state, including those that would be difficult to anticipate in an approach based on rational design. Nonetheless, the fact that the application of dynamic combinatorial chemistry for catalyst discovery has so far received much less attention compared to the development of receptors, sensors, and materials, indicates that the translation from transition-state recognition to reactivity is far from trivial.[18] Recently, we have introduced a dynamic covalent capture strategy that facilitates this process.[19] It relies on the covalent anchoring of the transition-state analogue to a scaffold (**1b**) equipped with an aldehyde group for the reversible covalent capture of hydrazides, which are able to develop stabilizing interactions with the transition-state analogue.[15] The major advantage is that self-selection is driven by intramolecular interactions, which permits the detection of weak energetic contributions that would go unobserved in a classical dynamic combinatorial library.[20] Additionally, the covalent attachment of the target precisely defines the position where the reaction has to occur. A similar mode of action is employed by enzymes that mechanistically rely on covalent enzyme catalysis.[21] Here, a transient covalent enzyme-substrate intermediate is formed, which is subsequently hydrolyzed to regenerate the enzyme (Figure 1a). Previously, we have shown that the phosphonate target (acting as a TSA for the hydrolysis of a carboxylic ester) in scaffold **1b** self-selects hydrazides **B** and **C** from a library of hydrazides (Figure 2).[15] It was indeed shown that a correspondence exists between the amplification factors and the ability of the selected hydrazides to accelerate the cleavage of a neighboring carboxylic ester through transition-state stabilization. Mechanistically, this resembles the regeneration step of an enzyme involved in covalent enzyme catal-

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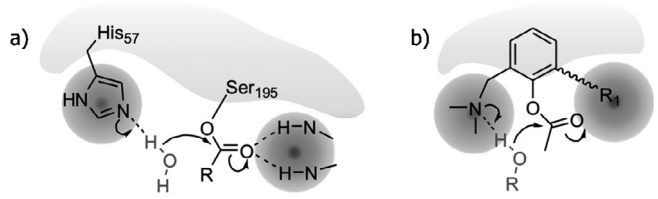

Figure 1. a) Regeneration of a serine protease upon solvolysis of the covalent enzyme-substrate intermediate, and b) the same process in a synthetic enzyme mimic.

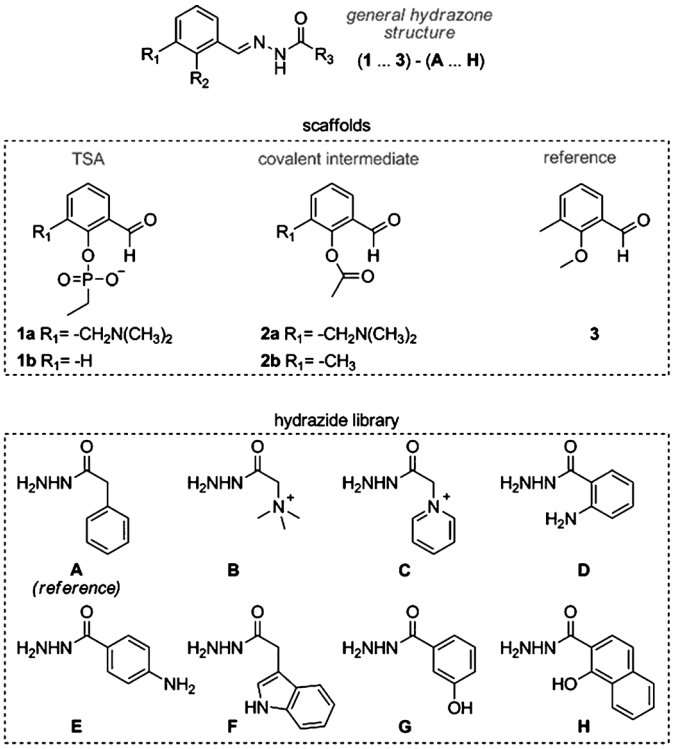

Figure 2. Scaffold molecules and hydrazide library.

