Cyclodextrin-Based Artificial Acyltransferase: Substrate-Specific Catalytic Amidation of Carboxylic Acids in Aqueous Solvent

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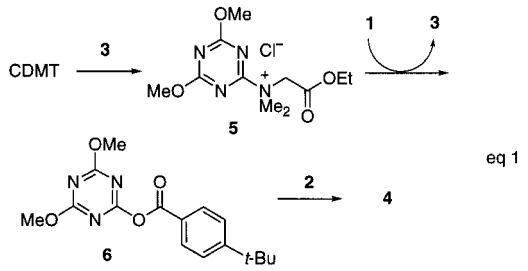
We report herein for the first time a new, cyclodextrin (CD)-based artificial enzyme that efficiently mimics an acyltransferase. The reaction involves substrate-specific condensation of aromatic carboxylic acids that possess a strong affinity for the CD cavity, with amines to give carboxamides. The artificial enzyme catalyzes in situ activation of the carboxylic acid in an aqueous solvent leading to the formation of an acyloxytriazine (activated ester), which undergoes aminolysis to give an amide.

In the biomimetic chemistry using CD or its derivatives, many studies on a hydrolase model, especially chymotrypsin, have been reported.[1,2] By contrast, artificial enzyme models catalyzing the reverse reaction, dehydrating condensation between carboxylic acids and amines, have not appeared. Aqueous media generally increase the hydrophobic effect, and therefore, promote the formation of an inclusion complex between CD and hydrophobic substances.[3] In fact, most reactions employing CD were carried out in aqueous media;[4] generally, CD-based enzyme-like reactions should tolerate and enjoy the use of aqueous solvent. This limitation is probably responsible for the lack of enzymatic models of dehydrating condensation between carboxylic acids and amines, which are generally carried out under dry conditions,[5] via in situ activation of an acid moiety in aqueous media.

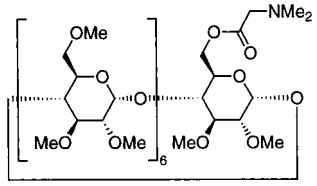
Recently, we have introduced 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) as a new dehydrating condensing agent,[6] which enables us to carry out the direct one-pot condensation of carboxylic acids and amines in water or alcohols.[7] In the course of the study, we now found a novel catalytic system for formation of carboxamides in aqueous solvent. Condensation of sodium 4-*tert*-butylbenzoate **1** with benzylamine hydrochloride **2** using 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) and a stoichiometric amount of *N*,*N*-dimethylglycine ethyl ester **3** in 5% aqueous MeOH afforded carboxamide **4** in 76% yield (Scheme 1). Use of a catalytic amount of **3** (0.2 equiv) also gave **4** in 71% yield. Without use of **3**, the condensation did not take place to a discernible extent. These findings clearly indicate that CDMT cannot react directly with the carboxylate anion **1** by itself in the absence of **3**, and therefore, that intervention of **3** as a catalyst is essential for the condensation. We believe that ammoniotriazine **5** should be generated from CDMT by the reaction with amine **3** as an actual condensing agent, as observed in the reaction of CDMT and *N*-methylmorpholine that produces reactive DMT-MM (eq 1).[6a,c] The reaction



eq 1

of **5** and **1** would afford reactive acyloxytriazine **6**,[6−8] which in turn undergoes aminolysis with amine **2** to give the carboxamide **4**.[9] Since **3** is regenerated by attack of carboxylate **1** on the triazino group of **5**, it enjoy the reuse in the next catalytic cycle for further activation of carboxylates.

On the basis of this finding, we have designed a substrate-specific, catalytic system mimicking an *N*-acyltransferase in which a *β*-CD **7** possessing *N*,*N*-dimethylglycyl group acts as an apoenzyme. As shown in Scheme 2, **7** is activated by coupling with CDMT (coenzyme) through the dimethylamino group to give a reactive holoenzyme **8** corresponding to **5**. An aromatic



carboxylate ion fitting the CD cavity preferentially interacts with **8** to form an inclusion complex (ES-complex). The included carboxylate ion in the resulting ES-complex is brought into close proximity with the triazino group and attacks it preferentially, giving an EP-complex. Final aminolysis of the resulting acyloxytriazine takes place to precipitate the produced amides with concomitant liberation of the apoenzyme **7**, which can be recycled.

