A Single Enzyme Oxidative "cascade" via a Dual-Functional Galactose Oxidase

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A single enzyme oxidative ‘cascade’ via a dual functional galactose oxidase

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ABSTRACT: The galactose oxidase (GOase) $M_{3\text{-}5}$ variant, previously engineered for enantioselective oxidation of (*R*)-secondary alcohols, is now shown to catalyze the sequential four electron oxidation of substituted benzylic and heteroaromatic benzylic alcohols to the corresponding carboxylic acids via the intermediate aldehyde. Aldehyde oxidation has been shown to occur on the hydrated (*gem*-diol) form of the aldehyde, and hence the activity of this second oxidation step is primarily determined by the effects of substituents on the aromatic ring. The demonstration of GOase for ‘through oxidation’ of alcohols to carboxylic acids represents a fusion of the activities of two distinct copper radical oxidases (galactose oxidase and glyoxal oxidase) into a single enzyme sequence with potential applications as an abbreviated oxidative cascade.

KEYWORDS: biocatalysis, through oxidation, galactose oxidase, dual functional enzyme, alcohol oxidation, aldehyde oxidation

INTRODUCTION

Biocatalytic oxidations offer many advantages over equivalent chemical methods such as regio- and stereoselectivity, a renewable resource for mild oxidation methods, and importantly, catalytic turnover rather than the need for stoichiometric inorganic oxidants. In some instances these transformations can be performed by organisms such as acetic acid bacteria,[1] however, bio-oxidations are more commonly achieved by a dehydrogenase or multiple dehydrogenase enzymes in sequence (Figure 1a). Although a dehydrogenase cascade can be quite successful, as in the case of production of 5-methylpyrazine-2-carboxylic acid (MPCA) from 2,5-dimethylpyrazine by Lonza,[2] there are some inherent shortcomings of nicotinamide-dependent oxidation cascades as they can often suffer from reversibility in an unfavourable equilibrium and are absolutely required to have a cofactor regeneration system to drive the reaction.

Instead, a cascade of oxygen-dependent enzymes may represent an irreversible and more efficient alternative for sequential substrate oxidation without the need of an expensive cofactor. A notable example of this is the biocatalytic production of furan-2,5-dicarboxylic acid (FDCA) from 5-hydroxymethylfurfural (HMF) by galactose oxidase (GOase) and periplasmic aldehyde oxidase (PaoABC)[3] (Figure 1b). However, even more useful would be the combination of alcohol and aldehyde oxidation in a single, dual-functional oxidase enzyme. Toward this, several FAD-dependent alcohol oxidases have been characterized as also catalysing the oxidation of a variety of aldehydes, although in general this secondary activity is fairly low.[4-7]

We were interested to determine if this combination, dual-oxidase activity could be established in galactose oxidase through cultivating activity of the related aldehyde oxidizing enzyme glyoxal oxidase (Figure 1c). While sequence

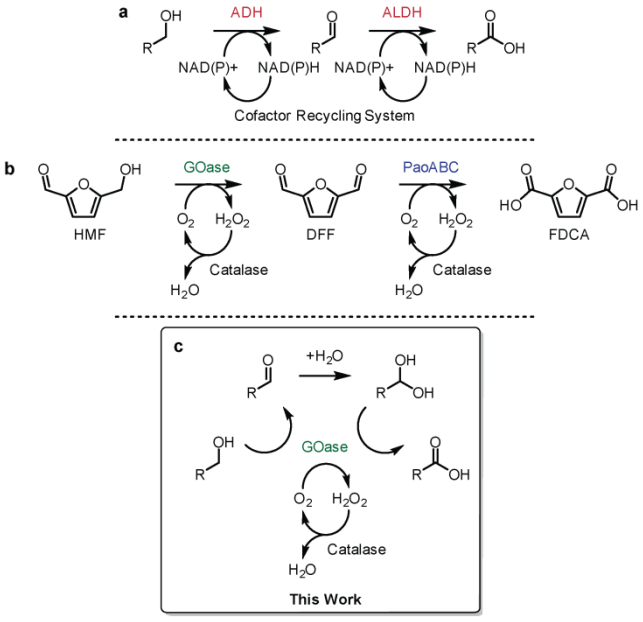


Figure 1. Stepwise oxidation of alcohols to carboxylic acids via (**a**) a dehydrogenase cascade, (**b**) a cascade consisting of galactose oxidase (GOase) and periplasmic aldehyde oxidase (PaoABC) and (**c**) a galactose oxidase only ‘cascade.’ ADH: alcohol dehydrogenase. ALDH: aldehyde dehydrogenase.

