ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 8 (2000) 111–120

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

www.elsevier.com/locate/molcatb

A spectroscopic and kinetic study of *Escherichia coli* amine oxidase

Simon de Vries [a,*], Rob J.M. van Spanning [b], Vincent Steinebach [a]

[a] *Department of Biotechnology, Enzymology Section, Delft University of Technology, Julianalaan 67, 2628 BC Delft, Netherlands*
[b] *Department of Molecular Cell Physiology, Faculty of Biology, BioCentrum Amsterdam, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, Netherlands*

Abstract

Escherichia coli amine oxidase has been overexpressed and characterized spectroscopically and by means of pre-steady state kinetics. The X-band EPR spectrum of *E. coli* amine oxidase prepared with isotopically pure ^{63}Cu and ^{65}Cu shows superhyperfine contributions of three slightly different nitrogen nuclei. The Q-band spectrum of the enzyme indicates the presence of two different copper signals in an approximate one to one stoichiometry. Furthermore, a signal ascribed to enzyme bound Mn^{2+} is observed. Both the X-band and Q-band EPR signal of the topasemiquinone as prepared in the presence of the substrate 2-phenylethylamine and KCN show multiple hyperfine lines. The Q-band spectrum of the semiquinone shows that the *g*-tensor is axial or slightly rhombic. The *g*-value of $g_{x,y} = 2.005$ is consistent with hydrogen bonding between the 5-C=N and/or 2-C=O atoms of the topasemiquinone with nearby acid/base groups of the protein. Equilibrium incubation experiments with substrate at different pH values and pre-steady state kinetic analysis indicate the presence of a species absorbing at 400 nm preceding the formation of the aminoquinol and the topasemiquinone intermediate. The amount of topasemiquinone formed in equilibrium is governed by a single acid/base group with p*K* 9.0, the relation between the amount of 400 nm species and pH being more complex. The 400 nm species is proposed to be the protonated product Schiff-base. The nature of other intermediates of the reductive part of the catalytic cycle is also discussed.

Keywords: Escherichia coli; Amine oxidase; Kinetics

1. Introduction

The first report on a copper-containing amine oxidase dates back to 1930 and describes a histaminase from porcine kidney [1]. In the sixties, when highly purified preparations of amine oxidases became available important progress on the structure of these types of enzymes was made. It was by then firmly established that these enzymes contain two copper atoms per dimer [2,3] but the identity of the covalently bound organic cofactor had already been disputed for several decades, riboflavin or pyridoxal phosphate being mentioned as the prime candidates [see, e.g., Refs. [4,5]]. This dispute ended in 1990 when the structure of the

Abbreviations: ECAO: *Escherichia coli* amine oxidase; AGAO: *Arthrobacter globiformis* amine oxidase; BSAO: bovine serum amine oxidase; PPAO: porcine plasma amine oxidase; PSAO: pea seedling amine oxidase; ESEEM: Electron spin echo envelope modulation; CW-ENDOR: Continuous wave-electron nuclear double resonance

* Corresponding author. Tel.: +31-15-278-5139; fax: +31-15-278-2355; E-mail: s.devries@stm.tudelft.nl

1381-1177/00/$ - see front matter
PII: S1381-1177(99)00074-0

cofactor was elucidated and identified as 2,4,5-trihydroxyphenylalanine (topa) in the reduced enzyme and as topaquinone or TPQ (2,4,5-trihydroxyphenylalaninequinone) in the oxidized enzyme [6]. Thus, the copper-containing amine oxidases (E.C. 1.4.3.6) can be defined as a class of enzymes which are homodimers of about 160–190 kDa containing per subunit one Cu-ion and one tyrosine residue post-translationally modified to a topaquinone (TPQ). They catalyse the oxidation — using molecular oxygen as oxidant — of primary amines to their corresponding aldehydes, in addition yielding hydrogen peroxide and ammonia. The copper-containing amine oxidases are widespread amongst prokaryotes (eubacteria) and lower and higher eukaryotes including yeast, plants and animals. The first prokaryotic amine oxidase has been purified in the group of prof. Duine by Van Iersel et al. [7]. So far, amine oxidase activity has not been reported to be present in the group of archaebacteria which is not surprising since there is no apparent sequence homology between the genomes of archaebacteria for which the sequence has been determined and the gene encoding, e.g., the *Escherichia coli* amine oxidase (ECAO).

There exists a plethora of experimental data suggesting that the overall reaction catalysed by the amine oxidases may be dissected into the following three reactions (cf. Refs. [8–10] for an overview and Fig. 6):

$$RCH_2NH_2 + TPQ/Cu^{2+} \rightarrow RCHO + TPQH_2 \cdot NH_2/Cu^{2+} \quad (1)$$

$$TPQH_2 \cdot NH_2/Cu^{2+} \leftrightarrow TPQH^{\cdot} \cdot NH/Cu^{1+} \quad (2)$$

$$H_2O + H^+ + TPQH^{\cdot} \cdot NH/Cu^{1+} + O_2 \rightarrow TPQ/Cu^{2+} + H_2O_2 + NH_4^+ \quad (3)$$

(1) The amine group of the substrate adds to the C-5 carbonyl group of TPQ, yielding the aminoquinol intermediate, a sequence of reactions in which water is first released and subsequently being taken up (not shown in the equations); (2) after release of the aldehyde, rapid internal electron transfer takes place producing the iminosemiquinone [11–13] form of TPQ and Cu^{1+}. (3) The latter intermediate is presumably the species that reacts directly and rapidly [14] with molecular oxygen. In this last step, ammonia is released from the enzyme [15,16].

