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Citation	Chemical Communications, 47(25): 7176-7178
Issue Date	2011-05-23
Type	Journal Article
Text version	author
URL	<a href="http://hdl.handle.net/10119/10865">http://hdl.handle.net/10119/10865</a>
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Description	

# Carbon nanotube structured biomimetic catalyst for polysaccharide degradation

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Received (in XXX, XXX) Xth XXXXXXXXXX 200X, Accepted Xth XXXXXXXXXX 200X

First published on the web Xth XXXXXXXXXX 200X

DOI: 10.1039/b000000x

A unique artificial catalyst that mimics the structure of active sites in real enzymes using functionalized carbon nanotubes is presented. This concept will allow for the potential construction of a library of biomimetic catalysts for enzyme active centers, for which the structure–catalysis relationships are well defined.

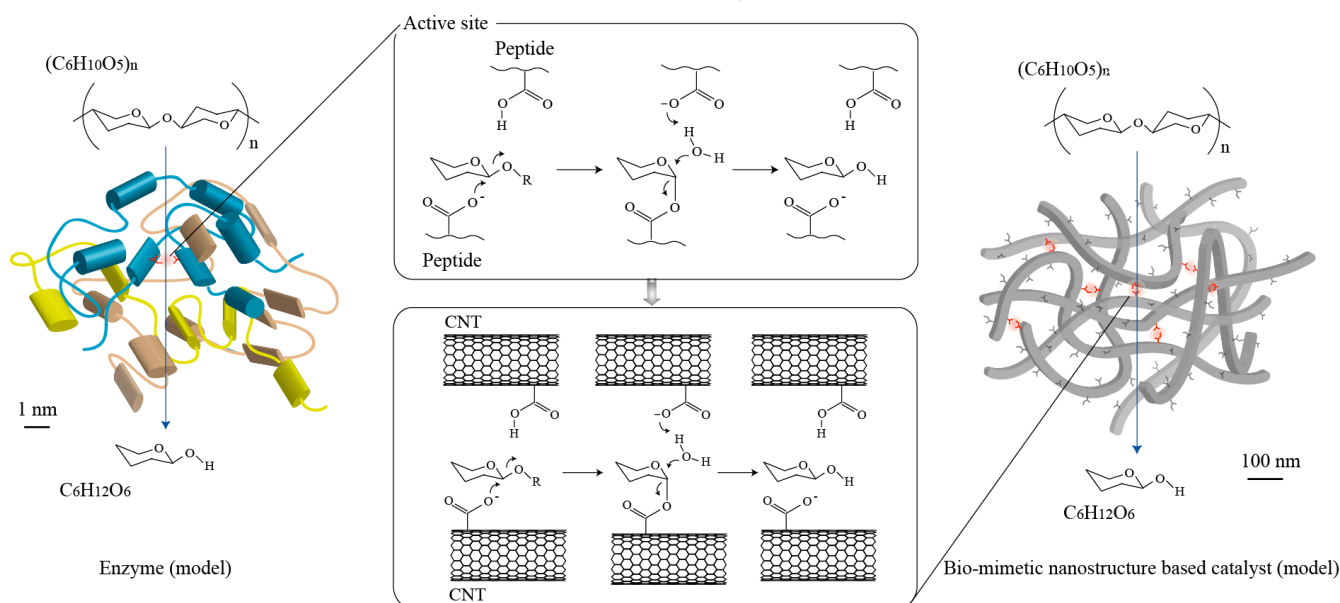
In nature, enzymes produced by microorganisms degrade biomass components - cellulose, for example, is degraded by different types of cellulases.<sup>1-3</sup> These enzymes, using their unique quaternary structure, demonstrate very specific catalytic degradation of specific biomass components.  $\beta$ -glycosyl hydrolases catalyze the degradation of cellulose by specifically breaking down the  $\beta$ -1,4-glycosyl bonds of sugar substrates.  $\beta$ -glycosyl hydrolases release their saccharide products, retaining the configuration at the anomeric center. In enzymes of this group, the catalytic nucleophile is a carboxylic sidechain while a second carboxylic sidechain typically provides acid-base catalytic assistance. This is achieved first by donating a proton to the linking oxygen of the scissile bond, and then by abstracting a proton from an incoming water molecule.<sup>4-6</sup> In this paper, we report the synthesis of a carbon nanotube (CNT)-based catalyst that mimics the catalytic center of  $\beta$ -glycosyl hydrolases and its catalytic function in sugar degradation (Scheme 1). Artificial catalysts made of metal and zeolite nano-materials have been well studied.<sup>7-11</sup>

However, to the best of our knowledge, there are no reports on bio-inspired catalysts that mimic the unique orientation and function of the active sites in enzyme conformations.

Although nano-scale control of nano-materials to mimic the exact structural conformation of an enzyme is inconceivable at current state of technology, it is possible to make functional catalysts by mimicking the active sites of biomass degrading enzyme structures stochastically. We have followed this probable approach in mimicking the active site cellulase.

