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Copper(II) Complexes as Functional Models for Lytic Polysaccharide Monooxygenase: (A Kinetic Report)

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ABSTRACT

Mononuclear copper(II) complexes based on the symmetric tridentate N3-donor ligands bis(pyridin-2-yl)methyl)amine (HBPA, L1) and its methylated counterpart, di(2-pyridylmethyl)amine (MeDPA, L2) have been prepared, characterized, and demonstrated as the functional models for lytic polysaccharide monooxygenase. These complexes disrupt the synthetic substrate, such as p-nitrophenyl-β-D-glucopyranoside (β-PNPG) into p-nitrophenol (PNP) and D-allose via oxidative cleavage as LPMOs do in nature. The observed spectroscopic and kinetic analysis have revealed that the reaction proceeds via copper(II) hydroperoxide as intermediate, whose electronic spectral signature has appeared at 350nm in the electronic absorption spectra with the formation rate of 1.61 and 9.06×10^{-3} s^{1} respectively for complexes 1 and 2. Especially the obtained product was newly appeared at 400nm, indicates the formation of p-nitrophenol with the rate of 7.52 and 5.45×10^{-3} s^{1} for complexes 1 and 2 respectively. These results affirm the ability of copper complexes as the functional models of LPMOs.

Keywords: Copper(II) complexes, Biomimicking, LPMO models, Oxidative cleavage, Formation of D-Allose.

INTRODUCTION

Natural carbohydrate polymers which include starch, cellulose, and chitin provide a big renewable alternative to fossil fuels as a source of fuels and materials.[1-3] Utilization of these polymers in large-scale industrial applications is still a difficult task due to their recalcitrant form for breaking into monomers.[4-8] The enzymatic degradation of recalcitrant plant biomass has become very challenging in enzyme development for biomass utilization.[9] A new enzyme called LPMOs are grouped in the enzyme families and termed Auxiliary Activities (AAs) in the Carbohydrate-Active Enzymes database (CAZy).[10-13] Vaaje-Kolstad *et al.,* uncover the capacity of

LPMOs to degrade the native polymers into monomers.[14] The LPMOs are more popular nowadays due to their ability to activate O_2 to cleave the glycosidic bonds of polysaccharides.[15-19] Vu *et al.,* have provided a detailed characterization of the active site of cupric ions in LPMOs.[20] In addition, to employ O_2 as an oxidant, Bissaro *et al.,* demonstrated that the preferred co-substrate of LPMOs is H_2O_2 instead of using O_2.[21-23] Although several articles have stated that the polysaccharide has been selectively oxidized only at the C4 carbon, and both C1, as well as C4 through intermediates such as cupric-superoxide and cupric hydroperoxide species respectively, the nature of the intermediate involved in the C-H hydroxylation, is still unknown.[24-27] Many functional models of copper complexes bearing N3 donor tridentate ligands have been demonstrated as catalysts for the degradation of β-PNPG to mimic the role of the native enzymes.[28-32] Based on these backgrounds, we have prepared two mononuclear copper(II) complexes, bearing N3 donor tridentate ligands which are displaying LPMOs functions through the breakdown of β-PNPG into corresponding products such as PNP and D-allose.

EXPERIMENTAL

Reagents and Techniques

2-pyridinecarboxaldehyde, sodium borohydride, formaldehyde, 2-aminomethyl pyridine, copper(II) perchlorate hexahydrate and *p*-nitrophenyl-β-D-glucopyranoside were obtained from Alfa Aesar. Hydrogen peroxide (30%), hydrochloric acid, sodium carbonate, magnesium sulfate, and methanol were obtained from Merck. The ligands were confirmed by the nuclear magnetic resonance (NMR) spectroscopic technique using Bruker 400MHz. The formation of complexes was confirmed by electrospray ionisation mass spectrometer (ESI-MS) (Agilent 6530 LC/Q–TOF). Electronic absorption spectra recorded on the Agilent diode array spectrometer (Agilent 8453). Electrochemical measurements were performed using computer-controlled CH-Instruments, model 440. Electron paramagnetic resonance (EPR) spectral data were obtained from JEOL Model JES FA200. The degradation products were confirmed by gas chromatography mass spectrometer (GC-MS) Agilent 5977E.