A few years ago, the 3D-structure of ECAO has been determined including a structure containing a covalently bound inhibitor [17,18]. Also, the 3D-structures of pea seedling amine oxidase [19] and of the apo- and holoenzyme forms of the enzyme from *Arthrobacter globiformis* have been established recently [20]. In all four structures, the copper-ion was found to be ligated to three histidine residues and two water molecules, one equatorially and the other one axially bound yielding an approximate square-pyrimidal symmetry around the Cu^{2+}-ion, exactly in the manner as predicted from the numerous spectroscopic analyses (cf. Refs. [21–28]). The active sites of the two monomers are apparently physically linked via antiparallel β-ribbon arms, but direct evidence that this interaction would provide the basis for the negative cooperativity observed in the reaction between, e.g., BSAO and PPAO and hydrazines is lacking [29–31]. Regarding TPQ, several differences between enzymes from different sources have been observed, the most important one being the relative orientation of TPQ with respect to the Cu-atom, being closest to the 2-C=O atom of TPQ in the active form of AGAO and closest to the 5-C=O atom in the active form of PSAO. Although this difference may be due to difference in pH at which the crystals were prepared (cf. Ref. [20]) this finding also supports the suggestion that the TPQ ring may rotate around the Cβ–Cγ bond (by about 180°) during the biogenesis of the cofactor [9,20,32–35]. More importantly, the TPQ seems to rotate around the Cβ–Cγ bond by about 150° in ECAO upon binding of the substrate analogue 2-hydrazinopyridine [18], a finding indicating

that a similar rotation may occur when convertible substrate becomes bound. Further, the residue that might act as the active-site base in abstracting the *pro-S* hydrogen from aromatic primary amines has been identified as an aspartic acid residue in all four crystal structures.

In spite of the impressive progress made over the last years, several general questions remain to be solved regarding the mechanism of action of the amine oxidases: (1) The precise protonation states of the various intermediates, (2) the putative role and identity of nearby acid–base groups in addition to the aspartic-acid residue alluded to above such as a conserved tyrosine residue that may aid in catalysis, (3) the fate of H_2O being formed and being taken up during the reaction, (4) the precise routes taken by substrates and products to and from the active site. In addition, little is known about the reduction of oxygen, for example the way of ligation of oxygen to copper (side-on or end-on?) and the characteristics of the electron transfer and protonation reactions.

We have recently cloned and sequenced the gene encoding the ECAO and characterized the purified, overexpressed, enzyme [36]. This preparation was shown to be heterogeneous as revealed by high-resolution anion exchange chromatography, although all species had identical N-terminal sequences. The purest fraction with the highest specific activity obtained by high-resolution anion exchange chromatography was, however, homogeneous on rechromatography and shown to be a dimeric enzyme containing two copper-ions and maximally 1.0–1.1 TPQ per mol of dimer, the latter value based on the reaction stoichiometry obtained upon derivatization with hydrazines. One should realise, however, that two TPQ per mol of dimer might actually be present, one being much more susceptible to reaction with hydrazines (cf. Refs. [17–20]). The high purity of the enzyme made it possible for the first time to perform a Resonance Raman study of the underivatized cofactor in situ [37]. Comparison with a model compound confirmed the presence of TPQ in ECAO [38,39]. Furthermore, although ECAO prefers aromatic amines as substrates, the enzyme very slowly converts methylamine. With this latter substrate considerable amounts of the topasemiquinone state are generated. The $^{14}N/^{15}N$ isotope dependence of the Resonance Raman spectrum directly confirmed the presence of the amine group in this semiquinone intermediate [37]. Pre-steady state kinetic analyses under anaerobic conditions revealed the formation of a novel intermediate absorbing at 400 nm which had been ascribed to a reduced and protonated form of the N-containing TPQ [40].

In contrast to the great amount of spectroscopic data available on many, mostly eukaryotic, amine oxidases, the ECAO has not been spectroscopically characterized in detail, this in spite of the fact that ECAO was the first of the amine oxidases for which a high resolution 3D-structure had become available [17]. In this paper, we describe X-band and Q-band EPR spectroscopic properties of ECAO. In the light of the progress made during the last years in the research field of the amine oxidases, we will also re-evaluate some of our conclusions drawn previously [40,41] concerning the identity of the 400 nm intermediate and consequently on the mechanism of action of the ECAO.

2. Materials and methods

Recombinant ECAO was overproduced and purified (enzyme species B obtained after Mono Q anion exchange chromatography) as described before [36]. The ^{63}Cu- and ^{65}Cu-containing ECAO were obtained by growing in a medium supplemented with 6 μM of the specific copper isotope (> 99% enrichment).

EPR spectroscopy (both X-band and Q-band) was performed on a Varian E9 EPR spectrometer equipped with home-built He-flow systems to obtain low-temperatures or in a flat cell for room-temperature X-band spectroscopy. Simulation of spectra was performed with a home

written program running on a Macintosh PowerPC computer. The program simulates spectra of $S_{\text{eff}} = 1/2$ systems up to second order in nuclear hyperfine-interaction and up to first order in superhyperfine-interaction, using Gaussian lineshapes and allowing rotation of the *g*-tensor with respect to the *A*-tensor. Normalized strain parameters, linear and quadratic in M_I, simulating *g*- and *A*-strain, can be varied. For the present purpose, it was sufficient to assume colinearity of the *g*- and *A*-tensors and visual inspection was used a criterion for the goodness of fit.

Quantization of EPR signals was performed by double integration of the respective signals appropriately correcting for differences in the spin states of the various systems.