similarity between the two enzyme groups is quite low, they share the same copper binding residues and the characteristic fused cysteine-tyrosine motif that creates the sequence derived redox cofactor.[8-10] GOase and glyoxal oxidase catalyse distinct reactions with very little overlap in accepted substrates.[10] Catalytic efficiency of glyoxal oxidase catalysed alcohol oxidation is nearly two orders of magnitude less than that of methyl glyoxal.[8] Similarly, wild type galactose oxidase has

been found to catalyse low level oxidation of galactose and galactose containing oligosaccharides to the corresponding galacturonic acid if under condition of long reaction time and high enzyme concentrations.[11-13] Despite the lack of global sequence similarity between these enzymes, evidence of promiscuous catalysis of two discrete oxidation reactions within the conserved enzyme active site *suggested a potential for converging both activities in one engineered biocatalyst*.

To facilitate detection of aldehyde oxidase activity in GOase and develop a system for efficient 'through oxidation' of alcohol to aldehyde to acid, it was necessary to approach from two directions. The first undertaking was adjusting reaction conditions to reach maximal activity, thereby enhancing any latent aldehyde oxidase character. This has now been accomplished via a systematic optimization of reaction conditions for GOase, primarily by addition of supporting enzymes.[14] The second point was in selecting an appropriate substrate by determining the requirements for aldehyde oxidation. Based on the substrate scope of glyoxal oxidase, aldehyde oxidation by this enzyme is proposed to proceed through the hydrated (*gem*-diol) form of the aldehyde,[10, 15] and we anticipated this to apply to GOase as well.

Accordingly, we now demonstrate that the GOase M_{3-5} variant previously engineered for oxidation of (*R*)-secondary benzylic alcohols[16] also has considerable aldehyde oxidase activity. This activity is dependent on aldehyde hydration, and therefore follows a series of trends in chemically acceptable substrates. The proficiency of this newly characterized trait of GOase is highlighted in the 'through oxidation' of *N*-heteroaromatic benzylic alcohols to carboxylic acids which serve as valuable pharmaceutical intermediates. The results presented show the potential of GOase as an oxygen-dependent, dual functional oxidase for production of carboxylic acids via a single enzyme 'cascade'.

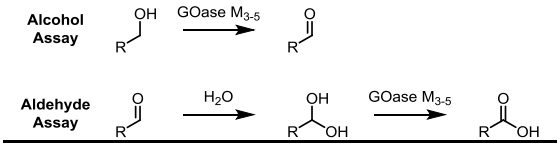
RESULTS AND DISCUSSION

Reaction conditions for GOase catalysed alcohol oxidation were previously optimized via addition of supporting enzymes to regenerate the active form of the enzyme (horseradish peroxidase) and to protect it from irreversible inactivation by the H_2O_2 byproduct (catalase).[14] Inclusion of these maintenance enzymes in biotransformations had dramatic effects on both the turnover rate and duration of GOase activity, ultimately leading to much higher conversions and space time yields.[14] In addition to providing better performance for alcohol oxidation, the greater activity found through the use of optimized conditions enabled straightforward detection of aldehyde oxidation by GOase.

To examine the scope of GOase aldehyde oxidase activity, a series of substituted benzaldehydes and *N*-heteroaromatic aldehydes were analyzed as substrates in a liquid phase assay. The panel consisted of a variety of electron donating and electron withdrawing groups (EDG and EWG, respectively) substituted at the *ortho*-, *meta*- or *para*- positions around the ring to cover a broad enough range of electronic effects to identify any trends in substrate properties required for aldehyde oxidation by M_{3-5}. The specific activity of M_{3-5} for each aldehyde is listed in Table 1, along with the activity for the corresponding alcohol substrate.

Surprisingly, most of the aldehydes tested did appear to be active substrates for GOase M_{3-5}. However, as anticipated, each had significantly lower specific activity compared to that of the equivalent alcohol. Based on these activities, some apparent correlations can be observed in relation to benzaldehyde substitution patterns. First, *meta*- and *para*-EWG substitutions (*para*- > *meta*-) lead to higher aldehyde oxidase activities, generally increasing in rate with the strength of the electron withdrawing group, and reaching a maximum

Table 1. Specific activities of GOase M_{3-5} toward substituted benzylic alcohols and aldehydes.



