Catalytic Turnover of Benzylamine by a Model for the Lysine Tyrosylquinone (LTQ) Cofactor of Lysyl Oxidase

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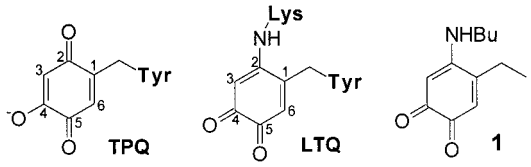
Abstract: Lysyl oxidase differs from other copper amine oxidases in that its active quinone cofactor reflects cross-linking of a lysyl residue into the tyrosine-derived quinone nucleus found in the plasma and other copper amine oxidases. A model for the lysyl oxidase cofactor (LTQ), 3,3-dimethyl-2,3-dihydroindole-5,6-quinone (**4**), was synthesized and found to be stable to both hydrolysis and oxidation events that prevent simpler models from functioning as turnover catalysts. We show that **4** catalyzes the aerobic oxidative deamination of benzylamine, though turnover eventually ceases on account of oxidation of the dihydrobenzoxazole tautomer of the "product Schiff base" to form a benzoxazole, a reaction that may be physiologically relevant. The mechanism of the overall reaction profile was elucidated by a combination of optical and NMR spectroscopy and O_2 uptake studies.

Introduction

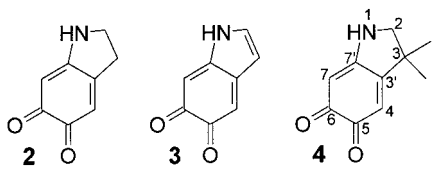
Interest in the mechanism of amine oxidation by the quinone-dependent copper amine oxidases has resulted in model systems[1,2] that successfully mimic both the single turnover and catalytic behavior of the 2,4,5-trihydroxyphenylalanine quinone (TPQ) cofactor found in most of these enzymes.[3] These reactions involve condensation of primary amine with the C^5 carbonyl of TPQ to give a "substrate Schiff base", followed by a rate-limiting imine shift to give a "product Schiff base" that hydrolyzes to release aldehyde product and a reductively aminated cofactor, which in turn is reoxidized ($O_2 \rightarrow H_2O_2$) to starting TPQ with release of NH_3. One member of the copper amine oxidase class, namely lysyl oxidase, exhibits different spectroscopic features from the TPQ-dependent enzymes, now understood in terms of it having a modified cofactor, termed lysine tyrosylquinone (LTQ), where an active site lysine ϵ-amino group has added into the tyrosine-derived quinone nucleus.[4] This enzyme plays an integral role in the posttranslational maturation of the mammalian connective-tissue proteins collagen and elastin: conversion of protein-based lysines to α-aminoadipic δ-semialdehydes initiates aldol and Schiff base condensation chemistry which evolves into mature intra- and intermolecular cross-links that stabilize the tissue matrix.

Although TPQ and LTQ might be expected to share a similar transamination capability utilizing the same electrophilic C^5 carbonyl, they are quite different in that the former exists as a resonance-stabilized anion whereas the latter is an amino-*o*-quinone. There have been no studies to date reporting transaminative activity of a viable LTQ model. Despite fairly complete characterization of the TPQ-dependent reactions, aided by the availability of several crystal structures and the behavior of key mutants, the reaction catalyzed by the key mammalian enzyme lysyl oxidase still awaits analogous investigation. Thus, model studies aimed at identifying the intrinsic chemical reactivity of the LTQ nucleus should provide important information.

The first LTQ model (**1**) was synthesized to permit spectroscopic comparison with the newly identified enzyme cofactor,[4,5]



but molecules such as **1** are known to be unstable under aerobic conditions[6] and thus seem unsuitable as turnover catalysts. To whatever extent molecules such as **1** might hydrolytically lose the nitrogen substituent during catalytic oxidation of primary amines, we considered that incorporation of this nitrogen into a bicyclic skeleton might provide a more robust model. A pertinent molecule in this regard (**2**) was previously described



in studies on the oxidative cyclization of dopamine and 6-hydroxydopamine, but it is also an inadequate LTQ model

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(1) (a) Wang, F.; Bae, J.-Y.; Jacobson, A. R.; Lee, Y.; Sayre, L. M. *J. Org. Chem.* **1994**, *59*, 2409−2417. (b) Lee, Y.; Sayre, L. M. *J. Am. Chem. Soc.* **1995**, *117*, 3096−3105. (c) Lee, Y.; Sayre, L. M. *J. Am. Chem. Soc.* **1995**, *117*, 11823−11828.

(2) (a) Mure, M.; Klinman, J. P. *J. Am. Chem. Soc.* **1995**, *117*, 8698−8706. (b) Mure, M.; Klinman, J. P. *J. Am. Chem. Soc.* **1995**, *117*, 8707−8718.

(3) Brown, D. E.; McGuirl, M. A.; Dooley, D. M.; Janes, S. M.; Mu, D.; Klinman, J. P. *J. Biol. Chem.* **1991**, *266*, 4049−4051.

(4) Wang, S. X.; Mure, M.; Medzihradszky, K. F.; Burlingame, A. L.; Brown, D. E.; Dooley, D. M.; Smith, A. J.; Kagan, H. M.; Klinman, J. P. *Science* **1996**, *273*, 1078−1084.

(5) Wang, S. X.; Nakamura, N.; Mure, M.; Klinman, J. P.; Sanders-Loehr, J. *J. Biol. Chem.* **1997**, *272*, 28841−28844.

(6) Borchardt, R. T.; Smissman, E. E.; Nerland, D.; Reid, J. R. *J. Med. Chem.* **1976**, *19*, 30−37.

