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Communication

Metalloglycosidase Mimics: Oxidative Cleavage of Saccharides Promoted by Multinuclear Copper Complexes under Physiological Conditions

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ABSTRACT: Degradation of saccharides is relevant to the design of catalytic therapeutics, the production of biofuels, inhibition of biofilms, as well as other applications in chemical biology. Herein, we report the design of multinuclear Cu complexes that enable cleavage of saccharides under physiological conditions. Reactivity studies with *para*-nitrophenyl (*p*NP)-conjugated carbohydrates show that dinuclear Cu complexes exhibit a synergistic effect and promote faster and more robust cleavage of saccharide substrates, relative to the mononuclear Cu complex, while no further enhancement is observed for the tetranuclear Cu complex. The use of scavengers for reactive oxygen species confirms that saccharide cleavage is promoted by the formation of superoxide and hydroxyl radicals through $Cu^{II/I}$ redox chemistry, similar to that observed for native copper-containing lytic polysaccharide monooxygenases (LMPOs). Differences in selectivity for di- and tetranuclear Cu complexes are modest. However, these are the first reported small multinuclear Cu complexes that show selectivity and reactivity against mono- and disaccharide substrates and form a basis for further development of metalloglycosidases for applications in chemical biology.

Saccharides play a vital role in multiple biological processes, including cell adhesion,[1] cell recognition,[2] and the host immune response,[3] while also serving a vital role in bacterial cell walls[4] and post-translational modification in eukaryotic cells.[5] Accordingly, catalysts that promote cleavage of the glycosidic linkage under physiological conditions may be developed into pharmaceutically active agents and as chemical tools to investigate cellular function and pathways.[3,6] Degradation of saccharides from biomass can also produce biofuels and other saccharide derivatives for use in food or synthetic industries.[7] Conventional approaches for these reactions include biomass hydrolysis that requires harsh acidic conditions,[8] while high temperatures and pressures are also usually required.[9] Enzymatic degradation is another option; however, reaction condition compatibility, product inhibition, and cost are all obstacles that have yet to be overcome.[7,10,11] For these reasons there is increasing interest in the development of milder and more efficient conditions to promote biomass degradation.

Previously, we have developed catalyst complexes that target DNA,[12,13] RNA,[14] proteins,[15,16] and other antimicrobial targets,[17] especially metallopeptides that selectively promote cleavage of L-fucose, a carbohydrate that commonly decorates cell surfaces.[18] The Cu^{II}/Cu^{I} couple is ubiquitous in copper-containing enzymes in biological systems, especially lytic polysaccharide monooxygenases (LPMOs) that degrade polymeric carbohydrates via reactive oxygen species (ROS).[19−22] We were therefore motivated to develop new metalloglycosidase mimics promoting efficient saccharide cleavage. Some mononuclear copper complexes have been developed to promote cleavage of saccharides through Cu^{II}/Cu^{I} chemistry in recent studies; however, a nonphysiological condition, such as a pH of 10.5, is usually required to facilitate efficient glycosidase activity of these mononuclear copper complexes.[23] Herein, we report novel copper complexes derived from the Cu-binding ligand, di(2-picolyl)amine,[24] which promotes Cu^{II}/Cu^{I} redox activity under physiological conditions (Figure 1). In addition to a mononuclear copper complex, **1a**, two dinuclear Cu complexes, **2** and **3**, were designed by use of a xylylenediamine linker, as well as a tetranuclear copper complex, **4**, that contains a diaminobenzidine core to tether four copper centers. These multinuclear Cu complexes demonstrate synergy in reactivity.[25]

Glycosidase activity was evaluated by use of *para*-nitrophenyl(pNP)-derivatized glucose and galactose mono- and disaccharides as model substrates (Figure S1), with hydrogen peroxide and ascorbate as physiologically relevant coreagents that stimulate redox activity. Following cleavage of these substrates, the release of p-nitrophenolate results in a distinct absorbance band that is used to monitor the reaction progress. In the presence and absence of both ascorbate and peroxide, but without a catalyst, no cleavage was observed (Figure S2 and Table S1). In the presence of a catalyst and ascorbate/dioxygen, the activity levels are ∼10%, reflecting a

