Combinatorial Chemistry

Catalytic Self-Screening of Cholinesterase Substrates from a Dynamic Combinatorial Thioester Library**

*Rikard Larsson, Zhichao Pei, and Olof Ramström**

Dynamic combinatorial chemistry (DCC) has proven to be a useful tool in the rapid identification of ligands and inhibitors for receptors and enzymes, as well as receptors for certain ligands.[1–6] The technique relies on reversible interconnections between building elements, assembled into discrete entities of interacting species, which constitute libraries of dynamically interchanging species. Different formats of the technique have been developed, including the adaptive approach,[7–11] the pre-equilibrated approach,[12,13] the iterative approach,[14] and the deletion approach,[15] all of which address specific challenges. The concept has also been demonstrated for a range of applications: from model systems to biological applications.

One major advantage with dynamic combinatorial libraries (DCLs) over their static counterparts is their potential susceptibility to change in response to an external selection pressure. Under such conditions the DCLs will reconstitute to adopt the best overall arrangement. For example, in the presence of a selective receptor, certain constituents of a DCL of potential ligands will be bound by the receptor, thereby forcing the remaining building blocks to establish a new equilibrium with more of the bound ligand being generated in the process. Thus, one or more of the DCL constituents may be favored and amplified—increased concentrations generated at the expense of other constituents—from binding to the receptor. This whole process is highly dependent on the binding constants, and high amplification is mainly achieved with strong binding of single constituents. If the binding event can be coupled to a secondary process, the selection can be enhanced by allowing the bound species to be removed from the equilibrating pool; this process generates more of this species by re-equilibration of the DCL. If, for example, the bound species is transformed in a catalytic process and expelled from the binding site, the site is free to host more of the DCL constituent and the dynamic system is thus forced to run to completion. An additional advantage with this approach is that only catalytic amounts of the target species need to be present.

An important challenge with DCC is the need for new means to generate reversible reactions. Until now, mainly imines,[7,10,16] acyl hydrazones,[12,17,18] and disulfides[9,13,19,20] have been used for DCLs since these formats have proven to be the most efficient in the systems studied. This is especially the case when biological systems are targeted, since these require reactions that are stable under mild conditions in the aqueous phase. New methods are needed to advance the technique, and new reaction types are necessary for rapid, yet sufficiently stable DCL generation and screening. In this respect, transthioesterification appears to be highly useful. The reaction type is reversible under mild conditions and relatively compatible with common target protein molecules since side reactions are considerably slower. In addition, thioester DCLs can be rapidly extended by simple means to larger libraries.

In the present study transthioesterification has been used to produce prototype DCLs, and it has further been demonstrated that an enzyme catalyst may be used to assist the dynamic self-screening of its substrates (Figure 1). Thus,

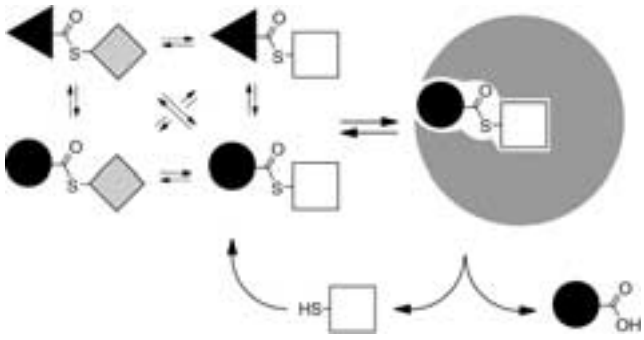

Figure 1. Schematic representation of the DCL catalytic self-screening process with thioesters.

acetylcholinesterase (AChE; see refs. [21,22] and references cited therein) in conjunction with transthioesterification allowed rapid generation and screening of the DCL, a process which results in an apparently complete amplification of the best substrates.

The dynamic combinatorial libraries were generated from a series of thioesters and thiols, respectively (Scheme 1; for further details see the Supporting Information). A homologous series of five different acyl groups was used, ranging from acetyl to hexanoyl and chosen for their resemblance to the native substrate of the enzyme, acetylcholine. The thioesters were all prepared from the same 3-sulfanylpropionic acid in order to keep all components soluble at neutral pH values. Two different thiol groups were also employed, sulfanylpropionic acid and thiocholine. During formation of the dynamic libraries these components may potentially form ten different thioesters, all of which are in exchange with the others during the whole process. If the process was started with equimolar amounts of all acyl components and five equivalents of thiocholine, the resulting concentrations of the formed constituents were relatively comparable and the libraries showed close to isoenergetic behavior, with the sulfanylpropionic acid derivatives formed in slightly higher amounts than their thiocholine counterparts (0.6:0.4).