Synthetic Procedures

Synthesis of N,N-bis(2-pyridylmethyl)amine (L1)

Both ligands HBPA (L1) and MeDPA (L2) were prepared by the early reported methods with slight modifications.[33,34] 2-pyridine carboxaldehyde (1.335 g, 12.5 mmol) was added to a solution containing 2-aminomethyl pyridine (1.35 g, 12.5 mmol) in MeOH (25 mL), the solution was undergoing colour change to dark brown. After 10 h sodium borohydride (0.945 g, 25 mmol) was slowly added, which turned the colour to a pale-yellow solution and the stirring was continued for another 3 hour. Remove the all the volatiles under reduced pressure. Distilled water (25 mL) was added and neutralized the resulting aqueous solution with 32% hydrochloric acid, followed by extraction with dichloromethane. The combined organic extract was dried over anhydrous $MgSO_4$ and put rotary evaporate to obtain the desired product, N,N-bis(2-pyridylmethyl)amine as yellow liquid. Yield, 0.6435 g (62.4%). ^{1}H NMR (400MHz): $CDCl_3$-d,δ, 8.52 (d, 2H), 7.60 (t, 2H), 7.25 (d, 2H), 7.12 (t, 2H), 3.96 (s, 4H), 3.06 (s, 1H). ^{13}C NMR: $CDCl_3$-d, 159.66, 149.34, 136.57, 122.38, 122.04, 54.78ppm

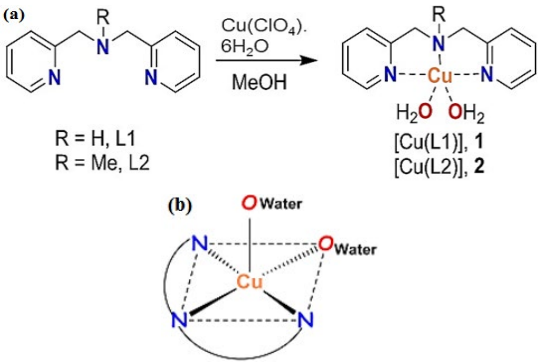
Synthesis of N-Methyl, N-bis(2-pyridylmethyl) amine (L2)

A reaction mixture containing bis[2-(2-pyridyl)methyl]amine (1.04 g, 5.225 mmol) and 1,2-dichloro-ethane (25 mL) was treated with 33% of aqueous formaldehyde (0.85 g, 10.45 mmol) under constant stirring. After 15 min, $NaBH(OAc)_3$ (2.21 g, 10.45 mmol) was slowly added to the stirred solution and further stirred for 24 h at room temperature and quenched with the addition of an aqueous NaOH (2 M, 50 mL), the organic layer was separated and extracted with CH_2Cl_2 (3 × 50 mL portions). Combine the organic fractions, and dried over $MgSO_4$, filtered and removed the solvent in vacuo. Taken up the obtained oily semisolid in diethyl ether (100 mL), filtered again and remove diethyl ether in vacuo to get the desired product as translucent golden coloured oil. Yield, 0.84 g (72.4%). ^{1}H NMR (400MHz): $CDCl_3$, δ, 2.31 (s, 3H), 3.77 (s, 4H), 7.15 (m, 2H), 7.52 (d, 2H), 7.66 (dt, 2H), 8.54 (d, 2H). ^{13}C NMR $CDCl_3$: 42.7, 63.6, 122.0, 123.1, 136.4, 149.0, 159.2ppm.

Synthesis of copper(II) complexes

Copper(II) perchlorate in methanol was

treated with the ligands (L1 or L2) in dichloromethane and the blue colour solids were separated and dried (Scheme 1).



Scheme 1. Synthesis of copper(II) complexes of L1 and L2 ligands (a) and structures model of LPMOs (b)

RESULTS AND DISCUSSION

Synthesis and Characterization

$[Cu(L1)(H_2O)_2](ClO_4)_2$ (1): $Cu(ClO_4)_2.6H_2O$ (0.37 g, 1 mmol) in 4 mL of methanol was drop-wisely added to a stirring solution of ligand, L1 (0.200 g, 1 mmol) in 4 mL of dichloromethane (DCM), this reaction mixture was stirred for 2 h at room temperature. The obtained blue colour solid product was precipitated, filtered, and dried for further purposes.

[Cu(L2)(H2O)2] (ClO4)2 (2): The complex 2, the above same procedure has been adopted using ligand L2 instead of L1.

These prepared complexes were used as functional models of LPMOs. The obtained blue colour crystalline solids were confirmed by ESI-MS in methanol, where the peak appears at m/z 297.0090 for $[C_{12}H_{16}CuN_3O_2]$+and m/z 307.0377 for $[C_{12}H_{14}CuN_3$+2Na] for complex 1 and ESI-MS: m/z 360.1803 for $[C_{13}H_{19}CuN_3O_2$+2Na] for complex 2 respectively (Figure 1).