Anaerobic rapid scan stopped-flow spectrophotometry was performed with Scientific PQ/SF-53 preparative stopped-flow equipped with an EG&G Princeton Applied Research 1024-element photodiode array detector (model 1461). Anaerobiosis was obtained by flushing the enzyme and substrate solutions with Argon followed by addition of glucose oxidase (two units), catalase (2.5 μg/ml) and glucose (4 mM) and transfer to the syringes.

3. Results and discussion

3.1. EPR spectroscopy at X-band and Q-band frequencies

The Q-band EPR spectrum of ECAO is shown in Fig. 1. This signal is similar to that observed in, e.g., PPAO [21,42]. Simulations suggest that the signal is an overlap of a rhombic and an axial signal in an approximate 1:1 ratio just as in the case of PPAO. In contrast to PPAO the values of A_z of the two copper signals are the same within experimental resolution. In addition to the copper signal, the spectrum shows a signal from Mn^{2+} which is not derived from the buffer. In the crystal structures of ECAO, PSAO and AGAO an additional metal center has been

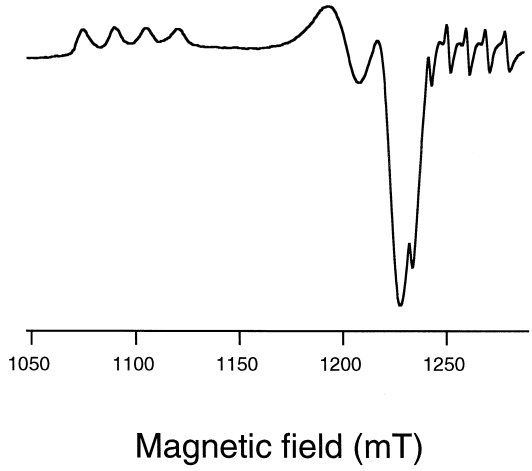

Fig. 1. Q-band EPR spectrum of ECAO (378 μM in 50 mM potassium phosphate buffer, pH 7). The spectrum shows signals due to type II Cu(II) and Mn (II), the latter represented by six sharp peaks at the right-hand side of the spectrum. Experimental conditions: Frequency: 35.334 GHz; modulation amplitude: 3.2 mT; microwave power: 14 mW; temperature: 42 K.

observed ascribed to Ca^{2+} and/or Mn^{2+}. The results of EPR at Q-band frequency where signals due to Mn^{2+} are usually more intense due to smaller second order effects than at X-band frequency (cf. the spectrum in Fig. 2) are consistent with Mn^{2+} as this second metal. However, this site is mostly empty or at least not occupied by an EPR detectable (paramagnetic) metal, since the concentration of Mn^{2+} relative to that of copper equals only about 0.2%.

The X-band EPR spectrum of oxidized ECAO with natural copper is very similar to that of AGAO and PPAO (data not shown). The X-band EPR spectra of the isotopically pure ^{63}Cu and ^{65}Cu ECAO preparations are shown in Fig. 2 together with enlargements of the $g_{x,y}$ region and the outermost hyperfine line of the g_z resonance of the ^{63}Cu-derivative. The superhyperfine interaction of copper with various nitrogen nuclei is clearly resolved in all these spectra, much more clearly than in other amine oxidases. Seven superhyperfine lines are seen which are due to interaction with three nitrogen nuclei. The observation that the copper signal is in fact an overlap of two slightly different signals as concluded from the Q-band spectra, the greatest

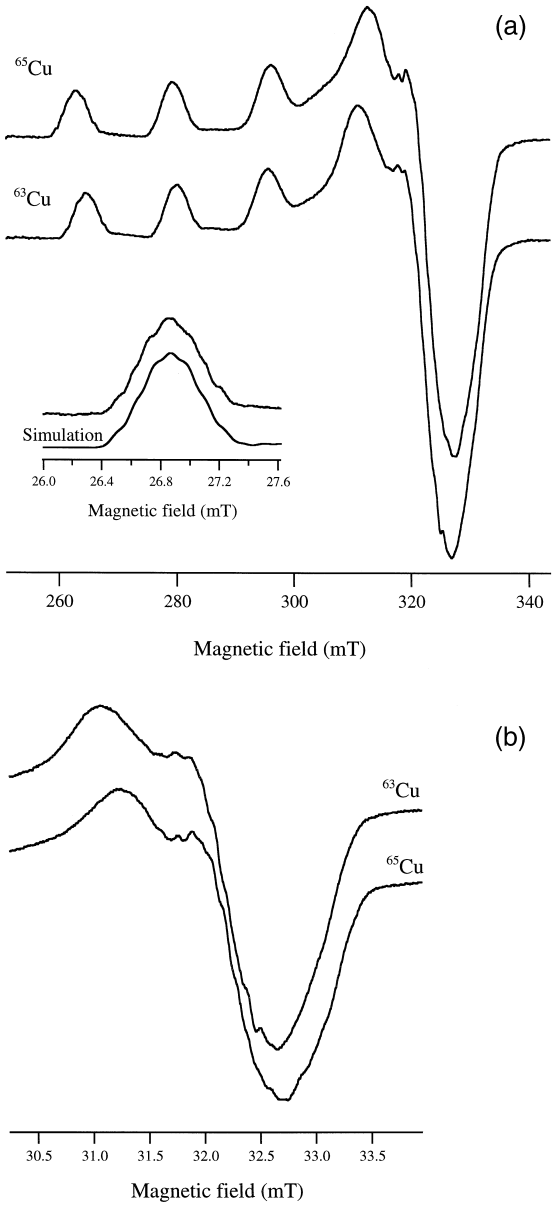

Fig. 2. X-band EPR spectra of ^{63}Cu- and ^{65}Cu-containing ECAO (375 μM in 10 mM MOPS buffer, 20 mM NaCl, 12% (v/v) glycerol, pH 7). The inset in (a) shows a blow up of the left outermost hyperfine line of the ^{63}Cu spectrum (modulation amplitude: 0.25 mT) and a simulation including three different nitrogen nuclei (see text). In b, the $g_{x,y}$ region of the spectrum of the two isotopically pure preparations is shown in expanded view (modulation amplitude: 0.20 mT). Other experimental conditions: Frequency: 9.236 GHz; modulation amplitude: 0.4 mT; microwave power: 12.6 mW; temperature: 60 K.