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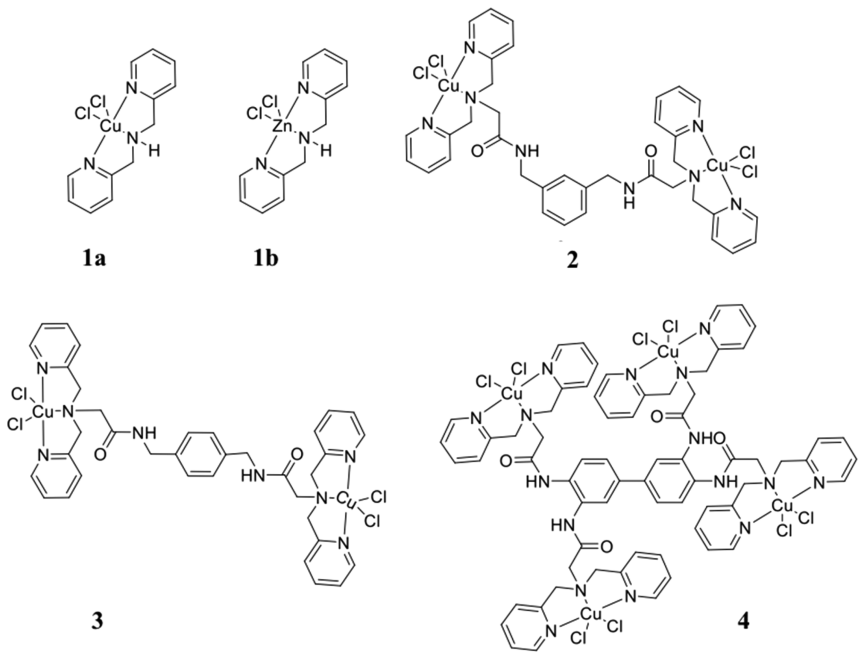

Figure 1. Metal complexes used in these studies.

Table 1. Michaelis–Menten Parameters for Saccharide Cleavage

Cu complexes	saccharides	K_M (mM)	k_{cat} (min^{-1})	k_{cat}/K_M (M^{-1} min^{-1})
1a	*p*NP-*α*-D-glucose	1.04 ± 0.19	0.41 ± 0.07	400 ± 100
	*p*NP-*β*-D-glucose	0.42 ± 0.030	0.42 ± 0.07	990 ± 180
	*p*NP-*α*-D-galactose	0.54 ± 0.109	0.30 ± 0.09	610 ± 220
	*p*NP-*β*-D-galactose	1.92 ± 0.69	0.57 ± 0.08	310 ± 120
	*p*NP-*β*-D-cellobiose	1.18 ± 0.2	1.1 ± 0.05	900 ± 160
	*p*NP-*β*-D-lactose	1.61 ± 0.45	1.4 ± 0.2	1000 ± 310
2	*p*NP-*α*-D-glucose	1.41 ± 0.11	15.3 ± 4.8	10800 ± 3500
	*p*NP-*β*-D-glucose	0.93 ± 0.090	5.4 ± 1.1	7100 ± 1600
	*p*NP-*α*-D-galactose	1.4 ± 0.34	9.7 ± 0.04	8230 ± 1900
	*p*NP-*β*-D-galactose	1.10 ± 0.20	3.0 ± 0.84	2740 ± 910
	*p*NP-*β*-D-cellobiose	0.51 ± 0.05	7.4 ± 0.2	14500 ± 1500
	*p*NP-*β*-D-Lactose	0.64 ± 0.09	10.0 ± 0.2	15700 ± 2200
3	*p*NP-*α*-D-glucose	1.20 ± 0.25	9.9 ± 1.1	8760 ± 2100
	*p*NP-*β*-D-glucose	0.90 ± 0.335	6.5 ± 0.35	9757 ± 3700
	*p*NP-*α*-D-galactose	1.4 ± 0.29	10.3 ± 1.1	7630 ± 1700
	*p*NP-*β*-D-galactose	0.41 ± 0.070	3.6 ± 0.2	9910 ± 1800
	*p*NP-*β*-D-cellobiose	0.52 ± 0.06	6.7 ± 0.2	13000 ± 1500
	*p*NP-*β*-D-lactose	0.82 ± 0.12	9.8 ± 0.4	11800 ± 1800
4	*p*NP-*α*-D-glucose	0.88 ± 0.173	6.6 ± 1.0	8400 ± 2100
	*p*NP-*β*-D-glucose	1.27 ± 0.01	12.4 ± 0.54	9800 ± 430
	*p*NP-*α*-D-galactose	0.66 ± 0.065	7.5 ± 0.36	11600 ± 1300
	*p*NP-*β*-D-galactose	0.61 ± 0.077	15.6 ± 0.7	16200 ± 2200
	*p*NP-*β*-D-cellobiose	0.72 ± 0.07	7.0 ± 0.2	9500 ± 960
	*p*NP-*β*-D-lactose	0.28 ± 0.04	5.8 ± 0.2	20600 ± 3000

