PERGAMON

Phytochemistry 51 (1999) 175–186

PHYTOCHEMISTRY

Aerobic oxidation of indole-3-acetic acid catalysed by anionic and cationic peanut peroxidase

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Received 6 October 1998; received in revised form 27 November 1998

Abstract

The catalytic properties of anionic and cationic peanut peroxidases with regards to the oxidation of indole-3-acetic acid (IAA) by molecular oxygen at low pH have been studied. Transient kinetic studies demonstrate that only cationic peroxidases (peanut and horseradish) but not anionic peroxidases (such as anionic tobacco and anionic peanut peroxidases) form a stable compound III in the course of IAA oxidation. The failure to observe inhibition in the presence of superoxide dismutase is consistent with the formation of compound III from a ternary complex comprising ferric enzyme, IAA and dioxygen at the initiation step. Product analysis by HPLC showed an enhanced rate of IAA oxidation in the presence of superoxide dismutase. Co-addition of superoxide dismutase and catalase demonstrates that this stimulation is not due to the formation of hydrogen peroxide. The correlation between initial rates of IAA degradation and product accumulation indicates that skatole hydroperoxide is a primary reaction product and indole-3-methanol is the product of its subsequent enzymatic reduction. The relative catalytic activities for IAA oxidation by tobacco:horseradish isoenzyme c:anionic peanut:cationic peanut peroxidase are 28:20:2:1.

Keywords: HPLC studies; Anaerobic stopped-flow kinetics; Reaction mechanism; Compound III; Catalase; Superoxide dismutase; Tobacco peroxidase; Horseradish peroxidase; Skatole hydroperoxide; Indole-aldehyde; Indole-methanol

1. Introduction

Indole-3-acetic acid (IAA) is a powerful plant hormone controlling plant growth and development at in vivo concentrations below 10^{-8} M. The regulatory relationship between IAA levels and plant peroxidase activities (EC 1.11.1.7), that include lignification (Goldberg, Le, & Catesson, 1985), cell differentiation (Fukuda, & Kokamine, 1982), plant stress (Mehlhorn, 1990) and pathogen response (Graham, & Graham, 1991), is still not clear after over 40 years of investigation.

Recent studies with transgenic tobacco plants which were either constructed to overproduce the anionic peroxidase under the control of a strong constitutive promoter or silenced in the expression of this peroxidase with antisense RNA have supported a role for peroxidases in the metabolism of IAA *in planta* (Lagrimini, Bradford, & Rothstein, 1990; Lagrimini, Joly, Dunlap, & Liu, 1997; Lagrimini, Gingas, Finger, Rothstein, & Liu, 1997). Numerous phenotypic changes were observed in the growth and development of these transgenic plants which could be explained by changes in IAA metabolism. More recently, the measurement by GC-MS of the steady-state levels of IAA in these transgenic plants revealed a close correlation between endogenous anionic peroxidase activity

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0031-9422/99/$ - see front matter
PII: S0031-9422(98)00758-4

and the accumulation of IAA in plant tissues (Wang and Lagrimini, unpublished results). Taken together, these data indicate that peroxidases may play an important role in the normal metabolism of IAA and hence plant growth. Clearly it is important to determine a mechanism of IAA degradation in vitro that can account for the metabolites found in plants. A recent study by Hu and Dryhurst showed that horseradish peroxidase catalysed oxidation of IAA gave oxindole-3-acetate which is a major IAA metabolite found in plants (Hu, & Dryhurst, 1997). A detailed mechanism of peroxidase catalysed oxidation of IAA (Krylov, & Dunford, 1996) has recently been extended by us following our identification and isolation of skatole hydroperoxide as a reaction intermediate (Gazarian et al., 1998). Unfortunately, under the separation conditions used we could not resolve oxindole-3-acetic acid from oxindole-3-carbinol. However, indole-3-methanol and indole-3-aldehyde, which are known to be IAA degradation products *in planta,* were identified as the main degradation products in vitro (Gazarian et al., 1998). The intermediate skatole hydroperoxide only yielded oxindole-3-carbinol and subsequently 3-methylene-oxindole (products not found *in planta*) after IAA exhaustion and/or in the absence of potential peroxidase electron donor substrates (Gazarian et al., 1998). However, in the presence of reducing substrates (e.g. phenol or ascorbate), skatole hydroperoxide was quantitatively converted by peroxidase to indole-3-methanol (Gazarian et al., 1998). Thus, the difference in IAA degradation products in vitro from those *in planta* is explained by the absence of peroxidase-reducing substrates in in vitro assays. These data together with our previous anaerobic spectrophotometric kinetic studies (Gazaryan, Lagrimini, Ashby, & Thorneley, 1996; Gazarian, & Lagrimini, 1998) give confidence in our proposed mechanism for the peroxidase catalysed removal of IAA *in planta* (Eqs. (1)–(9)). The reaction cycle is initiated via the formation of a ternary complex between a haem-containing peroxidase, IAA and oxygen. This complex yields an IAA cation radical and a superoxide radical. These intermediates remain bound close to the active site of horseradish peroxidase C (HRP-C) and react by Eqs. (1) and (2) to form compound III (Gazarian et al., 1998):

$$E + IAA \leftrightarrow [E\text{–}IAA] + O_2 \leftrightarrow [E\text{–}IAA\text{–}O_2] \quad (1)$$

$$[E\text{–}IAA\text{–}O_2] \leftrightarrow E\text{–}O_2^{\cdot -} + IAA^{\cdot +} \quad (2)$$

where E is ferric HRP-C, $E\text{–}O_2^{\cdot -}$ is its compound III, and $IAA^{\cdot +}$ is IAA cation radical.

