Oxygen-DePLETED Calixarenes as Ligands for Molecular Models of Galactose Oxidase

Matthias Keck,[a] Santina Hoof,[a] Christian Herwig,[a] Arkadi Vigalok,[b] and Christian Limberg*[a]

Abstract: A calix[4]arene ligand, in which two of the phenol functions are replaced by pyrazole units has been employed to mimic the His–Tyr (His: histidine, Tyr: tyrosine) ligand sphere within the active site of the galactose oxidase (GO). The calixarene backbone has formed the corresponding copper(II) complex in a see-saw-type structure, which is hitherto unprecedented in GO modelling chemistry. It undergoes a one-electron oxidation that is centered at the phenolate donor leading to a copper-coordinated phenoxyl radical like in the GO. Accordingly, the complex was tested as a functional model and indeed proved capable of oxidizing benzyl alcohol to the respective aldehyde using two phenoxyl-radical equivalents as oxidants. Finally, the results show that the calixarene platform can be utilized to arrange donor functions to mimic binding pockets that allow for the creation of novel types of model compounds.

Macrocyclic polyphenols—coined calixarenes by C. D. Gut sche—have been known for almost 80 years now.[1] Their high-yield preparation from cheap commercially available starting materials and easy post modifications have made them well-established and often utilized macromolecules in diverse fields of chemical research and applications.[2] Calixarenes were also employed as ligands in transition-metal chemistry, for instance, as a mimic of oxidic surfaces and hence for the modelling of the active sites of heterogeneous catalysts.[3] However, given that they provide a coordination platform that contains exclusively hard oxygen donors, they have hardly been used in bioinorganic studies. There are no enzymes with such binding pockets composed for obvious reasons: hard metal centers in high oxidation states (e.g. FeIII or FeV) mainly occur in reactive intermediates as part of catalytic cycles and in the course of turnover these are reduced to softer, low-oxidation-state metal centers (e.g. FeII), which prefer soft ligands. Metal-binding sites of metalloenzymes have to balance these two different demands and therefore often feature mixed ligand spheres, which have to be mimicked also in models. Hence, it is not surprising that calixarenes, so far, have rarely been employed in bioinorganic chemistry. Only modified forms in which the phenol units are functionalized by pendant N-donors were investigated, for instance, in biomimetic copper or zinc chemistry.[4] From the structural point of view, the introduction of the pendant groups makes the metal complex more flexible. Even though not necessarily detrimental to the desired activity, this flexibility eliminates one attractive feature of the calixarene platform, namely its potential to direct donors to a well-defined binding pocket, which renders calixarenes appealing as three-dimensional analogues of rigid multidentate systems, such as salens or porphyrins.

We were interested in exploiting calixarene-shaped binding sites also in bioinorganic investigations, which required the introduction of soft donor atoms directly in place of one or more oxygen atoms of the calixarene lower rim. Recently, some of us have developed the oxygen-depleted calixarene bispipyrazolyl-tert-butil-calix[4]arene ([H2(bpzCal]), Figure 1) featuring two pyrazole moieties beside two phenolic donors,[5] which is reminiscent of a His–Tyr coordination sphere found in certain enzymes. One of those is the galactose oxidase (GO) and hence we decided to test the potential of the [bpzCal]2⁺ ligand to construct molecular models of the GO.

Figure 1. Left: Molecular structure of [H2(bpzCal)].[5] Right: Similarities of the ligand’s donors with natural amino acid side chains.
The GO catalyzes the oxidation of primary alcohols to the corresponding aldehydes. In the resting state it contains a copper(II) ion coordinated by two tyrosine and two histidine amino acid residues (Figure 2). Previous attempts to mimic this coordination environment often used salen ligands, which, however, forces the central ion into an almost square-planar coordination environment. This rigidity has proven to impede changes in oxidation state, because the Franc–Condon barrier is high in energy as evidenced by model complexes that were oxidized most easily when a high degree of distortion at the metal center was given. In contrast to salens, calixarenes provide a more variable coordination site, which, however, is still well-defined and pre-organized for metal-ion complexation.