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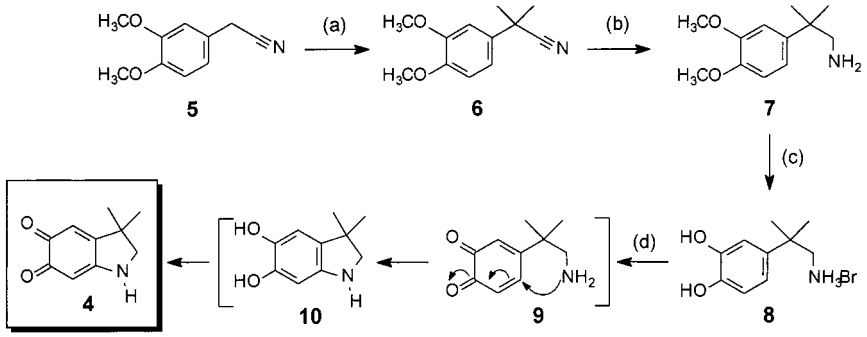
Scheme 1[a]



[a] Conditions: (a) $NaNH_2/NH_3(I)$, CH_3I; (b) $LiAlH_4/Et_2O$/room temperature; (c) 48% HBr/100 °C; (d) Ag_2O/CH_3OH.

due to its susceptibility to be oxidized to **3**.[7] However, for the gem-dimethyl analogue **4**,[8] such oxidation would be blocked, and the alternative potential self-Diels−Alder reaction would be sterically inhibited. Here we report the synthesis and catalytic functioning of the LTQ model **4**.

Results and Discussion

Synthesis and Properties of 4. Our synthesis of LTQ model **4** started from 3,4-dimethoxyphenylacetonitrile (**5**, Scheme 1). Using sodium amide as a deprotonation reagent[9] (phenylsodium has also been used,[10] but in our hands gave inferior yields), **5** was methylated to give **6** (optimal crude yields of 90% required a two-step methylation), which was subsequently reduced with $LiAlH_4$ to afford amine **7**.[9] Deprotection of **7** was achieved by refluxing with 48% HBr to give **8**.[10] The crude products **6** and **7** were both used without purification and the overall yield of the first three steps (from **5** to **8**) was 52%. The conversion of **8** to the target molecule **4**, which involves oxidation to the corresponding *o*-quinone **9**, intramolecular amine conjugate addition and tautomerization to give aminoquinol **10**, and oxidation of **10**, can be achieved in one pot without isolation of intermediates.[11] We found that the ammonium bromide **8** could be directly oxidized by excess silver oxide, acting as the base in generating the free amine **8** as well as the oxidant, to give **4** in moderate yield (35%).

Compound **4** was found to be quite stable in buffered aqueous acetonitrile over the pH range 7−10. At pH 7 it exhibits a λ_{max} of 485 nm ($\epsilon = 4110\ M^{-1}cm^{-1}$), 20 nm blue shifted from the model **1** that closely mimics the absorption properties of lysyl oxidase.[5] The shifted absorption of model **4** likely arises from a slight compromise in delocalization caused by the fused five-membered ring. Evidence that the LTQ cofactor exists as the neutral amino-*o*-quinone, contrasting the existence of TPQ as a delocalized anion (the vinylogous carboxylic acid TPQ has a pK_a of 4.5[12]), is that only the latter β-diketone/enolate system exhibits rapid exchange of the C^3-proton in D_2O at pH 7.[13] No exchange is observed for LTQ,[5] and likewise, **4** showed no exchange in 10 h in neutral CD_3OD, though exchange could be effected under basic conditions. On the basis of the C^6 carbonyl (^{13}C NMR δ 164) being part of a vinylogous amide, it is the C^5 carbonyl (^{13}C NMR δ 184), as with TPQ, that is the electrophilic center presumed to be the site of attack by amine substrates.

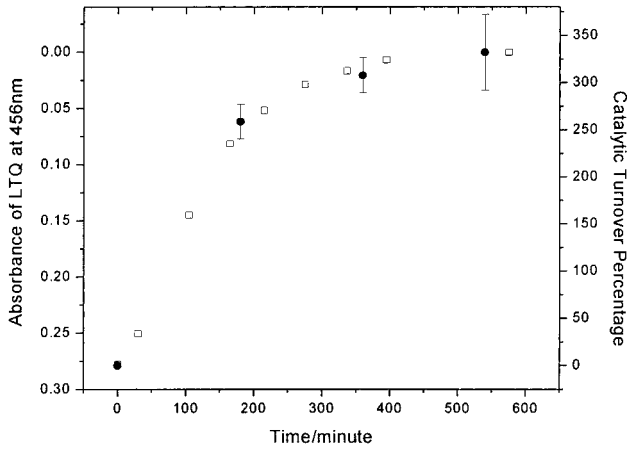


Figure 1. Progress of aerobic reaction of LTQ model compound **4** (1 mM) with benzylamine (50 mM) in 7:3 (v/v) pH 9 aqueous borate buffer−CH_3CN at 25 °C (□) plot for absorbance at 456 nm vs time; (●) plot for catalytic turnover percentage (±S.D.) vs time.

Catalytic Turnover. The ability of **4** to mediate the O_2-dependent oxidative deamination of benzylamine in buffered aqueous CH_3CN was studied in the pH range 7−9, as evaluated by quantifying the 2,4-dinitrophenylhydrazone of PhCH═O. The cumulative turnover yield plateaued over time, and the highest yield (~340%) was observed at pH 9 (borate buffer). Although the reactions were faster at higher than lower pH, the factor that governed the optimal yield at the point of plateau in every case was a competing deterioration of the catalyst, as evidenced by a gradual bleaching of the red color of **4**, which also occurred more rapidly at higher pH. Figure 1 shows the time course for catalytic oxidative deamination of benzylamine at pH 9.0, superimposed on the time course for deterioration of the absorbance for **4** under the same conditions. The coincidence of these plots implies that *the plateau in turnover yield is due to loss of the active form of the LTQ model 4*.