Substrate	Alcohol Activity[a]	Aldehyde Activity[a]	*gem*-Diol (%)[b]
Substituted Benzyl Alcohols/Benzaldehydes			
Electron Donating Groups			
2-OMe	12.7	0.0	0.0
3-OMe[c]	78.6	1.2	1.0
4-OMe	56.9	0.0	0.0
2-Me	2.2	0.0	0.0
3-Me	84.0	0.3	2.0
4-Me	56.1	0.1	0.0
H	65.3	0.1	1.0
Halogenated			
2-F	37.6	0.0	9.1
3-F	86.9	1.0	3.8
4-F	52.9	0.3	1.0
2-Cl	13.7	0.0	12.3
3-Cl	87.4	1.3	5.7
4-Cl	83.2	0.3	1.0
2-Br	11.8	0.0	13.8
3-Br	119.8	1.1	5.7
4-Br	89.8	0.3	5.7
Electron Withdrawing Groups			
2-Acetyl	n.a.[d]	n.d.[e]	43.2
3-Acetyl	n.a.[d]	3.9	4.8
4-Acetyl	n.a.[d]	5.8	9.1
2-CF_3	2.0	0.0	15.3
3-CF_3	94.4	1.6	4.8
4-CF_3	118.1	2.5	9.1
3-CN	86.8	1.9	6.5
4-CN	94.4	5.3	18.0
2-NO_2	24.5	0.3	27.5
3-NO_2	95.0	4.9	16.7
4-NO_2	103.5	5.9	23.1
***N*-Heteroaromatic Benzylic Alcohols/Aldehydes**			
2-Pyridine	71.2	8.1	41.9
3-Pyridine	49.6	1.1	13.8
4-Pyridine	n.d.[e]	14.2	60.3
2-Pyrazine	76.9	9.4	85.2
5-Me-2-pyrazine	78.0	10.2	69.9
2-Quinoline	n.a.[d]	11.9	58.7
3-Quinoline	n.a.[d]	1.8	8.3
4-Quinoline	n.a.[d]	2.4	65.9
2-Indole	n.d.[e]	0.1	0.0
3-Indole	n.d.[e]	0.1	0.0
4-Imidazole	n.d.[e]	0.0	0.0

[a]Activity provided in units of μmol/min/mg. [b]As determined by NMR (see Supporting Information). [c]3-OMe is electron withdrawing based on Hammett values, but was grouped with the matching substitutions for ease of activity comparison. [d]n.a. Not applicable. Substrate not commercially available. [e]n.d. Substrate reacted with the ABTS dye so activity measurement could not be collected.

at 5.9 μmol/min/mg for 4-nitrobenzaldehyde. Additionally, although *ortho*- and *para*- substituents impart comparable electronic effects on the aromatic ring, activity of the analogous substrate pairs was noticeably different as aldehyde substrates with *ortho*-substitutions do not seem to be tolerated by the enzyme regardless of substituent effect. Activities on the substituted alcohols similarly display these activity patterns as well, indicating the degree of effect caused by the substituents is not limited to aldehyde reactivity.

Unexpectedly, *meta*-EDG substituted benzaldehydes showed higher activity than the related *ortho*- and *para*-substitutions, which also appeared to increase with the strength of the electron donating group. While slightly noticeable in the methylbenzaldehyde series, this effect was most evident in the case the methoxybenzaldehyde set where activity toward *meta*-methoxybenzaldehyde was 1.2 μmol/min/mg while no activity was observed for the analogous *ortho*- or *para*- substitutions. Similarly, *meta*-halogenated aldehydes showed higher activity compared to the *para*-substituted counterparts, which is also mirrored in activity toward the respective alcohol substrates. This departure from the expected trend is due to the specific substituent positioning, since according to Hammett values, a methoxy- or halogen substitution at the *meta*-position will act as an EWG,[17] therefore giving the characteristics of a preferred substrate for GOase.

