Generation of Electronically Excited Aromatic Aldehydes in the Peroxidase Catalyzed Aerobic Oxidation of Aromatic Acetaldehydes

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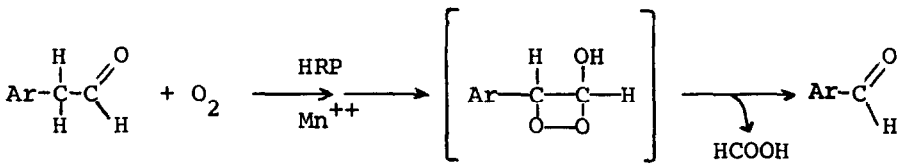
SUMMARY : The peroxidase catalyzed aerobic oxidation of aromatic acetaldehydes has been investigated with regard to the formation of electronically excited states because it generates the products expected from the cleavage of an intermediate dioxetane, that is, the aromatic aldehyde and formic acid. Emission was detected with the liquid scintillation counter. Integrated emission, indole-3-aldehyde formation, and O_2 uptake strictly correlate with each other, unequivocally indicating that the aromatic aldehyde is generated electronically excited. Although the quantum yield of emission is approximately 5×10^{-9}, the yield of chemiexcitation must be several orders of magnitude higher.

Most bioluminescent systems are peroxidase catalyzed reactions which appear to proceed through a dioxetane intermediate(1). One difficulty is that in general dioxetanes give as a result of their cleavage a much higher yield of triplet carbonyl compounds (non emissive) than of excited singlets (2-4). We have pointed out several biochemically important peroxidase catalyzed reactions which generate products as expected from the cleavage of an intermediate dioxetane (5-13). It is therefore legitimate to suspect that these systems may generate a non-emissive electronically excited product. These systems are aerobic, with the peroxidase acting as an oxidase; therefore, the triplet species may be quenched due to the presence of oxygen. Clearly, the identification of such non-emissive states is a difficult task,

Abbreviations: PAA, phenylacetaldehyde; HRP; horseradish peroxidase; IAAL, indole-3-acetaldehyde; IA, indole-3-carboxaldehyde; IAA, indole-3-acetic acid; DPAS, 9,10-diphenylanthracene-2-sulfonate; DBAS, 9,10-dibromo-anthracene-2-sulfonate; DMSO, dimethylsulfoxide; tert-BuOK, potassium tert-butoxide.

which nevertheless has been undertaken in this laboratory (5-14).

We have now investigated the oxidation of aromatic acetaldehydes. PAA may be formed in plants from β-phenylethylamine(15). It is oxidized by many plant saps, or simply by the HRP/Mn^{++}/O_2 system to benzaldehyde and formic acid (15). Similarly, IAAL can be oxidized to IA and formic acid (16,17). Therefore, these reactions may go through a dioxetane intermediate whereby the aldehyde may be generated electronically excited (2,18):



Additional interest in this reaction comes from the fact that IAAL, like IAA, is a plant hormone (19) and we have suggested that auxins might act by generating an electronically excited product (10,12).

Material and Methods

PAA from Aldrich, was redistilled twice under reduced pressure. IAAL.$NaHSO_3$, HRP (VI), and IA were from Sigma. Free IAAL, was prepared by the method of Brown and Purves (20). Eosin was from Fisher. DBAS (21,10) and DPAS (22) were prepared by standard procedures.

Oxygen consumption was determined with a YSI Model-53 oxygen monitor. To measure the emission from the enzymic systems, a Beckman Model LS-250 liquid scintillation counter, with the coincidence circuit turned off, was used. With DMSO solutions, the Aminco--Bowman spectrofluorometer was used. The quantum yield of emission was measured by a standard, the scintillation "cocktail" of Hastings and Weber (23).

RESULTS

PAA can be oxidized to benzaldehyde and formic acid, even in the absence of Mn^{++} ions, provided the peroxidase used is type VI.

