The free radical site in pea seedling copper amine oxidase probed by resonance Raman spectroscopy and generated by photolysis of caged substrate

Steven A. Johnson[a], Roger H. Bisby[a,*], Susan M. Tavender[b], Anthony W. Parker[b]

[a]*Department of Biological Sciences, University of Salford, Salford M5 4WT, UK*
[b]*Laser Support Facility, Rutherford Appleton Laboratory, Chilton, Didcot, Oxon OX11 0QX, UK*

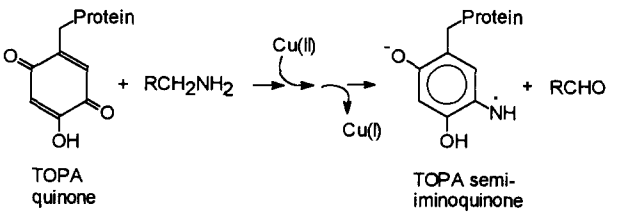
Received 13 December 1995

Abstract **Resonance Raman spectra were obtained of the free radical site in substrate reduced anaerobic samples of pea seedling copper amine oxidase (PSAO). The spectra differ significantly from those reported previously for *E. coli* copper amine oxidase [Moenne-Loccoz et al. (1995) Biochemistry 34, 7020]. The spectra were found to be independent of substrate (benzylamine, spermidine or methylamine) used to reduce the TOPA quinone cofactor, however, several of the peaks in the Raman spectrum displayed small shifts on using [^{15}N]benzylamine, proving incorporation of the substrate nitrogen atom onto the cofactor radical. Changes in the spectrum were also observed when measured in D_2O solution indicating a strongly bound proton in the radical. The spectra were independent of pH values between 5 and 9 and are interpreted as showing that the radical exists as a semiiminoquinone radical monoanion. Benzylamine and phenethylamine have been caged with 2-nitrobenzaldehyde and shown by laser flash photolysis to uncage on a sub-millisecond timescale. Preliminary experiments have shown the formation of the enzyme radical intermediate on laser flash photolysis of 2-nitrobenzyl-caged benzylamine in the presence of enzyme. This should permit time-resolved resonance Raman spectral investigations of the catalytic cycle of copper amine oxidases.**

Key words: Copper amine oxidase; Resonance Raman spectroscopy; Free radical; Caged substrate; Laser flash photolysis

1. Introduction

Copper containing amine oxidases (EC 1.4.3.6) have been shown to contain the quinone of TOPA (2,4,5-trihydroxyphenylalanine) as an active site cofactor [1–4]. This cofactor occurs in amine oxidases from yeasts, bacteria, plants and mammals. The TOPA quinone arises from posttranslational oxidative modification of an active site tyrosine residue [5]. During the catalytic cycle of copper amine oxidase, the TOPA quinone is reduced by the amine substrate, generating a semiquinone radical intermediate (Scheme 1) which is stable in anaerobic solutions [6,7]. Investigations of the free radical site in bovine amine oxidase using electron spin echo techniques indicate incorporation of the substrate nitrogen atom into the structure [6–8] and suggest that the radical exists as the dianion [9]. Confirmation of the formation of a nitrogen-containing semi-iminoquinone in *E. coli* amine oxidase (ECAO) was recently obtained by resonance Raman spectroscopy [10].



Scheme 1.

Photolabile precursors of a range of biologically active molecules, including enzyme substrates, have been developed [11,12] for a wide range of applications. 'Caged' compounds have a functional group modified with a suitable photoremovable protecting or caging group, such as the 2-nitrophenyl substituent [13]. Rates of uncaging of 2-nitrobenzyl-substituted molecules, including amines, via *aci*-nitro intermediates vary widely [13–16] but in some cases occur on a sub-millisecond timescale, enabling kinetic measurements to be undertaken on timescales similar to or faster than those using the stopped flow technique. We describe steady-state resonance Raman spectroscopy of the free radical site in pea seedling copper amine oxidase (PSAO). A preliminary study of caged amine substrates by laser flash photolysis is also reported, which may allow time-resolved detection of catalytic intermediates by Raman spectroscopy.

