Cooperative Effect in Carboxylic Acid Catalyzed Cellobiose Hydrolysis

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Introduction

Since glucose can be converted readily into many useful compounds, efficient conversion of cellulose to this building block is important to successful utilization of biomass for large-scale fuels and chemicals production, The conversion of cellulose to glucose is catalyzed by acid and by glycosidase enzymes. In the general acid catalyzed reaction, it has been shown that the rate of hydrolysis of cellobiose (a model compound for cellulose) is proportional to the proton concentration.[1] Thus, organic acids, being weaker than inorganic acids and possess higher pKa's, are less effective catalysts.

However, although carboxylic acid groups constitute their active sites, the glycosidase enzymes are very effective. From knowledge of their structures, it has been proposed that the high activities of glycosidase are due to cooperative action of two adjacent carboxylic acids groups as general acid/base catalysts, where one of the groups acts as an acid and the other as a base.[2] Extrapolating from this model, it would seem possible that two strategically placed carboxylic acid groups on designed structures could exhibit a similar cooperative effect and render the carboxylic acid much more active in cellobiose hydrolysis than that predicted from the acid concentration. In this study, we explored using carbosilane dendrimers as a vehicle to anchor these acid groups. These well-known molecules can be synthesized using the growth step synthesis with a high degree of control of size and functionality.[3] They make possible the investigation of the effect of separation between carboxylate groups. Here we report the results of such an investigation using a first generation dendrimer that possesses four peripheral carboxylic acid groups. The activity is compared with valeric acid, its free-acid equivalent, for the hydrolysis of cellobiose.

Materials and Methods

The first generation (1G-COOH) carbosilane dendrimer was synthesized by hydrosilylation of dimethylchlorosilane to tetravinylsilane, reduction with $LiAlH_4$, followed by hydrosilylation to trimethyl silyl 4-pentenoic ester, and finally deprotection with MeOH. The chemical equivalent free-acid, valeric acid, was used for comparison.

The hydrolysis tests were run in 15mL pressure vessels in an oven at 150 ºC with dimethylsulfoxide as the solvent, 18 v/v % H_2O, 0.5 M of total carboxylic acid, and ca. 20 mg/mL cellobiose. Samples were withdrawn and analyzed every 2 hours using a HPLC system with a Bio-Rad HPX-87H organic acid column at 60 ºC. The mobile phase was 5 mM sulfuric acid at a flow rate of 0.8 mL/min. During sampling, the vessels were removed from the oven, cooled in a water bath, and 1 mL of the sample was mixed with 1 mL of water and syringe filtered in order to remove the water-insoluble dendrimer catalyst. An external standard of 6 mg/mL of glucose was used for sample quantification.

Results and Discussion

Although the acid concentrations used in these experiments were the same, the pH of the reaction mixtures containing valeric acid was found to be lower than the ones containing G1-COOH. Thus, the proton concentration was higher in mixtures containing the free-acid. This implies that placing acid groups at the dendrimer periphery modified their acid dissociation constants. Both valeric acid and G1-COOH were active in catalyzing hydrolysis of cellobiose. Fig. 1 shows the cellobiose conversion with time. For valeric acid, the reaction rate was constant up to at least 60% conversion. Hydrolysis of cellobiose was faster in the mixture containing G1-COOH, in spite of the higher pH. The estimated initial hydrolysis rate using G1-COOH was at least 100% faster. The primary product was glucose (Fig. 2). But at higher conversions, further reaction of glucose was observed. Thus, the glucose concentration reached a maximum and then declined with further increase in reaction time. The maximum concentration was higher using G1-COOH, implying that degradation of glucose was less severe than using valeric acid. These differences between G1-COOH and valeric acid can be attributed to the cooperative effect of carboxylic acid groups in close proximity at the dendrimer periphery.

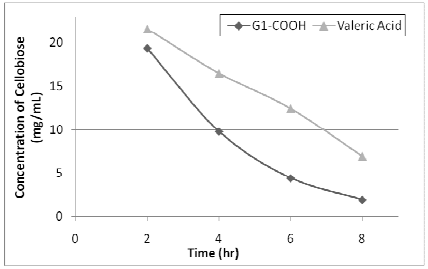

Figure 1. Changes in cellobiose concentration with time in mixtures containing valeric acid or G1-COOH.

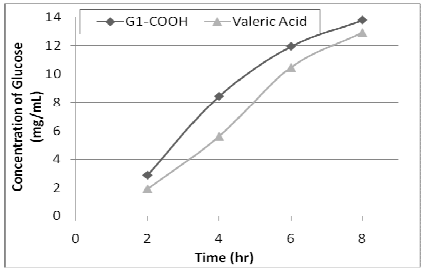

Figure 2. The time-profile of glucose in catalytic cellobiose hydrolysis of Figure 1.

Significance

Cooperative effect of carboxylic acid in cellobiose hydrolysis is demonstrated using functionalized dendrimers. The effect of separation between the acid groups on cooperativity can be examined. The results confirmed the potential of incorporating enzyme functionality in nonbiological systems.

References

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