Oxidation of Primary Amines

Oxidation of Unactivated Primary Aliphatic Amines Catalyzed by an Electrogenerated 3,4-Azaquinone Species: A Small-Molecule Mimic of Amine Oxidases

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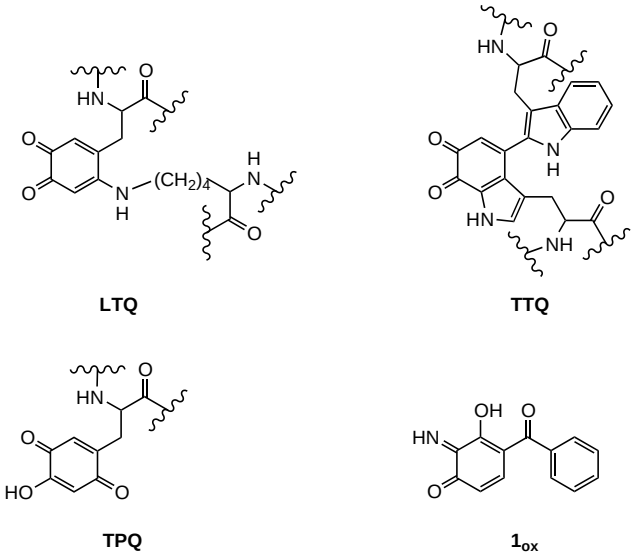
The copper amine oxidases are ubiquitous enzymes that occur in bacteria, yeast, plants, and mammals. These enzymes catalyze the two-electron oxidative deamination of a primary amine to produce the corresponding aldehyde and ammonia, with subsequent two-electron reduction of dioxygen to hydrogen peroxide. To carry out this reaction, copper amine oxidases, which belong to the new class of proteins designated quinoproteins, contain two cofactors: an organic cofactor, 2,4,5-trihydroxyphenylalanine quinone (TPQ, Scheme 1), and a cupric ion.[1] This class also includes bacterial methylamine oxidases, which use a peptide-bound tryptophan tryptophylquinone (TTQ)[2] and lysyl oxidase, a mammalian copper amine oxidase whose active site has been identified as lysine tyrosylquinone (LTQ).[3]



Scheme 1. Quino cofactors derived from protein-bound tyrosine (TPQ, LTQ), tryptophan (TTQ), together with 3,4-iminoquinone model cofactor $\mathbf{1}_{ox}$.

It is well established that TPQ catalyzes the conversion of an amine substrate into an aldehyde through a pyridoxal-like transamination mechanism, which results in the reduction of TPQ to an aminoquinol form.[4] Although there is no question regarding the crucial role of copper in the biogenesis of TPQ from its tyrosine precursor, the role of the copper cofactor during amine oxidation is, however, less well understood. Recent intensive studies have shown that an electron transfer from the cofactor to the metal is not required during the catalytic turnover, so that the metal essentially stabilizes a reduced oxygen intermediate.[5] These results are supported by the fact that the mature form of bovine lysyl oxidase retains its catalytic function in the absence of copper.[6]

Substantial mechanistic information has been obtained through catalytic aerobic deamination of benzylamine mediated by synthetic models of natural quinones which confirm the occurrence of the transamination mechanism.[7] However, these models failed to oxidize unactivated primary aliphatic amines under the same experimental conditions, with the exception of a recent study showing that the metal ion complex of the TTQ model compound was able to oxidize aliphatic amines in anhydrous organic media, whereas no reaction took place in the absence of the metal ion.[8]

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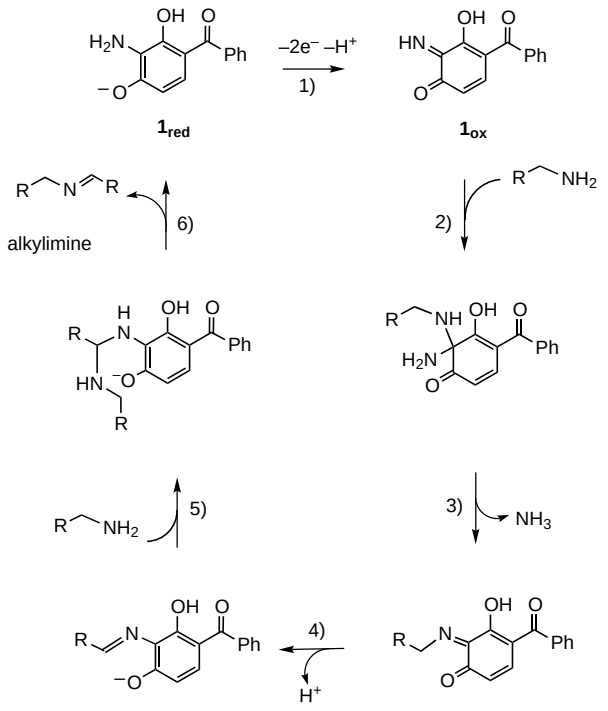
We recently showed that electrogenerated 3,4-iminoquinone $\mathbf{1}_{ox}$ (Scheme 1) acts as an efficient catalyst for the autorecycling oxidation of benzylamine through the transamination process reported for amine oxidase cofactors. The catalytic cycle produced the reduced catalyst $\mathbf{1}_{red}$ and *N*-benzylidenebenzylamine as the product of benzylamine oxidation in 3200% yield (based on the initial concentration of $\mathbf{1}_{red}$).[9] In contrast to other existing amine oxidase model cofactors, $\mathbf{1}_{ox}$ was also very active toward aliphatic amines. Herein, we report for the first time the catalytic oxidation of unactivated primary aliphatic amines by a small-molecule enzyme mimic in the absence of a metal ion.