GOase $M_{3\text{-}5}$ also showed good specific activity toward many of the nitrogen containing heterocyclic aldehydes (Table 1). Here as well the position of the electronegative nitrogen relative to the aldehyde had a large effect on activity, emulating an EWG substitution as much greater activity was observed for 4-pyridinecarboxaldehyde than 3-pyridinecarboxaldehyde. Interestingly, despite the lack of activity in the *ortho*-EWG substituted benzaldehydes, 2-pyridinecarboxaldehyde was a readily accepted substrate for $M_{3\text{-}5}$. It is likely that an *ortho*-substitution on the ring creates a steric clash with either an active site residue or possibly the catalytic Cu^{2+} centre and prevents appropriate binding for efficient catalysis for these substrates. However, the nitrogen atom within the ring effectively promotes the availability of this substrate for aldehyde oxidation without the apparent negative interactions of an *ortho*- ring substituent. Similarly, $M_{3\text{-}5}$ is highly active toward both pyrazine aldehydes tested, and shows some activity toward each quinoline substrate. The distinctly lower activity toward 4-quinolinecarboxaldehyde also agrees with the proposed negative steric effect of the *ortho*-substitution. Activity toward the indole and the imidazole aldehydes was very low in all cases.

'Through oxidation' of the substituted benzylic alcohols (or aldehydes where not available) to the corresponding carboxylic acids was measured in biotransformations to confirm the activities observed in liquid phase. Of the 38 substrates tested, 18 provided at least 50% conversion to the carboxylic acid after six hours, with 10 of those reaching greater than 90% conversion in the same timeframe (Figure 2 and Supporting Table S1). Parallel to what was observed in the liquid phase assay, the most active benzyl compounds had predominantly *meta*- or *para*- electron withdrawing substitutions, with a stronger EWG largely leading to higher conversions. As an unanticipated outlier based on liquid phase specific activity, the electron donating *meta*-methoxybenzyl alcohol reached 95% conversion to the acid despite the low specific activity of the aldehyde substrate. The *meta*-halogenated alcohols also showed greater conversion to the acid than expected (at least 70%) based on the low activity in the liquid phase assay. High conversion of several nitrogen-containing aromatic heterocycles was also demonstrated as five of the 11 substrates showed complete oxidation to the carboxylic acid. As mentioned above, a strong EDG (such as a methoxy- substituent) in the *meta*-position has Hammett values that characterize these substitutions as electron withdrawing. In a similar manner, *meta*-halogen substitutions also provide stronger EWG effects than the equivalent *para*-substitutions based on Hammett values. In both cases, the unique electronic effects imparted by the substituent when specifically at the *meta*-position help rationalize the higher activity and conversion levels observed for these substrates.

Oxidation of an aldehyde by GOase would seemingly challenge the accepted catalytic mechanism since alcohol oxidation begins with the removal of the substrate hydroxyl proton by the enzyme (Supporting Figure S1). However, the activity of the related enzyme glyoxal oxidase indicates that this reaction is catalytically possible within the shared active site. The hydrated form of the aldehyde could instead be used to justify this activity with the same sequence of events for alcohol oxidation (Supporting Figure S1), and in fact this *gem*-diol form is proposed to be the actual substrate for glyoxal oxidase.[10, 15] Several FAD-dependent alcohol oxidases, such as choline oxidase,[5] aryl alcohol oxidase,[6] alditol oxidase[7] and the recently reported 5-hydroxymethylfurfural oxidase (HMFO),[4] have also been characterized as able to catalyse aldehyde oxidation. Correspondingly, in each of these cases the *gem*-diol form has been demonstrated to be the active substrate, rather than the aldehyde itself.

To further investigate the relationship between *gem*-diol formation and aldehyde oxidation by GOase, the extent of hydration for all aldehyde substrates was estimated from the ^{1}H NMR spectra (Table 1 and Supporting Information). Overall, benzaldehydes with EDG substitutions at any position lead to low levels of hydration, with marginally greater levels noticeable at *meta*-positions. In contrast, EWG substitutions on benzaldehyde lead to significantly higher degrees of hydration overall, generally increasing with both the strength of the substituent and by placement at the *ortho*- or *para*-position, with *ortho*-EWGs promoting the highest levels of *gem*-diol formation. *N*-heteroaromatic aldehydes also displayed a greater degree of hydration when the ring nitrogen was at the *ortho*- or *para*-position, and proved to be much more effective at promoting hydration as over half of the substrates were greater than 40% hydrated.