difference being expressed in the $g_{x,y}$ region of the spectrum, precluded a reliable simulation of the complete spectrum. However, since the difference in the values of A_z between the two copper ions is very small the g_z region of the spectrum could be simulated. These simulations indicate the presence of three chemically slightly distinct nitrogen nuclei with superhyperfine coupling constants, $A_z^{N1-3} = 13.2$, 11.7 and 9.2 G. Thus, the symmetry at the copper atom is lower than square pyrimidal. The distortion may be caused by the inequivalence of the two water molecules bound to the copper as seen in the crystal structures of oxidized and underivatized ECAO and AGAO.

In ECAO incubated anaerobically or aerobically in the presence of KCN with aromatic amines such as 2-phenethylamine the TPQ is reduced to the semiquinone state. The properties of the TPQ semiquinone can be studied both optically and by EPR. In Fig. 3, the X-band and Q-band spectra of the TPQ semiquinone as obtained in the presence of KCN are compared. As we have shown previously, addition of KCN leads to a slight broadening of the lines of the semiquinone in the X-band EPR spectrum and

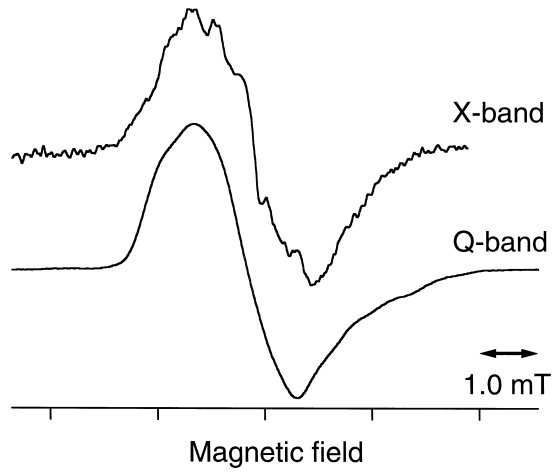

Fig. 3. X-band, Q-band EPR spectra of the topasemiquinone intermediate of ECAO. ECAO (378 μM in 50 mM potassium phosphate buffer, pH 7) was incubated with 2-phenylethylamine (3.7 mM) in the presence of KCN (6 mM). The X-band spectrum was recorded at room temperature, the Q-band spectrum at 116 K. Additional experimental conditions (X-band and Q-band, respectively): Frequency: 9.234 GHz and 35.185 GHz; modulation amplitude: 0.1 and 0.5 mT; microwave power: 2.0 and 1.6 mW.

to an increase in the rate of relaxation compared to the semiquinone signal obtained in the absence of inhibitors [40]. The Q-band spectrum of the semiquinone, in the presence of KCN, shows partially resolved hyperfine structure and further a non-isotropic *g*-tensor. This spectrum cannot be simulated completely, like the X-band spectrum [cf. Ref. [12]], because of the great number of nuclei contributing to the hyperfine pattern. Preliminary simulations, however, indicate that the *g*-tensor is axial ($g_{x,y} = 2.005$, $g_z = 2.002$) or perhaps slightly rhombic, not uncommon for organic radicals. The value of $g_z = 2.002$ is close to the free electron *g*-value and its direction is in all likelihood perpendicular to the plane of the topasemiquinone ring [cf. Refs. [43,44]]. In most if not all *para*-benzosemiquinones, either the g_x- or the g_y-axis coincides with the line joining the carbonyl groups of the quinone ring. Assuming a similar orientation for the g_x- or the g_y-component of the *g*-tensor in the topasemiquinone and ignoring the difference between N and O bound to C5, then the relatively low value of $g_{x,y}$ (2.005 compared to 2.007–2.009) for the topasemiquinone suggests a low spin density at 2-C=O and 5-C=N consistent with protonation of the semiquinone or the formation of a hydrogen bond at either or both of these positions [45–48]. This finding supports the results obtained in ESEEM and CW-ENDOR studies which indicate a hydrogen bonding between (unidentified) acid/base groups in the protein and the 5-C=N and 2-C=O atoms of the topasemiquinone in addition to hydrogen bonding at the 4-C=O position [12].