The IAA cation radical decarboxylates yielding skatole radical Eq. (3) which reacts with dioxygen Eq. (4) and is subsequently converted by reaction with a second molecule of IAA into skatole hydroperoxide Eq. (5):

$$IAA^{\cdot +} \longrightarrow InCH_2^{\cdot} + CO_2 \quad (3)$$

$$InCH_2^{\cdot} + O_2 \longrightarrow InCH_2O_2^{\cdot} \quad (4)$$

$$InCH_2O_2^{\cdot} + IAA \longrightarrow InCH_2OOH + IAA^{\cdot} \quad (5)$$

where $InCH_2^{\cdot}$, $InCH_2O_2^{\cdot}$ and $InCH_2OOH$ are skatole radical, skatole peroxy radical and skatole hydroperoxide, respectively. Skatole hydroperoxide switches on the peroxidase cycle generating two IAA radicals for each molecule of skatole hydroperoxide consumed (Eqs. (6)–(8)).

$$E + InCH_2OOH \longrightarrow EI + ROH \quad (6)$$

$$EI + IAA \longrightarrow EII + IAA^{\cdot} \quad (7)$$

$$EII + IAA + H^+ \longrightarrow E + IAA^{\cdot} + H_2O \quad (8)$$

where EI and EII are peroxidase compounds I and II, respectively. Two-electron sequential reduction of HRP-C compound III with IAA yielding compound II first described in (Ricard, & Job, 1974) was rationalised as follows Eq. (9):

$$E\text{–}O_2^{\cdot -} + 2IAA \longrightarrow EII + 2IAA^{\cdot} + H_2O. \quad (9)$$

The principal difference between tobacco peroxidase (TOP) and HRP-C is the relative amount and reactivity of their compound III forms with respect to IAA. This determines the extent of the second route to compound II Eq. (9) thereby making the oxygenase cycle independent of the peroxidase cycle and insensitive to catalase. The purpose of the present study was to extend our understanding of the mechanism of IAA oxidation and to clarify the role of compound III in the oxygenase cycle using anionic and cationic peanut peroxidases (PNP-A and PNP-C, respectively).

2. Results and discussion

2.1. Transient kinetic studies

Peanut anionic peroxidase (PNP-A) behaved in a similar manner to tobacco anionic peroxidase during indole-3-acetic acid oxidation (Gazaryan et al., 1996). Fig. 1 shows the traces obtained when PNP-A was mixed with an IAA-oxygen mixture in the stopped-flow spectrophotometer. Ferric enzyme was clearly present during the phase when oxygen was being consumed with ferrous enzyme only appearing on

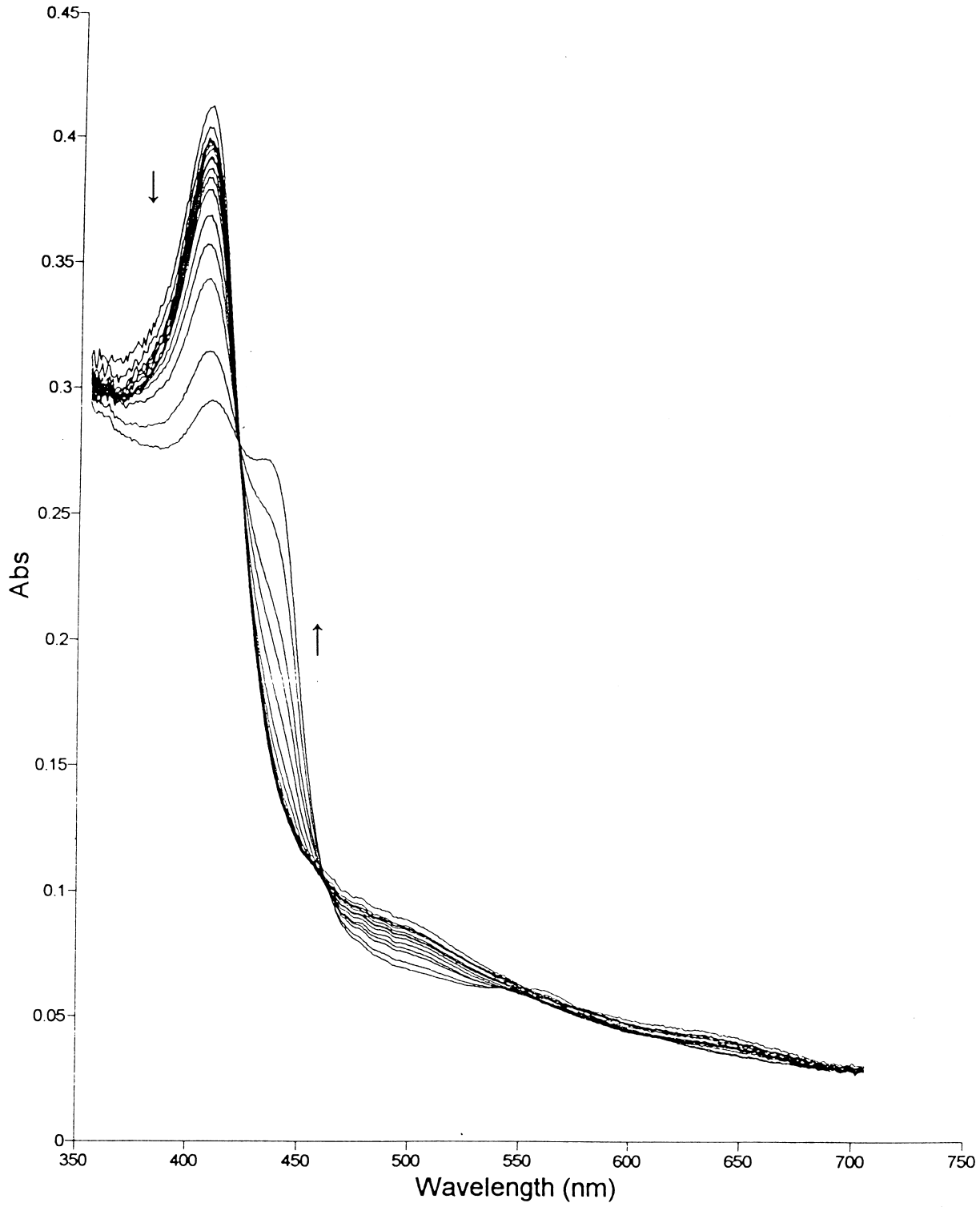

Fig. 1. Spectral changes occurring in the course of the reaction of 1 mM IAA with 4 μM peanut anionic peroxidase (PNP-A) in O_2-containing buffer (62.5 μM), 0.1 M Na–acetate buffer, pH 5.0, 22°. (A) 200 scans recorded in 600 s, with every tenth spectrum shown. The arrows indicate the direction of change in absorbance with time.

exhaustion of the oxygen. This contrasts to the traces shown in Fig. 2A for the same experiment with peanut cationic peroxidase (PNP-C), when compound III was clearly detected as an intermediate. The isosbestic point at 408 nm, diagnostic of the direct conversion of ferric enzyme to compound III, is clearly defined.