Consequently, the synthesis of a copper(II) complex of \([\text{H}_2\text{bpzCal}]\) was pursued, as well as corresponding nickel and zinc complexes for comparison. Deprotonation of the ligand precursor, followed by salt metathesis, only led to incomplete reactions and salt contamination resulting in purification issues. Hence, another strategy had to be chosen. The use of basic metal precursors has already proven a suitable approach to obtain pure calixarene complexes in previous metalation reactions. Therefore, \([\text{Ni(NPh}_2]_2\]) and \([\text{Cu(dmap)}]\) (dmap: 1-imethylamino-2-propanolate) were chosen as metalation reagents resulting in high yields of complexes \([\text{Ni(bpzCal)}]\) and \([\text{Cu(bpzCal)}]\) (Scheme 1), which were isolated as intensely colored analytically pure solids.

X-ray structure analyses of single crystals that were grown by slow evaporation of the volatiles from THF solutions revealed almost identical coordination spheres for the metal centers in the two complexes, composed of two phenolate and two pyrazole donors (Figure 3 and Figure S14 in the Supporting Information).

In both cases, there are two crystallographically independent molecules in the unit cell, the metrical parameters of which are almost identical. Regarding the \(\tau_0\) value, the coordination geometry of the metal ions can be described best as intermediate between tetrahedral and square-planar, commonly called the seesaw coordination. To the best of our knowledge, these two complexes are the first examples of structurally characterized mononuclear nickel and copper calixarene complexes in which phenolic oxygen donors directly coordinate the metal and therefore interact without any spacers.

Both compounds are in high-spin configurations with magnetic moments of \(\mu_{\text{eff}}=3.16\) and \(\mu_{\text{eff}}=1.91\) \(\mu_B\) for \([\text{Ni(bpzCal)}]\) (two unpaired electrons) and \([\text{Cu(bpzCal)}]\) (one unpaired electron) respectively, as determined by the Evans method on \(\text{CDCl}_3\) solutions of the complexes. Therefore only \([\text{Cu(bpzCal)}]\) is X-band EPR active (Figure S10 in the Supporting Information) in contrast to \([\text{Ni(bpzCal)}]\) for which the large zero-field splitting (ZFS) of the \(S=1\) spin state results in the absence of any observable signal.
Cyclovoltammetric (CV) measurements on both complexes surprisingly revealed variations in the electrochemical behavior: in contrast to [Ni(bpzCal)], which exhibits only one quasi-reversible oxidation wave at $E_{1/2} = 130 \text{ mV}$, [Cu(bpzCal)] can be oxidized a second time at a potential of $E^\prime = 670 \text{ mV}$ ($E'_{1/2} = 110 \text{ mV}$) although this event is highly irreversible (Figure 4). To understand the origin of the oxidation (metal- or ligand-based) and the differing behavior, the corresponding complex of the redox-inert metal zinc [Zn(bpzCal)] was analyzed. This compound also exhibits oxidation events in the CV in a similar region. However, in contrast to the cyclovoltammograms of the lighter analogues, the zinc complex exhibits two quasi-reversible oxidation waves ($E_{1/2} = 135$, $E_{1/2} = 340 \text{ mV}$) separated by only 205 mV (Figure 4). This indicates that the redox events found for the previously mentioned complexes likely are not metal-based but involve the phenolate functions.

The different appearances of the three CVs can then be explained by the different degrees of electronic delocalization and communication after the first oxidation event: the electronic communication between the oxidized and the remaining second phenolate cannot be mediated effectively by the central ion in the case of zinc because of its completely filled d$^{10}$ configuration, leading to two oxidations at potentials that do not differ significantly; indeed there are also examples reported in which both electrons are removed simultaneously.

