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Synthesis of a peptidocalix[4]arene library and identification of compounds with hydrolytic activity

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Abstract

A 120 member library of peptidocalix[4]arenes was synthesized and screened for catalysis of the hydrolysis of *p*-nitrophenyl acetate. His-Ser-His-calix[4]arene was found to catalyze this reaction with $v_0 = 3.24 \times 10^{-8}$ M/s, an increase of 1520% above background and 30% above the tripeptide (His-Ser-His) alone.

Keywords: Calixarenes; Catalysis; Solid-phase synthesis; Amino acids; Peptides

1. Introduction

Calix[4]arenes have been extensively studied for their ability to act as synthetic receptors and enzyme mimics. As hosts, calixarenes have been found to bind a wide range of compounds, including amino acids [1], carboxylic acids [2], and other small, organic molecules [3]. Calix[4]arenes substituted on the upper rim with ligands that

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chelate zinc [4] or copper [5] have been used to mimic hydrolytic enzymes and catalyze the hydrolysis and transesterification of phosphonate esters. Calix[4]arene-4-sulfonates [6] have been shown to facilitate the regioselective cleavage of cytidine 2′,3′-cyclic phosphate. Additionally, calix[4]arenes substituted with imidazoles on the upper rim have been found to catalyze the hydrolysis of *p*-nitrophenyl esters [7]. The success of these studies indicates that the functionality on the upper rim of a calix[4]arene can be manipulated to design catalysts for a variety of reactions. Peptides are an attractive choice for these substituents due to their wide range of functional groups and inherent chirality. The binding pocket formed by amino acids incorporated at the upper rim of a calix[4]arene can take on the characteristics of an enzyme-like active site, which is expected to confer selectivity and potency to the catalyst.

We have reported the solid phase synthesis of a peptidocalix[4]arene [8] using standard Fmoc peptide synthesis techniques, and we are currently investigating the ability of peptidocalix[4]arenes to act as molecular receptors and enzyme mimics. The development of these molecules is facilitated through the use of parallel, solid phase synthesis, which allows rapid preparation of large numbers of compounds. This paper details the synthesis and screening of a peptidocalix[4]arene library to identify compounds that catalyze ester hydrolysis.

2. Materials and methods

2.1. General methods

The solvents and all reagents used in this study were purchased from commercial suppliers and were used as received.

2.2. Tripropoxytetranitrocalix[4]arene anhydride 2

Tripropoxytetranitrocalixarene carboxylic acid **1** [8] (0.1 g, 0.12 mmol) and dicyclohexylcarbodiimide (0.012 g, 0.060 mmol) were stirred in 2 mL of CH_2Cl_2. After 3 h, the precipitate was removed by filtration and the filtrate was concentrated in vacuo to yield **2** (0.99 g, 99%) as a yellow solid. This compound was used without further purification.

2.3. Synthesis of library

Microtiter plates were amine functionalized [9] and the addition of tripropoxytetranitrocalix[4]arene anhydride **2** to the glass was accomplished using DMAP as a catalyst. The nitrocalix[4]arene **3** was treated with $SnCl_2 \cdot 2H_2O$ to provide the corresponding tetraamino derivative **4**. The appropriate amino acids were added sequentially using Fmoc-protected amino acids, HBTU, and DIPEA. Treatment with 20% piperidine removed the Fmoc protecting groups and with 95% TFA removed the side chain protecting groups.

2.4. Screening of library

The library was screened for the hydrolysis of *p*-nitrophenyl acetate (NPA) as follows. Into each well was added 74 μM NPA in 100 mM sodium acetate buffer, pH 5.0. Negative controls contained no calix[4]arene catalyst, while positive controls contained 0.2 mM KOH. The production of *p*-nitrophenol was monitored by the change in UV-Vis absorbance at 324 nm over 24 h.