3.2. Characterization of intermediates of the catalytic cycle obtained under equilibrium conditions and in the pre-steady state

Anaerobic incubation of amine oxidases with substrate leads to partial reduction of the enzyme. At room temperature, the reducing equivalents of the substrate are distributed both over the TPQ system and the copper atom yielding the iminosemiquinone intermediate state and Cu^{1+} [11,40]. In contrast, at low temperature, the redox equilibrium has shifted in a manner that copper remains Cu^{2+} and the TPQ is reduced to the aminoquinol state. At room temperature, the precise distribution of reducing equivalents is also dependent on pH (Fig. 4). At pH 5 and 7.5, a topasemiquinone is formed as indicated by the absorption peaks at 468 nm, 440 nm and around 350 nm, at pH 10, no peaks

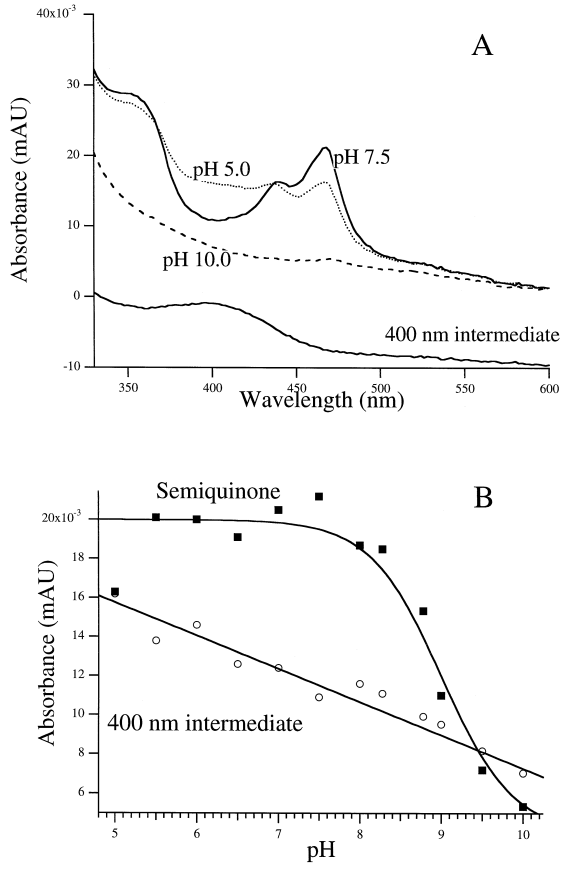

Fig. 4. Optical spectra showing the effect of reduction of ECAO (5.3 μM) anaerobically with 2-phenylethylamine (196 μM) at different pH values. (A) pH 5.0 (dotted line), pH 7.5 (continuous line) and pH 10 (broken line). The spectrum of the 400 nm intermediate was obtained by subtraction of the pH 7.5 spectrum, multiplied by 0.65 to cancel out the contribution of the semiquinone, from the pH 5.0 spectrum. (B) pH dependence of the absorbance at 468 nm (representing mainly the topasemiquinone) and 400 nm (representing for more than 55% the '400 nm intermediate'). The line through the points of the absorbance at 468 nm represents a simulation for an acid–base group with $pK = 9.0$.

are present between 300 and 600 nm, the TPQ in all likelihood being converted completely to the aminoquinol form. The amount of semiquinone is somewhat lower at pH 5 compared to pH 7.5 and titrates out with an apparent pK of 9.0 (Fig. 4).

It is further clear from Fig. 4A that the spectrum recorded at pH 5 contains another component with an absorption maximum around 400 nm. Our previous analysis has shown that this 400 nm intermediate is EPR silent [40]. The optical spectrum of this intermediate can be obtained by subtracting the pH 7.5 spectrum from that of the pH 5 spectrum appropriately correcting for the different amounts of topasemiquinone formed under these two conditions. The spectrum of the 400 nm intermediate thus obtained is shown in Fig. 4A. The amount of this species obtained under equilibrium conditions decreases more or less linearly with increasing pH values. Thus, the pH dependency of the amount of 400 nm species formed is probably linked to deprotonation of more than one acid/base group (Fig. 4B). This 400 nm intermediate observed under equilibrium conditions is essentially the same species as formed to its full extent within 6 ms after rapid mixing of, e.g., 2-phenylethylamine with ECAO [40]. The result of such an anaerobically performed stopped-flow experiment at pH 7.0 is shown in Fig. 5. It is clear that the formation of the 400 nm intermediate precedes that of the topasemiquinone. As a matter of fact, the traces suggest a direct conversion of the 400 nm intermediate into the topasemiquinone. However, one cannot rule out the formation of intermediates living shorter than the time difference between the most rapid measurements, 6 ms. Further-