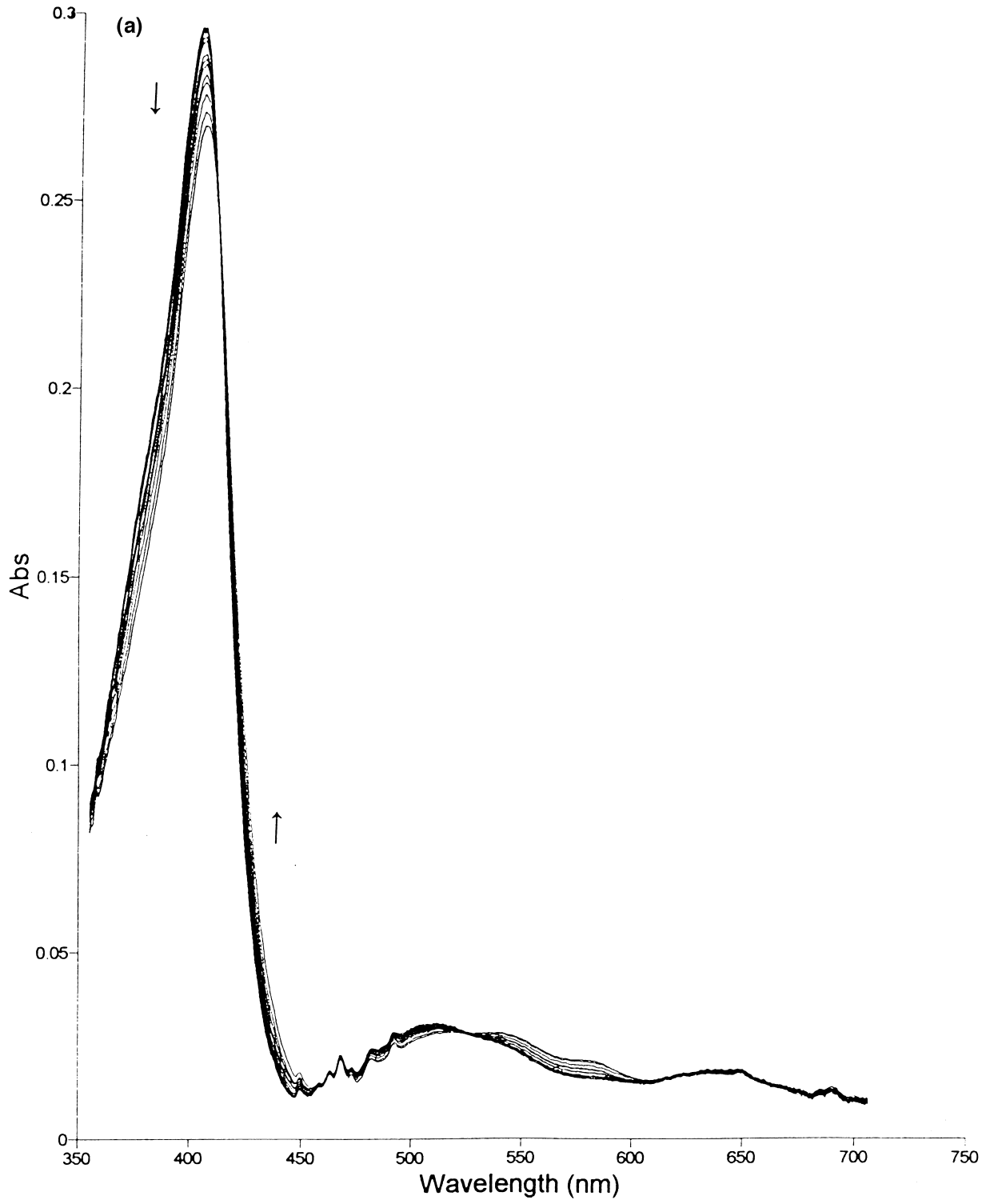

Fig. 2. Spectral changes occurring in the course of the reaction of 1 mM IAA with 4 μM peanut cationic peroxidase (PNP-C) in O_2-containing buffer (62.5 μM). 0.1 M Na–acetate buffer, pH 5.0 22°. (A) 200 scans recorded in 100 s, and (B) 200 scans in 500 s, with every tenth scan shown. The arrows indicate the direction of change in absorbance with time.

PNP-C also differs from HRP-C where the absence of a clearly defined isosbestic point was ascribed to the formation of both compounds II and III (Gazaryan et al., 1996). After oxygen exhaustion, compound III was converted back to ferrous enzyme (Fig. 2B). These data unequivocally confirm that compound III is