The electronic absorption spectra of the oxidized species have a new strong absorption band around 400 nm in common (402 for [Ni(bpzCal)]$^{+}$ and 392 nm for [Cu(bpzCal)]$^{+}$). Given that [Zn(bpzCal)]$^{+}$ also shows such a band at 401 nm, these absorptions (Figures S8 and S9 in the Supporting Information) can be assigned to the π–π$^*$ transition of a phenoxyl radical.

For further investigations, we headed towards chemical oxidation of the complexes. AgSbF$_6$ ($E_{1/2} = 650 \text{ mV}$ vs. Fc/Fc$^+$) proved to be a suitable one-electron oxidant to realize the first oxidation. Addition of one equivalent of the silver salt to solutions of dark-blue [Cu(bpzCal)] or deep-red [Ni(bpzCal)] in CH$_2$Cl$_2$ caused an immediate color change to dark-green or dark-orange, respectively, accompanied by precipitation of elemental silver. These oxidation products gave UV/Vis spectra identical to those of the electrochemically generated species.

In previous extensive studies resonance Raman (rR) spectroscopy has proven a powerful tool for the detection of phenoxyl radicals. When CH$_2$Cl$_2$ solutions of the oxidized compounds are excited with a 413 nm laser, which matches the electronic transitions of the radical at around 400 nm well, especially the modes $v_{3a}$ and $v_{6a}$ of the phenoxyl radicals are enhanced (Figure 6).

In contrast to the nickel derivative, [Cu(bpzCal)]$^{+}$ shows a strong band at 1484 cm$^{-1}$, which can be assigned to a phenolate vibration by comparison with the reduced complex (Figure S6 in the Supporting Information). This points towards a localization of the radical in this case. Clearly, the nickel compound features a more delocalized electronic structure and therefore lacks a phenolate band in its rR spectrum. Both complexes exhibit a band at around 1520 cm$^{-1}$ assignable to the $v_{3a}$ mode of the C–O vibration. The frequencies of these bands are somewhat higher than those found for other known metal-
coordinated phenoxy radical species, suggesting a larger contribution of the quinonoid canonical form in our case. The \( \nu_{ab} \) mode of the C_{ortho}–C_{meta} vibration appears at around 1600 cm\(^{-1}\) in both cases fitting well with reported values.\(^{[17]}\)

Galactose oxidase in its active form has a diamagnetic ground state through antiferromagnetic coupling of the ligand-radical spin with the unpaired d-electron of the copper(II) metal ion.\(^{[18]}\) Therefore, it was of interest to determine which spin state the phenoxy radical complexes described above adopt. Given the limited stability of the oxidized species in the solid state, no reliable SQUID data could be obtained. However, magnetic susceptibilities in solution could be determined by using Evans method: \([\text{Ni(bpzCal)}]^+\) has a magnetic moment of \( \mu_{\text{eff}} = 3.67 \mu_B \) fitting well with the expected value for three unpaired electrons, thereby suggesting a \( S = \frac{3}{2} \) spin state. In contrast, \([\text{Cu(bpzCal)}]^+\) has a moment of \( \mu_{\text{eff}} = 2.66 \mu_B \) which is close to two unpaired electrons of a \( S = 1 \) spin state, definitely excluding a diamagnetic ground state in our case.

To further corroborate the actual spin states, X-band EPR measurements of frozen solutions were performed: in contrast to its neutral precursor, \([\text{Ni(bpzCal)}]^+\) is EPR active and exhibits a rhombic signal that was simulated best with \( g_x = 2.36 \), \( g_y = 2.34 \), and \( g_z = 2.24 \) (Figure S12 in the Supporting Information). The \( g \)-factors of typical Ni\(^{II}\) species are usually somewhat lower at around 2.1, thus a metal centered oxidation in our case can again be excluded. The signals are not well resolved, which may be attributed to the general difficulty of collecting EPR spectra of \( S = \frac{3}{2} \) systems at liquid-nitrogen temperatures. The original EPR signal of \([\text{Cu(bpzCal)}]\) is attenuated in the course of the oxidation leaving only a residual signal for an organic radical (\( g \approx 2 \)) together with one for copper(II) (Figure S11). They account for less than 10% of the total spin concentration, likely originating from a disproportionation reaction yielding Cu\(^{0}\) and a twofold oxidized species. \([\text{Zn(bpzCal)}]^+\) is the most unstable complex of the series, thus only traces (\( \approx 10 \%) of an organic radical with \( g_{\text{iso}} = 2.00 \) were detected by EPR (Figure S13).