Electronic Spectra, Redox Properties, and EPR Studies

Both these complexes, **1** and **2** have shown intense and broad absorptions at 645nm in water (Fig. 2a and Table 1) which corresponds to d-d transitions, interestingly they were closely related to the absorption of previously reported LPMO model (655nm) complexes.[35] Their well-defined Cu(II)/Cu(I) redox potentials (-0.18 and -0.19 V vs normal hydrogen electrode (NHE) respectively for complexes 1 and 2 (Fig. 2b Table1) have been observed in aqueous carbonate buffer, which are found to be lower than the previously reported enzymatic systems.[36-38] Furthermore, their square pyramidal geometries and oxidation states were confirmed by EPR spectra (Fig. 2c) dimethylforamide (DMF) (8:2) at 70 K in methanol and the obtained axial EPR parameters ($g_{||}$=2.2572, $A_{||}$=158×10^{-4} cm^{-1} and $g_{\perp}$ =2.0204) for complex 1 and ($g_{||}$=2.2604, All =179×10^{-4} cm^{-1} and $g_{\perp}$ =2.0221) for complex 2 were in good agreement with those obtained for LPMOs.[39-41]

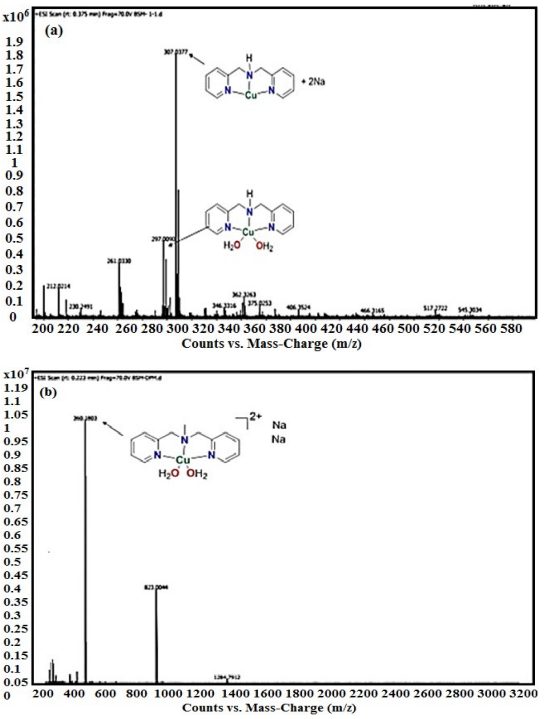


Fig. 1. ESI-MS spectra of complex 1 (a) and 2 (b) in methanol solution

Table 1: Electronic spectral and redox data for complexes 1 and 2 in carbonate buffer at pH 10

Complex	Electronic spectra λ_{max} nm, (ε_{max} M^{-1} cm^{-1})	Redox data				
		E_{pc}(V)	E_{pa}(V)	ΔE(V)	$E_{1/2}$(V)	$E_{1/2}$(V) vs NHE
1	645 (432.9)	-0.46	0.-31	-0.15	-0.39	-0.18
2	645 (435.1)	-0.45	-0.33	-0.12	-0.39	-0.19

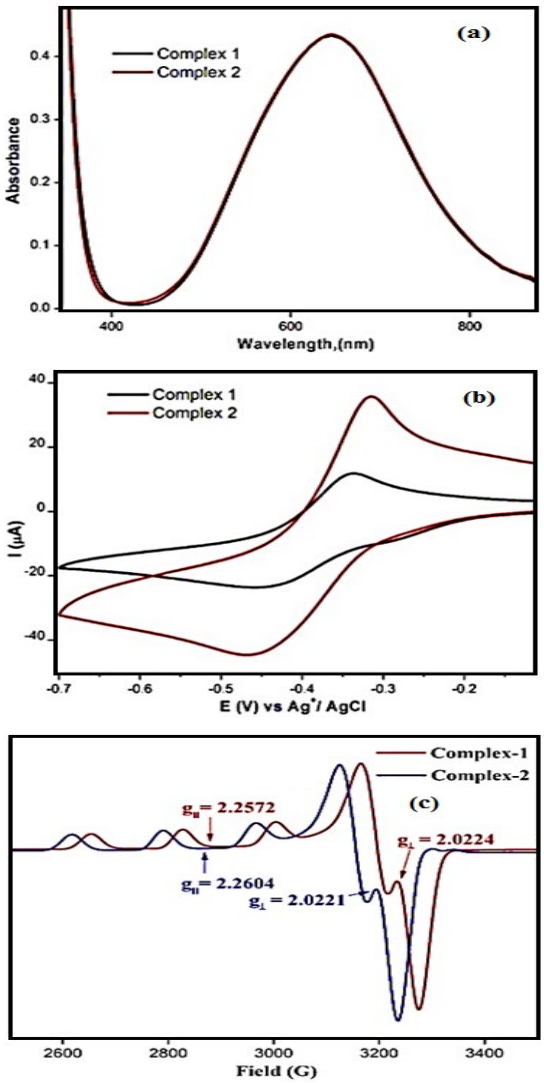


Fig. 2. Electronic spectra (a), cyclic voltammograms (b) and EPR (c) of complexes 1 and 2 (1 x10^{-3} M) in the presence of carbonate buffer at 25°C. The scan rate

Formation of Cu-OOH species

The formation of copper(II) hydroperoxide species in both complexes, **1** and **2** were obtained by the addition of H_2O_2 solution in carbonate buffer at pH 10 (Fig. 3), which appeared around 350nm in the electronic absorption spectra. The complex containing water molecules, which was deprotonated at basic medium using carbonate buffer at pH 10 to form Cu(II) hydroxyl, which was treated with hydrogen peroxide to form copper hydroperoxide (CuII-OOH) species.

