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Carboxylate-enhanced reactivity in the oxygenation of copper flavonolate complexes

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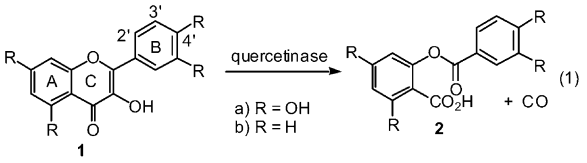
Abstract

The oxygenolytic cleavage of flavonolate coordinated to copper(II) with the auxiliary ligand 3,3′-iminobis(*N*,*N*-dimethylpropylamine), $[Cu^{II}(fla)(idpa)]ClO_4$, is enhanced by the addition of acetate and benzoate. Monodentate coordination of the flavonolate forced by the carboxylate ligands is believed to be the reason for the higher reaction rates.

Keywords: Quercetinase; Model; Dioxygenation; Copper; Dioxygen

1. Introduction

Quercetin 2,3-dioxygenase is a copper-containing metalloenzyme, which catalyzes the oxygenolytic cleavage of the heterocyclic ring of quercetin at the C_2–C_3 carbon bonds yielding the depside (carboxylic acid ester) and carbon monoxide (Eq. (1)) [1–4]. The substrate is believed to bind to the copper(II) ion through its 3OH and 4C=O groups [5–7].



Model studies on copper flavonolate complexes with various ligands have established this binding mode of the substrate quercetin to copper(II) by disclosing their molecular structures by X-ray diffractometry [8–13]. Oxygenation of these (flavonolato)copper complexes led to the (*O*-benzoylsalicylato)copper complexes and CO in almost all cases mimicking the enzyme reaction [14–20]. However, the rates of the reactions were rather slow and higher temperatures were necessary. Recent characterization of the enzyme quercetinase from *Aspergillus japonicus* by X-ray crystallography has shown that in the coordination environment of copper(II) at the active site beside histidine carboxylate coordination of glutamate is also possible [21]. This fact has prompted us to check at model reactions whether carboxylate coordination enhances the reaction rates of the ring scission reaction of flavonol. We used the formerly studied (flavonolato)copper(II) complex $[Cu^{II}(fla)(idpa)]ClO_4$ (fla = flavonolate; idpa = 3,3′iminobis(*N*,*N*-dimethylpropylamine)) (**3**) [13] for the oxygenation reactions by adding carboxylic acids or carboxylate anions in order to see whether these coordinate to the copper(II) ion or enhance the reactivity of the complex.

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2. Experimental

All manipulations were carried out using standard Schlenk technique under purified argon [22]. Anhydrous DMF was purchased from Aldrich and used as supplied. $[Cu^{II}(fla)(idpa)]ClO_4$ was prepared as described earlier [13]. Gaseous dioxygen from Messergriesheim was 99.6% and passed through P_2O_5 and Blaugel in order to remove traces of water and other impurities. Electronic spectra were recorded on a Shimadzu UV-160A spectrophotometer using quartz cells.

2.1. Oxygenation of $[Cu^{II}(fla)(idpa)]ClO_4$

$[Cu^{II}(fla)(idpa)]ClO_4$ (0.294 g, 0.5 mmol) in DMF (50 cm^3) was treated with dioxygen (0.1 MPa) at 100 °C for 8 h, the solution was cooled to room temperature and diethyl ether was layered to afford blue powder of $[Cu^{II}(O\text{-bs})(idpa)]ClO_4$ (0.183 g, 62%) [13].

2.2. Kinetic measurements

Reactions of the flavonolato copper complex with O_2 were performed in DMF solution. $[Cu^{II}(fla)(idpa)]ClO_4$ was dissolved under argon in a thermostatically controlled reaction vessel with an inlet for taking samples with a syringe, and connected to a mercury manometer to maintain a constant pressure. The solution was then heated to 100 °C, a sample was taken by syringe, and the initial concentration of complex was determined by UV/Vis spectroscopy measuring the absorbance of the reaction mixture at 426.5 nm (λ_{max} of a typical band of $[Cu^{II}(fla)(idpa)]ClO_4$). The argon was then replaced by dioxygen, and the consumption of $[Cu^{II}(fla)(idpa)]ClO_4$ was analyzed periodically. Experimental conditions are summarized in Table 1. The O_2 concentration was calculated from literature data [23] taking into account the partial pressure of DMF [24] and assuming the validity of Dalton's law. The rate of consumption was independent of the stirring rate, excluding eventual diffusion control effects.