2. Materials and methods

Peas *Pisum sativum* (orb C2) were obtained from W.W. Johnson and Son (Boston, UK). [^{15}N]Potassium phthalimide (98 atom % ^{15}N), benzyl bromide, spermidine, methylamine and phenyl methyl sulfonyl fluoride (PMSF) were obtained from Sigma/Aldrich. All other reagents used were of reagent grade purity.

2.1. Enzyme purification

PSAO was purified following procedures described by Kleutz et al. [17] and McGuirl et al. [18]. Briefly, the aqueous extract from homogenization of 10-day-old pea seedlings was precipitated by ammonium sulfate and then by ethanol/$CHCl_3$. After chromatography on DE52-cellulose (Whatman) and Ultrogel AcA34 (Pierce Chromatography), the pink fractions containing amine oxidase activity were pooled and used for spectroscopy. Only on overloaded SDS–PAGE gels was it possible to detect a small amount of a 30-kDa protein as previously described by McGuirl et al. [18] in addition to the 150-kDa amine oxidase band. Enzyme activity was determined spectrophotometrically

*Corresponding author. Fax: (44) (161) 745 5210.
E-mail: r.h.bisby@biosci.salford.ac.uk

A preliminary account of this work has been published in the Annual Report of the Central Laser Facility, Rutherford Appleton Laboratory (TR-95-025, pp.158–159) 1995.

SSDI 0014-5793(96)00045-2

using benzylamine as substrate and defining 1 U of activity as 1 μmol min^{-1} of benzaldehyde produced ($\Delta\varepsilon$(250 nm) = 13,100 M^{-1} cm^{-1} [17]). The final enzyme preparation had a specific activity of 1.61 U/mg and a $K_m = 1.6 \times 10^{-4}$ M when measured at 25°C in phosphate buffer (50 mM, pH 8.5). For spectroscopy of enzyme samples at different pH values and in D_2O, the purified enzyme sample was twice diluted 10-fold with the desired buffer and reconcentrated to the original concentration (~2 mg cm^{-3}) using an Amicon 8010 ultrafiltration cell with a PM30 membrane. Samples for resonance Raman studies were prepared by flushing with argon for 30 min in an Atmosbag (Aldrich) before adding an aliquot of anaerobic substrate solution and sealing the cell.

2.2. Resonance Raman spectroscopy

Resonance Raman spectra were obtained using a Coherent Innova 90–5 Argon ion laser. Wavelengths of 457.9 nm (~65–140 mW) and 476.5 nm (~360 mW) were used. Samples were contained in a rotating (10 revolution s^{-1}) cylindrical quartz cell (1.5 cm diameter, 1.5 mm high) at 20°C. Back scattering collection geometry was employed. The Raman signal was focused onto the entrance slit of a Spex (1877) Triplemate spectrograph and detected using a Princeton Instruments (TK1024) CCD detector. Spectra were typically accumulated over 400 s. Frequency calibration was performed using the Raman spectra of toluene measured under identical conditions with values accurate to ±2 cm^{-1}.

2.3. Laser flash photolysis

The output of a Lumonics HE460 XeCl excimer laser, giving up to 60 mJ/10-ns pulse at the sample, was used for flash photolysis. The sample was contained in a thermostatted quartz cuvette forming part of a single-beam spectrometer comprising a high-pressure Xe discharge lamp, monochromator and photomultiplier detector (Hammamatsu IP28).

2.4. Synthesis of [^{15}N]benzylamine

[^{15}N]Benzylamine was synthesised using a modified Gabriel synthesis [19] by reaction of potassium [^{15}N]phthalimide and benzyl bromide in DMF at 100°C for 3 h. The resulting benzylphthalimide was reduced by $NaBH_4$ in propan-2-ol with stirring for 24 h at room temperature [20]. Remaining $NaBH_4$ was quenched by addition of glacial acetic acid (2.5 cm^3). The propan-2-ol was removed under reduced pressure and product extracted to give the HCl salt of [^{15}N]benzylamine.