We synthesised the catalyst by functionalizing CNT with carboxylic group, using acid treatment method.<sup>12</sup> We also tried to create an environment conducive for the functionalized CNT matrix catalyst to attain a functional conformation analogous to that of the real enzyme. This was done by adjusting the pH of the reaction environment to the pK value so that the carboxylate modified CNT (COOH-CNT) matrix could contain both nucleophilic and acid-base catalytic centers on CNT backbone in a suitable steric orientation for catalysis.

In SEM observations of the nano-structure based matrix, there were virtually no differences between CNT and COOH-CNT matrix surface structures (Fig. 1). In these matrices, there are complex CNT conformations within close proximity to each other. These conformations contribute to the expression of the catalytic reaction of the bio-mimetic nano-structure based catalysts.



Scheme 1 Hypothesis of sugar degradation by carbon nanotube structured biomimetic catalyst.

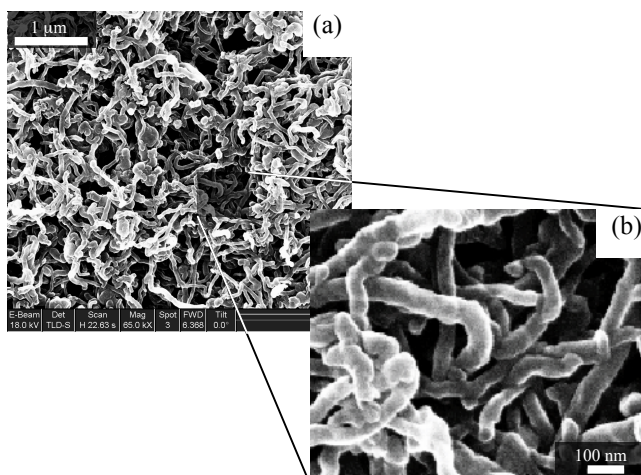


Fig. 1 SEM images of CNT based matrix. (a) 1  $\mu\text{m}$  scale, (b) 100 nm scale.

The surface structures of carboxylate modified and unmodified CNT were analyzed by IR spectroscopy. There were some bands derived from carboxylic group in the case of modified CNT. Four major bands around (1430, 1460), 1640 and 2980  $\text{cm}^{-1}$ , which are attributed to the bending vibrations of  $>\text{C}=\text{O}$ , C-O-H and OH derived from carboxylic group, respectively (SI. 1). Our results are in agreement with previous reports that a shift to lower bands is observed when COOH is decorated on CNT backbone.<sup>12</sup>

When COOH-CNT matrix was used for catalysis against the degradation reaction of cellobiose – a dimer of glucose connected with  $\beta$ -1,4-glycoside – as a substrate at pH below 3.0, the concentration of glucose product increased. Additionally, the catalytic effect of COOH-CNT matrix was higher than unmodified CNT matrix (SI. 2). This result unequivocally proves the cellulase-like catalytic role of this bio-mimetic catalyst. We investigated the changes in glucose concentration in the 24 h reacted from cellobiose under the citrate-phosphate buffer different pH environments: 2.6, 3.0, 4.2, 5.0 and 6.2.

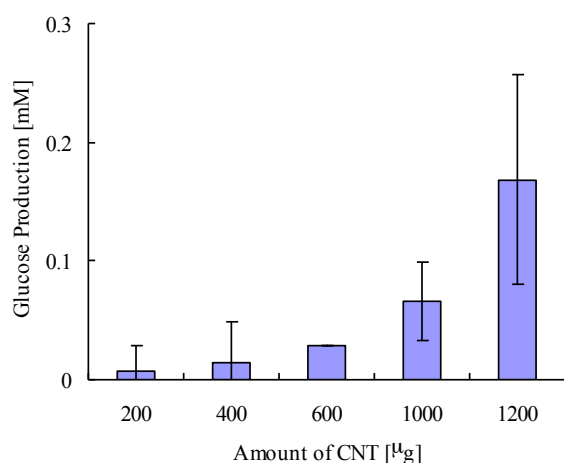


Fig. 2 Concentration of glucose produced against the amount of COOH functionalized CNT on a glass substrate. The 24 h reacted cellobiose solutions (10 mM) in citrate-phosphate buffer at pH 3.0, under the ambient temperature were used as substrates.

In the case of the unmodified CNT nano-structure, as hypothesized, pH dependent changes in glucose concentration was not observed at all. For COOH-CNT structured catalyst, glucose concentrations varied with pH, peaking at between pH 3.0 and 4.0. Since the theoretical pK value of carboxylic group is around 3.1~4.4, the degradation of cellobiose was the highest at pH  $\sim$ 3.0. It is likely that this specific pH environment allowed for the artificial catalyst to better mimic the steric orientation of the nucleophilic and acid-base catalytic active sites of the sugar degradable enzyme more closely. The performance of the proposed nano-structure was analyzed at different catalyst concentrations in various sugar degradation reaction. The result showed increase in glucose concentration with increasing amount of the COOH-CNT catalyst (Fig. 2). These observations confirmed the attainment of comparable active site conformation and catalytic functionality of the proposed CNT based biomimetic enzyme to its natural peer in the degradation of cellobiose into glucose.