Mechanism of Benzylamine Deamination. To learn about the mechanism of the deamination reaction effected by **4**, its reaction with benzylamine was monitored under anaerobic single-turnover conditions in degassed CD_3CN by ^1H NMR spectroscopy.[15] Using 1.5 equiv of benzylamine, suspended **4** was completely dissolved in 12 h (25 °C), yielding a spectrum

(7) Hikosaka, A.; Kumanotani, J. *Bull. Chem. Soc. Jpn.* **1970**, *43*, 2620−2622.

(8) Compound **4** has been described (without characterization) to form from oxidative cyclization of 5-(2-amino-1,1-dimethylethyl)benzene-1,2,4-triol: Borchardt, R. T.; Reid, J. R.; Thakker, D. R.; Liang, Y. O.; Wightman, R. W.; Adams, R. N. *J. Med. Chem.* **1976**, *19*, 1201−1209.

(9) Knabe, J.; Kubitz, J. *Naturwissenschaften* **1961**, *48*, 669.

(10) Finkelstein, J.; Chiang, E.; Brossi, A. *J. Med. Chem.* **1971**, *14*, 584−588.

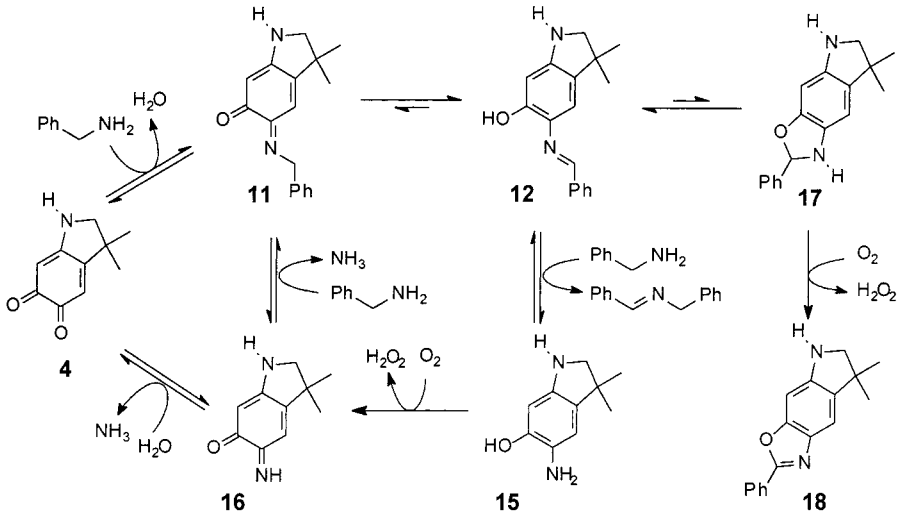
(11) Powell, W. S.; Heacock, R. A.; Smith, D. G.; McInnes, A. G. *Can. J. Chem.* **1973**, *51*, 776−779.

(12) Mure, M.; Klinman, J. P. *J. Am. Chem. Soc.* **1993**, *115*, 7117−7123.

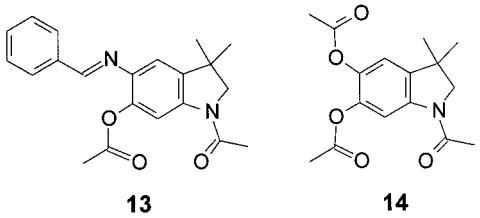
(13) This proton is readily exchangeable (in D_2O) at pH 7 both for TPQ in the *E. coli* amine oxidase[14] and for TPQ models.[14,1a]

(14) Moenne-Luccoz, P.; Nakamura, N.; Steinebach, V.; Duine, J. A.; Mure, M.; Klinman, J. P.; Sanders-Loehr, J. *Biochemistry* **1995**, *34*, 7020−7026.

Scheme 2

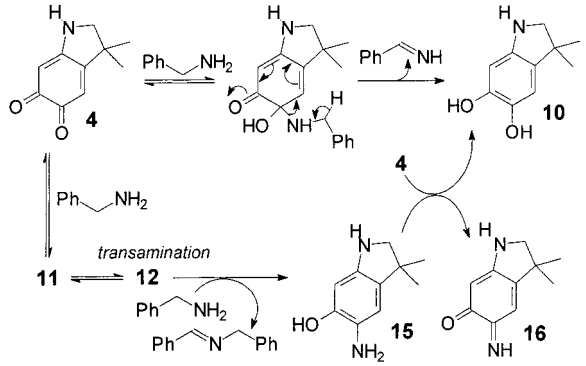


dominated (~92% from **4**) by the product Schiff base **12** (see Scheme 2). Also present to a lesser degree were the product of interception of **12** by benzylamine, PhCH═NCH$_2$Ph, and trace amounts (~8% from **4**) of reduced cofactor **10**. Both **10** and **12** were unstable with respect to routine chromatographic isolation in air, but their structures were confirmed by isolation and characterization of their acetylated derivatives, **13** (88% yield) and **14** (trace), obtained by anaerobic quenching of the reaction with Ac_2O.