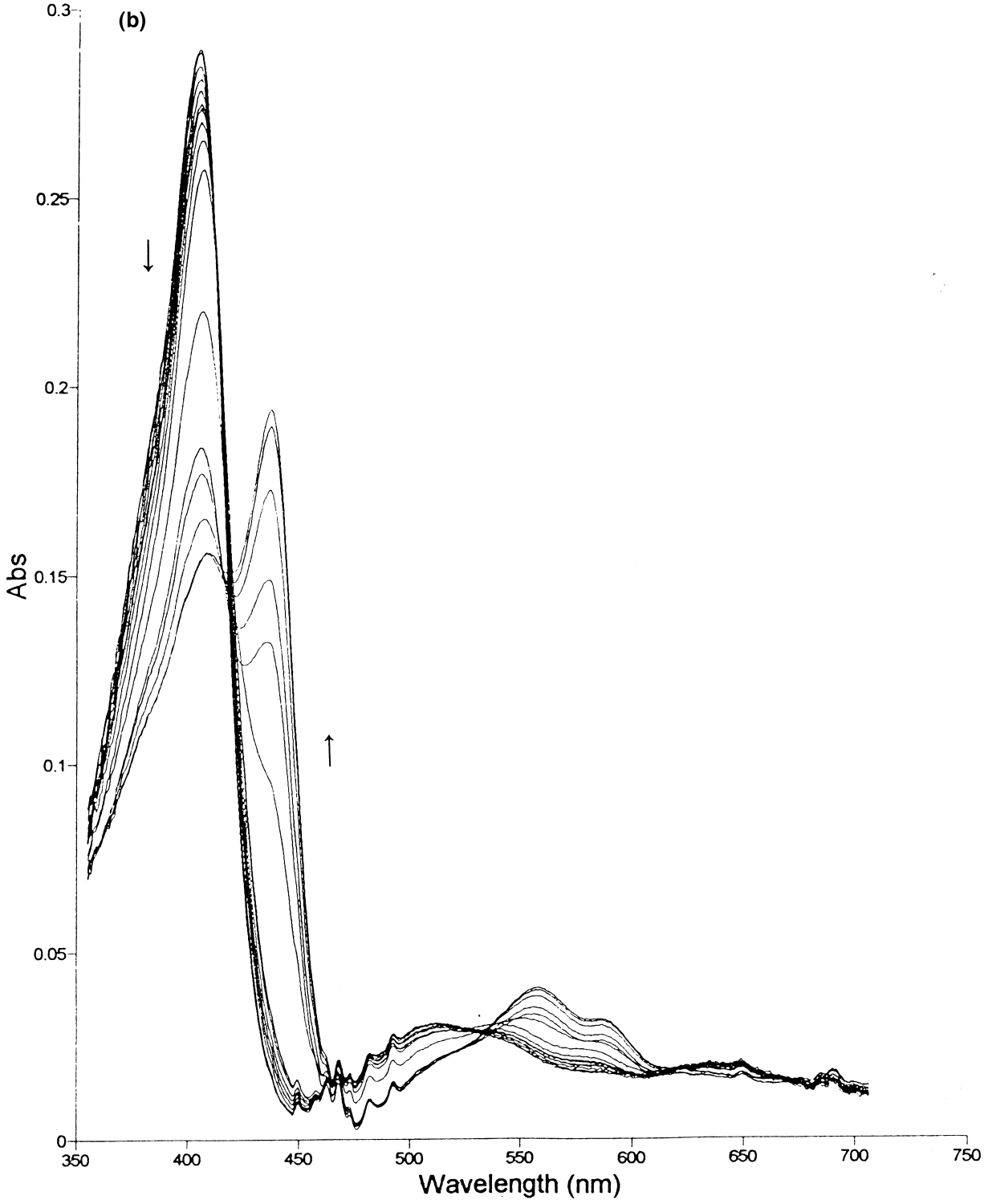

Fig 2 (*continued*)

formed directly from ferric enzyme with Eqs. (2) and (10) representing two possible mechanisms. Eq. (10) shows the production of a protonated superoxide radical via the disproportionation of a skatole peroxy radical. The superoxide anion would then react with ferric enzyme to form compound III Eq. (11).

$$\mathrm{InCH_2O_2^{\cdot}} \longrightarrow \text{methylene–3–indolenine} + \mathrm{HO_2^{\cdot}} \quad (10)$$

$$\mathrm{E} + \mathrm{O_2^{\cdot -}} \longrightarrow \mathrm{EO_2^{\cdot -}}. \quad (11)$$

The observation that cationic peroxidases, but not anionic peroxidases such as TOP and PNP-A, form stable compound III in the course of IAA oxidation, may be related directly to the difference in p*I* values. A possible explanation is the inherent instability of the compound III forms of anionic plant peroxidases when produced in the absence of added hydrogen peroxide. There are no published data on stability of compound III of anionic peroxidases. We have been unable to prepare TOP compound III by mixing ferrous enzyme with oxygen in the stopped-flow rapid scan spectrophotometer (Gazaryan and Thorneley, unpublished) although similar experiments with wild-type and recombinant HRP variants have previously allowed the rate constants for compound III formation and subsequent decay to be determined (Rodriguez-Lopez, Smith, & Thorneley, 1997).

It is also possible that the formation of compound III by the reaction of ferric enzyme with superoxide radical anion Eq. (11), could be inhibited by the overall negative charge of anionic peroxidases. However, local charge distribution at the entrance to, or within, the distal haem cavity are likely to be more important that the overall charge of the protein. Compound III by this mechanism Eq. (11) should be inhibited by superoxide dismutase. This has not been observed in stopped-flow experiments with either HRP-C (data not shown) or peanut cationic peroxidase (Fig. 3). The absence of any inhibition by SOD favours superoxide anion radical formation at the enzyme active site Eq. (2) since it is unlikely that cationic peroxidases could compete effectively for free superoxide radical anion with SOD which reacts at close to the diffusion limit. This is consistent with the established formation of compound III at the initiation step of IAA oxidation catalysed by peanut and horseradish cationic peroxidases (Gazaryan et al., 1996). The reactivities and stabilities of the compound III forms of the various peroxidases are predicted to be the major determinants of mechanism by which IAA is oxidised. IAA oxidation studies with HRP-C variants with altered compound III properties will be useful in providing further evidence for this hypothesis.

2.2. HPLC studies

Anionic peanut peroxidase was more active than the cationic isozyme in the IAA degradation assay although the former does not form compound III. The nature of the products formed from IAA (indole-3-methanol, indole-3-aldehyde, skatole hydroperoxide, oxindole-3-carbinol, 3-methylene-oxindole) were similar and independent of the enzyme used (PNP-A, PNP-C, HRP-C or TOP). The effect of catalase on the PNP-C and PNP-A-catalysed reactions was similar to that for HRP-C and TOP (Gazarian et al., 1998), respectively. The PNP-C catalysed process was insensitive to the addition of catalase while the PNP-A-catalysed process was inhibited (data not shown).

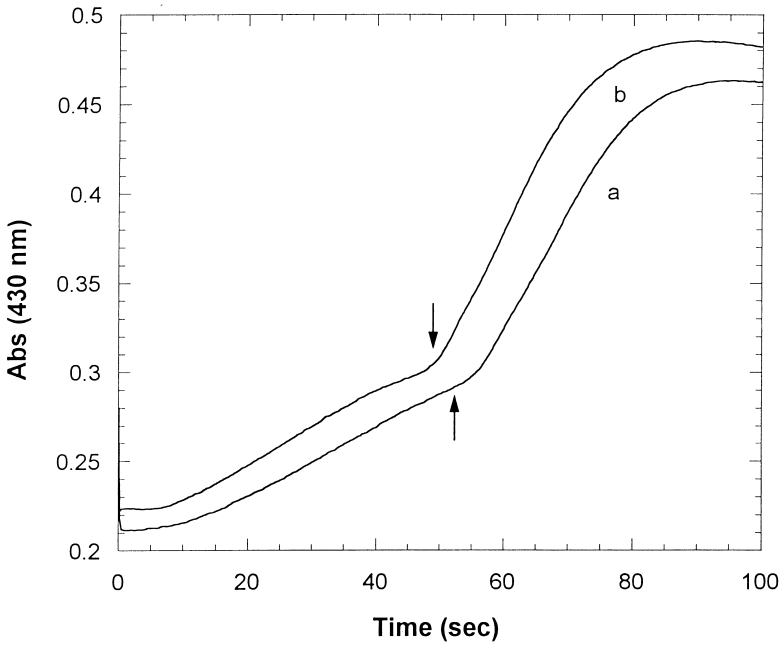

Fig. 3. The effect of superoxide dismutase (SOD) on the transient kinetics of IAA oxidation by molecular dioxygen catalysed by cationic peanut peroxidase (PNP-C) in 0.1 M Na–acetate buffer, pH 5.0, 22°. Traces recorded at 430 nm. (A) no additives; (B) 3 μM SOD. Reagent concentrations: 8 μM PNP-C, 5 mM IAA and 62.5 μM O_2. The enzyme was shot against IAA-O_2 mixture, SOD was subsequently added to the IAA/O_2 solution. The arrows indicate the time at which ferrous enzyme begins to appear which corresponds to dioxygen exhaustion.