ysis. Here, we show an evolution of our system towards a model that mechanistically operates in a way that is more similar to that of an enzyme. It is demonstrated that decoration of the scaffold molecule with an additional (tertiary) amine functionality renders the system susceptible to nucleophilic attack by bland solvent molecules. Furthermore, the presence of an additional functionality on the scaffold enhances the catalytic efficiency by directing the reaction center towards the self-selected functionality, thus reinforcing transition-state stabilization. Finally, a quantitative correspondence is observed between the amplification factors and catalytic activity.

2. Results and Discussion

Our previous system relied on the use of the highly active methoxide anion as a nucleophile for deacylation. This is much different from serine proteases, which rely on a histidine residue to activate water for nucleophilic attack on the acylated serine residue in the covalent enzyme intermediate (Figure 1a).[21] Similar observations have also been made in synthetic systems, in which neighboring amines activate alcohols for nucleophilic attack on carboxylic esters.[22–25] We argued that, in a similar manner, the insertion of a flanking tertiary amine near the carboxylic ester group would enable deacylation in methanol, thus eliminating the necessity of using the methoxide anion (Figure 1b). In order to test this hypothesis we prepared phenyl acetate derivative **2a**, containing a dimethylaminomethyl substituent in the *ortho* position to the ester. On the other *ortho* position an aldehyde group was inserted for reversible covalent bond formation with hydrazides. The stability of the ester moiety in reference hydrazone **2a-A** was studied by following the changes in the absorbance of a solution of **2a-A** in CH_3OH at 25°. Transesterification occurred highly efficiently, with a pseudo-

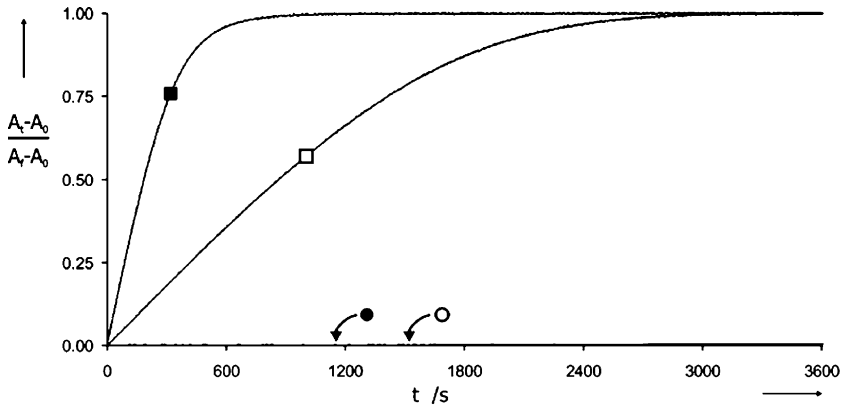

Figure 3. Changes in the absorbance at 340 nm as a function of time for the deacylation of **2a-A** (□) and **2a-B** (■) in CH_3OH (0.6 mM) at 25°. Under the same conditions, deacylation of **2b-A** (●, 0.6 mM) in the presence of one equivalent of Et_3N could not be detected in the same interval. The addition of one equivalent of trifluoroacetic acid completely inhibited deacylation of **2a-A** (○). Rate constants for the latter two deacylations were calculated from the initial rates of prolonged measurements (see Supporting Information).