Oxidation cleavage of β-PNPG

The oxidative cleavage of β-PNPG was examined by UV-Visible spectroscopic technique. as well as GC-MS. The oxidized products such as PNP and D-Allose were monitored in different temperatures with varying concentrations of catalyst, substrate, and oxidant (H_2O_2) at 400nm (Scheme 2).

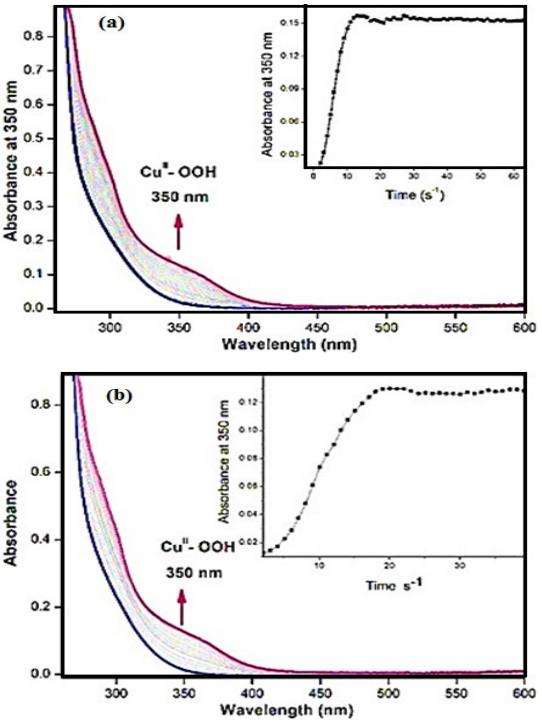
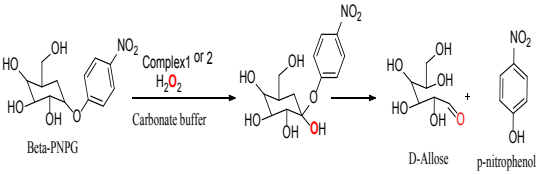


Fig. 3. The absorption spectra for the formation of hydroperoxo intermediates of complexes 1 (a) and 2 (b) (0.05 mM) with H_2O_2 (0.5 mM) in carbonate buffer (0.1 M) at 30°C. Insert figures: Plot of 1+Abs. vs Time



Scheme 2. Oxidative cleavage of β-PNPG and hydrogen peroxide with catalyst

Kinetic reactions were conducted in the presence of β-PNPG as well as aqueous H_2O_2 with complexes **1** and **2** for a ratio of 1:10:10 mixture in carbonate buffer at pH 10 to confirm the LPMO-like reactivity of the complexes. Thus, the kinetic spectral results lead to observing an absorption band at 400nm that may correspond to PNP (Fig. 4, 5). In addition, the significant shift in the electronic spectra indicates that the oxidative cleavage reaction will be

initiated when the substrate is combined with both complexes, either **1** or **2** in the presence of hydrogen peroxide (Fig. 6). The reaction did not go as planned if either complex or hydrogen peroxide were missing. The final catalytic solution consisting of a catalytic amount of the model complex **1** or **2** (20 mmol), the model substrate (200 mmol), with 30% aqueous hydrogen peroxide (200 mmol) in carbonate buffer (2.0 mL) were passed over a silica column after 2 h, in order to confirm the product analysis (PNP and D-allose) by GC-MS (Fig. 7). A blank reaction was also carried out under the same conditions, which did not yield any product as we anticipated.