Comparisons of the catalytic activities of isoenzymes are usually based on their specific activities. However, this approach assumes a linear dependence of the catalytic rate on the enzyme concentration. In the case of the peroxidase catalysed degradation of IAA, the comparison is complicated by the occurrence of both enzymatic reactions (Eqs. (1), (2) and (6)–(8)) and non-enzymatic radical reactions (Eqs. (3)–(5)). The situation is illustrated in Fig. 4 with a pronounced effect of PNP-C concentration on the composition and rates of production of IAA degradation products. The rate of IAA degradation depends linearly on the enzyme concentration only at comparatively high concentrations of PNP-C (>0.4 μM) when no lag-period can be detected under the experimental conditions (rates of 0.2, 2 and 6.5 μM/min per 0.07, 0.40 and 1.4 μM PNP-C, respectively, Fig. 4A). At low enzyme concentrations (<0.4 μM) the initial rate of skatole hydroperoxide production (0.2 μM/min, Fig. 4B) is equal to that of IAA degradation (Fig. 4A) but the final enzymatic conversion to indole-3-methanol is only 5% compared to 20% at the higher enzyme concentrations (Fig. 4D). The products of the non-enzymatic degradation of skatole hydroperoxide, e.g. oxindole-3-carbinol and 3-methylene-oxindole, make a major contribution to the IAA degradation products at low enzyme concentrations. At high enzyme concentrations (0.4 and 1.4 μM), the rates of skatole hydroperoxide degradation (1.6 and 5.0 μM/min, respectively) and indole-3-methanol production (0.4 and 1.3 μM/min, respectively) are proportional to the enzyme concentration. We conclude that skatole hydroperoxide is the primary reaction product and it is subsequently enzymatically reduced to indole-3-methanol.

Comparison of the rates of indole-3-aldehyde production (Fig. 4C) with those for skatole hydroperoxide (Fig. 4B) and indole-3-methanol production (Fig. 4D) demonstrate that the overall contribution of the indole-3-aldehyde production route increased with increasing enzyme concentration. Therefore, indole-3-aldehyde is unlikely to be a degradation product of skatole hydroperoxide. Indole-3-aldehyde production is thought to be catalysed by ferrous enzyme, however, the mechanism is still unclear (Gazarian et al., 1998).

Since the rate of formation and relative amounts of IAA degradation products depend on enzyme concentration, we have chosen to compare the catalytic activities of different peroxidases at those enzyme concentrations that yield equal rates of IAA degradation. Both isozymes of peanut peroxidase were less active than either TOP or HRP-C. The initial rates of IAA degradation were equal to 7.5 μM/min as catalysed by 1.4 μM PNP-C, 0.7 μM PNP-A, 0.05 μM TOP and 0.07 μM HRP-C under the above experimental conditions. Thus, the absence of compound III as a detectable intermediate does not necessarily mean a lower activity for a given peroxidase catalysing the oxygenase reaction. The detection of compound III, although indicative of a particular reaction mechanism (as defined by its sensitivity to catalase), does not necessarily mean a higher rate of IAA oxidative degradation. The low catalytic activity of PNP-C in the oxygenase reaction and the clear detection of compound III in the rapid scan transient kinetic experiments can be rationalised by consideration of the recently solved crystal structures for PNP-C (Schuller, Ban, van Huystee, McPherson, & Poulos, 1996) and HRP-C (Gajhede, Schuller, Henriksen, Smith, & Poulos, 1997). Although the structural elements of PNP-C and HRP-C are essentially identical the loop insertion between helixes F and G that is characteristic of class III peroxidases is shorter in PNP-C (27 residues) than in HRP-C (34 residues). This loop region is also highly variable amongst class III peroxidases and defines part of the substrate access channel to the haem edge and is thought to be at least in part responsible for substrate specificity. In the case of HRP-C, this insertion contains Phe179 which is involved in binding aromatic substrates (Smith, & Veitch, 1998). In addition, PNP-C has Gly at this position with the Phe shifted by one residue. PNP-C has a glycosylation site at Asn60 located at the exposed end of the loop while the nearest potential glycosylation site in HRP-C is Asn 57 positioned to one side of the loop. These structural differences may decrease the accessibility to the haem-binding pocket of PNP-C for skatole hydroperoxide and therefore favour compound III over compound I and II formation at the initiation step. Compound III although more easily formed, is less active than compound II, resulting in a lower catalytic activity for PNP-C in the oxidative degradation of IAA.

In order to understand in more detail the mechanism of compound III formation, we studied the effect of SOD in the presence and absence of catalase on the kinetics and product distribution during IAA degradation (Fig. 5). Any rate enhancement observed in the presence of SOD resulting from hydrogen peroxide production formed by superoxide radical dismutation, should be decreased by the co-addition of catalase and SOD. An enhanced rate of IAA degradation was still observed in the presence of SOD and catalase Fig. 5. This observation is consistent with our proposal that a ternary complex involving peroxidase, IAA and oxygen is initially formed that subsequently releases superoxide anion and IAA cation radicals (Eqs. (1) and (2)). In control experiments 1 h incubation with SOD resulted in the appearance of traces ($<0.1\%$) of oxindole-3-carbinol and indole-3-aldehyde (not shown).