3. Results and discussion

The X-ray structure determination of quercetin 2,3-dioxygenase revealed that the type copper(II) center at the active site in the enzyme isolated from *A. japonicus* displays two distinct geometries; a distorted tetrahedral coordination, formed by three histidine and a water molecule, and a distorted trigonal bipyramidal environment, which additionally comprises a carboxylate coordination of glutamate. In the course of our earlier studies on quercetin 2,3-dioxygenase model systems we prepared (flavonolato)copper(II) complexes (fla = flavonolate) with the auxiliary ligand 3,3′-iminobis(*N*,*N*-dimethylpropylamine) (idpa). The molecular structure of $[Cu^{II}(fla)(idpa)]ClO_4$, has been determined by X-ray diffractometry to have a distorted trigonal bipyramidal geometry and the kinetics of its oxygenation has been disclosed to give a second order rate law [13,19]. The tridentate N_3-ligand idpa seemed to model the histidine environment leaving

Table 1
Kinetic data for the oxygenation of $[Cu^{II}(fla)(idpa)]ClO_4$ in DMF solution in the presence of carboxylates[a]

Expriment no.	[Cu] ($\times 10^4$; mol dm^{-3})	[Additive] ($\times 10^3$; mol dm^{-3})	$10^4 k'$ ($\times 10^3$; s^{-1})	R^2[b] (%)	k_{obs} ($\times 10^2$; mol dm^{-3})
1	5.23	–	0.52	99.09	0.67 ± 0.03
2	5.16	Na(OAc), 1.63	1.83	98.28	2.38 ± 0.29
3	5.06	Na(OAc), 1.87	1.88	99.32	2.45 ± 0.11
4	5.34	Na(OAc), 5.43	3.01	99.42	3.91 ± 0.14
5	5.17	Na(OAc), 10.46	3.47	99.23	4.51 ± 0.16
6	5.30	Na(OAc), 15.55	3.19	99.61	4.14 ± 0.11
7	5.16	Na(OAc), 20.67	3.39	99.63	4.40 ± 0.10
8	5.48	Na(OBenz), 5.46	1.21	99.72	1.57 ± 0.01
9	5.31	HOAc, 5.31	0.47	99.65	0.61 ± 0.01

[a] *Conditions*: 50 cm^3 DMF, $T = 100$ °C, $[O_2] = 7.7 \times 10^{-3}$ mol dm^{-3}.
[b] Correlation coefficients of least-squares regressions.

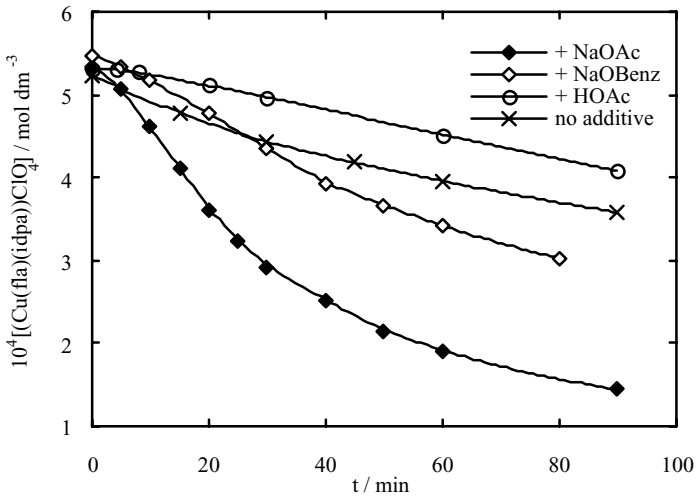
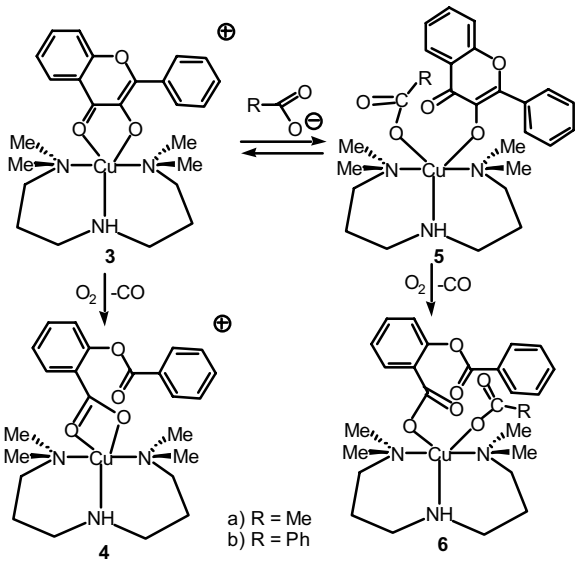


Fig. 1. Influence of additives on the oxygenation of [Cu^{II}(fla)(idpa)]ClO_4 (**3**). Conditions: 50 cm^3 DMF, $T = 100\,°C$, $[O_2] = 7.7 \times 10^{-3}$ mol dm^{-3}, (×) 0.026 mmol **3**, (○) 0.026 mmol ***3*** + 0.26 mmol HOAc, (◇) 0.027 mmol ***3*** + 0.273 mmol NaOBenz, (◆) 0.026 mmol ***3*** + 0.272 mmol NaOAc (experiments 1, 9, 8, and 4; Table 1).

one or two coordination sites free for the coordination of solvent and/or the substrate flavonolate. In order to mimic the carboxylate coordination in the model system, we added sodium acetate to the complex [Cu^{II}(fla)(idpa)]ClO_4 and looked at the kinetics of the reaction.