2.5. Synthesis of caged substrates

2-Nitrobenzyl cages of benzylamine and phenethylamine were prepared by reaction of the amine with 2-nitrobenzaldehyde at room temperature for 12 h, followed by reduction with $NaBH_4$ at 0°C according to the procedures described by Billington et al. [14]. The products were extracted and recrystallised from methanol as the HCl salts. Melting points of *N*-(2-nitrobenzyl)benzylamine (I) and *N*-(2-nitrobenzyl)phenethylamine (II) were 234 and 183°C, respectively.

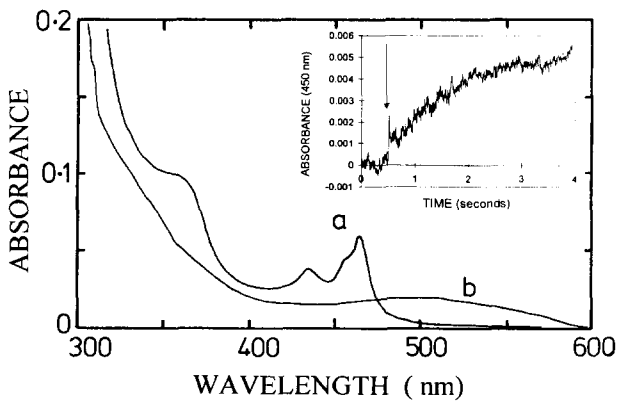


Fig. 1. Spectra of pea seedling amine oxidase (2.35 mg cm^{-3}) in anaerobic phosphate buffer (pH 7.2, 50 mM) before (b) and after (a) addition of benzylamine (1 mM) in a sealed cuvette. Inset: formation an absorbing species at 450 nm after laser flash photolysis (308 nm, 45 mJ/pulse) of an anerobic solution of PSAO (1.2 mg cm^{-3}) and *N*-(2-nitrobenzyl)benzylamine (I, 0.5 mM) in phosphate buffer (0.1 M, pH 7.2) at 25 C.

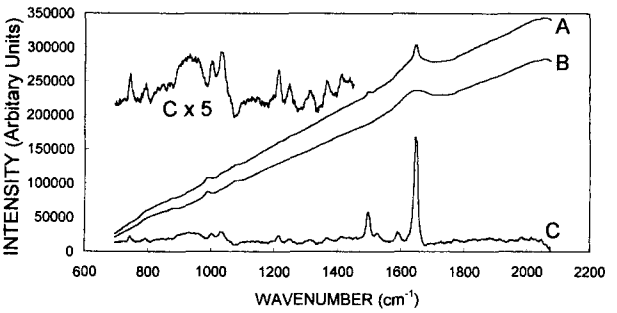


Fig. 2. Resonance Raman spectra of pea seedling amine oxidase (2.35 mg cm^{-3}) in anaerobic phosphate buffer (pH 7.2, 50 mM) before (B) and after (A) addition of benzylamine (1 mM). The difference spectrum after baseline correction, corresponding to the substrate-induced radical, is shown as (C). Laser wavelength 457.9 nm.

3. Results and discussion

3.1. Spectroscopy of the free radical site in PSAO

The sample of PSAO possessed a distinct pink colouration due to the 490–500 nm absorption of the anionic form of the TOPA quinone cofactor as previously reported [5–7,10,18] and illustrated in Fig. 1. Anaerobic addition of benzylamine (or another suitable substrate) caused a bleaching of this absorption band and the appearance of new peaks at 464 and 438 nm with a shoulder at about 360 nm. This spectrum is very similar to that reported for the enzyme free radical in PSAO by McGuirl et al. [18] but differs slightly from that of the *E. coli* enzyme obtained by Moenne-Loccoz et al. [10] with absorbance at the red edge of the spectrum decreasing more rapidly in the pea enzyme. The laser wavelength of 457.9 nm was chosen as most suitable for obtaining the resonance Raman spectrum of the radical (Fig. 2). The background spectrum measured before substrate addition contains only bands due to water and buffer components. The overall slope of the baseline is caused by weak sample fluorescence. Addition of benzylamine gave new bands in the spectrum which could be more clearly resolved after correction of the spectrum. This involved subtraction of a normalised background spectrum and removal of the fluorescence curve by subtracting a polynomial fitted to the spectrum baseline. The corrected spectrum contains predominant bands at 1647 and 1496 cm^{-1}, together with weaker bands tabulated in Table 1.