requirement to generate peroxide by an electron donor to promote robust Fenton-type chemistry. All complexes displayed Michaelis–Menten profiles, demonstrating formation of a prereaction complex with the catalyst. Complex **1a** yielded a k_{cat} in the range of 0.3 to 0.56 min^{-1} for the four monosaccharides and around 2-fold higher for the disaccharide substrates. The k_{cat} for Cu complexes with two copper centers, **2** and **3**, increased up to 37-fold depending on the substrates (Table 1, Figures S5 and S6). The significant increase in k_{cat} for the dinuclear Cu complexes most likely reflects synergism between the copper centers in promoting the formation of ROS or facilitate substrate binding. Alternatively, enhanced reactivity may reflect slight shifts in the reduction potentials between the multinuclear complexes in aqueous solutions, although previous reports on multinuclear di(2-picolyl)amine scaffolds only show minor change.[26] Interestingly, further addition of two more copper centers to yield a tetranuclear copper complex exhibited only a marginal change in k_{cat} values. This may be due to steric hindrance or suboptimal orientation of metal centers toward the substrate.

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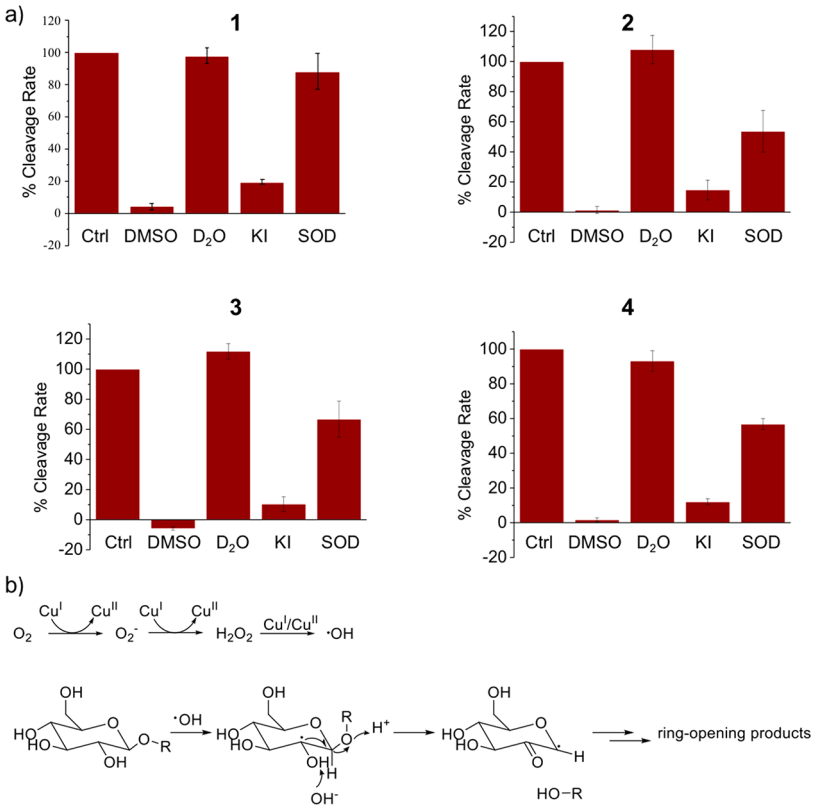