The artificial apoenzyme **7** was readily prepared from mono-6-hydroxy permethylated *β*-cyclodextrin[10] by coupling with *N*,*N*-dimethylglycine.[11] Substrate specificity of the condensation with amines was examined using a competitive reaction between **1** and sodium 3,5-di-*tert*-butylbenzoate **9a**. As shown in Table 1, a methanolic solution of ammonium salt **2** (1.0 equiv) and CDMT (1.0 equiv) was added to an aqueous solution of sodium

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(9) We separately synthesized and isolated **5** and found that **5** was able to act as a condensing agent giving **4** in 76% yield.

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(11) For experimental details, see Supporting Information.

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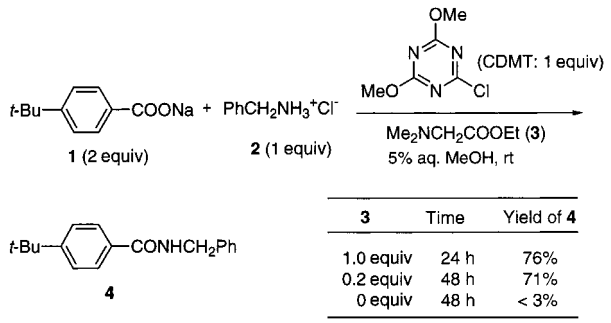
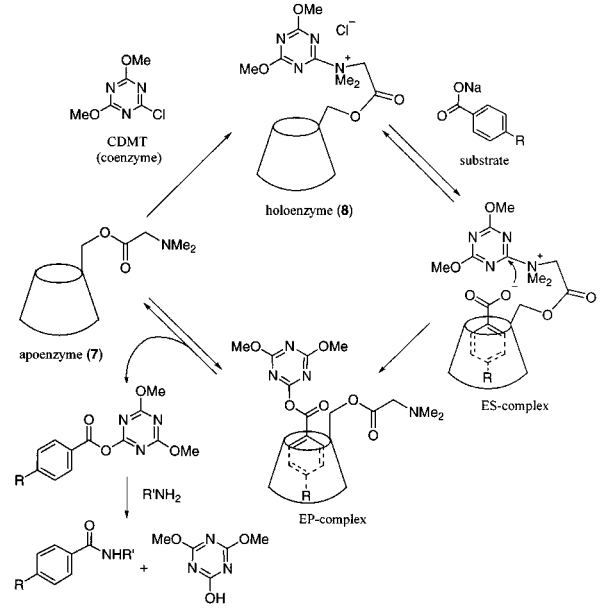
Scheme 1



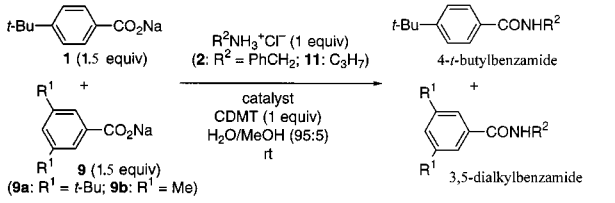
3	Time	Yield of 4
1.0 equiv	24 h	76%
0.2 equiv	48 h	71%
0 equiv	48 h	< 3%