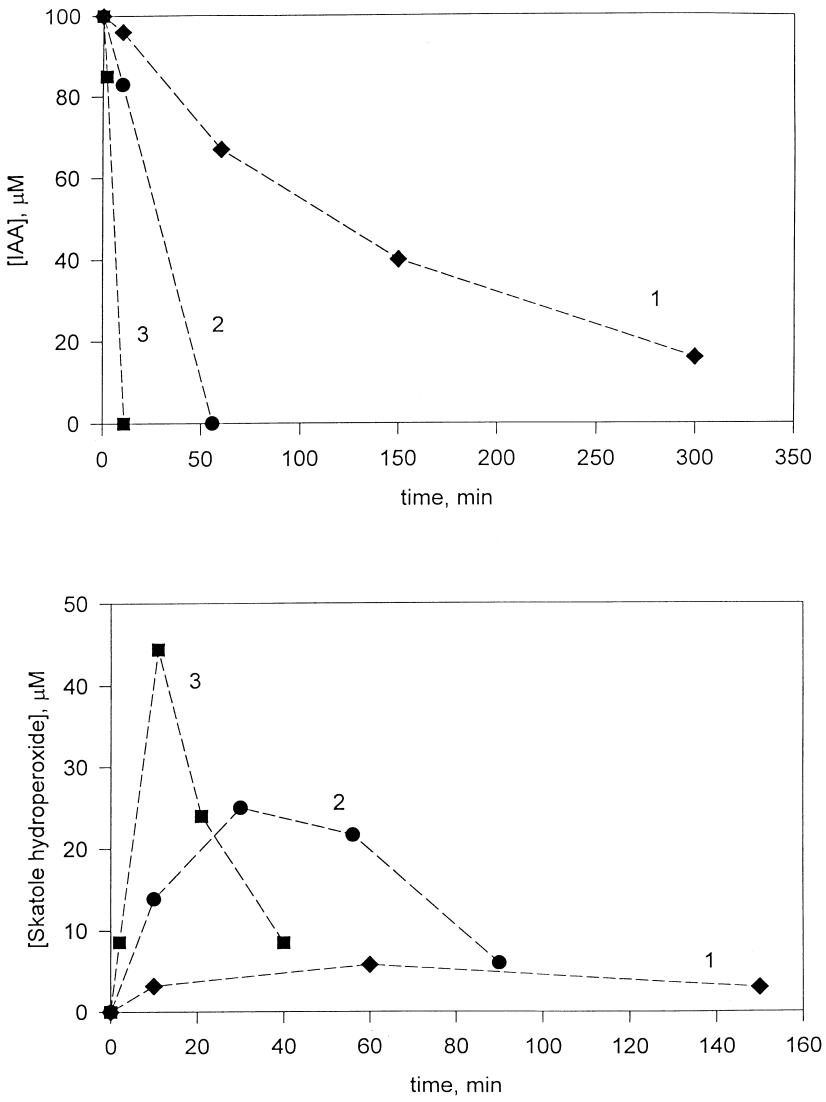

Fig. 4. The effect of PNP-C concentration on the kinetics of: (A) IAA degradation; (B) skatole hydroperoxide formation; (C) indole-3-aldehyde formation; (D) indole-3-methanol formation. All the reactions were in 0.1 M Na–acetate buffer, pH 5.0 with PNP-C concentrations of **1**, 0.07 μM; **2**, 0.4 μM; **3**, 1.4 μM.

3. Conclusions

The in vitro study of novel plant peroxidase structure–function relationships is contributing to our understanding at atomic resolution of a physiological reaction catalysed by plant peroxidases, the oxidation of the plant growth hormone indole-3-acetic acid by dioxygen. The data obtained in this study are consistent with the proposal that compound III formation occurs at the initiation stage. The insensitivity of the oxygenase reaction to catalase is due to compound II formation by the reduction of compound III which provides an alternative route into the common peroxidase cycle. Transient compound III formation at detectable concentrations appears to be a characteristic of cationic peroxidases. However, its detection cannot be used as a diagnostic of a high activity for a particular peroxidase in the oxygenase reaction with IAA e.g. anionic peanut peroxidase is twice as active in catalysing IAA oxidative degradation than the cationic isozyme. This finding is consistent with the power reactivity of PNP-C (K_m = 0.8 μM, V_m = 0.4 μg/min per mg protein) toward IAA in a common peroxidase cycle initiated by hydrogen peroxide compared to PNP-A (K_m = 0.2 μM and V_m = 1.6 μg/min per mg protein) (Zheng, & van Huystee, 1992). However, both of the peanut isozymes studied are poor catalysts of the reaction compared to anionic tobacco and cationic horseradish peroxidase.

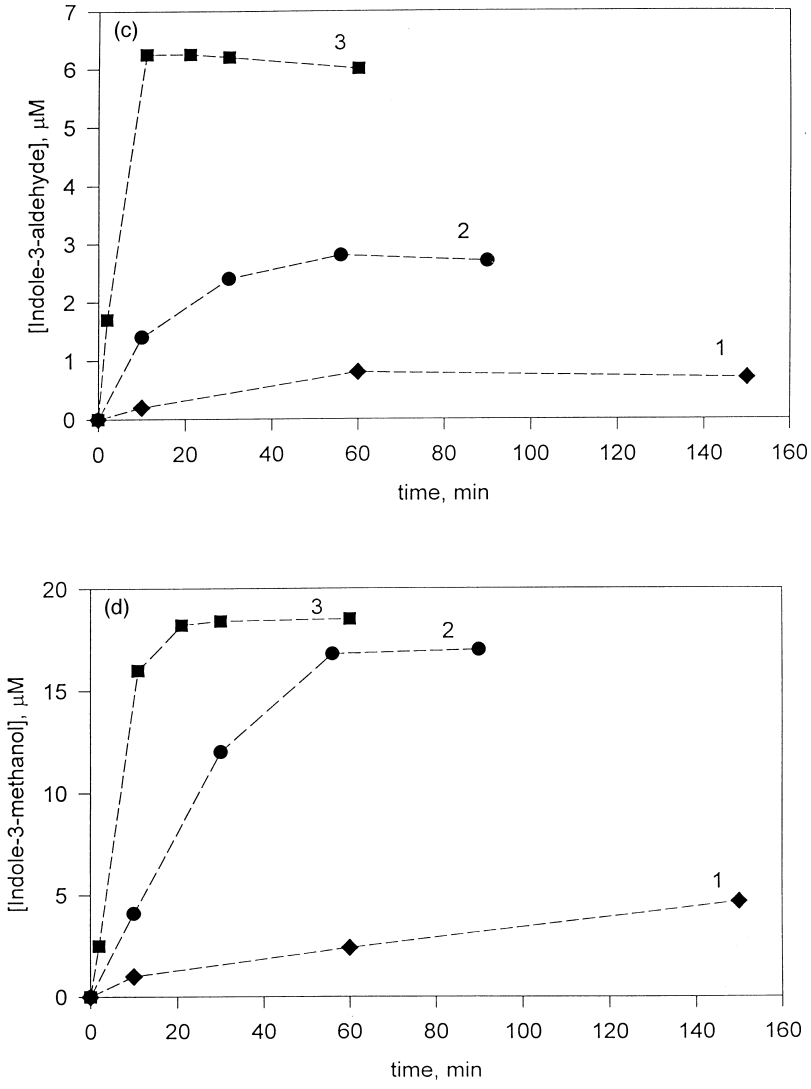

Fig 4 (*continued*)

4. Experimental

4.1. Chemicals

Indole-3-acetic acid (IAA) was purchased from Sigma (Poole, Dorset, UK), indole-3-methanol and indole-3-aldehyde were from Aldrich (Gillingham, Dorset, UK), salts were from BDH Merck (Poole, Dorset, UK). All solns were made up with Milli Q water.