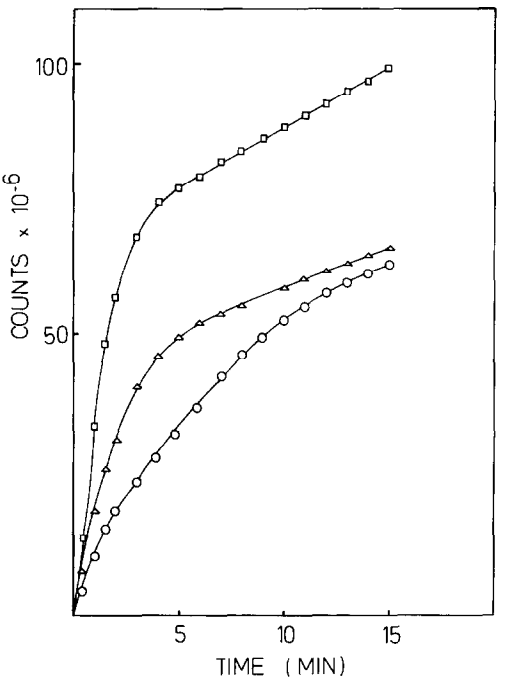


Fig. 1. Emission from PAA oxidation in 0.067M phosphate buffer, pH 7.5, containing 0.84 μM HRP. The PAA concentration was: 6mM (-O-O-O-); 14 mM (-Δ-Δ-Δ-) and 27 mM (-□-□-□-).

Substituting air by oxygen did not alter the rate of O_2 uptake. IAAL is partially oxidized in the HRP/Mn^{++}/O_2 system. Using IAAL.$NaHSO_3$, however, IA was formed in good yields (above 70%) and was the only product found besides formic acid. Peroxidatically there is also formation of 4-hydroquinoline (16,17).

Emission. Photon emission could be observed from the PAA/HRP/Mn^{++} system; it increased with increasing PAA concentration until oxygen dissolution became limiting (Fig.1). The initial rate of photon emission was the same in air and under O_2. DPAS which "counts" excited singlets, did not significantly affect the rate of emission or the rate of O_2 uptake at the 8.0 μM, level. DBAS which "counts" triplets, doubled the initial rate of photon emission; at the level tested,

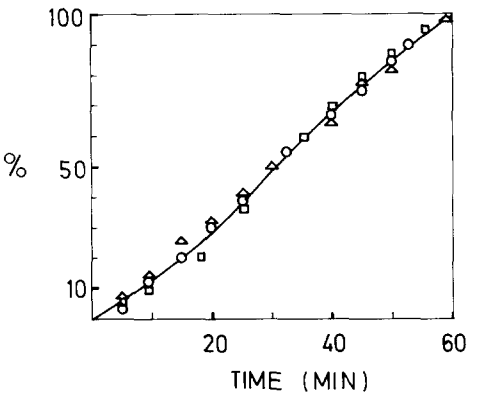


Fig. 2. Correlation between O_2 uptake (-Δ-Δ-Δ-), IA formation (-□-□-□-) and integrated photon emission (O-O-O-O) when 0.1 mM IAAL.$NaHSO_3$ was oxidized in 0.05 M acetate buffer, pH 4.6, in the presence of 1.5 μM HRP and 0.3 mM $MnSO_4$.

8.0 μM, DBAS slightly inhibited the rate of O_2 uptake. Eosin, 18 μM, increased 4-fold the emission. Also fluorescein, 50 μM, increased the emission. Neither eosin (18 μM) nor fluorescein (52 μM) had any influence upon the rate of O_2 uptake.

Emission was also observed with IAAL.$NaHSO_3$. Using 0.3mM Mn SO_4 and 1.6 μM HRP in 0.2M acetate, pH 4.6, the rate of emission was of the same order of magnitude as with PAA, despite the 200-fold smaller concentration. The quantum yield of emission was roughly $5x10^{-9}$. Neither 1.7 μM DPAS nor 0.14 μM DBAS sensitized the emission; higher concentrations could not be used here due to an inhibitory effect. Eosin, 15 μM, doubled the total emission and accelerated O_2 uptake and IA formation. This catalytic effect of eosin was supressed by 0.5 μM iodide ion.