The general trend of activity toward benzaldehydes with *para*-EWG substitutions, as well as an increase in activity coinciding with an increase in strength of the EWG, appears to be the direct result of the hydration equilibrium of the particular substrate. Barring the *ortho*-substituted benzaldehydes which showed little to no activity with $M_{3\text{-}5}$, the degree of hydration has a clear positive correlation to the $M_{3\text{-}5}$ specific activity for aldehyde oxidation across the full panel of aldehyde substrates. In addition to further supporting the *gem*-diol as the active substrate, this also implies that aldehyde oxidation by GOase is mechanistically dependent on formation of the hydrate prior to substrate binding, rather than

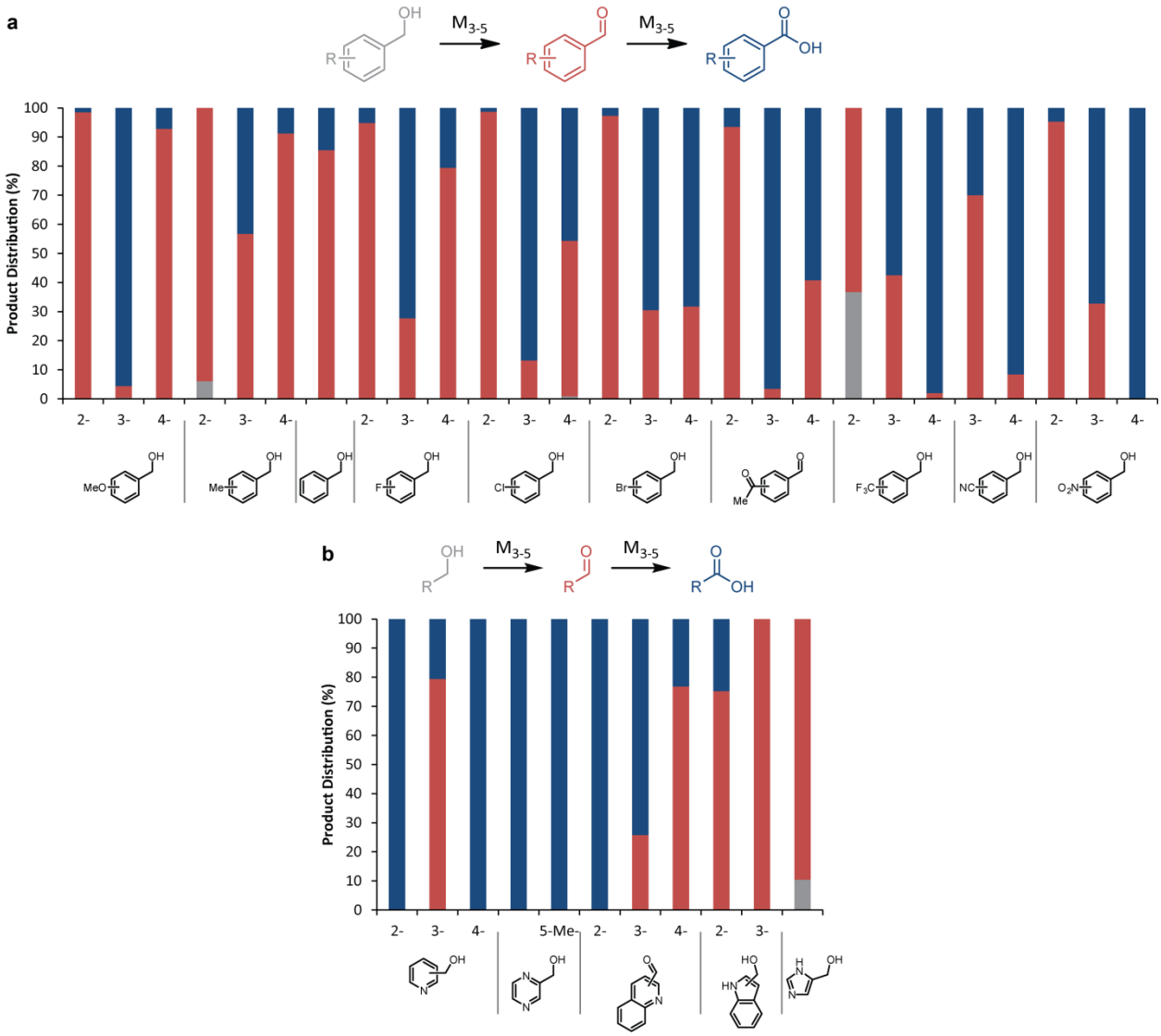


Figure 2. Distribution of products from oxidation of (**a**) benzylic and (**b**) *N*-heteroaromatic benzylic alcohols and aldehydes to carboxylic acids by GOase $M_{3\text{-}5}$ in analytical scale biotransformations. Alcohol composition shown in grey, aldehyde in red and acid in blue. Analytical scale (250 µL) reactions containing 100 mM NaPi pH 7.4 plus 10% DMSO (except for 4-imidazolecarboxaldehyde, which did not contain DMSO) with 0.25 mg/mL purified GOase $M_{3\text{-}5}$, 8.4 U/mL (0.032 mg/mL) HRP, 440 U/mL catalase and 10 mM substrate were shaken for 6 h at 25°C at 250 rpm. Numerical data presented in Supporting Table S1.

the enzyme additionally catalysing the hydration through activation of a water molecule preceding oxidation.

While it is clear that the identity and position of the substituent are critical for regulating the availability of the aldehyde for oxidation by GOase, unfortunately no fully encompassing trend line could be fit to the specific activities or 'through oxidation' results plotted against the core electron binding energy shift (ΔCEBE), Hammett parameters or contributions of resonance or inductive effects for each of the specific substituents on benzaldehyde.[17] However, general descriptions based on resonance and inductive effects can be used to explain the activity and hydration patterns. *Ortho*- and *para*-EWG substituents as well as the electron withdrawing effect of the *meta*-EDG substituents promote a δ+ charge on the *ipso*-carbon via resonance, activating the polarized carbonyl for hydration. While the *meta*-EWG substitutions do not provide this direct activation, the induced electron deficiency in the ring creates the partial positive charge necessary for activation. In