Both the single-turnover anaerobic and aerobic catalytic turnover results can be understood in terms of Scheme 2. Condensation of benzylamine at the most electrophilic C^5 carbonyl of **4** and subsequent imine shift leads to product Schiff base **12**. Although **12** would undergo hydrolysis in enzyme-catalyzed turnover to release PhCH═O, in the model turnover reaction **12** is mainly trapped by the excess benzylamine to give PhCH═NCH$_2$Ph (which gives the same 2,4-dinitrophenylhydrazine derivative). In the enzyme reaction, the generated aminophenol **15** undergoes oxidation to **16** accompanied by reduction of O_2 to H_2O_2. For the model aerobic turnover reaction, **15** and any reduced cofactor **10** arising from reduction of **4** by **15** undergoes O_2-dependent autoxidation, as confirmed by measuring O_2 consumption with a Clark-type oxygen electrode in a closed chamber. Consumption of O_2 implicates generation of H_2O_2 either directly or indirectly (via superoxide), and since H_2O_2 is suspected to be a more reactive oxidant of **15** and/or **10** than O_2, it is not expected to accumulate in solution. Consistent with this suspicion, addition of catalase at various times into the reaction of **4** with benzylamine being

Scheme 3



followed polarographically did not result in any increase in [O_2], indicating that H_2O_2 does not build up in the reaction mixture.

The finding that product Schiff base **12** is nearly the exclusive fate of **4** in its anaerobic reaction with benzylamine provides unambiguous evidence that model **4** reproduces the transamination mechanism assumed to transpire for lysyl oxidase, as opposed to an alternate addition−elimination mechanism (leading to PhCH═NH and quinol **10**, Scheme 3, top). The exclusivity of **12** is noteworthy in that the product Schiff base for the analogous anaerobic reactions of benzylamine with TPQ models was a minor component of a very complex equilibrium product mixture reflecting redox and Schiff base interchanges.[1c] Small amounts of the product Schiff base could alternatively arise from the addition−elimination pathway by condensation of PhCH═NH with the aminoresorcinol resulting from reduction of quinone imine generated from the starting quinone and released NH_3. Thus, unambiguous confirmation of a transamination mechanism in the TPQ model case required reliance on more complex NMR kinetics arguments and the use of special mechanistic probes.[1c,16] In the present case, the predominance of product Schiff base **12** obviates additional studies to confirm a transamination mechanism. The high yield of **12** indicates that its interception by benzylamine (giving PhCH═NCH$_2$Ph) is *slower* than its generation from **4** and benzylamine, unlike the case for the TPQ-derived product Schiff base.[15] The small amount (~8%) of the reduced LTQ (**10**) formed undoubtedly arises *not* from a minor competing addition−elimination pathway, but from reduction of **4** under anaerobic conditions

(15) When the anaerobic reaction of **4** with benzylamine was conducted in methanol, the reaction outcome was more complicated and was dominated by formation of a phenoxazine, resulting from condensation of starting quinone **4** with the aminophenol **15** generated according to Scheme 2. Apparently in methanol but not acetonitrile, interception of product Schiff base **12** by benzylamine is competitive with reaction of benzylamine with **4**.

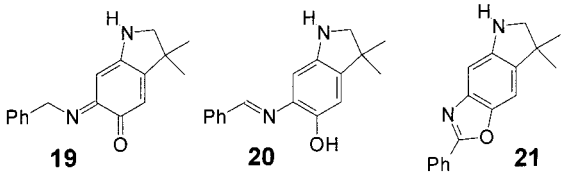
(16) Lee, Y.; Huang, H.; Sayre, L. M. *J. Am. Chem. Soc.* **1996**, *118*, 7241−7242.

by the low level of aminophenol **15** released when **12** *is* intercepted by benzylamine (Scheme 3, bottom).

To find out the cause of catalyst destruction in the aerobic turnover reactions, the reaction products were analyzed at the first point of bleaching of the red color. The only isolable product was the benzoxazole derivative **18**, which presumably arises from oxidation of the dihydrobenzoxazole **17** in equilibrium with the product Schiff base **12** (see Scheme 2). It was of interest to determine what factors govern the partitioning of **12**/**17** between productive turnover to **15** and irreversible oxidation to **18**, since the latter pathway must normally be suppressed in the enzyme case. Such determination is closely tied to the question of what serves as the oxidant of **17**. Our finding that running the aerobic catalytic turnover reaction in the presence of catalase neither inhibited the formation of **18** nor extended the lifetime of the catalyst indicates that oxidation of **17** is not principally mediated by the H_2O_2 generated during turnover. Another candidate for the oxidant is the starting quinone **4**. However, when the anaerobic model reaction (CD_3CN) was carried out using a deficiency (0.5 equiv) of benzylamine, unreacted **4** was now present alongside the product Schiff base **12**, and no **18** was detected even after several days. Thus, even though the **12** ⇆ **17** equilibrium is unfavorable, as indicated by nonobservance of **17** by ^{1}H NMR spectroscopy, the failure of **12** + **4** to be converted to **18** over time indicates that the responsible oxidant is not starting quinone **4**.

Nonetheless, >90% formation of benzoxazole **18** was observed when **12** (formed in situ in 92% yield from the reaction of **4** and 1.5 equiv of benzylamine in CD_3CN under argon) was exposed to either O_2 (slower) or H_2O_2 (faster). Thus the oxidation of **17** is at least in part an O_2-dependent autoxidation. Although H_2O_2 is clearly a more competent oxidant, we believe that the lack of effect of catalase on the formation of **18** must reflect the fact that any H_2O_2 generated during turnover is rapidly consumed in oxidizing **15**, whereas **17** is always present in very low concentration, but is eventually oxidized by the plentiful O_2.