The oxidation of PAA was also studied in DMSO/tert-BuOK. A small chemiluminescence emission peak was observed in the blue region. Both formic acid and benzaldehyde (24) were detected in the spent reaction mixture.

Correlation between oxygen uptake, aldehyde formation and photon emission. Oxygen uptake and benzaldehyde formation correlate in the initial stage of PAA oxidation; however no correlation exists with photon emission, either in the absence or presence of sensitizer or Mn^{++} ions (0-30 mM). In the case of IAAL.$NaHSO_3$ oxidation, the three parameters nicely correlated with each other (Fig. 2). In the presence of eosin, however, emission correlated only with IA formation. A small fraction of the O_2 consumed in the system of IAAL.$NaHSO_3$ is due to a non-enzymic reaction(25).

Self-damage. In contrast to the acetoacetate/myoglobin/Mn^{++}(9,8), IAA/HRP (10) and vanylpyruvate/HRP/Mn^{++} systems (11), the spectrum of the hemeprotein catalyst was only very slightly affected as a result of the reaction which it catalyzed. Yet the changes followed the same pattern.

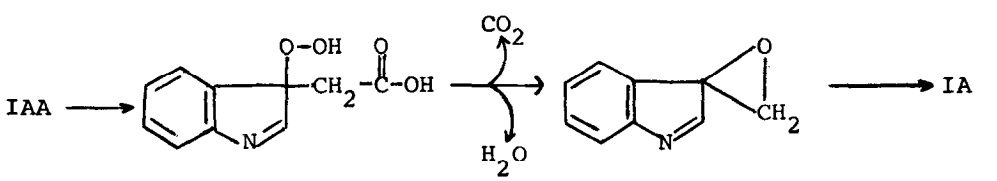
Quenching of fluorescence of the aromatic aldehyde. Under the conditions of the experiments, no fluorescence from IA is detectable with conventional equipment.

DISCUSSION

The weak emission observed in the enzymic oxidation of aromatic acetaldehydes is consistent with, and may be considered an indication of the generation of the aromatic aldehyde in an excited electronic state which is essentially non-emissive. Unequivocal evidence (26) that the chemienergized species is the aromatic aldehyde and not a luminescent product formed in a minor side reaction is the strict correlation between IA formation, photon emission and oxygen uptake (Fig. 2). The triplet is likely to be the main excited species because a high ϕ^3/ϕ^1 ratio is usually observed in the cleavage of dioxetanes (2,4,18) and the aldehyde singlet state undergoes very fast intersystem crossing. In aerated aqueous solutions, the triplet state is not expected to emit, unless exceptio-

nally (27). The emission may come from excited singlet IA or from the heme after energy transfer. The latter possibility is however unlikely in view of characteristics of the photomultiplier. Although the quantum yield of emission is very low, ca. 5×10^{-9}, the chemiexcitation yield must be greater, by a factor as high as 10^{8}, in view of the very low efficiency of the aldehyde fluorescence and of the preferred formation of triplets in the dioxetane decomposition. In the presence of eosin, IA should also be formed excited; however, other O_2 consuming processes should also occur. The lack of correlation in the case of PAA may be accounted for if the intermediate dioxetane is relatively stable. The formation of triplet species is attested to by the increased emission observed with DBAS in the initial stages of the reaction. A substantial yield of chemienergized benzaldehyde is suggested by the emission from the "model" system in aprotic solvent (12).

It is conceivable that in the case of IAAL the reaction may also take place at the indole nucleus; IA would than be generated through epoxide formation as suggested by Ricard and Job (28) for IAA oxidation



Epoxide formation, however, is unlikely on energetic grounds. Thus, while presumably still energetically possible in the case of IAA oxidation where epoxide generation is accompanied by the formation of CO_2 and H_2O, it is not energetically feasible in

the case of IAAL and indolepyruvic acid oxidation where there is formation of formic acid and oxalic acid respectively.

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