both cases, the effect generally coincides with the electron donating/withdrawing strength of the substituent. The observation of some activities that don't strictly fit these electronic properties (Hammett values, CEBE, or resonance or inductive effects) implies that in addition to the substituent effects, substrate acceptance is also limited by active site structure and enzyme-substrate interactions, which is also evident by the inability of GOase to accept the readily hydrated *ortho*-substituted benzaldehydes.

In order to directly confirm that GOase catalyses aldehyde oxidation via the *gem*-diol, oxidation of 4-nitrobenzaldehyde was performed in 30% ^{18}O-labelled water.[18] If the hydrated aldehyde is oxidized by GOase $M_{3\text{-}5}$, a portion of the carboxylic acid product will contain an ^{18}O-labelled oxygen

atom, or potentially two ^{18}O-labelled oxygen atoms from a double incorporation (Figure 3a). Incorporation of ^{18}O-labelled oxygen into 4-nitrobenzaldehyde in the absence of enzymes was observed via GC-MS (Supporting Figure S2). LC-MS analysis of the full biotransformation performed in unlabelled water revealed production of 4-nitrobenzoic acid with the expected mass of 166 *m/z* (Figure 3b). The identical reaction performed in 30% $H_2{}^{18}O$ gave the expected unlabelled product at 166 *m/z* with additional products with increased masses of 168 *m/z* (*M*+2) and 170 *m/z* (*M*+4) also observed (Figure 3c). Detection of these labelled products confirms that the additional oxygen of the carboxylic acid originated from water rather than O_2. Additionally, presence of the *M*+4 species gives further evidence to support the hydrated aldehyde being the catalytically relevant substrate as a double incorporation can only be observed via the *gem*-diol intermediate.

In a previous study it was found that because the *pro*-(*R*) hydrogen of the alcohol substrate is not involved in the GOase oxidation mechanism[19-20] (Supporting Figure S1), replacement of this hydrogen by a methyl group, such as in 1-phenylethanol, was readily accepted by the $M_{3\text{-}5}$ variant as an (*R*)-selective secondary alcohol oxidase.[16] Consequently, it is therefore also fitting that the sterically similar *gem*-diol aldehyde could be shown to be oxidized by this enzyme via the same mechanism as (*R*)-alcohol oxidation (Supporting Figure S1). This mechanism demonstrated for GOase is also expected to apply to the catalytic cycle of aldehyde oxidation by glyoxal oxidase based on active site similarity.