Although the cellobiose degradation mechanism of this bio-mimetic catalyst (i.e., whether it can form the complex with substrate or not) is not investigated, it can be considered that the carboxyl group on CNT contributes to the cellobiose degradation. We simply evaluated the cellobiose degradation efficiency of this bio-mimetic catalyst. The specific activity of this bio-mimetic catalyst was 3.82 nmol/min/mg of CNTs. This value was found to be lower than that of natural cellulase.<sup>13,14</sup> One of the probable reasons for the low efficiency of our catalyst was the presence of a low mean number of nano-constructs possessing the ideal structural conformation. They may have interfered with the functioning of the ideal nucleophilic and acid-base catalytic center orientation, which, for effective catalysis, should be close to each other (one should be protonated, the other should be deprotonated). In addition, our catalyst mimicked only the active site conformation of the natural cellulase while, in reality, natural enzymes have other important functions attributed by structures capable of recognizing the substrate with high specificity, promote the active site, and fix the substrate in the ideal conformation for catalytic reaction. That is, the efficiency of an enzyme lies in the entire conformation, not only in the conformation of active sites.

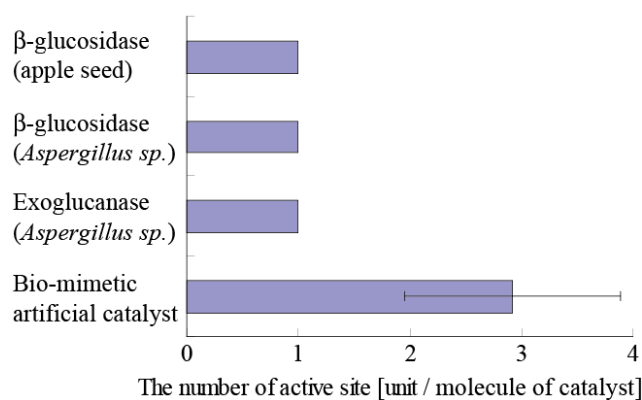


Fig. 3 The number of active sites per molecule of catalyst. One CNT structure was considered as one molecule of catalyst.

Therefore, the catalytic effect of our bio-mimetic catalyst was only partial. However, the number of the catalytic sites per molecule of bio-mimetic catalyst was higher than other sugar degradable catalysts (Fig. 3). This result indicates that the artificial catalyst possesses more active sites per one unit of catalyst. In generally, sugar degradation enzymes of the cellulose type may be greater than hundreds of kilodaltons in size, and there is often only one catalytic active site in each protein complex.<sup>13,14</sup> Therefore, this bio-mimetic nano-structure catalyst is beneficial due to the high number of active sites per weight of catalyst. Although this artificial catalyst is not yet as efficient as the natural enzyme it mimics, it holds the potential for further nano-scale improvement of the catalyst in terms of the number and orientation of COOH on the CNT backbone to achieve a desirable scale of degradation of sugar components in biomass.

We claim successful construction of a bio-inspired catalyst based on the CNT matrix functionalized with carboxylic groups. This nanostructure is capable of catalyzing sugar degradation reaction by cleaving the  $\beta$ -1,4-glycosyl bonds of the sugar substrate by the same way as the natural cellulase. Our construct successfully mimicked the active center of the sugar degrading  $\beta$ -glycosyl hydrolases. The catalyst functioned optimally at pH  $\sim$ 3.0. Our work will allow for the construction of an array of artificial catalysts that mimic the conformation of the active centers of enzymes, for which the structure-catalysis relationships are already known or at least can be partially speculated. With some improvement, such bio-inspired catalysts can be used in bioprocess industries, especially in bio-energy conversion, thereby avoiding the use of hazardous microbial cultures or expensive natural enzymes. Recently, for on-site utilization of biomass, a unique fuel cell was developed capable of generating electricity directly from cellulose.<sup>15</sup> We propose that the integration of this artificial catalyst with a direct energy conversion system that will enable us to use biomass from ubiquitous sources, for example, wastes, as a fuel for generating electricity. Moreover, such constructs can be used for systematic evaluation of the functional individual parts of complex enzyme conformations to gain a better insight into the mechanisms of enzymatic activity.

This novel bio-mimetic approach can enhance the scientific understanding of synthetic catalysis, natural catalytic processes, which will, in turn, provide information related to macroscopic biological networks.

The authors thank Professor Yoshihiro Kobayashi (Osaka University) for constructive and meaningful discussions. We highly appreciate Dr. Ha Minh Hiep, Mr. Mohammad M. Hossain, Ms. Hoa Le Quynh (Osaka University) and Mr. Anthony Veloso (University of Toronto) for their technical supports.

## Notes and references

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