first order rate constant of $1.55\times10^{-3}\,s^{-1}$ (□, Figure 3). Deacylation was quantitative after one hour, as evidenced by the fact that ESI-MS showed exclusively the presence of the corresponding phenol. The importance of the presence of the tertiary amine was evident from the fact that the analogous compound **2b-A**, which lacks the tertiary amine, showed deacylation with a pseudo-first order rate constant of $1.44\times10^{-6}\,s^{-1}$ even in the presence of one equivalent of triethylamine (●, Figure 3). Furthermore, the addition of one equivalent of trifluoroacetic acid to **2a-A** completely inhibited transesterification ($k_{obs}=7.07\times10^{-7}\,s^{-1}$; ○, Figure 3). The absence of large chemical shifts for the methyl groups in the ^1H NMR spectrum of **2a-A** and the absence of charge transfer bands in the UV/Vis spectrum indicated that intramolecular acyl transfer to the amine did not appear to take place, suggesting that the amine is indeed involved in nucleophile activation. Thus, the mere presence of the *o*-dimethylaminomethyl substituent in **2a-A** increases the efficiency of the acyl transfer to methanol by more than three orders of magnitude.

Motivated by the positive effect of the tertiary amine on the deacylation rate of **2a-A**, we then focused on the effect of the dimethylaminomethyl substituent on the self-selection experiments. Recent studies on this system had shown that substituents on the aromatic ring of the phosphonate scaffold play a large role in determining the strength of the intramolecular interaction between the phosphonate group and the functional group present in the hydrazide.[26] This behavior is very similar to the way in which even remote groups in enzymes influence enzyme activity by changing the orientation of functional groups in the active site. Therefore, we repeated the screening of the previously studied hydrazides **B**-**H** against reference hydrazide **A** using the new phosphonate-containing scaffold **1a** and a reference scaffold **3** with a neutral methoxy group. The concentration of hydrazones at thermodynamic equilibrium was determined by UPLC. To each hydrazide **B**-**H**, an amplification factor was assigned, corresponding to the ratio of the equilibrium constants using scaffolds **1a** and **3**. The obtained amplification factors are given in Figure 4, together with the values that we had obtained earlier under the same conditions for scaffold **1b** lacking the dimethylaminomethyl substituent.[15] The data reveal two important features. First, the obtained amplification profile is identical for both phosphonate scaffolds **1a** and **1b** and shows a significant amplification for hydrazides **B** and **C**, which contain positively charged ammonium or pyridinium groups, respectively. Second, much higher amplification factors are observed for scaffold **1a** compared to **1b**. In fact, for the most strongly amplified hydrazide **B**, the amplification factor increases from 1.8 to 4.2, the latter corresponding to a $\Delta\Delta G^0$ between **1a**-**A** and **1a-B** of 3.8 kJ mol^{-1}. We ascribe this to the substituent-induced orientation of the phosphonate group in the direction of the positive charge.

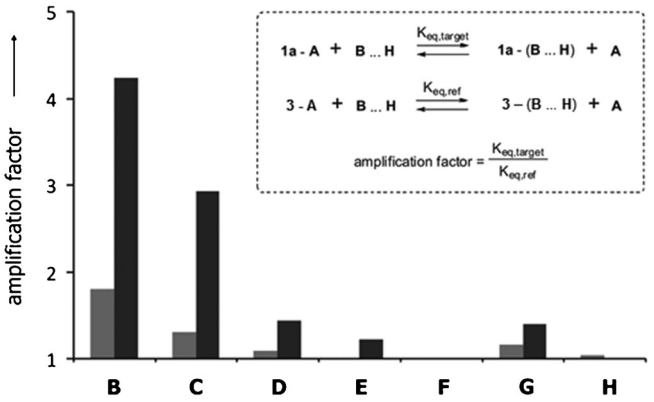

Figure 4. Amplification factors for hydrazides **B**-**H** obtained from competition experiments with reference hydrazide **A** using scaffold **1a** (dark gray). Samples were kept at 50° until the thermodynamic equilibrium was reached (typically 12 hours), which was confirmed by the absence of changes in the peaks of the UPLC chromatogram. The previously reported amplification factors obtained using scaffold **1b** (light gray) have been added for comparison. All measurements were performed in CH_3OH at 50° using five equivalents of each hydrazide (25 mM) compared to the scaffold (5 mM).