Performance of GOase $M_{3\text{-}5}$ for production of *N*-heteroaromatic carboxylic acids was examined in scaled up biocatalytic oxidations of the most active aldehyde substrates. Reactions were performed at 10 mL volume with 25 mM of 4-pyridinemethanol, 5-methyl-2-pyrazinemethanol or 2-quinolinecarboxaldehyde, and conversion was monitored over time (Figure 4). Both alcohol substrates showed full depletion of the starting material within the first 30 min of reaction, and notably, despite the low catalyst loading (3.6 µM), all three reactions had reached nearly maximal conversion to the respective carboxylic acid by the two hour time point. Final conversions in these reactions reached >99% for 5-methyl-2-pyrazinemethanol and 2-quinolinecarboxaldehyde and 87% (at 24h) for 4-pyridinemethanol, demonstrating the feasibility of using GOase $M_{3\text{-}5}$ as the sole biocatalyst for production of *N*-heteroaromatic acids from the corresponding alcohol. Of the two reactions that went to full conversion, 2-quinoline carboxylic acid could be purified at 80% isolated yield. Although 5-methyl-2-pyrazine carboxylic acid was not able to be purified at this scale, industrial methods of isolation by precipitation are quite successful.[21]

Given the degree of success with these three substrates as proof of concept, biocatalytic accessibility to a large variety of other *N*-heteroaromatic carboxylic acids by GOase could also be envisioned. *N*-heterocycles are widely found in pharmaceuticals and agricultural products, where the benefit of reducing byproduct formation via an enzymatic route could be of great value. Production in this way could be especially effective if the GOase 'cascade' is added in tandem with a monooxygenase enzyme that selectively hydroxylates a methyl group on aromatic *N*-heterocycles, such as in the biocatalytic preparation of MPCA.[2] The spectrum of accessible products could be easily diversified to include variously substituted ring

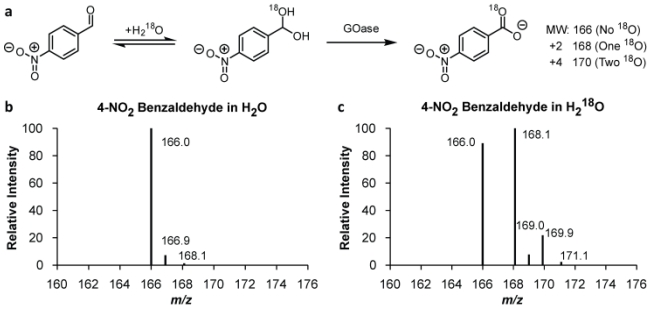


Figure 3. Negative mode LC-MS analysis of 4-nitrobenzoic acid produced by GOase $M_{3\text{-}5}$. (**a**) Scheme of aldehyde labelling via hydration, then oxidation of the *gem*-diol by GOase. (**b**) Spectrum of the $M_{3\text{-}5}$ oxidation product formed from 4-nitrobenzaldehyde in H_2O. (**c**) Spectrum of the product formed from 4-nitrobenaldehyde oxidation by GOase $M_{3\text{-}5}$ in 30% $H_2{}^{18}O$, showing the increased masses of 168 *m/z* (M+2) and 170 *m/z* (M+4) to indicate single and double incorporation of the labelled water. Analytical scale (250 µL) reactions containing 0.25 mg/mL purified GOase $M_{3\text{-}5}$, 8.4 U/mL (0.032 mg/mL) HRP, 440 U/mL catalase and 10 mM 4-nitrobenzaldehyde in DMSO (10% DMSO final concentration) in 100 mM NaPi pH 7.0 with or without 30% ^{18}O-labelled water were shaken for 2 h at 25°C at 250 rpm.

structures as well, some of which are versatile intermediates in pharmaceuticals or drug library production and can be made either biocatalytically by other oxidizing enzymes or even chemically after formation of the acid.[22-24]

Additionally, the finding that GOase had little to no activity toward *ortho*-substituted benzaldehydes and differences in activity based on substitution patterns creates a potential means for selectively oxidizing dialdehyde substrates. Targeted, single oxidation of substituted tere- or isophthalaldehyde derivatives by GOase would produce aromatic mono-acid/mono-aldehyde building blocks with a third unique chemical handle on the ring. Furthermore, the high level of activation of *N*-heteroaromatic aldehydes for oxidation also suggests a possible biocatalytic route to formation of *N*-heteroaromatic diacids via GOase oxidation.