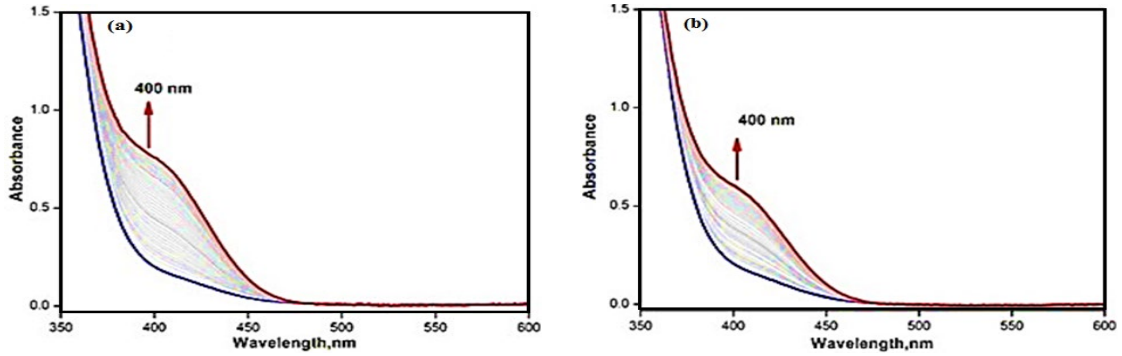


Fig. 4. The electronic spectra for the kinetic reaction of complexes 1 (a) and 2 (b) (0.05 mM) with the substrate (*p*-nitrophenyl-β-D-glucopyranoside, 0.5 mM) in the presence of H_2O_2 (0.5 mM) in carbonate buffer (0.1 M) at 30°C

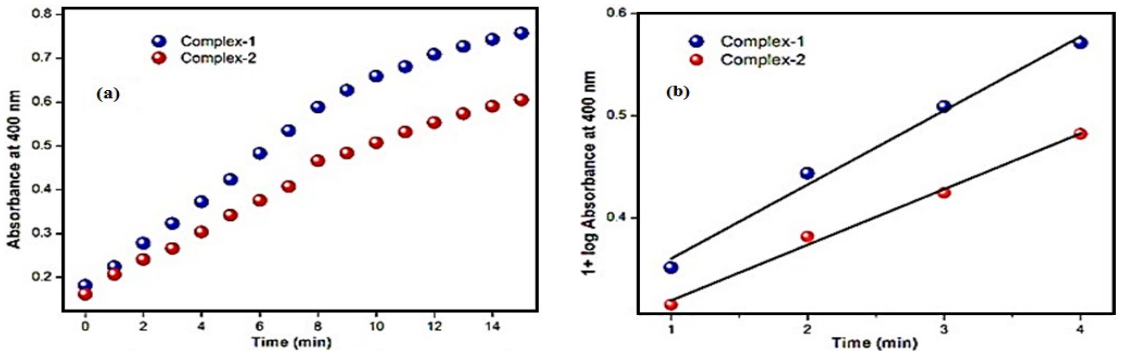


Fig. 5(a). Time dependant absorption spectra for the kinetic reaction of complexes 1 and 2 (0.05 mM) with the substrate (*p*-nitrophenyl-β-D-glucopyranoside, 0.5 mM) in the presence of H_2O_2 (0.5 mM), and (b) Plot of 1+log (Abs) vs Time in carbonate buffer (0.1M) at 30°C

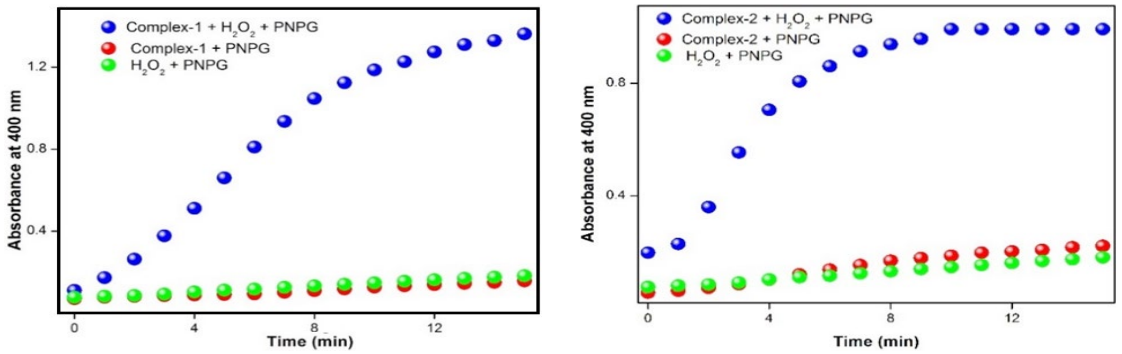


Fig. 6. The absorption spectra for the reaction between complexes 1 or 2 (0.05 mM) with the substrate (*p*-nitrophenyl-β-D-glucopyranoside, 0.5 mM) in the presence of H_2O_2 (0.5 mM) in carbonate buffer (0.1M) at 30°C