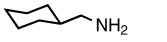
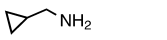
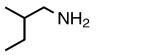
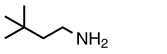
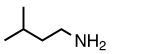
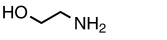
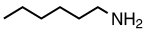
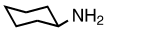
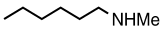
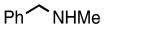
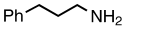
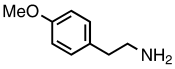
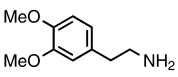
Unstable 3,4-iminoquinone $\mathbf{1}_{ox}$ was electrogenerated from $\mathbf{1}_{red}$ by using anodic-controlled potential electrolysis, at a platinum electrode in deaerated methanol that contained an excess of primary aliphatic amine. At a potential at which $\mathbf{1}_{red}$ could be oxidized to the iminoquinone form $\mathbf{1}_{ox}$ (see Experimental Section),[10] the anodic current remained unchanged for a long time, consistent with steady-state catalytic behavior. Accordingly, a high value was found for the total number of electrons (n) transferred per molecule of $\mathbf{1}_{red}$ in the catalytic process. These results indicated that the $\mathbf{1}_{red}/\mathbf{1}_{ox}$ system behaved as a redox mediator for the indirect electrochemical oxidation of unactivated primary aliphatic amines to the corresponding alkylimines, according to the transamination mechanism reported in Scheme 2.[9] After exhaustive electrolysis, the catalyst $\mathbf{1}_{ox}$ was lost irreversibly, as corroborated by the anodic current, which remained negligible upon further addition of amine substrate. Unstable alkylimine was isolated in terms of the corresponding aldehyde 2,4-dinitrophenylhydrazone (DNPH) obtained upon workup of the oxidized solution with 2,4-dinitrophenylhydrazine. Table 1 shows the effects of varying the structure of the amine substrate.[11] The most efficient substrates for $\mathbf{1}_{ox}$ were primary alkyl amines (Table 1, entries 1–6), with high yields of isolated DNPH ranging from 1000 to 2300% (relative to the mediator). With *n*-hexylamine (Table 1, entry 7), although a high value of 48 was found for n, the catalytic oxidation of more hydrophobic longer-chain amine produced DNPH in only 305% yield (based on $\mathbf{1}_{red}$), along with 255% of a second product identified as the osazone (1,2-bis-DNPH). Interestingly, as for the enzymes themselves, α-branched amines such as cyclohexylamine (Table 1, entry 8) were found to be poor substrates for the model catalyst $\mathbf{1}_{ox}$, whereas secondary amines (Table 1, entries 9 and 10) were not reactive at all.

Primary amines bearing aromatic substituents also underwent deamination, though the turnover was much lower on account of a more rapid deterioration of the catalyst. With phenylpropylamine (Table 1, entry 11), a value of 38 was found for n. Similarly to *n*-hexylamine (Table 1, entry 7), both DNPH (650%) and 1,2-bis-DNPH (100%) could be isolated at the end of the catalytic process. Further investigations would be necessary to justify the formation of the osazone,



Scheme 2. Mechanism of catalytic oxidation of primary aliphatic amines mediated by electrogenerated 3,4-iminoquinone model cofactor $\mathbf{1}_{ox}$.

Table 1: Catalytic oxidation of primary aliphatic amines mediated by the electrogenerated 3,4-iminoquinone $\mathbf{1}_{ox}$ model cofactor.