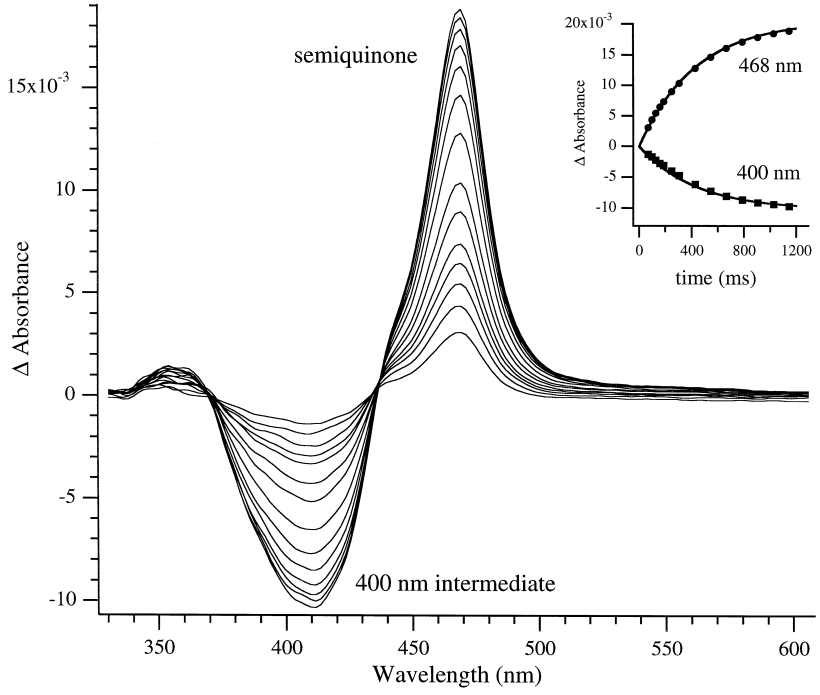

Fig. 5. Rapid-scanning stopped-flow spectrophotometry of ECAO mixed anaerobically with 2-phenylethylamine. Reaction temperature: 10°C. All spectra shown are difference spectra from which the spectrum obtained after 6 ms (the first spectrum obtained) had been subtracted. The first difference spectrum shown was obtained 66 ms after mixing, the following four in 30 ms increments, the next two in increments of 60 ms, the remaining seven in increments of 120 ms. The inset shows the kinetics of the absorbance changes at 468 and 400 nm. The lines through the points are simulations of single exponentials both with half times of 430 ms. The maximal absorbance change at 468 nm was 0.0205, that at 400 nm equalled 0.0103.

more, not all putative intermediates are necessarily optically detectable (between 350 and 600 nm). We have previously ascribed this 400 nm intermediate to a protonated form of the aminoquinol [40], but here we wish to revise this proposal.

Combining the pre-steady state kinetic data and the equilibrium measurements the following picture emerges. After anaerobic addition of the substrate 2-phenylethylamine to ECAO an equilibrium is set up between the 400 nm intermediate, the topasemiquinone and the aminoquinol. At high pH (pH 10), only a single species apparently prevails under equilibrium conditions, the aminoquinol. At lower pH values, all three species may be present, but only the 400 nm intermediate and the topasemiquinone are detectable optically or by EPR. However, as

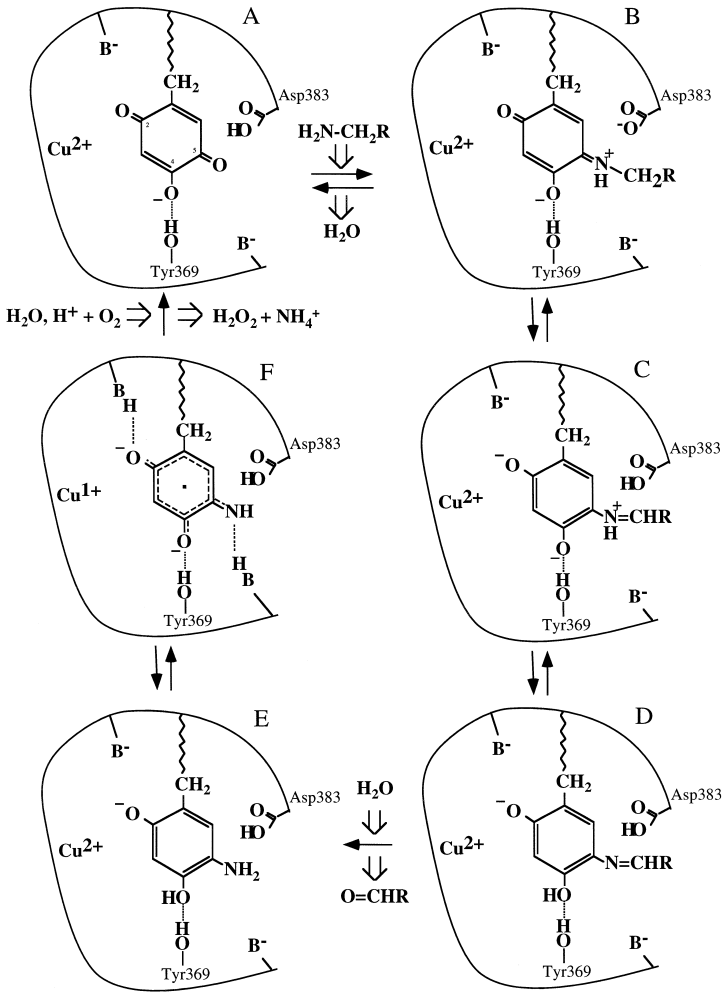

Fig. 6. Protonation states and redox states of various TPQ intermediates occurring in the catalytic cycle of ECAO. Asp383 (ECAO numbering) is the active site base proposed to be involved in abstraction of the *pro-S* proton of the substrate [18,20]. Tyr369 forms a tight hydrogen bond with 4-C=O of the TPQ [18]. Two different, unidentified, active site bases 'B^-' mainly involved in hydrogen bonding to the aminoquinol and topasemiquinone [12] forms are also shown. (A) oxidized enzyme as isolated in the 4-C=O deprotonated form; the *para*-benzoquinone state may be in equilibrium with the *ortho*-benzoquinone state. (B) Substrate Schiff base. (C) Protonated product Shiff base, the 400 nm intermediate. (D) Neutral product Schiff base. (E) Aminoquinol. (F) Topasemiquinone. Details of copper ligation, active site water residues and of reactions steps with oxygen have been omitted.

reasoned above, formation of the topasemiquinone must be preceded by formation of the aminoquinol. Since the kinetic traces show that the 400 nm intermediate is formed before the topasemiquinone (Fig. 5) the reaction sequence is: 400 nm intermediate, aminoquinol, topasemiquinone (Fig. 6). The steady-state turnover time of amine oxidase under conditions of the stopped-flow experiment is about 400–600 ms and because the 400 nm intermediate is formed within 6 ms it represents an early transient reaction intermediate. The time needed for 'internal equilibration', that is to form half of the topasemiquinone from the 400 nm intermediate, is about 430 ms (see Fig. 5) and, therefore, this process includes the major rate determining step(s) in the complete catalytic cycle.