Comparison with the spectrum of the radical site in ECAO [10] shows that the only similarity is the 1647 cm^{-1} band. Other bands present in the spectrum from PSAO are absent in that from ECAO and vice versa. Although obtained with only 2.35 mg cm^{-3} (15.7 μM) PSAO (molecular mass 150,000 [18]) compared with a concentration of 0.4 mM ECAO (molecular mass 170,000 [10]) used by Moennes-Loccoz et al. [10], the spectrum obtained here is positively attributed to the radical site in the PSAO, since it is probed specifically within the absorption band of the radical and is observed only after substrate addition. The resonance Raman spectrum of the PSAO radical was obtained under a range of conditions. Excitation at 476.5 nm as used by Moennes-Loccoz et al. [10], although only weakly in resonance

Table 1
Frequencies of observed bands in the resonance Raman spectra of the radical site in pea seedling amine oxidase

[^{14}N]Benzylamine in H_2O	[^{15}N]Benzylamine in H_2O	[^{14}N]Benzylamine in D_2O	Assignment (and approximate Wilson description)
1647	1643	1636	C–C stretch ($\nu 8_a$)
1590	1584	–	C–C stretch ($\nu 13$)
1524	1523	1505	C–C stretch ($\nu 19_a$)
1496	1496	1494	C–O/C–N stretch ($\nu 7_a$)
1411	1407	–	C–C stretch ($\nu 19_b$)
1367	1368	1375	C–C stretch ($\nu 8_b$)
1315	1317	–	C–C stretch ($\nu 14$)
1249	1247	–	C–C stretch ($\nu 3$)
1214	1215	–	C–O stretch ($\nu 7_b$)
1035	1034	1042	C–H bend ($\nu 18_a$) or C–C ring breathing ($\nu 9_b$)
1001	–	–	C–C ring breathing ($\nu 12$)
793	792	–	C–C ring deformation ($\nu 18_b$)
743	743	–	Ring deformation ($\nu 1$)

with the PSAO radical absorption, produced a spectrum virtually identical with that shown in Fig. 2. This eliminates any variation which may arise from differences between Raman cross-sections at the two wavelengths. Variation of pH values between 5 and 9 was also found to have no effect on the spectrum and essentially identical spectra were obtained using either methylamine (as in [10]), benzylamine or spermidine as substrates. We therefore conclude that there is some significant difference between the radical and/or its environment in PSAO and ECAO.