Recognition of GOase $M_{3\text{-}5}$ catalysed aldehyde oxidation introduces a new feature to an already well-studied alcohol oxidase, and identifies this enzyme as one of the few dual functional oxidoreductases able to catalyse oxidation of both alcohol and aldehyde moieties. Like the other alcohol oxidases previously mentioned to catalyse both reactions, this is a very substrate dependent activity for GOase and is determined primarily through substituent activation of the aldehyde for hydration rather than by enzyme reduction potential alone. Unlike these other dual functional oxidases, GOase is the first flavin-independent enzyme to demonstrate this activity at a significant level, and thus offers a unique new mechanistic and structural template for the development of more efficient 'through oxidation' biocatalysts. Additionally, the presence of aldehyde oxidation activity at such a high level in GOase provides some advantages over other enzymes with this two-step oxidation activity. For example, demonstration of aldehyde oxidase activity in GOase provides a more accessible route to application as this enzyme has been extensively engineered for laboratory use through expression, stability and substrate optimization,[16, 25-28] and as such has found use in many different fields.[29-34] Recognition of this secondary

activity on the known robust scaffold should enable an easier translation to practical implementation as a potential alternative oxygen-dependent oxidative cascade, and is particularly interesting for biocatalytic production of *N*-heteroaromatic acids.

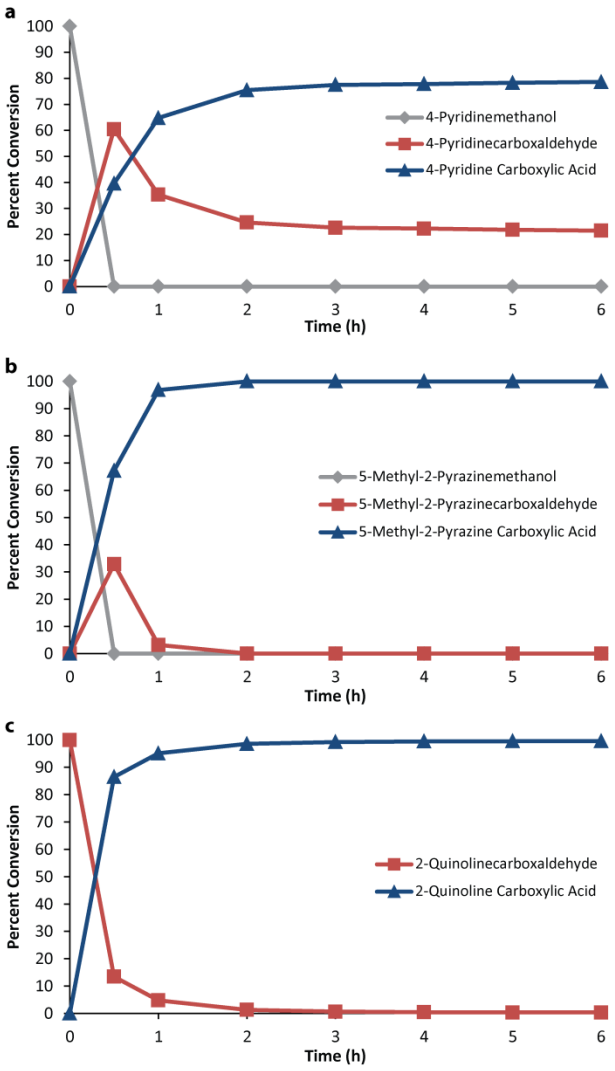


Figure 4. Product distribution over time for GOase M_{3-5} oxidation of (**a**) 4-pyridinemethanol, (**b**) 5-methyl-2-pyrazinemethanol and (**c**) 2-quinolinecarboxaldehyde. Reactions (10 mL) were performed in 200 mM NaPi pH 7.4 plus 10% DMSO with 0.25 mg/mL (3.6 µM) purified GOase M_{3-5}, 8.4 U/mL (0.032 mg/mL) HRP, 880 U/mL catalase and 25 mM substrate with shaking at 250 rpm at 25°C. Samples were removed for analysis at indicated time points.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge online at http://pubs.acs.org.

Experimental and analytical methods, NMR spectra, Figures S1-4, Tables S1-2

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Notes:

The authors declare no competing financial interests.

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