Figure 2. Cleavage of pNP β-D-glucose inhibited by ROS scavengers. Cleavage rates were measured in the absence (control, normalized to 100%) or the presence of 1% (v/v) DMSO, 10% (v/v) D_2O, 5 mM KI, or 10 U SOD.

The α- and β-glycosidic linkages appear to play an important role in defining relative k_{cat} for Cu complexes **2**, **3**, and **4**, but not **1a**. The Cu complexes **2** and **3** exhibit higher k_{cat} values toward cleavage of α substrates relative to β substrates, while **4** promotes faster cleavage of β substrates than α substrates. The absence of this trend for copper complex **1a** indicates that the preference for a specific structural isomer over another may require two copper centers in close proximity. The copper centers of **4** may be positioned in a different orientation from Cu complexes **2** and **3**, which in turn results in an opposite preference for glycosidic linkage, or the steric effect of benzidine may undermine cleavage of the α isomer.

All Cu complexes exhibit similar K_M values, ranging from 0.5 to 1 mM for monosaccharides, and as low as 0.28 mM for Cu complex **4** toward *p*NP-lactose (Figure S1), reflecting a weak interaction between the copper complex and saccharide substrate. The incorporation of phenyl or diphenyl rings into the Cu complexes does not improve binding affinity to the substrate, relative to the mononuclear analogue **1a**, indicating either the absence of $\pi-\pi$ stacking between the p-nitrophenyl ring and aromatic linker of the Cu complexes or favorable π-bonding with the axial C−H bonds of the saccharide moiety.[27] A slight increase in catalytic efficiency is observed for Cu complex **2** toward α- and β-galactose, and for Cu complex **4** for lactose over cellobiose. These results suggest that the appropriate linking of multiple copper centers can improve cleavage selectivity against a specific structural isomer; however, a more effective target recognition domain would be necessary for use as a molecular probe.

The reduction potential of **1a** has previously been reported as 230 mV vs RHE,[26] while other similar di- and trinuclear di(2-picolyl)amine compounds shift by 10 to 20 mV.[26] Given that robust activity is observed using ascorbic acid and hydrogen peroxide (potentials of −66 mV and 380 mV vs NHE respectively),[28,29] it is not expected that our synthetic modifications would cause further deviations outside of this electrochemical potential window. We were therefore interested in exploring if redox cooperativity can account for the drastic increase in catalytic activity, which was assessed using solution-phase electrochemistry. A single, irreversible reduction process is observed in the redox-inactive zinc control complex **1b** that is attributed to a ligand-centered transition, with a cathodic peak at −794 mV and an anodic peak at −882 mV (Figure S3). In addition to the ligand-based peak, a pronounced electrochemical signal is observed for the mononuclear copper complex **1a** with a midpoint potential of −749 mV, while smaller signals seen at −872 and −1214 mV are attributed to impurities or oligomerization products.[30] The dinuclear copper compounds **2** and **3** each also show three quasi-reversible couples, with peaks shifted slightly from each other and from the monometallic species (Figure S3 and Table S2). The redox transitions of the tetranuclear compound **4** exhibit greater irreversibility, as indicated by a greater separation between the cathodic and anodic peaks. This suggests that there is little electronic communication between the two dimer units, as otherwise additional peaks would be expected. The quasi-reversible nature of compounds **2** and **3** may contribute to the enhanced reactivity over the monomer, and the irreversibility of **4** may contribute to the absence of enhanced reactivity over the dinuclear complexes.