The resonance Raman spectrum of the radical in PSAO is strikingly similar to those of semiquinone radicals (reviewed by Tripathi [21]). It may be interpreted by analogy with resonance Raman spectra of *para*-substituted phenoxyl [22–25], including *p*-aminophenoxyl and benzosemiquinone anion, and *meta*-semiquinone radicals [26] in addition to radicals derived from one-electron oxidation of 1,2,4-benzenetriol [27]. The strong band at 1647 cm^{-1} is clearly the Wilson ν_{8a} C–C stretch which is strongly enhanced in phenoxyl radicals having *para*-substituents, such as –OMe, $-NH_2$ and –OH, with pπ electrons interacting with the phenoxyl radical π-electron system. In such radicals, the ν_{8a} vibration is observed at relatively high frequency, such as 1620 cm^{-1} in the *p*-benzosemiquinone anion [25] and 1636 cm^{-1} in the *p*-aminophenoxyl radical [23], compared with 1552 cm^{-1} in the phenoxyl radical [28]. The resonance Raman spectrum of the *m*-benzosemiquinone radical anion shows a very weak band at 1570 cm^{-1} assigned to ν_{8a} and two equally intense bands at 1093 and 1519 cm^{-1}. The latter are both assigned to C–O stretching vibrations and indicate the C–O groups are non-equivalent. In the 3-hydroxyphenoxyl radical, the second C–O band occurs at 1240 cm^{-1} and is weak. Since no strong band is observed in the region of 1200 cm^{-1} in the spectrum of the enzyme-derived radical, the observations point to a *para*-semiquinone radical with an unionised *meta*-substituted hydroxyl group. The weak band at 1210 cm^{-1} is assigned to the C–O stretching vibration of the *m*-hydroxyl substituent. Further comparisons with radicals from 1,2,4-benzenetriol support these conclusions. The mono- and dianion radicals from 1,2,4-benzenetriol have very similar absorption spectra with maxima at 430 nm. However, they are clearly distinguished by the frequency of the ν_{8a} C–C stretching vibration. In the 3-hydroxy-*p*-benzosemiquinone monoanion, which is isoelectronic with the enzyme-derived radical as shown in Scheme 1, this occurs at 1625 cm^{-1} whilst in the 2-oxy-*p*-benzosemiquinone radical dianion the band is at 1587 cm^{-1}. The neutral 3-hydroxy-*p*-benzosemiquinone radical ($pK_a = 4.75$) also has a relatively high-frequency ν_{8a} vibration at 1617 cm^{-1}, but in this case the absorption spectrum is shifted to 401 nm. Assuming that the enzyme radical has a similar pK_a value, this may explain why we fail to observe a resonance Raman spectrum below pH 5, since then the enzyme radical would be off reso-

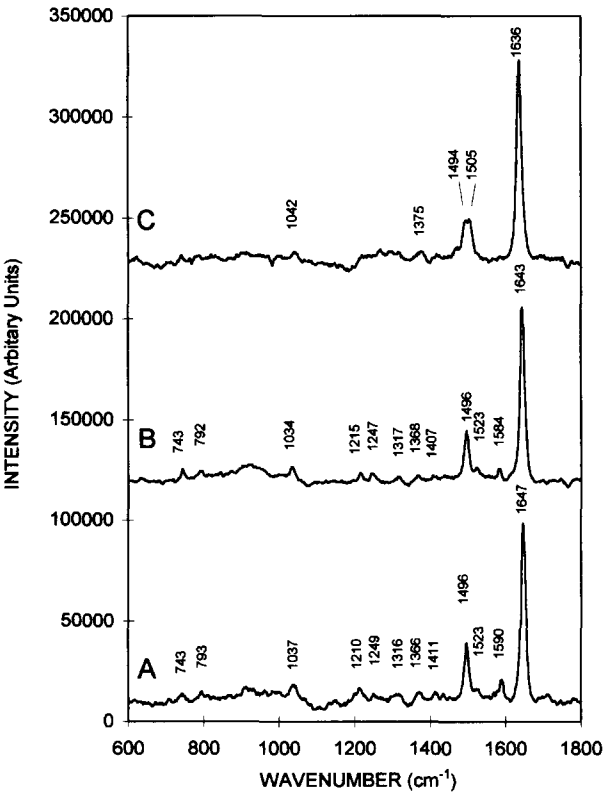


Fig. 3. Resonance Raman spectra of anaerobic solutions of pea seedling amine oxidase (1.5 mg cm^{-3}) after subtraction of background and baseline correction. (A) After reduction with spermidine (500 μM) in H_2O containing phosphate buffer (pH 7.2, 50 mM); (B) After reduction with [^{15}N]benzylamine (500 μM) in H_2O containing phosphate buffer (50 mM, pH 7.2); (C) After reduction with [^{14}N]benzylamine in D_2O containing phosphate buffer (50 mM, pD 7.8).