We ascribe the 400 nm intermediate to the so-called product Schiff base, most likely in the protonated form (see Fig. 6). This new assignment is based, inter alia, on the recent chemical synthesis of putative intermediates of the catalytic cycle of amine oxidases [49–51] and the description of a yeast mutant enzyme in which a similar intermediate, derived from methylamine as substrate accumulated [35,52]. Both the absorbance maximum (400–404 nm) and the estimated extinction coefficient (11.0 ± 1 mM^{-1} cm^{-1} estimated from the difference spectrum in Fig. 4A, assuming that it represents 20% of the enzyme concentration, i.e., the difference in semiquinone content at pH 5 and 7.5) are very similar to that of the protonated product Schiff base analogue which has been synthesized recently [51]. The decrease in the equilibrium amount of the 400 nm species with increasing pH suggests that it is a protonated intermediate, but more protonation of more than one acid/base group is probably involved in the determination of its amount. This is in contrast to the topasemiquinone, the amount of which is directly determined by a group with a pK of 9.0. While this might be the pK of the phenolic OH-group of Tyr369, deprotonation of which would lead to destabilization of the topasemiquinone, the participation of another acid/base group or active site water with a similar pK value involved in stabilization of the topasemiquinone cannot be ruled out at present.

In the E406N yeast mutant [35] the unprotonated product Schiff base of methylamine has been proposed to be stabilized. The mechanism of this stabilization involves rotation of the TPQ ring (possible because of the small size of methylamine) to a position away from the mutated active site base (E406 corresponding to D383 in *E. coli*). In native BSAO and AGAO also, the unprotonated product Schiff bases of methylamine have been proposed to be stabilized [34,52]. In contrast, in ECAO the topasemiquinone is stabilized when methylamine is used as substrate [37]. Although all amine oxidases show considerable sequence homology, in particular regarding the active site residues, small differences in for example the pK values of these active site residues may lead to specific stabilization of one or the other intermediate. This may explain why in the one type of amine oxidase formation of the substrate Schiff base is apparent and in the other stabilization of the product Shiff base occurs in the reductive part of the catalytic cycle [40,53–55].

References

[1] E.A. Zeller, Helv. Chim. Acta 21 (1930) 1645–1650.
[2] H. Yamada, K.T. Yasunobu, J. Biol. Chem. 237 (1962) 1511–1516.
[3] B. Mondovi, G. Rotilio, M.T. Costa, A. Finazzi-Agro, E. Chiancone, R.E. Hansen, H. Beinert, J. Biol. Chem. 242 (1967) 1160–1167.
[4] J. Hawkins, Biochem. J. 51 (1952) 399–404.
[5] H. Yamada, K.T. Yasunobu, J. Biol. Chem. 237 (1962) 3077–3082.
[6] S.M. Janes, D. Mu, D. Wemmer, A.J. Smith, S. Kaur, D. Maltby, A.L. Burlingname, J.P. Klinman, Science 248 (1990) 981–987.
[7] J. Van Iersel, R.A. van der Meer, J.A. Duine, Eur. J. Biochem. 161 (1986) 415–419.
[8] W.S. McIntire, C. Hartmann, in: Davidson, V.L. (Ed.), Principles and Applications of Quinoproteins, Dekker, New York, 1993, pp. 97–171.
[9] J.P. Klinman, D. Mu, Rev. Biochem. 63 (1994) 299–344.
[10] P.F. Knowles, D.M. Dooley, in: H. Seigel, (Ed.), Metals in biological systems, New York, NY, 1994, Vol. 30, pp. 361–403.

