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A NEW SOURCE OF CARBON OXIDES IN BIOCHEMICAL SYSTEMS. IMPLICATIONS REGARDING DIOXETANE INTERMEDIATES

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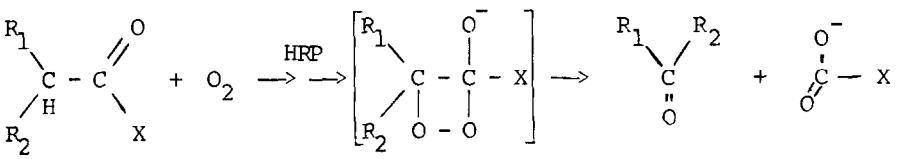
SUMMARY: The peroxidase catalyzed aerobic oxidation of aromatic pyruvates produces the aromatic aldehyde, oxalate, CO and CO_2 and a weak light emission. This indicates the preliminary formation of a peroxy anion which can cyclizes to a four-membered ring dioxetane and to a five membered ring α-keto-β-peroxylactone intermediates.

The possible importance of this new source of carbon oxides in biological systems is pointed out. The results considerably strengthen the view that a dioxetane is formed in side chain of the plant hormone indoleacetic acid during the oxidation catalyzed by peroxidase.

We reported that HRP‡ may act upon suitable substrates as an internal monooxygenase, generating products of the type expected from the cleavage of an intermediate dioxetane(1,2):



As a result one of the carbonyl groups may be generated electronically excited (3-5). A substrate of this type is VPA, which is converted into vanillin and oxalate during the degradation of lignin by fungi (6). HRP does indeed promote the reaction and excited states are generated (7).

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‡ Abbreviations: HRP, horseradish peroxidase; VPA, vanylpyruvate; IPA, indolepyruvate.

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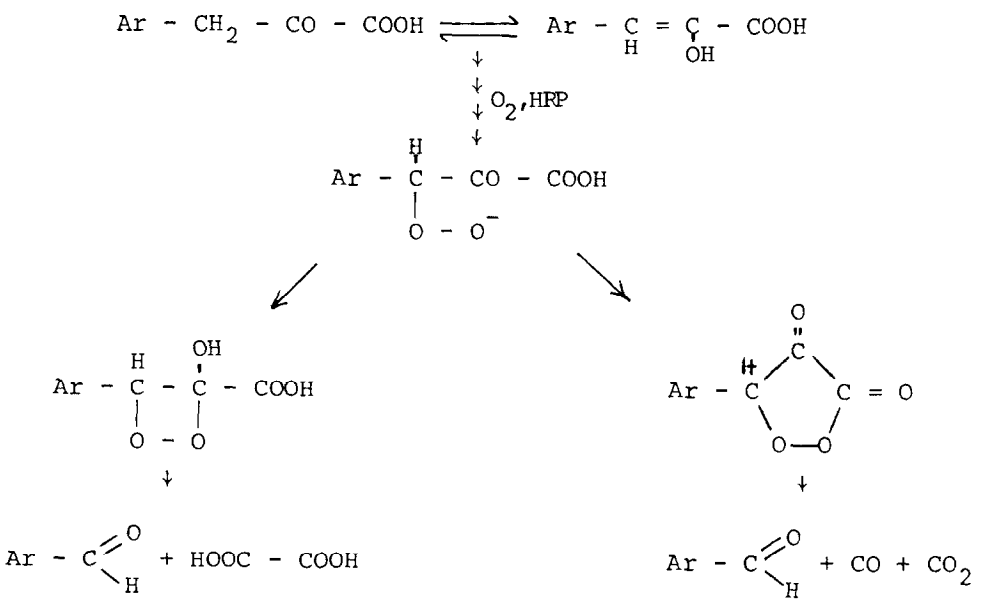
A similar reaction should also occur with IPA because it generates "in vivo" indole-3-carboxaldehyde (8) and oxalic acid (9). Indeed, earlier we had investigated these pyruvates in model systems, i.e. in dimethylsulfoxide containing potassium tert-butoxide, and found that the aromatic aldehyde and oxalate are formed with concomitant light emission (7,10,11); more recently, Jefford et al. (12,13) reported that this chemical reaction also produces CO and should produce CO_2. This indicates that the peroxy anion intermediate can also attack the carboxylic carbon atom:



where Ar- is an aromatic group.

It became therefore of great interest to verify if CO and CO_2 are formed in the peroxidase catalyzed aerobic oxidation of aromatic pyruvates. Their formation would reveal a new pathway for the generation of carbon oxides in biochemical systems and would also provide solid albeit, indirect evidence for the biochemical generation of dioxetanes.