Scheme 2



carboxylates **1** (1.5 equiv) and **9a** (1.5 equiv) and a stoichiometric amount (1.0 equiv) of the catalyst **7** at room temperature. Highly selective condensation toward carboxylate **1** over **9a** in a ratio of 88:12 was observed (run 1). For comparison, a control experiment using a combination of **3** and heptakis(2,3,6-trimethyl)-β-CD **10** instead of **7** was carried out, in which no selectivity (**1**:**9a** = 52:48) was obtained. The results indicate that the substrate binding site (CD moiety) and the catalytic site (dimethylamino group) must be linked to each other to achieve the substrate-specific activation of **1**. When the amount of **7** was reduced to 0.2 equiv, both the selectivity and the yields of amides were found to be almost unchanged (run 3). Thus, the present artificial enzymatic system involves turnover of the catalyst. A competitive reaction of **1** and sodium 3,5-dimethylbenzoate **9b** with either **2** or propylamine hydrochloride **11** also showed high selectivities of 92:8 or 91:9, respectively, whereas a control experiment showed no selectivity (runs 4−6). The observed selectivity can be attributed to the differences in affinity of the substrates to the CD cavity of **7**.[12]

Table 1. Substrate-Specific Formation of Carboxamide by CD Catalyst



run	ammonium salt	carboxylates	catalyst (equiv)	time (h)	yield[c] (%)	ratio (4-*t*-Bu-: 3,5-dialkyl-)
1[a]	**2**	**1, 9a**	**7** (1)	9	90	88:12
2[a]	**2**	**1, 9a**	**3** (1), **10** (1)	16	54	52:48
3[a]	**2**	**1, 9a**	**7** (0.2)	20	87	87:13
4[b]	**2**	**1, 9b**	**7** (0.2)	48	80	92:8
5[b]	**2**	**1, 9b**	**3** (0.2), **10** (0.2)	48	80	51:49
6[b]	**11**	**1, 9b**	**7** (0.2)	48	77	91:9

[a] A methanolic solution of CDMT and **2** was added over a period of 5 h by a syringe pump. [b] CDMT in MeOH was added to an aqueous solution of other reactants by one portion. [c] Total yields of amides.

Table 2. Pseudo-First-Order Rate Constants for Catalytic Acylation of **11**[a]

carboxylates	catalyst	k_{obsd} (min^{-1})
1	**7**	9.0×10^{-3}
1	**3**	0.7×10^{-3}
9b	**7**	1.8×10^{-3}

[a] Performed at 50 °C in 1 M Et_3N−HCl (pH 9) containing 10% MeOH. [carboxylate] = 22.5 mM; [**11**] = 15 mM; [catalyst] = 3 mM; [CDMT] = 15 mM.

Table 2 summarizes the pseudo-first-order rate constants for acylation of **11** with **1** or **9b** at 50 °C, pH 9. Most importantly, the CD-based catalyst **7** accelerates the condensation of **11** and **1** by a factor of 13, compared to simple catalyst **3**. Condensation rate of carboxylate **1** with **11** catalyzed by apoenzyme **7** is 5 times greater than that of **9b**, which combined with differences in competitive affinity of these substrates **1** and **9b** toward **7** may result in the observed selectivity (ca 9:1) in the competitive reaction (Table 1, run 6).[14]

It is noteworthy that product inhibition,[15] caused by a competitive binding of a hydrophobic part of the product to the cavity of catalyst **7**, can be avoided by precipitation of the produced amides under the conditions, because the amides are almost insoluble in the solvent used (5% aqueous MeOH) while other reactants are soluble. In fact, when the competitive reaction between **1** and **9b** was carried out under the same conditions as Table 1, run 4, except for use of amide **4** (1 equiv) as an additive, the selectivity remained unchanged (92:8, 75% yield).

There are several reports on enzyme models catalyzing the formation of carboxamides.[16] All of the reported reactions employed reactive acylating compounds such as activated esters that had been prepared separately prior to use in catalytic reactions, in which catalysts hold both the activated acyl moiety and an amine in proximity to accelerate their coupling. In contrast, our system provides a new conception of the acyltransferase reaction, in which the substrate specificity is achieved in the activation step of carboxylic acids in an aqueous solvent. Further kinetic characterization on the enzyme model reaction is under investigation.

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Supporting Information Available: Experimental details including synthesis and characterization of **7** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) According to the literature,[13] the dissociation constant K_d at 50 °C for the complex of **1** and **10** was determined to be 3.1 mM by a NMR method, whereas the complex of **9b** and **10** was too unstable to determine the K_d value.

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