Experiments showing the time course of the oxygenation reactions of [Cu^{II}(fla)(idpa)]ClO_4 (**3**) and those with added sodium acetate, sodium benzoate, and acetic acid are shown in Fig. 1. It can be seen that the addition of acetic acid did not have any effect on the reaction rate of the oxygenation. The addition of sodium acetate and benzoate, however, accelerate the reaction rates compared to the reaction with no additives. If we alter the ratio of sodium acetate to the complex **3** we can see that at higher acetate concentration the oxygenation reaction proceeds faster (Fig. 2). The second order reaction rate constants (k_{obs}) are compiled in Table 1. The kinetic date demonstrate clearly that the addition of acetic acid does not have any influence on the reaction rate of the oxygenation of [Cu^{II}(fla)(idpa)]ClO_4, however carboxylate addition (acetate, benzoate) enhances the reaction rates. Higher carboxylate concentration results in higher reaction rates. At a 20:1 acetate to (flavonolato)copper(II) complex ratio leads to almost one order of magnitude higher reaction rate.

The explanation of the larger reaction rates of the oxygenation of [Cu^{II}(fla)(idpa)]ClO_4 in the presence of added acetate or benzoate can be explained as shown in the Scheme 1. In complex [Cu^{II}(fla)(idpa)]ClO_4 (**3**) the geometry around the



Scheme 1.

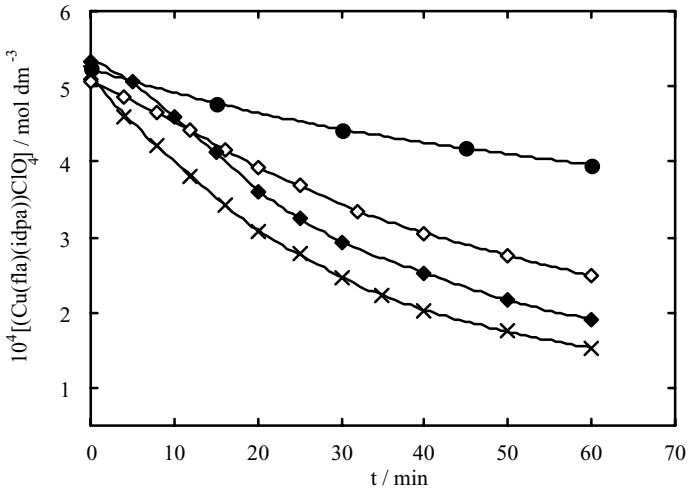


Fig. 2. Time course for the oxygenation of $[Cu^{II}(fla)(idpa)]ClO_4$ (**3**) in the presence of sodium acetate. Conditions: 50 cm^3 DMF, $T = 100\,^{\circ}C$, $[O_2] = 7.7 \times 10^{-3}$ mol dm^{-3}, (●) 0.026 mmol **3**, (◇) 0.025 mmol ***3*** + 0.093 mmol NaOAc, (◆) 0.026 mmol ***3*** + 0.272 mmol NaOAc, (×) 0.025 mmol ***3*** + 0.523 mmol NaOAc (experiments 1, 3, 4, and 5; Table 1).

copper(II) ion can be regarded as a distorted trigonal bipyramidal with a τ value of 0.61 [13]. The two nitrogen atoms of the N_3-ligand and one oxygen atom of the flavonolate ligand occupy basal positions. The remaining N-atom of the N_3-ligand and the other oxygen atom of the flavonolate ligand lie in apical positions. The flavonolate ligand in **3** is bidentate, however on addition of the carboxylate ions to the complex they coordinate to the copper(II) in a monodentate fashion just displacing one of the oxygen atoms of the flavonolate ligand. According to that **3** is converted to **5**, where the flavonolate ligand becomes monodentate. This may be the reason for the enhanced reactivity towards dioxygen. So **5** is converted to **6** by incorporating both O-atoms of dioxygen into the substrate with the concomitant release of carbon monoxide, just in agreement with the enzyme reaction. Seemingly, carboxylate coordination, as found in the structure of quercetin 2,3-dioxygenase from *A. japonicus*, is necessary for the enzyme reaction in order to elicit reasonable reaction rates. On the bases of these experiments we believe to have the first evidence that carboxylate coordination plays an important role in the enzyme reaction, and these reactions may be regarded as the first best mimicking models for the enzyme. Further work is in progress to work out structural and functional models of quercetin 2,3-dioxygenase in light of the recent knowledge of its structure.

Acknowledgements

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