Our assignment of the structure of substrate Schiff base **11** and all structures derived from it (e.g., **12** and **18**) was predicated on the assumption that benzylamine condenses with the more electrophilic of the two possible quinone carbonyl groups of **4**. Nonetheless, our results could not unambiguously rule out the possible alternative formation of substrate Schiff base **19**,



product Schiff base **20**, and benzoxazole **21**. To confirm the structures as those shown in Scheme 2, we synthesized ^{15}N-labeled benzoxazole **18** by air exposure of Schiff base **12** generated anaerobically from [^{15}N]benzylamine. The ^{15}N in **18** was found to be coupled only to the downfield (113.1 ppm) of the two CH carbons in the benzoxazole aryl ring, which is the one *meta* to the aniline-like nitrogen (Figure 2). The upfield CH carbon (91.3 ppm [91.4 ppm in [^{15}N]-**18**]) is the one *ortho* to the aniline-like nitrogen, as confirmed by the fact that this CH is the one that undergoes base-induced solvent deuterium exchange in the starting quinone **4**, permitting synthesis of the 7-deuterio benzoxazole **18-*d*** (Scheme 4), containing a weak positive signal at 91.4 ppm in place of the negative APT ^{13}C NMR signal at 91.3 ppm in **18**. Two bond ^{15}N−^{13}C coupling

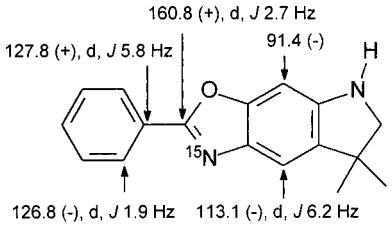
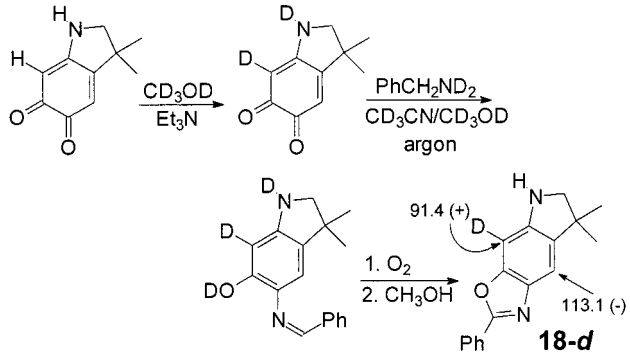


Figure 2. Selected ^{13}C−^{15}N coupling data for ^{15}N-labeled benzoxazole **18**.

Scheme 4



constants are known to be large when the carbon atom is close to the nitrogen lone pair.[17,18] If the benzoxazole had structure **21** rather than **18**, then the ^{15}N should have been coupled to the upfield rather than downfield benzoxazole aryl CH carbon.

Conclusions

We have shown that **4**, a stable model for the LTQ cofactor of lysyl oxidase, effects transaminative conversion of benzylamine anaerobically in acetonitrile. Thus, despite the difference in structures between the TPQ and LTQ nuclei, the two quinone cofactors possess the same intrinsic capability of deaminating amines by a transamination as opposed to the addition−elimination mechanism. The LTQ model **4** also achieves a *catalytic* aerobic deamination of benzylamine in buffered aqueous acetonitrile. In the latter case, an optimal 3.4 turnovers at pH 9 (borate buffer) is observed, on account of the O_2-dependent conversion of the catalyst to a benzylamine-derived benzoxazole derivative. Although the enzyme must normally avoid this side reaction, the same phenomenon was observed previously for the reaction of benzylamine with TPQ models.[19] In this latter case, the benzoxazole-forming side reaction is more than a chemical artifact in that it was shown to be responsible for the benzylamine-dependent inactivation of bovine plasma amine oxidase and other amine oxidases that occurs when the byproduct H_2O_2 reaches significant concentrations.[19] It should be pointed out that syncatalytic inactivation of lysyl oxidase has also been observed, and may represent a regulatory mechanism for this enzyme in vivo.[20] Although the nature of the latter inactivation has not been determined, benzoxazole formation may be a common physiologically

(17) Levy, G. C.; Lichter, R. L. *Nitrogen-15 nuclear magnetic resonance spectroscopy*; Wiley: New York, 1979.

(18) (a) Axenrod, T.; Mangiaracina, P.; Watnick, C. M.; Wieder, C. M.; Bulusu, S. *Org. Magn. Reson.* **1980**, *13*, 197−199. (b) Boyd, D. R.; Stubbs, M. E.; Thompson, N. J.; Yeh, H. J. C.; Jerina, D. M.; Wasylishen, R. E. *Org. Magn. Reson.* **1980**, *14*, 528−533.

(19) Lee, Y.; Shepard, E.; Smith, J.; Dooley, D. M.; Sayre, L. M. *Biochemistry* **2001**, *40*, 822−829.

(20) Kagan, H. M.; Soucy, D. M.; Zoski, C. G.; Resnick, R. J.; Tang, S.-S. *Arch. Biochem. Biophys.* **1983**, *221*, 158−167.

important mechanism that regulates the activity of the copper amine oxidases.

Experimental Section

General Methods. Unless otherwise stated the solvents and reagents were of commercially available analytical grade quality. Catalase (20 000 units/mg) was from Sigma Chemical Co. (St. Louis, MO). Reactions in aqueous CH_3CN were carried out using Millipore purified water, and all evaporations were carried out at reduced pressure with a rotary evaporator. ^{1}H NMR spectra (200 or 300 MHz) and ^{13}C NMR spectra (75 MHz) were recorded on Varian Gemini instruments. In all cases, tetramethylsilane or the solvent peak served as an internal standard for reporting chemical shifts, expressed on the δ scale; Attached Proton Test (APT) designations for ^{13}C NMR spectra are given in parentheses. High-resolution mass spectra (HRMS) were obtained at 20 eV on a Kratos MS-25A instrument. Optical spectra were obtained with Perkin-Elmer model Lambda 3B or 20 spectrophotometers fitted with a water-jacketed multiple cell holder for maintenance of constant temperature.