Interestingly, these data point to a double role for the dimethylaminomethyl substituent both as a nucleophile activator *and* as a steering group. The observed stabilizing interaction between phosphonate and ammonium groups in hydrazone **1a-B** should lead to an enhanced rate of deacylation of phenyl acetate derivative **2a-B** compared to the reference compound **2a-A**, because of transition-state stabilization by the ammonium group. In particular, as illustrated in Figure 5, in the case of a quantitative correspondence between TSA recognition in the self-selection experiments and transition-state stabilization in catalysis, the expected rate acceleration should correspond directly to the amplification factor (4.2). For that reason, we were very excited to find out that a threefold rate enhancement was measured for the deacylation of **2a-B** compared to **2a-A** ($k_{obs,\mathbf{2a\text{-}B}}=4.64\times10^{-3}\,s^{-1}$) in CH_3OH at 25° (■, Figure 3). Compared to the reference compound **2b**-**A**, which lacks the dimethylaminomethyl substituent, this implies a total rate acceleration of more than 3200 times.

3. Conclusions

In conclusion, we have developed a system that mimics some key features of the regeneration step of an enzyme involved in covalent enzyme catalysis. This system contains functional groups involved in transition-state stabilization and nucleophile activation. Furthermore, the tertiary amine substituent has a steering function by directing the reaction center towards the functional group that stabilizes the transition state. The result is an enhanced catalytic efficiency. From a more general point of view, this work illustrates the integration of a self-selection proce-

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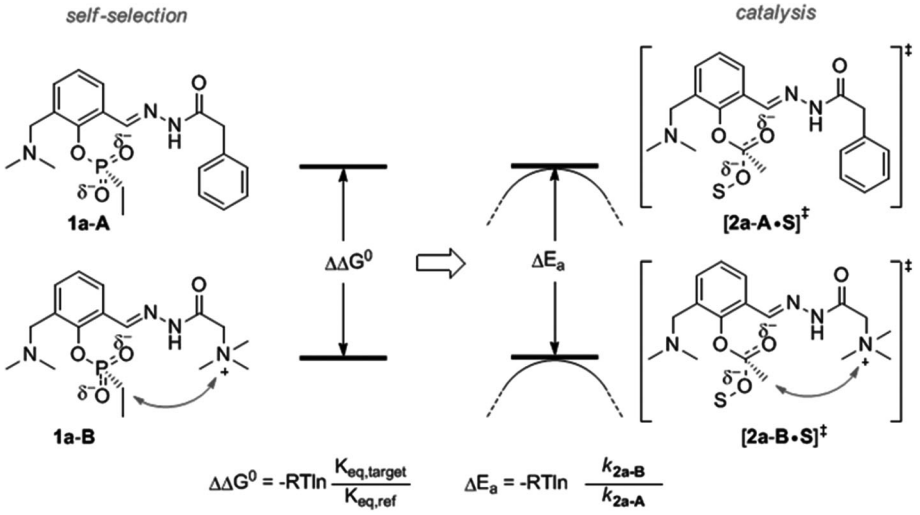

Figure 5. Correlation between the amplification factors in the self-selection experiments and rate acceleration in catalysis. S = solvent.

dure in catalyst development. In particular, the results show that the application of a dynamic covalent capture strategy gives an unprecedented access to the experimental quantification of very small energy variations at the transition state. Importantly, a neat correspondence is observed between amplification factor and rate enhancement. In our opinion, the advantage of the dynamic covalent capture strategy is twofold. First, the covalent attachment of the TSA to the scaffold molecule locates it precisely at the position where the actual transition state is formed. Consequently, during the self-selection and catalysis the functional groups are positioned at identical positions, which permits a quantitative correspondence between amplification factor and rate enhancement. Second, the fact that amplification is a result of intramolecular stabilization gives an unprecedented sensitivity to the selection process. This is perfectly in line with related approaches aimed at quantifying weak energetic contributions in (bio)molecular recognition events.[19,27,28]