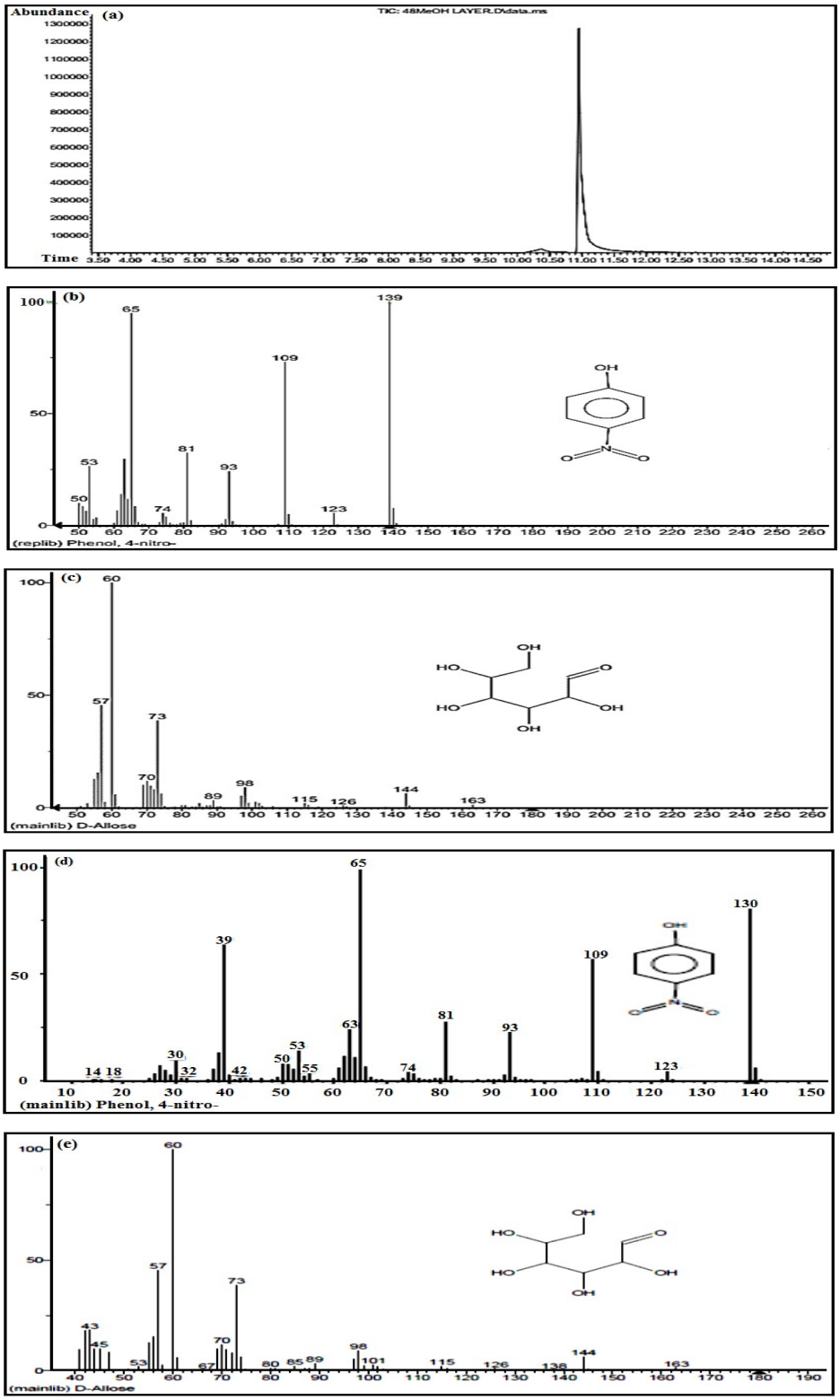


Fig. 7. GC-MS chromatogram of *p*-nitrophenyl-β-D-glucopyranoside (a) and their respective products, *p*-nitrophenol (b) and D-allose (c). Authentic GC-MS spectra of *p*-nitrophenol (d) and D-allose (e)

CONCLUSION

A simple mononuclear copper(II) complexes were synthesized, characterized, and demonstrated as functional models for lytic polysaccharide monooxygenase. Their geometrical and physicochemical properties were similar to those of the active sites of LPMOs. The catalytic activity of the copper(II) complexes were successfully evaluated using the polysaccharide model substrate. This model catalytic reaction proceeded via copper(II) hydroperoxide as an intermediate which is responsible for the degradation of the β-PNPG leading to the formation of oxidized products such as PNP and D-allose via oxidative cleavage. The formation of *p*-nitrophenol declares the ability of copper complexes to mimic the biological processes of LPMOs. This makes the present complexes excellent models which illustrate both the structural properties and reactivity of the active sites of LPMOs. In order to assess the contributions of N-H structural alterations and quantification products derived from the model complex, more research is being done.