3,3-Dimethyl-2,3-dihydroindole-5,6-quinone (4). (a) Methylation. Liquid ammonia (500 mL) was collected in a 3 L three-neck round-bottom flask cooled by a dry ice−acetone bath. To the mechanically stirred solution was added ferric nitrate (0.1 g) and then 5.06 g (0.22 mol) of finely divided sodium, and the mixture was vigorously stirred until the dark blue solution turned to gray. To this sodium amide suspension was added within 30 min a clear solution of 3,4-dimethoxyphenylacetonitrile (**5**, 35.4 g, 0.20 mol) in 1 L of ether. Some of the nitrile **5** began to precipitate and the mixture was vigorously stirred until all the nitrile **5** was dissolved. To the resultant orange mixture was added a solution of 28.4 g (0.2 mol) of iodomethane in 50 mL of ether, and the reaction mixture was further stirred for 2 h. After evaporation of ammonia, the solution was washed with water (3 × 500 mL) to remove NaI. The ether layer was separated, dried over anhydrous Na_2SO_4, and evaporated to dryness to afford crude α-methyl-3,4-dimethoxyphenylacetonitrile. The above methylation procedure was repeated with 4.6 g (0.2 mol) of sodium and 28.4 g (0.2 mol) of iodomethane, resulting in crude α,α-dimethyl-3,4-dimethoxyphenylacetonitrile (**6**) as a light yellow oil which solidified on standing: ^{1}H NMR ($CDCl_3$) δ 1.72 (s, 6H), 3.88 (s, 3H), 3.92 (s, 3H), 6.86 (d, 1H, J = 8.0 Hz), 6.98 (s, 1H), 6.99 (d, 1H, J = 8.0 Hz).

(b) Reduction. To a solution of diethyl ether (1 L) in a 3 L three-neck round-bottom flask fitted with a mechanical stirrer and a $CaCl_2$-drying-tube-protected condenser was added $LiAlH_4$ (38 g, 1 mol). The suspension was vigorously stirred while a solution of the crude nitrile **6** in 100 mL of ether was slowly added over 30 min, and the mixture was further stirred for 2 h, then cooled in an ice bath, at which point 150 mL of ethyl acetate was carefully added to decompose any unreacted $LiAlH_4$. The mixture was then poured into a mixture of ice and aqueous sodium tartrate with continuous stirring. The ether layer was separated and the aqueous layer was further extracted with ether (3 × 200 mL). The combined organic layer was dried (Na_2SO_4) and evaporated to give crude 2-(3,4-dimethoxyphenyl)-2-methylpropylamine (**7**) as a light yellow oil: ^{1}H NMR (of HCl salt **7** in D_2O) δ 1.31 (s, 6H), 3.14 (s, 2H), 3.73 (s, 3H), 3.77 (s, 3H), 6.89−6.94 (3H).

(c) Deprotection. To a 1 L flask were added the above prepared crude amine **7** and 320 mL of 48% aqueous HBr. The mixture was heated on a steam bath for 3 h and then evaporated to dryness in vacuo at 60 °C. To the residue was added 500 mL of anhydrous ethanol, the mixture was concentrated to 50 mL, and then 350 mL of ethyl acetate was added. Cooling of the mixture resulted in deposition of the first crop (23.24 g) of 2-(3,4-hydroxyphenyl)-2-methylpropylamine hydrobromide (**8**). The mother liquor was treated as above to give a second crop of product **8** (3.68 g). The total product recovery corresponded to an overall yield of 52% for the first three steps (from **5** to **8**). Compound **8**: mp 247−249 °C dec; ^{1}H NMR (D_2O) δ 1.26 (s, 6H), 3.08 (s, 2H), 6.81−6.87 (3H); ^{13}C NMR (D_2O) δ 40.6 (−), 51.0 (+), 65.5 (+), 128.9 (−), 131.1 (−), 133.4 (−), 151.2 (+), 157.4 (+), 158.7 (+).

(d) Oxidation. To a vigorously stirred solution of **8** (26.03 g, 0.099 mol) in 1 L of methanol was added 93 g (0.4 mol) of Ag_2O powder. The mixture was stirred for 8 min and then passed through a Buchner funnel packed with anhydrous Na_2SO_4 and NaCl. The funnel was further washed with methanol (200 mL), and the combined dark red solution was evaporated to dryness to yield a residue that was purified by silica gel flash chromatography (EtOAc−acetone as eluent), affording model compound **4** (6.22 g, 35%). The crude product was further purified by crystallization in methanol−ethyl acetate to yield dark red microscopic needles: mp 160−161 °C; ^{1}H NMR (DMSO-d_6) δ 1.31 (s, 6H), 3.58 (s, 2H), 5.48 (br, s, 1H), 6.43 (s, 1H), 9.44 (br, s, 1H); ^{13}C NMR (CD_3-OD) δ 27.4 (−), 41.3 (+), 63.9 (+), 93.8 (−), 124.7 (−), 164.3 (+), 164.7 (+), 184.7 (+); HRMS (EI) m/z calcd for $C_{10}H_{11}NO_2$ 177.0790, found 177.0792 (relative intensity 82).

Catalytic Aerobic Deamination of Benzylamine Mediated by LTQ Model 4. Quantitative analysis was conducted at pH 7−9 as follows. A mixture of 7.5 mmol of benzylamine, 0.15 mmol of LTQ model **4**, and 0.75 mmol of sodium phosphate (pH 7 and 8) or sodium borate (pH 9) buffer in 150 mL of 30% aqueous CH_3CN was adjusted to the desired pH with HCl. The solution was magnetically stirred vigorously in an open 250 mL Erlenmeyer flask at 25 °C with monitoring of the pH, the reaction volume being maintained by periodic addition of CH_3CN, which evaporates somewhat at long reaction times. Three 50 mL aliquots were worked up at different reaction times by addition to each of 18 mL of standard 2,4-dinitrophenylhydrazine (150 mM) reagent (in 15 mL of H_2SO_4, 70 mL of EtOH, and 20 mL of water). After being cooled to 0 °C for 1 h, the solution was filtered, and the precipitate was dried to constant weight to obtain the yield of the benzaldehyde 2,4-dinitrophenylhydrazone, the identity and purity of which were confirmed by TLC and ^{1}H NMR.