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References

[1] *Comprehensive Asymmetric Catalysis, Vol. I–III* (Eds.: E. N. Jacobsen, A. Pfaltz, H. Yamamoto), Springer, New York, **1999**.
[2] M. T. Reetz, *Angew. Chem., Int. Ed.* **2001**, *40*, 284.
[3] C. Gennari, U. Piarulli, *Chem. Rev.* **2003**, *103*, 3071.
[4] J. G. de Vries, A. H. M. de Vries, *Eur. J. Org. Chem.* **2003**, 799.
[5] M. T. Reetz, *Angew. Chem., Int. Ed.* **2008**, *47*, 2556.
[6] M. J. Wilkinson, P. van Leeuwen, J. N. H. Reek, *Org. Biomol. Chem.* **2005**, *3*, 2371.
[7] B. Breit, *Angew. Chem., Int. Ed.* **2005**, *44*, 6816.
[8] A. J. Sandee, J. N. H. Reek, *Dalton Trans.* **2006**, 3385.
[9] *Supramolecular Catalysis* (Ed.: P. W. N. M. van Leeuwen), 1st edition, Wiley-VCH, Weinheim, **2008**.
[10] J. Meeuwissen, J. N. H. Reek, *Nat. Chem.* **2010**, *2*, 615.
[11] M. J. Wiester, P. A. Ulmann, C. A. Mirkin, *Angew. Chem., Int. Ed.* **2011**, *50*, 114.
[12] G. Gasparini, M. Dal Molin, L. J. Prins, *Eur. J. Org. Chem.* **2010**, 2429.
[13] B. Brisig, J. K. M. Sanders, S. Otto, *Angew. Chem., Int. Ed.* **2003**, *42*, 1270.
[14] L. Vial, J. K. M. Sanders, S. Otto, *New J. Chem.* **2005**, *29*, 1001.
[15] G. Gasparini, L. J. Prins, P. Scrimin, *Angew. Chem., Int. Ed.* **2008**, *47*, 2475.
[16] M. Matsumoto, D. Estes, K. M. Nicholas, *Eur. J. Inorg. Chem.* **2010**, 1847.
[17] J. Wassenaar, E. Jansen, W. J. van Zeist, F. M. Bickelhaupt, M. A. Siegler, A. L. Spek, J. N. H. Reek, *Nat. Chem.* **2010**, *2*, 417.
[18] P. T. Corbett, J. Leclaire, L. Vial, K. R. West, J. L. Wietor, J. K. M. Sanders, S. Otto, *Chem. Rev.* **2006**, *106*, 3652.
[19] L. J. Prins, P. Scrimin, *Angew. Chem., Int. Ed.* **2009**, *48*, 2288.
[20] M. Martin, G. Gasparini, M. Graziani, L. J. Prins, P. Scrimin, *Eur. J. Org. Chem.* **2010**, 3858.
[21] A. Fersht, *Structure and Mechanism in Protein Science*, 1st edition, W. H. Freeman and Company, **1998**.
[22] J. Hine, M. N. Khan, *J. Am. Chem. Soc.* **1977**, *99*, 3847.
[23] K. A. Wayman, T. Sammakia, *Org. Lett.* **2003**, *5*, 4105.
[24] G. T. Notte, T. Sammakia, P. J. Steel, *J. Am. Chem. Soc.* **2005**, *127*, 13502.
[25] L. A. Wessjohann, M. Z. Zhu, *Adv. Synth. Catal.* **2008**, *350*, 107.

[26] M. Dal Molin, G. Gasparini, P. Scrimin, F. Rastrelli, L. J. Prins, *Chem. Commun.* **2011**, *47*, 12476.
[27] D. A. Erlanson, A. C. Braisted, D. R. Raphael, M. Randal, R. M. Stroud, E. M. Gordon, J. A. Wells, *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 9367.
[28] M. G. Woll, S. H. Gellman, *J. Am. Chem. Soc.* **2004**, *126*, 11172.

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