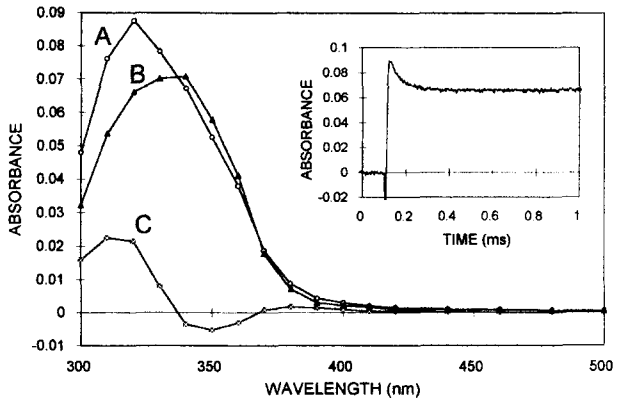


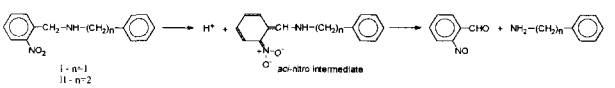
Fig. 4. Spectra from laser flash photolysis (308 nm) of compound II (0.5 mM) in aqueous phosphate buffer (25 mM, pH 7.3) at 45°C, measured 36 (A) and 300 μs (B) after the laser pulse delivering 50 mJ to the sample. The difference spectrum is shown as (C). The inset shows the transient absorption at 320 nm under the same conditions.

nance with the laser wavelengths used in this work. However, it is also possible that this may be ascribed to the very low activity of the enzyme in this region, since an investigation of the activity of PSAO towards benzylamine as a function of pH showed that activity decreased with decreasing pH correlating with a single pK_a of 8.3 ± 0.1. The strong band in the PSAO radical spectrum at 1496 cm^{-1} is most likely to be the ν_{7a} C–O stretch on the basis of the strong resonance enhancement of this mode in phenoxyl and related radicals [28]. This mode occurs in the enzyme radical at a higher frequency than in the resonance Raman spectrum of *p*-aminophenoxyl (1434 cm^{-1}) where it is mixed with the C–N due to near equivalence of the C–O and C–N bonds. Indeed, both experiment [23] and calculation [24] show that there is significant mixing of modes in radicals such as these. From the above discussion we have made further assignments based on the work of Tripathi et al. [21] and our conclusions are shown in Table 1. The equivalent Wilson modes given in Table 1 may only be taken as approximate because of the non-normal mode character of the vibrations [24] and the lack of symmetry in the TOPA-derived free radical.

Resonance Raman spectra were also obtained using [^{15}N]benzylamine as substrate in H_2O and [^{14}N]benzylamine in D_2O. The results shown in Fig. 3 indicate that some of the bands listed in Table 1 are sensitive to such changes. The shifts obtained using [^{15}N]benzylamine confirm again that the substrate nitrogen atom is incorporated into the radical structure. The observation of a change in the ring C–C stretching frequency on changing the mass of the –NH substituent is further evidence of mixing of modes referred to above. Although the hydrogen atom at C3 has also been shown to be labile and to become deuteriated [29] under conditions similar to those employed here, the changes in D_2O solution indicate a strong interaction of the radical with a proton (or deuteron) and are consistent with the interpretation given above in which the *m*-OH substituent is unionised.