Spectral monitoring was conducted at pH 9 as follows. To a solution of benzylamine (50 mM) in 25 mL of 5 mM sodium borate buffered 30% aqueous acetonitrile in an open 50 mL Erlenmeyer flask was added **4** (1 mM), and the mixture was stirred vigorously at 25 °C. At regular time intervals, 0.3 mL aliquots of the reaction were transferred into 1 mm path length cuvettes and the absorbance was recorded at 456 nm.

Oxygen uptake was monitored in a closed chamber using a Yellow Springs Instruments 5300 biological oxygen meter at 30 °C, equipped with magnetic stirring and a Clark-type oxygen electrode. A solution of benzylamine (10 mM final) in 10 mM pH 9 borate buffered 10% aqueous acetonitrile (2.8 mL) was first equilibrated in the chamber following insertion of the electrode. Reactions were initiated by injecting 200 μL of a 37.5 mM stock solution of **4** in CH_3CN through the side vent in the chamber, followed by commencement of monitoring of O_2 consumption. When 50% of oxygen consumption was reached in the reaction system, 50 μL of a solution of catalase (Sigma C-10 from bovine liver, 2 mg/mL) was added to the reaction; no change in the O_2 concentration was seen, indicating the absence of H_2O_2 at this point in the reaction. Confirmation of the ability of catalase to release O_2 from H_2O_2 under these conditions was achieved by the addition of H_2O_2 (100 μM) following the catalase addition and noting the expected increase in the O_2 concentration. For these experiments the ambient concentration of O_2 was taken to be 223 μM.

Product Study for the Aerobic Deamination of Benzylamine Catalyzed by LTQ Model 4. To a stirred solution of boric acid (124 mg, 2 mmol) in 380 mL of water were added 2.14 g (20 mmol) of freshly distilled benzylamine and 120 mL of acetonitrile. The solution was adjusted to pH 9.00 using aqueous HCl. To this buffered solution was added 70.8 mg (0.4 mmol) of **4**. The resultant dark red solution was continuously stirred for 24 h in an open flask (the color turned from red to orange after 5 h and then to yellow), at which time the pH had dropped to 8.5. The acetonitrile was removed in vacuo at 30 °C and the aqueous solution was extracted with dichloromethane (3 × 100 mL). The combined organic layer was dried (Na_2SO_4) and evaporated, and the residue was subjected to silica gel flash chromatographic separation (eluant hexanes−EtOAc), affording as the only isolable **4**-derived product benzoxazole **18** (23.3 mg, 22%), which was crystallized from hexanes−acetone as colorless plates: mp 133−135 °C; ^{1}H NMR ($CDCl_3$) δ 1.36 (s, 6H), 3.39 (s, 2H), 3.93 (br s, 1H, NH), 6.76 (s, 1H), 7.36 (s, 1H), 7.40−7.60 (3H), 8.20 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 27.9 (−), 41.4 (+), 62.2 (+), 91.3 (−), 113.1 (−), 126.8 (−), 127.9 (+), 128.8 (−), 130.5 (−), 134.9 (+), 136.9 (+), 149.1 (+), 151.2 (+), 160.8 (+); HRMS (EI) calcd for $C_{17}H_{16}N_2O$ 264.1263, found 264.1267. The aqueous layer was divided into two

portions. One was adjusted to pH 11 and the other to pH 2. Both solutions were extracted again with dichloromethane. TLC analysis revealed that the alkaline extract mainly contained unreacted benzylamine while the acidic extract contained a mixture of strongly polar intractable materials. Benzoxazole **18** gradually decomposed in aerobic solution to give intractable products, probably accounting in part for the low yield isolated from the deaminative turnover reaction.

Anaerobic Reaction of LTQ Model 4 with Benzylamine in Acetonitrile. A suspension of **4** (9 mg, 0.05 mmol) in CD_3CN (0.07 mL) in an NMR tube was bubbled with dry argon for 30 min before sealing the tube with a septum. Degassed freshly distilled benzylamine (7.9 μL, 0.075 mmol) was added by syringe. The mixture was allowed to stand at room temperature until the color turned from dark brown to bright yellow (12 h). ^{1}H NMR showed that benzylamine was nearly consumed and a mixture of Schiff base **12** (92% from **4** by integration) and $PhCH{=}NCH_2Ph$ was obtained along with traces of **10** (8% from **1** by integration). Compound **12**: ^{1}H NMR (CD_3CN) δ 1.28 (s, 6H), 3.28 (s, 2H), 6.16 (s, 1H), 7.26 (s, 1H), 7.2−7.5 (3H, overlapped with those of $PhCH{=}NCH_2Ph$ and benzylamine), 7.94 (dd, 2H, J = 7.68, 2.19 Hz), 8.70 (s, 1H). The appearance of only one singlet for CH=N indicated that only one isomer of **12** (probably anti) was formed. **$PhCH{=}NCH_2Ph$** (syn and anti isomers): ^{1}H NMR (CD_3CN) δ 4.77 and 4.78 (2s, 2H total), 7.2−7.5 (8H total, overlapped with those of **12** and benzylamine), 7.75−7.79 (2H total), 8.46 and 8.47 (2s, 1H total). Compound **10**: ^{1}H NMR (CD_3CN) δ 1.18 (s, 6H), 3.12 (s, 2H), 6.14 (s, 1H), 6.51 (s, 1H).