4.2. Enzymes

HRP-C (RZ 3.0) was purchased from Biozyme Ltd. (Blaenavon, Gwent, UK) and used without further purification. The concentration of HRP-C was determined spectrophotometrically ($\varepsilon_{403\ nm}$ = 102 mM^{-1} cm^{-1}) (Ohlsson, & Paul, 1976). Cationic and anionic peanut peroxidases (RZ 3.0) were purified as described previously (Chibbar, & van Huystee, 1984; Sesto, Chibbar, & van Huystee, 1989). Anionic tobacco peroxidase (RZ 3.5) was isolated from leaves of *Nicotiana sylvestris* plants overexpressing the enzyme (Gazaryan, & Lagrimini, 1996). Catalase with a specific activity of 2800 U mg^{-1} solid and bovine superoxide dismutase (SOD) with a specific activity of 3,000 U mg^{-1} protein were purchased from Sigma (USA) and used without further purification (units of activity and assay conditions as described by the supplier).

4.3. Transient kinetic studies

Anaerobic stopped-flow studies were performed using a Hi-Tech SF-61 DX-2 stopped-flow rapid-scan spectrophotometer (Salisbury, UK) in both rapid-scan and single wavelength modes. The latter was used to

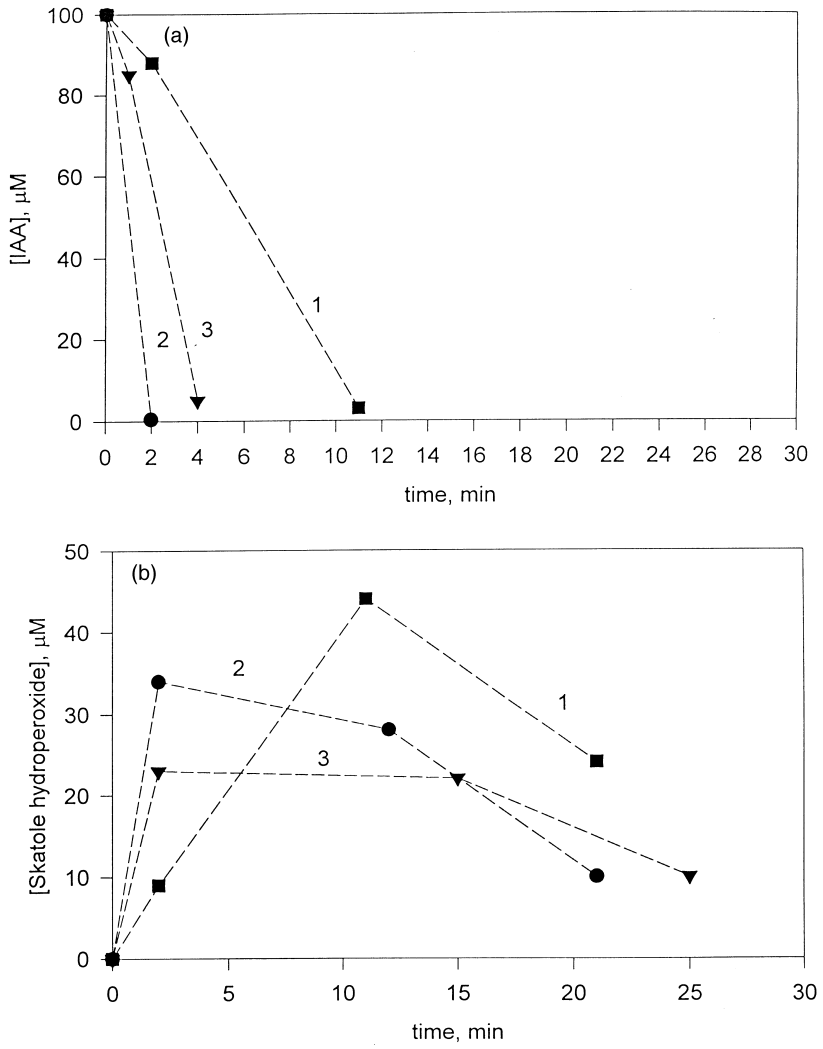

Fig. 5. Effect of superoxide dismutase (SOD, 0.3 μM) and/or catalase (0.05 mg/ml) on the PNP-C (1.4 μM) catalysed kinetics of: (A) IAA degradation; (B) skatole hydroperoxide formation; (C) indole-3-methanol formation; (D) indole-3-aldehyde formation. **1**. No additives; **2**, addition of SOD; **3**, co-addition of catalase and SOD.

study the effect of SOD on transient kinetics followed at 430 nm (the isosbestic point between the native enzyme and compound I). The stopped-flow apparatus was installed in an anaerobic glove box operating under N_2 with less than 1 ppm of O_2. Temperature was controlled at 25° using a Techne-400 circulating bath with a heater–cooler also installed in the anaerobic box. Lyophilised enzymes and solid substrates were placed into the hermetically sealed serum vials and deoxygenated for 1 h before being placed into the glove box. Tris–HCl buffer (0.1 M, pH 8.7) or Na–acetate buffer (0.1 pH 5.0), was used in all the experiments after deoxygenation by overnight sparging with N_2 in the glove box. IAA stock soln (50 mM) and enzyme solns were prepared anaerobically under N_2 in 0.1 M Tris–HCl buffer (0.1 M, pH 8.7), and Na–acetate buffer (0.1 M, pH 5.0), respectively. Oxygen-saturated (1.25 mM) Na–acetate buffer solns (0.1 M, pH 5.0), were transferred into the anaerobic glove box in hermetically sealed serum vials.

HRP-C (PNP) in O_2-containing buffer (syringe A) was mixed in the stopped-flow apparatus with IAA (syringe B). SOD (3 μM) was subsequently added to syringe A.