3.2. *Caged substrates for amine oxidases*

Laser photolysis of *N*-(2-nitrobenzyl)benzylamine (I) and *N*-(2-nitrobenzyl)phenethylamine (II) are expected to proceed via the *aci*-nitro intermediate, releasing the free amine and 2-nitrosobenzaldehyde (Scheme 2) [11]. Spectra obtained from laser flash photolysis using the intense (50 mJ) unfocussed output at 308 nm from a XeCl excimer laser gave the spectra shown in Fig. 4. The spectrum at 36 μs appeared within a μs. Subsequently, there was a decay between 300–330 and 380–420 nm and a small increase in absorption at 340–360 nm. The spectrum measured 300 μs after the laser pulse (Fig. 4) shows no decay in the flash photolysis experiment and corresponds to the permanent changes in the spectrum of the solution measured conventionally in a double beam spectrometer, representing formation of the 2-nitrosobenzaldehyde product. The short-lived spectrum, measured 36 μs after the laser pulse, is that of the *aci*-nitro intermediate. Considerable evidence [11] indicates that the rate of decay of this intermediate corresponds to the rate of release (uncaging) of the amine product. The first-order rate constant for this process was determined from transient absorptions, such as that shown in the inset to Fig. 4. Identical results were obtained from decays at 320 and 380–420 nm. At 25°C in phosphate buffer (25 mM, pH 7.3), the first-order rate constants for decay of transients from I and II were $(4.0 \pm 0.3) \times 10^3$ s^{-1} (τ = 250 μs) and $(6.2 \pm 0.3) \times 10^3$ s^{-1} (τ = 161 μs), respectively. Measurements over the range of 15–55°C gave linear Arrhenius plots and activation energies of 40 ± 2 kJ mol^{-1} for both compounds. These uncaging rates are approximately an order of magnitude faster than those for *N*-(2-nitrobenzyl) cages of glycine [14], glutamine [15] and γ-aminobutyric acid [16], but slower than for the uncaging of *N*-(1-(2-nitrophenyl)ethyl)carbamoylcholine iodide [13]. The activation energy for decay of the *aci*-nitro intermediates of I and II is slightly smaller than the value of 55 kJ mol^{-1} measured for caged ATP [11] under similar conditions. Some preliminary experiments have been undertaken to observe the formation of the enzyme free radical after laser flash photolysis (308 nm) of an anaerobic solution of PSAO in the presence of I. The inset to Fig. 1 shows formation of a species absorbing at 450 nm. This was identified as the enzyme-derived free radical by the steady-state spectrum, obtained after illumination, which was similar to that shown as Fig. 1a. The formation of the radical in this system is slower than observed by stopped flow for the reaction of lentil seedling amine oxidase with the chromogenic substrate *p*-dimethylaminomethylbenzylamine (*p*-DABA) [30,31], consistent with *p*-DABA being a better substrate than benzylamine. The preliminary result for PSAO and benzylamine does not show the sigmoidal curve observed with lentil amine oxidase and *p*-DABA.



Scheme 2.

The laser flash photolysis results demonstrate that I and II offer the opportunity for time-resolved studies of the catalytic cycle of copper amine oxidase on a slightly faster timescale than available with conventional stopped-flow instruments. They are, however, ideally suited to situations, such as the measurement of time-resolved resonance Raman spectra, where signal accumulation and averaging over several hundred experiments are required. In such experiments, a 'pump' laser pulse would be used to uncage the substrate, followed at a precisely variable time delay by the 'probe' laser pulse for measurement of the

Raman scattering. It is hoped that the observations reported here will soon lead to such studies.

Acknowledgements: This work was supported by the BBSRC (Grant GR/J71175) and the EPSRC. The Raman spectroscopy was undertaken at the Laser Support Facility, Rutherford Appleton Laboratory. We thank Dr. P.F. Knowles (University of Leeds) for helpful discussions.