Entry	Amine	n[a]	Turnover	Yields[%][b] (A)	(B)
1		54	20.0	2000	40.0
2		52	17.5	1750	35.0
3		55	19.5	1950	39.0
4		56	23.0	2300	46.0
5		48	10.0	1000	20.0
6		46	10.0	1000	20.0
7		48	3.0[c]	305	6.1
8		8	1.1	110	2.2
9		4	–	–	–
10		3	–	–	–
11		38	6.5[c]	650	13.0
12		10	2.1	210	4.2
13		9	1.3	130	2.6

[a] Total number of electrons transferred per molecule of mediator. [b] Determined by weight of the aldehyde derivative DNPH, relative to the mediator (A) and to the amine substrate (B), respectively. [c] The 1,2-bis-DNPH derivative was also formed; turnover: 2.5 (entry 7); 1.0 (entry 11).

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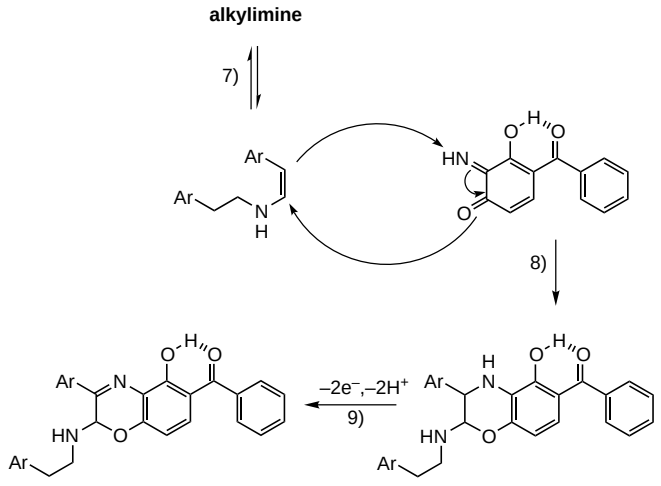

Scheme 3. Inactivation of model cofactor $\mathbf{1}_{ox}$ by ring-substituted phenylethylamine substrates, leading to 1,4-benzoxazine derivatives **2** and **3**.

which seems favored when starting amines are not sterically encumbered by β and γ branching.

With ring-substituted phenylethylamines, the catalytic process ceased after roughly 2 turnovers (Table 1, entries 12 and 13). Close inspection of the exhaustively oxidized solution revealed that electrogenerated 3,4-iminoquinone $\mathbf{1}_{ox}$ was trapped with the tautomeric enamine form of the alkylimine extruded during the catalytic process (Scheme 3, step 7) through an inverse-electron-demand Diels–Alder reaction,[12] affording unstable 1,4-benzoxazine derivatives (Scheme 3, step 8). However, these compounds could be isolated as stable products **2** and **3** in 50 and 65% yields, respectively, after a subsequent two-electron oxidation reaction (Scheme 3, step 9). Interestingly, this multistep one-pot electrochemical procedure could lead to novel neuroprotective agents as a consequence of the structural similarity of **2** and **3** with earlier reported 1,4-benzoxazine derivatives.[13]

In summary, we have demonstrated for the first time that unactivated primary aliphatic amines can be efficiently oxidized by a synthetic model cofactor of amine oxidases in the absence of a metal ion. The catalyst $\mathbf{1}_{ox}$ exhibits the same substrate specificity as the copper amine oxidases themselves, that is, poor reactivity with α-branched primary amines and no reactivity toward secondary amines. The reaction displays two features that are most often associated with enzymatic systems: a) the reaction is likely enhanced through the participation of neighboring substituents, as they prevented the competing formation of Michael adducts (in this case, the benzoyl and 2-hydroxy groups); b) the presence of an active hydroxy proton very probably constitutes an essential component of the catalytic activity (in this case, the 2-hydroxy proton, analogous to that of TPQ).[14] We are currently developing further analogues to confirm the substantial role of the 2-hydroxy group to convert a catalytic inert species into a highly effective enzymatic prosthetic group.