4.4. HPLC studies

Peanut peroxidases (0.07–1.4 μM) were incubated with IAA (100 μM) in Na–acetate buffer (0.1 M, pH 5.0) at 25° for periods up to 6 h. The reaction was initiated by enzyme addition. The effect of varying the concentration of PNP-C over the range 0.07–1.4 μM on the composition of the IAA degradation products was determined. The effect of SOD (0.3 μM) and catalase (0.05 mg/ml) on IAA degradation kinetics was studied with 1.4 μM PNP-C. SOD and/or catalase were

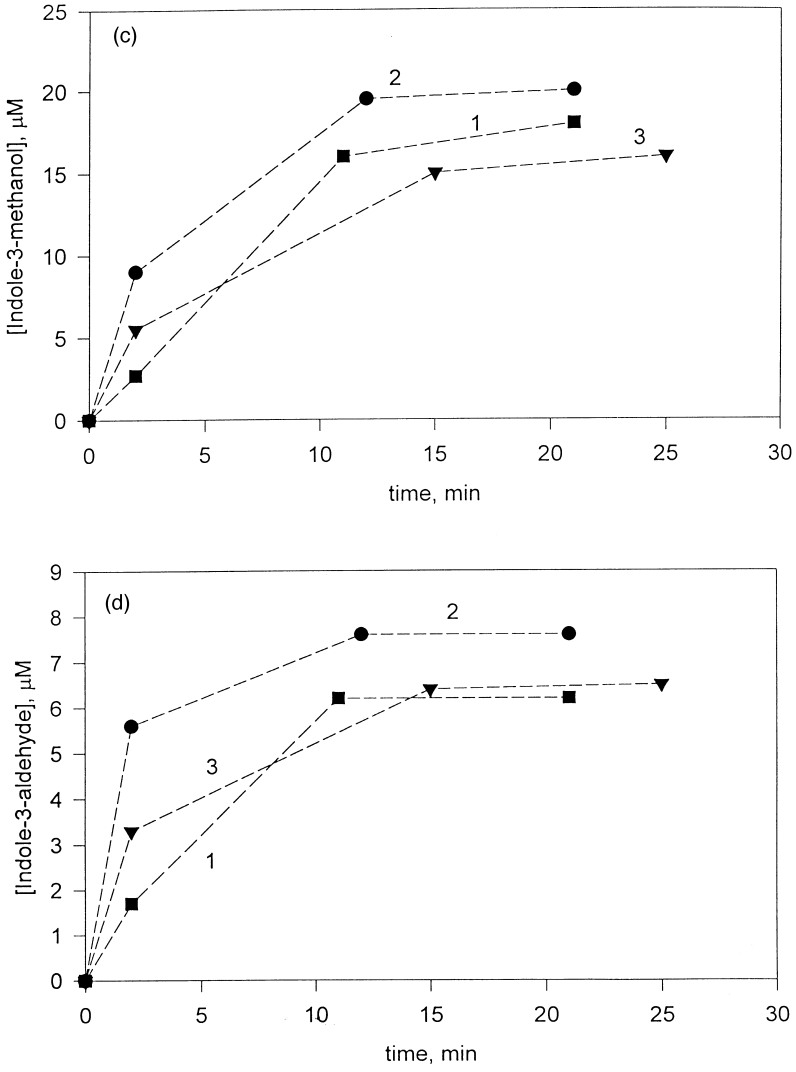

Fig 5 (*continued*)

added before the initiation of the reaction. After various times of incubation, 200 μl aliquots of the reacting mixture were taken and analysed using a Shimadzu LC-5A HPLC instrument by reverse phase chromatography on a C18 Columbus column, 300 A, 5 μM, 150 × 4.6 mm (Phenomenex, USA) using isocratic elution in a MeOH–1% HoAc mixture (40:60 v/v) at a flow rate 1.0 ml/min with absorbance monitoring at 250 nm. The incubation experiments were performed in triplicate. The concentrations of IAA and its degradation products were determined as described in (Gazarian et al., 1998).

References

Chibbar, R. N., & van Huystee, R. B. (1984). *Plant Physiology*, *75,* 956.

Fukuda, H., & Kokamine, A. (1982). *Planta*, *155,* 423.

Gajhede, M., Schuller, D. J., Henriksen, A., Smith, A. T., & Poulos, T. P. (1997). *Nature Structural Biology*, *4,* 1032.

Gazarian, I. G., & Lagrimini, L. M. (1998). *Biophys. Chem.*, *72,* 231.

Gazarian, I. G., Lagrimini, L. M., Mellon, F. A., Naldrett, M. J., Ashby, G. A., & Thorneley, R. N. F. (1998). *Biochem J.*, *333,* 223.

Gazaryan, I. G., & Lagrimini, L. M. (1996). *Phytochemistry*, *41,* 1029.

Gazaryan, I. G., Lagrimini, L. M., Ashby, G. A., & Thorneley, R. N. F. (1996). *Biochem J.*, *313,* 841.

Goldberg, R., Le, T., & Catesson, A. M. (1985). *J. Exptl. Bot.*, *36,* 503.

Graham, M. Y., & Graham, T. L. (1991). *Plant Physiol.*, *97,* 1445.

Hu, T., & Dryhurst, G. (1997). *J. Electroanalytical Chemistry*, *432,* 7.

Krylov, S. H., & Dunford, H. B. (1996). *J. Phys. Chem.*, *100,* 913.

Lagrimini, L. M., Bradford, S., & Rothstein, S. (1990). *Plant Cell*, *2,* 7.

Lagrimini, L. M., Joly, R. J., Dunlap, J. R., & Liu, T. -T. Y. (1997). *Plant Mol. Biol.*, *33,* 887.

Lagrimini, L. M., Gingas, V., Finger, F., Rothstein, S., & Liu, T. Y. (1997). *Plant Physiol*., *114,* 1187.
Mehlhorn, H. (1990). *Plant Cell Environ*., *13,* 971.
Ohlsson, P. -I., & Paul, K. G. (1976). *Acta Chem Scand. B*, *30,* 373.
Ricard, J., & Job, D. (1974). *Eur. J. Biochem*., *44,* 359.
Rodriguez-Lopez, J. N., Smith, A. T., & Thorneley, R. N. F. (1997). *J. Biol. Chem*., *272,* 389.
Schuller, D. J., Ban, N., van Huystee, R. B., McPherson, A., & Poulos, T. L. (1996). *Structure*, *4,* 311.
Sesto, P. A., Chibbar, R. N., & van Huystee, R. B. (1989). *Plant Sci*., *61,* 163.
Smith, A. T., & Veitch, N. C. (1998). *Current Opinion in Struct. Biol*., *2,* 269.
Zheng, X., & van Huystee, R. B. (1992). *Phytochemistry*, *31,* 1895.