References

[1] Janes, S.M., Mu, D., Wemmer, D., Smith, A.J., Kaur, S., Maltby, D., Burlingame, A.L. and Klinman, J.P. (1990) Science 248, 981–987.
[2] Janes, S.M., Palcic, M.M., Scaman, C.H., Smith, A.J., Brown, D.E., Dooley, D.M., Mure, M. and Klinman, J.P. (1992) Biochemistry 31, 12147–12154.
[3] Klinman, J.P. and Mu, D. (1994) Annu. Rev. Biochem. 63, 299–344.
[4] Knowles, P.F. and Dooley, D.M. (1994) Metal Ions Biol. Syst. 30, 361–403.
[5] Matsuzaki, R., Suzuki, S., Yamaguchi, K., Fukui, J. and Tanizawa, K. (1995) Biochemistry 34, 4524–4530.
[6] Dooley, D.M., McGuirl, M.A., Brown, D.E., Turowski, P.N., McIntyre, W.S. and Knowles, P.F. (1991) Nature (London) 349, 262–264.
[7] Pedersen, J.Z., El-Sherbini, S., Finazzi-Agro, A. and Rotilio, G. (1992) Biochemistry 31, 8–12.
[8] McCracken, Peisach, J., Cote, C.E., McGuirl, M.A. and Dooley, D.M. (1992) JACS 114, 3715–3720.
[9] Warncke, K., Babcock, G.T., Dooley, M.A., McGuirl, M.A. and McCracken, J. (1994) JACS 116, 4028–4037.
[10] Moenne-Loccoz, P., Nakamura, N., Steinebach, V., Duine, J.A., Mure, M., Klinman, J.P. and Sanders-Loehr, J. (1995) Biochemistry 34, 7020–7026.
[11] McCray, J.A. and Trentham, D.R. (1989) Annu. Rev. Biophys. Biophys. Chem. 18, 239–270.
[12] Adams, S.R. and Tsien, R.Y. (1993) Annu. Rev. Physiol. 55, 755–784.
[13] Walker, J.W., McCray, J.A. and Hess, G.P. (1986) Biochemistry 25, 1799–1805.
[14] Billington, A.P., Walstrom, K.M., Ramesh, D., Guzikowski, A.P., Carpenter, B.K. and Hess, G.P. (1992) Biochemistry 31, 5500–5507.
[15] Ramesh, D., Wiebolt, R., Billington, A.P., Carpenter, B.K. and Hess, G.P. (1993) J. Org. Chem. 58, 4599–4605.
[16] Weibolt, R., Ramesh, D., Carpenter, B.K. and Hess, G.P. (1994) Biochemistry 33, 1526–1533.
[17] Kleutz, M.D., Adamsons, K. and Flynn, J.E. (1980) Prep. Biochem. 10, 615–631.
[18] McGuirl, M.A., McCahon, C.D., McKeown, K.A. and Dooley, D.M. (1994) Plant Physiol. 106, 1205–1211.
[19] March, J. (1992) Advanced Organic Chemistry, 4th Ed., Wiley, New York, NY.
[20] Osby, J.O., Martin, M.G. and Ganem, B. (1984) Tetrahedron Lett. 25, 2093–2096.
[21] Tripathi, G.N.R. (1989) in: Time Resolved Spectroscopy (Clark, R.J.H. and Hester, R.E., Eds.) pp. 157–218, Wiley, New York, NY.
[22] Tripathi, G.N.R. and Schuler, R.H. (1988) J. Phys. Chem. 92, 5129–5133.
[23] Tripathi, G.N.R. and Schuler, R.H. (1984) J. Phys. Chem. 88, 1706–1710.
[24] Raymond, K.S. and Wheeler, R.A. (1993) J. Chem. Soc. Faraday Trans. 89, 665–670.
[25] Tripathi, G.N.R. and Schuler, R.H. (1987) J. Phys. Chem. 91, 5881–5885.
[26] Tripathi, G.N.R., Chipman, D.M., Miderski, C.A., Davies, H.F., Fessenden, R.W. and Schuler, R.H. (1986) J. Phys. Chem. 90, 3968–3975.
[27] Qin, L., Tripathi, G.N.R. and Schuler, R.H. (1987) J. Phys. Chem. 91, 1905–1910.
[28] Tripathi, G.N.R. and Schuler, R.H. (1984) J. Chem. Phys. 81, 113–121.
[29] Mure, M. and Klinman, J.P. (1993) J. Am. Chem. Soc. 115, 7117–7127.
[30] Bellelli, A., Brunori, M., Finazzi-Agro, A., Floris, G., Giartosi, A. and Rinaldi, A. (1985) Biochem. J. 232, 923–926.
[31] Bellelli, A., Finazzi-Agro, A., Floris, G. and Brunori, M. (1991) J. Biol. Chem. 266, 20654–20657.