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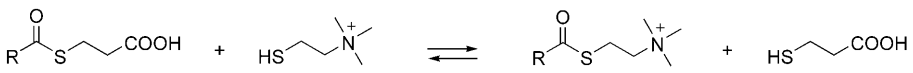

R = Me, Et, *n*Pr, *n*Bu, *n*Pe

Scheme 1. DCL generation by transthioesterification.

The transthioesterification reaction took place effectively under very mild conditions in aqueous media by simple mixing all the components. The ^{1}H NMR spectrum of a mixture of the five original acyl components is displayed in Figure 2a and the spectrum after formation of the full library is shown in Figure 2b. A pD value of 7.0 and room temperature (298 K) were used for all measurements; under these conditions, the libraries were generated with a $t_{1/2}$ value of about 50 minutes. The catalytic action of the enzyme acetylcholinesterase was relatively slow under the same set of conditions. This lower rate was chosen to ensure pseudoequilibrium conditions of the libraries during the enzymatic action, with scrambling being faster than enzyme catalysis.

Treatment of the thioester library with acetylcholinesterase resulted in the best substrate being immediately recognized by the enzyme and hydrolyzed. This resulted in loss of the acyl component from the library, which forced the library to reconstitute to accommodate the increasing amounts of free thiol and to generate more of the hydrolyzed species. Over time, two of the acyl functionalities, the acetyl and propionyl groups, proved to be mainly acted upon by the enzyme, with the acetyl species being more rapidly hydrolyzed than the propionyl counterpart (Figure 2c). The overall rate of formation of acetic acid ($t_{1/2} = 210$ min) proved to be around 20% faster than the rate of formation of propionic acid ($t_{1/2} = 270$ min, Figure 3). Only after substantial hydrolysis of the two main substrates (> 600 min) did the enzyme start hydrolyzing the butyrate ester, and even then, the reaction was considerably less efficient ($t_{1/2} =$ ca. 1100 min). This long lag phase may be caused by inhibitory activities of the present esters.[23] All the other acyl groups remained untouched by the enzyme, a result which is in accordance with the known specificity of acetylcholinesterase. Addition of the enzyme after complete equilibration of the library, as compared to addition of enzyme from the beginning, did not result in any major differences and the same distribution of compounds was recorded, as had been anticipated. In both cases, the combined library interchange and catalytic processes resulted in the complete hydrolysis of