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REFERENCES

1. Meier, K. K.; Jones, S. M.; Kaper, T.; Hansson, H.; Koetsier, M.J.; Karkehabadi, S.; Solomon, E. I.; Sandgren, M.; Kelemen, B. *Chem. Rev.*, **2018**, *118*, 2593–2635.
2. Hemsworth, G.R.; Johnston, E.M.; Davies, G.J.; Walton, P.H. *Trends Biotechnol.*, **2015**. *33*, 747–761.
3. Quinlan, R. J.; Sweeney, M. D.; Lo Leggio, L.; Otten, H.; Poulsen, J. C. N.; Johansen, K. S.; Krogh, K. B. R. M.; Jorgensen, C. I.; Tovborg, M.; Anthonsen, A.; Tryfona, T.; Walter, C. P.; Dupree, P.;Xu, F.; Davies, G. J.; Walton, P. H. *Proc. Natl. Acad. Sci. U. S. A.*, **2011**, *108*, 15079–15084.
4. Beeson, W. T.; Phillips, C. M.; Cate, J. H. D.; Marletta, M. A. *J. Am. Chem. Soc.*, **2012**, *134*, 890–892.
5. Hemsworth, G. R.; Davies, G. J.; Walton, P. H. *Curr. Opin. Struct. Biol.*, **2013**, *23*, 660–668.
6. Beeson, W. T.; Vu, V. V.; Span, E. A.; Phillips, C. M.; Marletta, M. A. *Annu. Rev. Biochem.*, **2015**, *84*, 923–946.
7. Chylenski, P.; Bissaro, B.; Sorlie, M.; Rohr, A. K.; Varnai, A.; Horn, S. J.; Eijsink, V. G. H. *ACS Catal.*, **2019**, *9*, 4970–4991.
8. Forsberg, Z.; Sorlie, M.; Petrovic, D.; Courtade, G.; Aachmann,F. L.; Vaaje-Kolstad, G.; Bissaro, B.; Rohr, A. K.; Eijsink, V. G. H., *Curr. Opin. Struct. Biol.*, **2019**, *59*, 54–64.
9. Sandra T. M .; *Joel Cherry. Adv Biochem Engin/Biotechnol.*, **2007**, *108*, 95–120.
10. Solomon, E. I.; Heppner, D. E.; Johnston, E. M.; Ginsbach, J. W.; Cirera, J.; Qayyum, M.; Kieber-Emmons, M. T.; Kjaergaard, C. H.; Hadt, R. G.; Tian, L. *Chem. Rev.*, **2014**, *114* (7), 3659–3853.
11. Hemsworth, G. R.; Henrissat, B.; Davies, G. J.; Walton, P. H. *Nat. Chem. Biol.*, **2014**, *10*, 122-126.
12. Cantarel, B. L.; Coutinho, P. M.; Rancurel, C.; Bernard, T.; Lombard, V.; Henrissat, B. *Nucleic Acids Res.,* **2009**, *37*, D233–D238.
13. Frandsen, K.E.H.; Lo Leggio, L. *IUCrJ.*, **2016**, *3*, 448–467.
14. Vaaje-kolstad, G.; Westereng, B.; Horn, S. J.; Liu, Z.; Zhai, H.; Sorlie, M.; Eijsink, V. G. H. *Science.*, **2010**, *330*, 219–223.
15. Walton, P. H.; Davies, G. *J. Curr. Opin. Chem. Biol.*, **2016**, *31*, 195–207.
16. Lo Leggio, L.; Simmons, T. J.; Poulsen, J.-C. N.; Frandsen, K. E. H.; Hemsworth, G. R.; Stringer, M. A.; von Freiesleben, P.; Tovborg, M.; Johansen, K. S.; De Maria, L.; Harris, P. V.; Soong, C.-L.; Dupree, P.; Tryfona, T.; Lenfant, N.; Henrissat, B.; Davies, G. J.; Walton, P. H. *Nat. Commun.*, **2015**, *6*, 5961–5969.
17. Vu, V. V.; Beeson, W. T.; Span, E. A.; Farquhar, E. R.; Marletta, M. A., *Proc. Natl. Acad. Sci. U. S. A.*, **2014**, *111*, 13822–13827.
18. Lo Leggio, L.; Simmons, T. J.; Poulsen, J. C. N.; Frandsen, K. E.H.; Hemsworth, G. R.; Stringer, M. A.; von Freiesleben, P.; Tovborg, M.; Johansen, K. S.; De Maria, L.; Harris, P. V.; Soong, C. L.; Dupree, P.; Tryfona, T.; Lenfant, N.; Henrissat, B.; Davies, G. J.; Walton, P. H., *Nat. Commun.*, **2015**, *6*, 5961.
19. Simmons, T. J.; Frandsen, K. E. H.; Ciano, L.; Tryfona, T.;Lenfant, N.; Poulsen, J. C.; Wilson, L. F. L.; Tandrup, T.; Tovborg,M.; Schnorr, K.; Johansen, K. S.; Henrissat, B.; Walton, P. H.; LoLeggio, L.; Dupree, P., *Nat. Commun.*, **2017**, *8*, 1064