2.5. Synthesis of peptidcalixarenes on beads

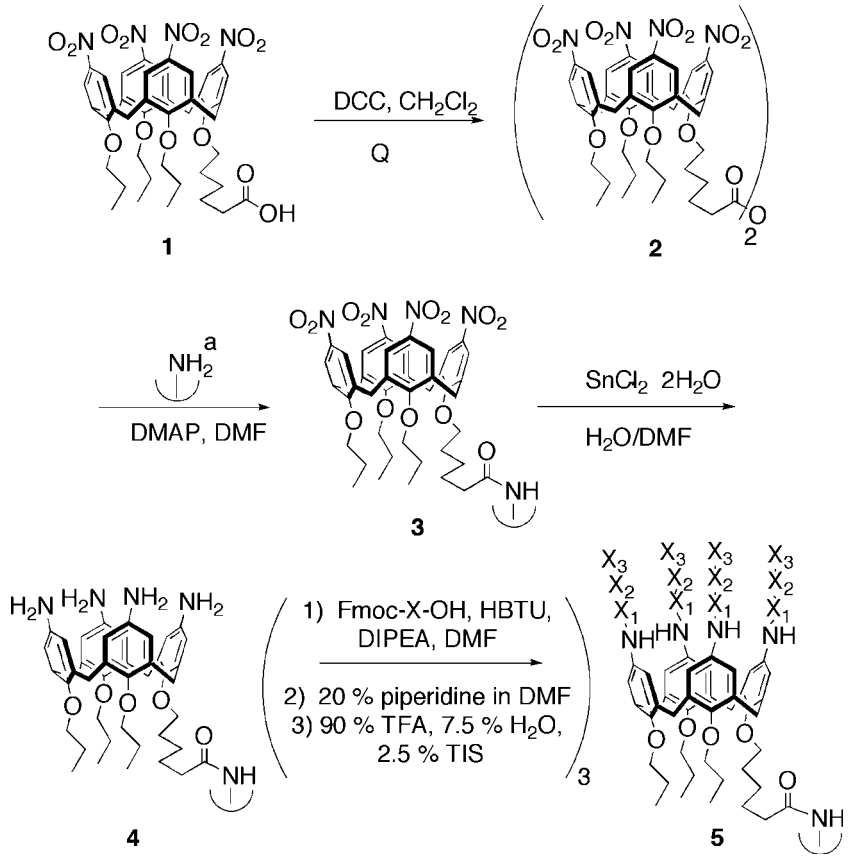
The peptidocalix[4]arenes were synthesized according to literature procedures [8] with exception that the anhydride derivative **2** of tripropoxytetranitrocalixarene carboxylic acid **1** was used to facilitate addition of the calixarenes to the solid support. The loading was determined by measuring Fmoc release from the terminal amino acids [10].

3. Results and discussion

A calix[4]arene library with four identical tripeptides on the upper rim was synthesized on the surface of wells of 96-well glass microtiter plates. Each tripeptide contained serine and histidine, two of the amino acids found in the catalytic triad of serine proteases and esterases. The third amino acid was varied and all possible sequences—HSX, SHX, HXS, SXH, XHS, and XSH, where X, any of the 20 common amino acids—were prepared, giving a library of 120 compounds. The preparation of the calix[4]arene scaffold and the synthesis of the peptidocalix[4]arenes on solid support are shown in Scheme 1.

This screen revealed six compounds that catalyzed the hydrolysis: SerGluHis-calix[4]arene (SerGluHisCX), HisSerHisCX, GluSerHisCX, AsnSerHisCX, GluHisSerCX, and HisSerGluCX. These six compounds were synthesized on a larger scale on Argopore-NH_2 beads and were studied for their ability to catalyze the hydrolysis of *p*-nitrophenyl acetate (shown in Scheme 1). The beads were screened by incubating them with 61.8 mM NPA in 100 mM sodium acetate buffer (pH 5.0)/acetonitrile 50/50 to give approximately equimolar amounts of catalyst and substrate. Controls included either unmodified beads or beads with AlaAlaAlaCX. Samples were taken every 30 min for 8 h. These results are shown in Fig. 1. UV–Vis spectroscopy indicated that these peptidocalix[4]arenes were capable of facilitating the hydrolysis with SerGluHisCX, HisSerHisCX, and GluSerHisCX being the most efficient. AlaAlaAlaCX showed no hydrolytic activity, as did the unmodified beads.

The initial rates for the hydrolysis of NPA by SerGluHisCX, HisSerHisCX or GluSerHisCX were determined and compared to the initial rates of reaction for the analogous tripeptides directly attached to the Argopore-NH_2 beads (SerGluHis, HisSerHis, and GluSerHis). The tripeptides were prepared directly attached to the beads using standard Fmoc-protected peptide coupling techniques without the calix[4]arene scaffold. This comparison was meant to determine if the preorganization



Scheme 1. Synthesis of peptidocalix[4]arenes on solid support. [a]Amine functionalized well of a microtiter plate.