Given that no crystals could be grown to investigate the molecular structures of these compounds due to the high instability of the oxidized complexes, DFT calculations were performed to gain insights into possible structures and spin states: for \([\text{Cu(bpzCal)}]^+\) two isomers were found, one with the same symmetry as its neutral precursor and one asymmetric version, both in a singlet and a triplet state (Figure 7). With exception of the symmetric closed-shell singlet state, all structures and states are very close in energy (Table S1 in the Supporting Information), with the asymmetric triplet as ground state, matching the results described above.

Both in the symmetric and the asymmetric isomer of \([\text{Cu(bpzCal)}]^+\) the Cu atom was then replaced by Ni followed by re-optimization. Given that Ni has one electron less than Cu, doublet and quartet states result. For the doublet state, a symmetric and an asymmetric isomer were found, however, for the quartet state all optimizations resulted in a symmetric structure, which is the ground state of the molecule (Table S2 and Figures S19–S21 in the Supporting Information), which is in agreement with the experimental findings.

Hence, from the above data it becomes very clear that a complex with copper(II) ion in a coordination sphere resembling the one in the active site of the GO can be generated and that it can be oxidized at the phenolate donor to yield a radical complex with a triplet ground state. This complex compares well with the active state of the GO, with the difference that the latter has a singlet ground state. To that extent it was now of much interest to test the reactivity of our complexes towards a primary alcohol.

Accordingly, chemically generated solutions of \([\text{Cu(bpzCal)}]^+\) and, for comparison, also \([\text{Ni(bpzCal)}]^+\) in CH\(_2\)Cl\(_2\) were treated with benzyl alcohol as the model substrate. The formation of the two-electron oxidation product benzaldehyde was indeed detected through \(^1\)H NMR spectroscopy; however, unlike in case of the enzymatic paragon, formation of only half an equivalent of the aldehyde per equivalent complex was observed (Scheme 2).

Although in the enzymatic catalytic cycle the Cu\(^{II}\) center performs the second oxidation step (→Cu\(^{III}\)) its oxidation potential in the complex is obviously not sufficiently positive, so that a second equivalent of the complex is needed.\(^{[19]}\) Indeed, the CV of \([\text{Cu(bpzCal)}]\) exhibits a redox event assigned to the Cu\(^{2+}/\)

![Figure 6. Resonance Raman spectra (Kr\(^+\) laser, \( \lambda_{exc} = 413 \text{ nm}, 5 \text{ mW}\)) of \([\text{Ni(bpzCal)}]^+\) (top) and \([\text{Cu(bpzCal)}]^+\) (bottom) in CH\(_2\)Cl\(_2\) (4 mW) at 203 K.](image)

![Figure 7. Optimized molecular structures and Mulliken spin-density distributions of the asymmetric triplet state of \([\text{Cu(bpzCal)}]^+\). Hydrogen atoms are omitted for clarity.](image)

**Scheme 2.** Benzyl alcohol oxidation with \([\text{M(bpzCal)}]^+\) (M = Ni, Cu).
The copper(I) state becomes more oxidizing, future attempts will focus on the stabilization of the copper(II) state through the ligand system so that the copper(II) state becomes more oxidizing.

**Acknowledgements**

We are grateful to the German Israeli Foundation for Scientific Research and Development (G.I.F.) and the Humboldt-Universität zu Berlin for financial support. We also would like to thank Deniz Ar for recording EPR spectra and Dr. Eckhard Bill for trying to obtain SQUID data.