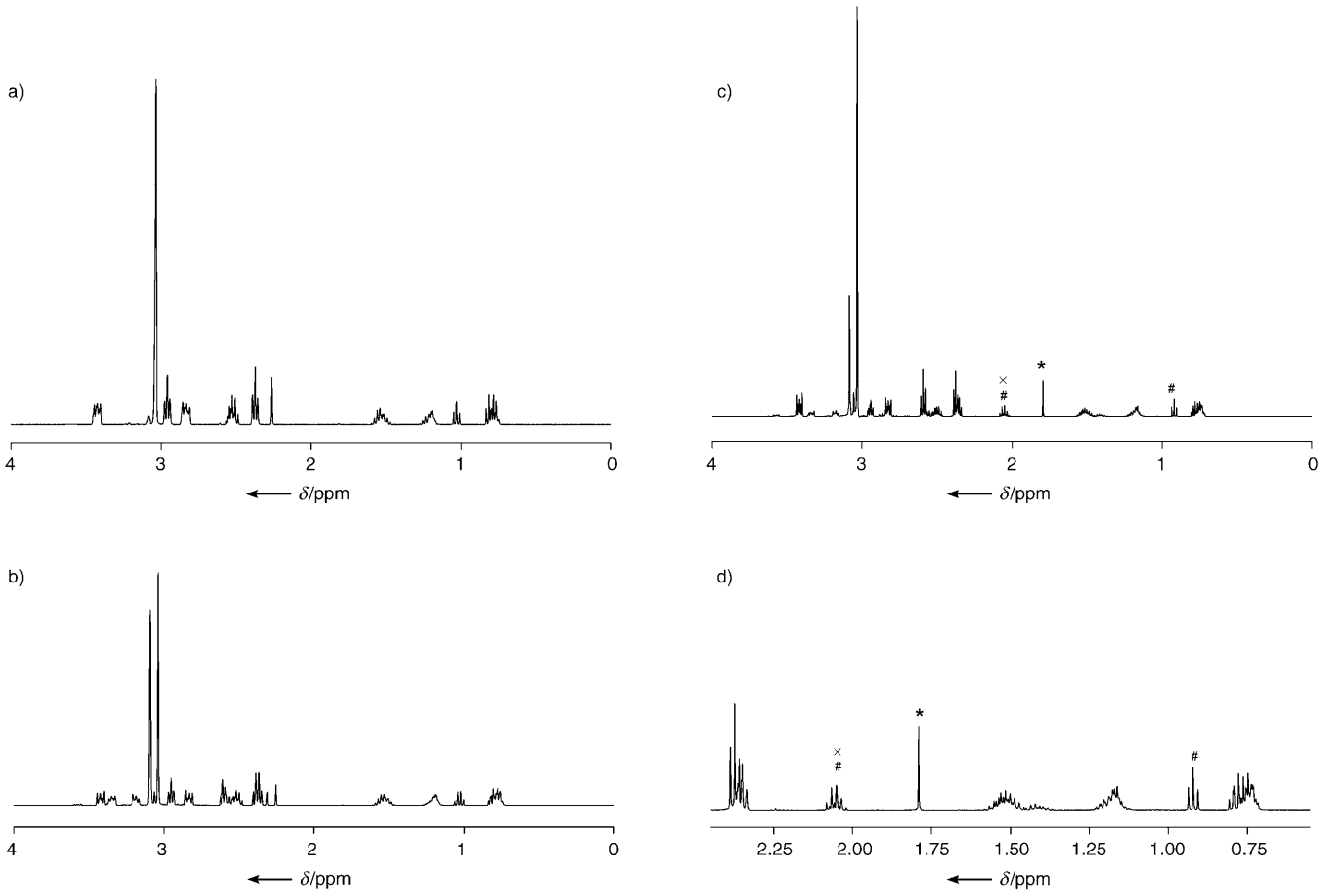

Figure 2. ^{1}H NMR spectra of library components/constituents: a) Before library generation, b) DCL in the absence of AChE, c) DCL in the presence of AChE, d) enlarged section of (c). ⋆, #, and × indicate the signals for acetate, propionate, and butyrate groups, respectively.

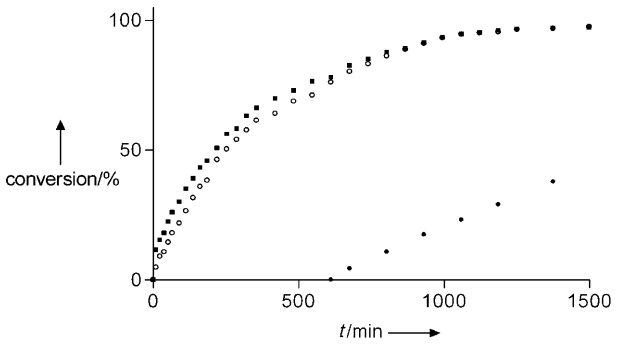

Figure 3. Formation of acetate (■), propionate (○), and butyrate (●) hydrolysis products.

certain acyl thioesters to form the corresponding acids and thiols. Thus, this catalysis assistance could be used to fully amplify the selected acyl groups. It was rapidly shown for the thiol components that removal of thiocholine failed to generate any hydrolytic products under the same conditions, thus demonstrating that thiocholine was essential for the reaction to occur. The screening of the DCL thus proved straightforward, and the selection effect of the combined process can be estimated as the ratio of the amount of hydrolytic products formed versus the amount of substrate fragment added. In the present case, 100% selection/amplification was achieved for the acetate and propionate groups, respectively.

The present study has demonstrated that catalysis can be used as a means to self-screen substrates from a dynamic combinatorial library. Acetylcholinesterase could thus be targeted to allow rapid identification of the most efficient substrate in the library. This system is however not restrained to enzyme catalysis; it may be extended to any catalytic system, including organic and inorganic catalysts, and may be employed to rapidly screen reactions of catalysts for new substrates. In particular, catalysts of unknown specificity may be easily mapped with such systems, for example, in proteomics and catalyst-development campaigns. This approach also enables screening of complex DCLs without the necessity of using equimolar amounts of targets. In addition, it has been shown that transthioesterification can be readily employed to generate DCLs that are compatible with mild conditions in aqueous media.

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Keywords: combinatorial chemistry · enzyme catalysis · hydrolysis · proteomics · transesterification

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