To explore the mechanism of saccharide cleavage, ROS scavengers or enhancers were added to reaction mixtures. Since peroxide can be an intermediate generated by Cu complexes, only ascorbate was used to facilitate copper

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reduction in order to unambiguously determine the identity of ROS produced. D_2O, which extends the lifetime of singlet oxygen, exhibited no significant enhancement of cleavage rate (<10%). The addition of either 1% DMSO or 5 mM KI significantly decreased the cleavage rate of β-D-glucose by (>90%), indicating that a hydroxyl radical and peroxide is formed during the cleavage reaction, respectively, which is independent of nuclearity (Figure 2a). These observations are in line with cleavage of DNA via a hydroxyl radical mechanism[31] by previously reported coumarin-di(2-picolyl)-amine complexes.[32] Superoxide dismutase, which converts superoxide (O_2^{*-}) to molecular oxygen and hydrogen peroxide, was also added to determine if superoxide was a likely candidate for saccharide cleavage. No significant inhibition in the mononuclear compound **1a** was observed; however, for all other compounds tested, regardless of nuclearity, the relative activity was diminished by approximately 40−50%. These results suggest that hydroxyl radicals are produced by Fenton-like chemistry, and facilitate hydrogen abstraction from the saccharide ring, promoting cleavage and further degradation (Figure 2b), similar to the mechanisms employed by copper-containing LPMOs to degrade their native substrates.[22]

While robust chemistry is observed, it was of interest to determine if this was due to freely diffusible ROS or if it resulted from close proximity of the Cu complex to the saccharide substrate, consistent with prereaction Michaelis complex formation, or a metal-associated mechanism. Therefore, the rate of formation of freely diffusible ROS was determined for each Cu complex using a CELLROX probe, which only fluoresces in the presence of ROS. Results are shown in (Table S3) with relatively low levels of diffusible ROS produced in the presence of hydrogen peroxide, while even less was observed in its absence. More interestingly, the level of diffusible ROS generated is quite low (<1%) relative to the amount of *p*NP liberated from the *p*NP-saccharide (Figures S2 and S4−S7 and Tables S1 and S3). This result is not surprising given the relatively short half-life of ROS in solution[33−35] and is consistent with the proposed reaction mechanism.

In summary, we have reported the design and characterization of multinuclear Cu complexes that promote cleavage of saccharides under physiological conditions. Robust saccharide cleavage by these Cu complexes requires the formation of ROS through Cu^{II}/Cu^{I} redox chemistry, as peroxide and hydroxyl radicals are the main ROSs participating in saccharide degradation, while superoxide is also generated and used as a cosubstrate. All Cu complexes exhibit similar K_M values in the mid-micromolar to low millimolar range, reflecting relatively weak interaction. A synergistic effect on saccharide cleavage was observed for compounds **2** and **3** with two copper centers, while there is no further enhancement for complex **4**. Electrochemical characterization shows that the multinuclear compounds lack redox cooperativity, however, there does appear to be a synergistic effect in the rate of cleavage with regard to having two copper centers as opposed to one. While other natural glycosidases[36−38] exhibit enhanced activity relative to these Cu complexes, natural enzymes lose activity at an exponential rate above pH 6.0, while activity is quite robust for Cu complexes at physiological pH. This robust activity at physiological pH could be beneficial depending upon the application and further development of these metalloglycosidases could result in activity that surpasses natural enzymes at neutral pH. These findings will be applied to the design of novel multinuclear Cu complexes for saccharide cleavage in future studies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.inorgchem.0c01193.

Materials and instruments, Tables S1−S3, Figures S1−S7 (PDF)

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Notes

The authors declare no competing financial interest.

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