Experimental Section

General procedure: Controlled-potential electrolysis was carried out in a cylindrical, three-electrode, divided cell (9 cm diameter), using an electronic potentiostat. In the main compartment, a platinum grid (area = 60 cm^2) served as the anode (working electrode). A platinum sheet was placed in the concentric cathodic compartment (counter-electrode), which was separated from the main compartment with a glass frit. The reference electrode was an aqueous saturated calomel electrode (SCE), which was isolated from the bulk solution in a glass tube with a fine-porosity frit. The electrolyte solution (0.04 mol L^{-1} lithium perchlorate or tetraethylammonium perchlorate in methanol) was poured into the anodic and the cathodic compartments, as well as into the glass tube that contained the SCE electrode. 3,4-Aminophenol[15] $\mathbf{1}_{red}$ (0.1 mmol) and an excess of primary aliphatic amine (5 mmol) were added to the solution in the main compartment (250 mL), and the resulting solution was then oxidized under nitrogen at room temperature at +600 mV vs. SCE (initial current 30–40 mA), that is, at a potential following the anodic peak observed in cyclic voltammetry, characteristic of the two-electron oxidation of $\mathbf{1}_{red}$ to $\mathbf{1}_{ox}$. After exhaustive electrolysis (6–10 h, n = 8–56), that is, when a negligible value of the current was recorded (0.5–1.0 mA), the solution was worked up by addition of 2.5 mmol of 2,4-dinitrophenylhydrazine reagent (in 5 mL of H_2SO_4, 15 mL of EtOH and 5 mL of water) because 5 mmol of primary amine gave 2.5 mmol of alkylimine (Scheme 2). After 1 h, the resulting solution was concentrated to 40 mL. The solid was collected by filtration, washed with water, and dried in a vacuum desiccator. The identity and purity of DNPH was confirmed by thin-layer chromatography (TLC) and ^{1}H NMR spectroscopy, after comparison with an authentic sample. In the case of *n*-hexylamine or phenylpropylamine, the solid was purified by chromatography on silica gel to separate DNPH from 1,2-bis-DNPH. Control studies indicated that the amount of aldehyde produced either from simple autoxidation or from electrochemical oxidation of the starting amine in the absence of catalyst was negligible.

Isolation of **2** and **3**: After exhaustive electrolysis (6 h, n = 9 or 10), the electrolysis solution was poured into an acetic acid buffered aqueous solution of pH 4.8 (1 mol L^{-1}, 50 mL). The resulting hydroalcoholic solution was concentrated to remove methanol. The yellow solid, identified as the 1,4-benzoxazine derivative, was collected by filtration, washed with water, and dried in a vacuum desiccator.

2: M.p. 162 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.25 (m, 1H), 2.75 (t, J = 6 Hz, 2H), 3.15(m, 2H), 3.75 (s, 3H), 3.90 (s, 3H), 5.90 (d, J = 8 Hz, 1H), 6.55 (d, J = 9 Hz, 1H), 6.75 (d, J = 8 Hz, 2H), 6.95 (d, J = 8 Hz, 2H), 7.05 (d, J = 8 Hz, 2H), 7.45 (d, J = 9 Hz, 1H), 7.55 (m, 3H), 7.70 (d, J = 8 Hz, 2H), 8.00 (d, J = 8 Hz, 2H), 13.10 ppm (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 35.7, 46.2, 55.2, 55.3, 81.2, 108.3, 113.9, 114.2, 122.7, 127.9, 128.3, 128.9, 129.2, 129.6, 131.2, 131.6, 133.2, 138.2, 150.7, 154.5, 158.1, 160.3, 161.9, 202.2 ppm; DCI (desorption chemical ionization) MS: m/z: 509 [MH$^+$].

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[10] The cyclic voltammogram of $\mathbf{1}_{red}$ (0.1 mmol), recorded under our experimental conditions (see Experimental Section), showed an oxidation peak Pa at +500 mV vs. SCE, the sweep rate being 0.5 $V s^{-1}$. The addition of 5 mmol of primary amine had two effects: 1) a shift of the peak Pa to 0.0 mV vs. SCE as a result of the ionization of the 4-hydroxy group; 2) an increase in the anodic peak intensity which suggests that 3,4-iminoquinone $\mathbf{1}_{ox}$ can act competitively as a catalyst (even on the time scale of cyclic voltammetry) with the succeeding oligomerization. For similar behavior of quinonoid species, see: K. Kano, M. Nakagawa, K. Takagi, T. Ikeda, *J. Chem. Soc. Perkin Trans. 2* **1997**, 1111 – 1119, and references therein.

[11] In some cases, the yield of DNPH was lower than that expected from the value of *n* as a result of the partial conversion of unstable alkylimine into volatile aldehyde on the time scale of anodic electrolysis.

[12] We recently showed similar inverse-electron-demand Diels–Alder reactions of $\mathbf{1}_{ox}$ with $R^1R^2CHCH_2NH_2$ amines, but under different experimental conditions, which have been defined as optimal for the synthesis of 1,4-benzoxazine derivatives; see: M. Largeron, A. Neudorffer, M. Vuilhorgne, E. Blattes, M.-B. Fleury, *Angew. Chem.* **2002**, *114*, 852 – 855; *Angew. Chem. Int. Ed.* **2002**, *41*, 824 – 827.

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[15] Reduced catalyst $\mathbf{1}_{red}$ was synthesized from commercially available 2-nitroresorcinol through a four-step sequence previously reported; see: R. Larget, B. Lockhart, B. Pfeiffer, A. Neudorffer, M.-B. Fleury, M. Largeron, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2929 – 2934.