MATERIALS AND METHODS

HRP (type VI) and VPA were from Sigma; IPA was from K and K. Spectral distributions of low-light-emitting reactions were measured on a spectrometer of our own construction (1) provided with a Hamamatsu HTV-R-562 photomultiplier unit. Intensities are measured with and without filters, normalized and corrected for differences in transmittance between successive filters and for photomultiplier spectral response. For the short-lived emissions, each of the intensities corresponds to a complete reaction.

Vanillin and indole-3-carboxaldehyde were detected on a Varian 8500 high pressure liquid chromatograph. CO_2 and CO were measured with an Instrumentos Científicos CG Modêl 2527 gas chromatograph employing Chromosorb (CO_2) or molecular sieve (CO) columns.

The oxidation of IPA (0.62 mM) promoted by HRP (0.16 μM) was investigated in 0.2 M acetate buffer, pH 5.6. In the case of VPA (5.5 mM), the HRP concentration was 15 μM; 0.05 M acetate buffer, pH 4.0 was used.

RESULTS AND DISCUSSION

HRP promotes the oxidation of IPA; oxygen is consumed and indole-3-carboxaldehyde is formed in 97% yield. An emission, whose spectral distribution is shown in fig. 1, is detectable with a photon counter. Carbon monoxide is formed in an amount which indicates that roughly 50% of the oxidation proceeds through the 5-membered ring route. Carbon dioxide was also detected.

In the case of VPA, the aromatic aldehyde vanillin is formed in somewhat lower yield (64%). More CO is formed at pH 3.8 than at pH 4.2 and the yield decreases still further at pH 5.0. The amount formed is much greater than can be accounted for in terms of HRP decomposition and is not influenced by the concentration of the enzyme. The formation of CO_2 was investigated only at pH 4.0, its presence being confirmed.

Although CO and CO_2 are formed during the autoxidation of these pyruvate substrates, much less CO and CO_2 is evolved in

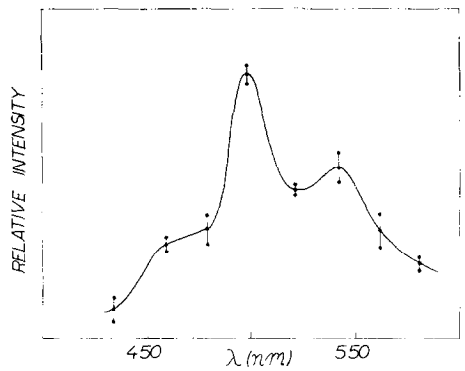


Fig. 1 - Chemiluminescence spectrum of the system IPA (0.6 mM)/ HRP (0.15 μM)/O_2 in 0.2 M acetate buffer pH 5.6.

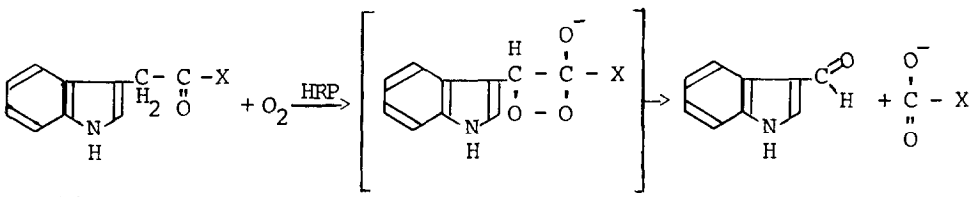
the absence of enzyme. Most important, when indole-3-acetic acid (I; X = OH) was used as substrate as a control experiment, no CO was formed, either spontaneously or enzymically.

Despite the fact that production of CO is a widespread phenomenon - microbia, plants, fungi and animals, including man, can produce it - in most cases the biochemical pathways that are associated with its production are not known (14). The peroxidase-catalyzed aerobic oxidation of aromatic pyruvates is obviously of potential importance in relation to the origin of CO "in vivo". In addition it may explain why L-dihydroxy-phenylalanine (L-DOPA) evolves CO at much higher rates during enzymatic oxidation than during autoxidation (15).

It should be noted that CO_2 has been reported to be formed along with benzaldehyde during the oxidation of phenyl-pyruvic acid by mitochondrial preparations from higher plants (16).

The formation of CO and CO_2, and therefore of a α-keto-β-peroxylactone, implies that the dioxetane should also be formed competitively. This inference is specially important for the case of IPA (I; X = COOH) because it solidly strengthens our already well-substantiated hypothesis that a dioxetane

intermediate is formed in the side chain of the plant hormones indole-3-acetic acid (I; X = OH)(17) and indole-3-acetaldehyde (I; X = H)(18) when they are oxidized, in the presence of peroxidase, to indole-3-carboxaldehyde and to CO_2 or formic acid, respectively.



(I)

At present it is premature to speculate upon the exact nature of the emitter(s) responsible for chemiluminescence from the enzymic reaction.

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