**Conflict of interest**

The authors declare no conflict of interest.

**Keywords**: alcohol oxidation · biomimetic chemistry · calixarenes · copper · nickel

[1] C. D. Gutsche, Calixarenes, Royal Society of Chemistry, Cambridge, 2008.
[2] a) E. S. Espaço, M. M. Villamil, Biomolecules 2019, 9, 90; b) R. Rodik, S. Cherenok, O. Kalchenko, O. Yesypenko, J. Lipkowski, V. Kalchenko, Curr. Org. Chem. 2018, 22, 2200; c) A. Osvyannikov, S. Soloviev, I. Antipin, S. Ferlay, Coord. Chem. Rev. 2017, 352, 151.
[3] a) C. Limberg, Eur. J. Inorg. Chem. 2007, 3303; b) C. Floriani, Chem. Eur. J. 1999, 5, 19; c) U. Radius, Z. Anorg. Allg. Chem. 2004, 630, 957.
[4] a) N. Le Poul, Y. Le Mest, J. Jabin, O. Reinaud, Acc. Chem. Res. 2015, 48, 2097; b) N. Segaud, C. de Thomasson, C. Daverat, K. Sénéchal-David, A. Dos Santos, V. Steinmetz, P. Maitre, J.-N. Reibilly, F. Banse, O. Reinaud, Chem. Eur. J. 2017, 23, 2894; c) A. Parrot, S. Collin, G. Bruylants, O. Reinaud, Chem. Sci. 2018, 9, 5479.
[5] V. Rawat, K. Press, I. Goldberg, A. Vigalok, Org. Biomol. Chem. 2015, 13, 11189.
[6] N. Itô, S. E. Phillips, C. Stevens, Z. B. Ogel, M. J. McPherson, J. N. Keen, K. D. Yadav, P. F. Knowles, Nature 1991, 350, 87.
[7] Y. Wang, T. D. P. Stack, J. Am. Chem. Soc. 1996, 118, 13097.
[8] a) M. M. Olmstead, G. Sigel, H. Hope, X. Xu, P. P. Power, J. Am. Chem. Soc. 1985, 107, 8087; b) J. Zeller, S. Koenig, U. Radius, Inorg. Chim. Acta 2004, 357, 1813.
[9] H. Hope, M. M. Olmstead, B. D. Murray, P. P. Power, J. Am. Chem. Soc. 1985, 107, 712.
[10] S. C. Goel, K. S. Kramer, M. Y. Chiang, W. E. Buhro, Polyhedron 1990, 9, 611.
[11] L. Yang, D. R. Powell, R. P. Houser, Dalton Trans. 2007, 60, 955.
[12] I. Przyjomska-Ray, D. Zornik, M. Pätz, K. B. Krause, L. Grubert, B. Braun-Gula, S. Hecht, C. Limberg, Chem. Eur. J. 2018, 24, 5341.
[13] O. Roththaus, F. Thomas, O. Jarjaye, C. Philouze, E. Saint-Aman, J.-L. Pierre, Chem. Eur. J. 2006, 12, 6953.
[14] D. M. D’Alessandro, F. R. Keene, Chem. Soc. Rev. 2006, 35, 424–440.
[15] F. Thomas, Eur. J. Inorg. Chem. 2007, 2379.
[16] N. G. Connelly, W. E. Geiger, Chem. Rev. 1996, 96, 877.
[17] R. Schnepf, A. Sokolowski, J. Müller, V. Bachler, K. Wieghardt, P. Hildebrandt, J. Am. Chem. Soc. 1998, 120, 2352.
[18] J. Müller, T. Weyhermüller, E. Bill, P. Hildebrandt, L. Ould-Moussa, T. Glaser, K. Wieghardt, Angew. Chem. Int. Ed. 1998, 37, 616; Angew. Chem. 1998, 111, 637.
[19] R. C. Pratt, T. D. P. Stack, J. Am. Chem. Soc. 2003, 125, 8716.