To confirm the above ^{1}H NMR assignment, a mixture of 44.3 mg (0.25 mmol) of **4** in acetonitrile (0.35 mL) in an NMR tube was degassed and sealed. Then 39.5 μL (0.375 mmol) of degassed freshly distilled benzylamine was slowly added by syringe. After 12 h, 0.25 mL of degassed Ac_2O and 0.5 mL of degassed triethylamine were added, and the mixture was allowed to stand for 12 h. The resultant mixture was partitioned between water and dichloromethane (50 mL each). The organic layer was separated, washed with water (2 × 20 mL), dried (Na_2SO_4), and evaporated, and the residue was subjected to silica gel flash chromatography (hexanes−EtOAc) to afford diacetyl derivative **13** (77.2 mg, 88%) as yellow prisms from hexanes−ethyl acetate: mp 218−220 °C; ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 6H), 2.21 (s, 3H), 2.29 (s, 3H), 3.80 (s, 2H), 6.94 (s, 1H), 7.44−7.47 (3H), 7.86 (m, 2H), 8.01 (s, 1H), 8.45 (s, 1H, CH=N); ^{13}C NMR ($CDCl_3$) δ 20.7 (−), 24.1 (−), 28.7 (−), 40.3 (+), 63.9 (+), 111.7 (−), 112.5 (−), 128.8 (−), 131.5 (−), 136.3 (+), 138.8 (+), 139.7 (+), 140.3 (+), 143.9 (+), 159.9 (−), 168.6 (+), 169.5 (+); HRMS (EI) calcd for $C_{21}H_{22}N_2O_3$ 350.1630, found, 350.1627 (rel intensity 49). A trace amount of the triacetyl derivative **14** was also observed, as verified by synthesis of an authentic sample. Thus, reduction of **4** with sodium borohydride in acetonitrile under argon followed by treatment with degassed Ac_2O/triethylamine gave **14** in 96% yield as colorless needles from hexanes−ethyl acetate: mp 154−156 °C; ^{1}H NMR ($CDCl_3$) δ 1.36 (s, 6H), 2.20 (s, 3H), 2.26 (s, 3H), 2.28 (s, 3H), 3.80 (s, 2H), 6.93 (s, 1H), 8.03 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 20.6 (−), 20.7 (−), 24.0 (−), 28.7 (−), 40.3 (+), 63.9 (+), 112.1 (−), 116.6 (−), 138.2 (+), 138.6 (+), 139.6 (+), 141.0 (+), 168.4 (+), 168.5 (+), 168.6 (+); HRMS (EI) calcd for $C_{16}H_{19}NO_5$ 305.1263, found 305.1263 (relative intensity 20).

Preparation of 7-*d*-benzoxazole 18. To a clear solution of 44.3 mg (0.25 mmol) of **4** in 2 mL of CD_3OD was added 0.2 mL of triethylamine, and the mixture was stirred for 10 min with warming to 40 °C, when ^{1}H NMR showed that the 7-H in **4** was completely exchanged with deuterium. The solvent was removed in vacuo and the residue was dried thoroughly. The resultant 7-*d*-**4** was suspended in 4 mL of CD_3CN−CD_3OD (5:1, v/v), and dry argon was bubbled through the mixture for 10 min while deuterium-exchanged benzylamine (C_6H_5-CH_2ND_2, 39 μL, 0.38 mmol) was added by syringe. The mixture was further bubbled with dry argon for 20 min and then sealed. After 8 h, the mixture was purged with dry oxygen for 5 min, sealed, and allowed to stand for 2 h. Evaporation of the solvent afforded a dark brown residue that was subjected to silica gel flash chromatography (hexanes−EtOAc as eluent) to afford 7-*d*-**18** (40.3 mg, 61%) as colorless needles: ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 6H), 3.42 (s, 2H), 3.5 (br, s, 1H, NH), 7.37 (s, 1H), 7.46−7.50 (m, 3H), 8.14−8.19 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 27.9 (−), 41.4 (+), 62.2 (+), 91.4 (+), 113.1 (−), 126.9 (−), 127.9 (+), 128.8 (−), 130.5 (−), 135.1 (+), 137.0 (+), 148.7 (+), 151.1 (+), 160.9 (+).

Preparation of [3-^{15}N]-Benzoxazole 18. A suspension of 44.3 mg (0.25 mmol) of **4** in 4 mL of CD_3CN was bubbled with dry argon for 10 min while ^{15}N-benzylamine (Isotec, >99% ^{15}N, 39 μL, 0.38 mmol) was added by syringe. The mixture was further bubbled with dry argon for 20 min and then sealed. After 12 h, the mixture was purged with dry oxygen for 5 min, sealed, and allowed to stand for 2 h. Workup as for 7-*d*-**18** above afforded [3-^{15}N]-**18** (54.1 mg, 83%) as colorless needles: ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 6H), 3.43 (s, 2H), 3.7 (br, s, 1H, NH), 6.83 (s, 1H), 7.37 (s, 1H), 7.4−7.5 (m, 3H), 8.1−8.2 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 27.9 (−), 41.4 (+), 62.2 (+), 91.4 (+), 113.1 (−) (d, J = 6.2 Hz), 126.8 (−) (d, J = 1.9 Hz), 127.8 (+) (d, J = 5.8 Hz), 128.8 (−), 130.5 (−), 135.0 (+), 137.0 (+) (d, J = 2.0 Hz), 150.0 (+), 151.2 (+), 160.8 (+) (d, J = 2.7 Hz).

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