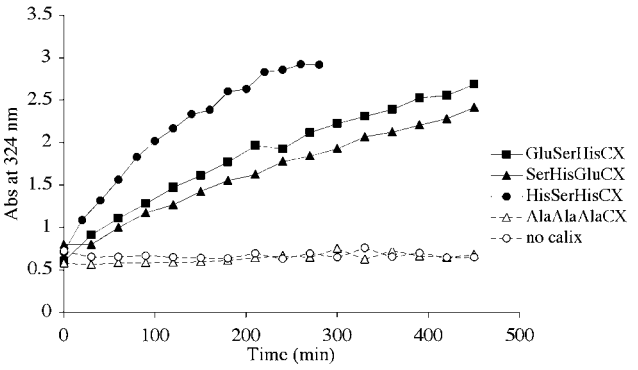


Fig. 1. Initial release of *p*-nitrophenol from PNA catalyzed by peptidocalix[4]arenes, monitored by UV–Vis spectroscopy.

Table 1
Initial rates for the hydrolysis of NPA in 50/50 acetonitrile/buffer (pH = 5.0)

Catalyst	v_0 (10^{-8} M/s)	v_0(cat)/v_0(bkgd)	v_0(pep-CX)/v_0(pep)
—	0.2 ± 0.1	—	—
GluSerHis	1.7 ± 0.2	8.5 ± 0.5	—
SerGluHis	1.7 ± 0.4	8.4 ± 0.6	—
HisSerHis	2.4 ± 0.3	12.2 ± 0.5	—
GluSerHisCX	1.2 ± 0.4	6.2 ± 0.6	0.7 ± 0.3
SerGluHisCX	0.8 ± 0.3	4.1 ± 0.6	0.5 ± 0.4
HisSerHisCX	3.2 ± 0.1	16.2 ± 0.5	1.3 ± 0.1

[catalyst]/tripeptide = 0.48 mM. [NPA]/[cat] = 500. Each point is the average of three runs.

of tripeptides on the upper rim of the calix[4]arene scaffold enhanced the catalytic ability of these compounds over the tripeptides alone. Therefore, the amount of catalyst used for the determination of the initial rates was based on the concentration of the tripeptides and not on the concentration of the whole calixarene compound, which allowed the comparison of the tripeptide's ability to catalyze the hydrolysis with and without calixarene scaffold. The initial rates for all six compounds were determined using 0.48 mM ([catalyst]/tripeptide) of SerGluHisCX, HisSerHisCX or GluSerHisCX or 0.48 mM SerGluHis, HisSerHis, or GluSerHis and 240 mM NPA ([NPA]/[catalyst] = 500). These data are shown in Table 1. HisSerHisCX proved to be the most efficient catalyst with $v_0 = 3.2 \times 10^{-8}$ M/s at 240 mM NPA. Under these conditions, the presence of the calix[4]arene scaffold results in a 30% increase in the initial rate of reaction over that of HisSerHis alone and a 1520% increase over that of background hydrolysis (without catalyst). Interestingly, the tripeptides attached directly to the beads also proved to be quite effective at catalyzing this reaction in all cases, and even better than the calix[4]arene catalysts for the tripeptides GluSerHis and SerGluHis. Therefore, in only one case (HisSerHisCX) the calix[4]arene scaffold enhanced the catalytic ability of the active tripeptide. Most importantly, all six compounds showed a dramatic increase in reaction rate (from 310 to 1520%) over that of the background.

4. Conclusions

We have synthesized a peptidocalix[4]arene library and screened it for the hydrolysis of NPA. This revealed three compounds (SerGluHisCX, HisSerHisCX, and GluSerHisCX) capable of hydrolyzing NPA. Further analysis indicated HisSerHisCX is the most efficient catalyst, giving an initial rate that is 1520% higher than the background and 30% greater than that of HisSerHis in the absence of calix[4]arene scaffold. This is the first step in the development of these compounds as novel, recyclable catalysts. We are currently preparing calix[4]arenes that contain two or more different peptides at the upper rim and we expect these more diverse peptidocalix[4]arenes to have improved catalytic ability. Additionally, we are continuing to study the ability of the calix[4]arene scaffold to improve the catalytic efficiency of the tripeptides.

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