20. Vu, V. V.; Ngo, S. T., *Coord. Chem. Rev.*, **2018**, *368*, 134–157.
21. Bissaro, B.; Rohr, A. K.; Muller, G.; Chylenski, P.; Skaugen, M.;Forsberg, Z.; Horn, S. J.; Vaaje-Kolstad, G.; Eijsink, V. G. H., *Nat. Chem. Biol.*, **2017**, *13*, 1123–1128.
22. Filandr, F.; Man, P.; Halada, P.; Chang, H. H.; Ludwig, R.;Kracher, D. *Biotechnol. Biofuels.*, **2020**, *13*, 37.
23. Hangasky, J. A.; Iavarone, A. T.; Marletta, M. A. *Proc. Natl. Acad. Sci. U. S. A.*, **2018**, *115*, 4915–4920.
24. Hedegard Dhar, D.; Tolman, W. B., *J. Biol. Inorg. Chem.*, **2017**, *22*, 1–9.
25. Neira, A. C.; Martínez-Alanis, P. R.; Aullón, G.; Flores-Alamo, M.; Zerón, P.; Company, A.; Chen, J.; Kasper, J. B.; Browne, W. R.;Nordlander, E. *ACS Omega.*, **2019**, *4*(6), 10729–10740.
26. Kim, S.; Stahlberg, J.; Sandgren, M.; Paton, R. S.; Beckham, G.T., *Proc. Natl. Acad. Sci. U. S. A.*, **2014**, *111*(1), 149–154.
27. Elwell, C. E.; Gagnon, N. L.; Neisen, B. D.; Dhar, D.; Spaeth, A.D.; Yee, G. M.; Tolman, W. B., *Chem. Rev.*, **2017**, *117*(3), 2059–2107.
28. Concia, A. L.; Beccia, M. R.; Orio, M. ; Ferre, F. T.; Scarpellini, M.; Biaso, F.; Guigliarelli, B.; Reglier M.; Simaan, A. *J. Inorg. Chem.,* **2017**, *56*, 1023-1026.
29. Muthuramalingam, S.; Maheshwaran, D.; Velusamy, M.; Mayilmurugan, R., *J. Catal.,* **2019**, *372*, 352-361.
30. Fukatsu, A.; Morimoto, Y.; Sugimoto, H.; Itoh, S., *Chem. Commun.*, **2020**, *56*, 5123-5126.
31. Gagnon, N.; Tolman, W.B., *Acc Chem. Res.,* **2015**, *48*, 2126–2131.
32. Itoh, S., *Acc. Chem. Res.*, **2015**, *48*, 2066–2074.
33. Liu, Y.; Xiang, R.; Du, X.; Ding, Y.; Ma, B., *Chem. Comm.*, **2014**, *50*, 12779-12782.
34. Budweg, S.; Junge, K.; Beller, M. Chem. Comm., 2019, 55, 4143-14146.
35. Solomon, E.I., *Proc. Natl. Acad. Sci. U.S.A.* **2014**, *111,* 8797–8802.
36. Hemsworth, G. R.; Johnston, E.M.; Davies, G.J.; Walton, P.H., *Trends Biotechnol.*, **2015**, *33*, 747–761.
37. Beeson, W.T.; Vu V. V.; Span, E. A.; Phillips, C. M.; Marletta M.A., *Annu. Rev. Biochem.*, **2015**, *84*, 923–946.
38. Garajova, S.; Mathieu, Y.; Beccia, M.R.; Bennati-Granier, C.; Biaso, F;. Fanuel, M.; Ropartz, D.; Guigliarelli, B.; Record, E;. Rogniaux, H.; Henrissat, B.; Berrin, J. G., *Sci. Rep.*, **2016**, *6*, 28276.
39. Frandsen, K. E. H.; Simmons, T. J.; Dupree, P.; Poulsen, J.-C.N.; Hemsworth, G. R.; Ciano, L. E. M.; Johnston, M.; Tovborg, K. S.; Johansen, P.; von Freiesleben, L.; Marmuse, S.; Fort, S.; Cottaz, H.; Driguez, B.; Henrissat, N.; Lenfant, F.; Tuna, A. Baldansuren,; G.J. Davies, L.; Lo Leggio,; P. H. Walton., *Nat. Chem. Biol.*, **2016**, *12*, 298–303.
40. Halfen,, J.A.; Uhan J.M,; Fox, D.C.; Mehn, M.P;. Que, L. *Inorg. Chem.*, **2000**, *39,* 4913–4920.
41. Zhu, Q. ; Lian, Y.; Thyagarajan, S.; Rokita, S. E.; Karlin. K. D.; Blough; N. V., *J. Am. Chem. Soc.*, **2008**, *130*, 6304–6305.