[11] D.M. Dooley, M.A. McGuirl, D.E. Brown, P.N. Turowski, W.S. McIntire, P.F. Knowles, Nature 349 (1991) 262–264.
[12] K. Warncke, G.T. Babcock, D.M. Dooley, M.A. McGuirl, J. McCracken, J. Am. Chem. Soc. 116 (1994) 4028–4037.
[13] J. McCracken, J. Peisach, C.E. Cote, M.A. McGuirl, D.M. Dooley, J. Am. Chem. Soc. 114 (1992) 3715–3720.
[14] P.N. Turowski, M.A. McGuirl, D.M. Dooley, J. Biol. Chem. 268 (1993) 17680–17682.
[15] F.X. Rius, P.F. Knowles, G. Petterson, Biochem. J. 220 (1984) 767–772.
[16] S.M. Janes, J.P. Klinman, Biochemistry 30 (1991) 4599–4604.
[17] M.R. Parsons, M.A. Convery, C.M. Wilmot, K.D.S. Yadav, A.S. Corner, S.E.V. Philips, M.J. McPherson, P.F. Knowles, Structure 3 (1995) 1171–1184.
[18] C.M. Wilmot, J.M. Murray, G. Alton, M.R. Parsons, M.A. Convery, V. Blakeley, A.S. Corner, M.M. Palcic, P.F. Knowles, M.J. McPherson, S.E.V. Philips, Biochemistry 36 (1997) 1608–1620.
[19] V. Kumar, D.M. Dooley, H.C. Freeman, J.M. Guss, I. Harvey, M.A. McGuirl, M.C.J. Wilce, V.M. Zubak, Structure 4 (1996) 943–955.
[20] M.C.J. Wilce, D.M. Dooley, H.C. Freeman, J.M. Guss, H. Matsunami, W.S. McIntire, C.E. Ruggiero, K. Tanizawa, H. Yamaguchi, Biochemistry 36 (1997) 16116–16133.
[21] R. Barker, N. Boden, G. Caylry, S.C. Charlton, R. Henson, M.C. Holmes, I.D. Kelly, P.F. Knowles, Biochem. J. 177 (1979) 289–302.
[22] R.A. Scott, D.M. Dooley, J. Am. Chem. Soc. 107 (1985) 4348–4350.
[23] D.M. Dooley, M.A. McGuirl, Inorg. Chim. Acta 123 (1986) 231–236.
[24] G.J. Baker, P.F. Knowles, K.B. Pandeya, J.B. Rayner, Biochem. J. 237 (1986) 609–612.
[25] J. McCracken, J. Peisach, D.M. Dooley, J. Am. Chem. Soc. 109 (1987) 4065–4072.
[26] P.F. Knowles, R.W. Strange, N.J. Blackburn, S.S. Hasnain, J. Am. Chem. Soc. 111 (1989) 102–107.
[27] D.M. Dooley, M.A. McGuirl, C.E. Cote, J.L. Bates, J. Am. Chem. Soc. 112 (1990) 2782–2789.
[28] D.M. Dooley, W.S. McIntire, M.A. McGuirl, C.E. Cote, P.F. Knowles, I. Singh, M. Spiller, R.D. Brown III, S.H. Koenig, J. Am. Chem. Soc. 113 (1991) 754–761.
[29] L. Morpurgo, O. Befani, S. Sabatini, B. Mondovi, M. Artico, F. Corelli, S. Massa, G. Stefanchich, L. Avigliano, Biochem. J. 256 (1988) 565–570.
[30] L. Morpurgo, E. Agostinelli, B. Mondovi, L. Avigliano, R. Silvestri, G. Stefanchich, M. Artico, Biochemistry 31 (1992) 2615–2621.
[31] D. De Biase, E. Agostinelli, G. De Matteis, B. Mondovi, L. Morpurgo, Eur. J. Biochem. 237 (1996) 93–99.
[32] R. Matsuzaki, T. Fukui, H. Sato, Y. Ozaki, K. Tanizawa, FEBS Lett. 351 (1994) 360–364.
[33] R. Matsuzaki, S. Suzuki, K. Yamaguchi, T. Fukui, K. Tanizawa, Biochemistry 34 (1995) 4524–4530.
[34] N. Nakamura, R. Matsuzaki, Y.-H. Choi, K. Tanizawa, J. Sanders-Loehr, J. Biol. Chem. 271 (1996) 4718–4724.
[35] D. Cai, J. Dove, N. Nakamura, J. Sanders-Loehr, J.P. Klinman, Biochemistry 36 (1997) 11472–11478.
[36] V. Steinebach, J.A.E. Benen, R. Bader, P.W. Postma, S. de Vries, J.A. Duine, Eur. J. Biochem. 237 (1996) 584–591.
[37] P. Moënne-Loccoz, N. Nakamura, V. Steinebach, J.A. Duine, M. Mure, J.P. Klinman, J.A. Sanders-Loehr, Biochemistry 34 (1995) 7020–7026.
[38] V. Steinebach, B.W. Groen, S.S. Wijmenga, W.M.A. Niessen, J.A. Jongejan, J.A. Duine, Anal. Biochem. 230 (1995) 159–166.
[39] V. Steinebach, in: Structural and mechanistic aspects of copper-quinone amine oxidase from *Escherichia coli*, PhD Thesis Delft University of Technology, 1995, ISBN 90-9009005-3.
[40] V. Steinebach, S. de Vries, J.A. Duine, J. Biol. Chem. 271 (1996) 5580–5588.
[41] V. Steinebach, G.A.H. de Jong, S.S. Wijmenga, S. de Vries, J.A. Duine, Eur. J. Biochem. 238 (1996) 683–689.
[42] D. Collison, P.F. Knowles, F.E. Mabbs, F.X. Rius, I. Singh, D.M. Dooley, C.E. Cote, M. McGuirl, Biochem. J. 264 (1989) 663–669.
[43] R.C. Sealy, L. Harman, P.R. West, R.P. Mason, J. Am. Chem. Soc. 107 (1985) 3401–3406.
[44] P.J. O'Malley, G.T. Babcock, J. Am. Chem. Soc. 108 (1986) 3995–4001.
[45] B.S. Prabhanada, J. Phys. Chem. 79 (1983) 5752–5757.
[46] C.C. Felix, B.S. Prabhanada, J. Phys. Chem. 80 (1984) 3078–3081.
[47] C.W. Hoganson, G.T. Babcock, Biochemistry 31 (1992) 11874–11880.
[48] G.J. Gerfen, B.F. Bellew, S. Un, J.M. Bollinger Jr., J. Stubbe, R.G. Griffin, D.J. Singel, J. Am. Chem. Soc. 115 (1993) 6420–6421.
[49] M. Mure, J.P. Klinman, J. Am. Chem. Soc. 115 (1993) 7117–7127.
[50] M. Mure, J.P. Klinman, J. Am. Chem. Soc. 117 (1995) 8698–8706.
[51] M. Mure, J.P. Klinman, J. Am. Chem. Soc. 117 (1995) 8707–8718.
[52] N. Nakamura, P. Moënne-Loccoz, K. Tanizawa, M. Mure, S. Suzuki, J.P. Klinman, J. Sanders-Loehr, Biochemistry 36 (1997) 11479–11486.
[53] A. Bellelli, A. Finazzi-Agrò, G. Floris, M. Brunori, J. Biol. Chem. 266 (1991) 20654–20657.
[54] C. Hartmann, P. Brzovic, J.P. Klinman, Biochemistry 32 (1993) 2234–2241.
[55] R. Medda, A. Padiglia, J.Z. Pedersen, G. Rotillio, A. Finazzi-Agrò, G. Floris, Biochemistry 34 (1995) 16375–16381.