EMISSION FROM SINGLET OXYGEN DURING THE PEROXIDASE-CATALYZED OXIDATION OF MALONALDEHYDE

C. VIDIGAL-MARTINELLI, K. ZINNER, B. KACHAR, N. DURÁN* and G. CILENTO
Department of Biochemistry, Instituto de Química, Universidade de São Paulo, Caixa Postal 20.780, São Paulo
and **Instituto de Química, Universidade Estadual de Campinas, C. P. 1170, Campinas, São Paulo, Brasil*

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1. Introduction

Malonaldehyde is formed during lipid peroxidation [1] and is further metabolized [2]. The study of reactions that involve malonaldehyde is of multiple biochemical interest [3]. Because it has a highly activated $-CH_2$ group, malonaldehyde qualifies very well as a substrate for peroxidase (acting as an oxidase). As a consequence, peroxidase may convert malonaldehyde to electronically excited products [4], which in turn, may be involved in detrimental effects.

2. Experimental

Malonaldehyde (sodium salt) was prepared by hydrolysis of 1,1,3,3-tetraethoxypropane (Aldrich), employing the procedure in [5]. HRP (type VI) was from Sigma. All other reagents were of analytical grade.

Oxygen consumption was measured in a Yellow Springs Instruments Oxygen Monitor. Light emissions and spectral distributions were measured with both a Hitachi Perkin-Elmer MPF-4 spectrofluorimeter and a spectrometer of our own construction provided with a Hamamatsu HTV-R-562 photomultiplier. In the latter case, intensities are measured with and without filters, normalized and corrected for differences in transmittance between the filters and for the photomultiplier's spectral response. Due to technical reasons (availability of filters), the upper wavelength limit is presently 677 nm.

A calibrated PPO–POPOP 'cocktail' [6] was employed as the light standard.

3. Results and discussion

Provided that both Mn^{2+} and HRP are present, malonaldehyde consumes O_2 in a reaction which emits light; neither myoglobin nor hemoglobin could be substituted for HRP. The principal emission peak is just above 700 nm (fig.1); occasionally a smaller peak was observed at 630 nm. The spectral distribution has also been determined with a highly sensitive spectrometer (fig.2).

In addition to the spectral evidence, the emitting species has been further identified as $^1O_2 \,.\, ^1O_2$ ($^1\Delta_g\, ^1\Delta_g$) on the basis of the following data. The emitter cannot be a product or by-product of the reaction because no red fluorescence is observed from the spent reaction mixture. Emission from singlet oxygen is confirmed by the effect of known singlet oxygen traps and quenchers. Histidine [7,8] (1 mM) quenches ~66% and triethylamine [9] (1 mM) 50% of the emission without affecting the rate of O_2 uptake. As anticipated , the effect of the N_3^- anion [10] could not be

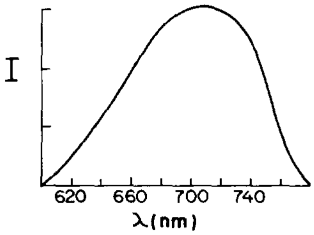

Fig.1. Chemiluminescence spectrum of the system malonaldehyde (0.67 mM) /Mn^{2+} (1.6 mM) /HRP (6.7 μM) /O_2 in 0.2 M acetate buffer (pH 4.8) determined with a spectrofluorimeter.

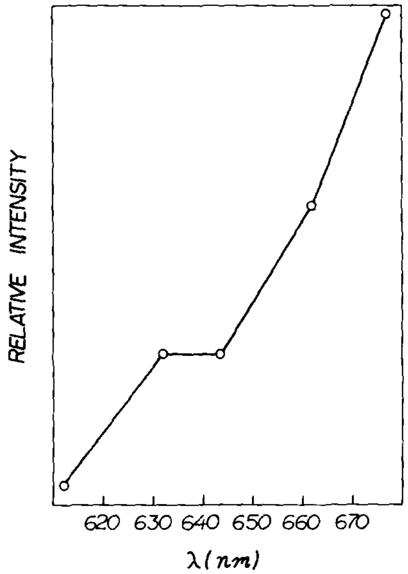

Fig.2. Chemiluminescence spectrum of the system malonaldehyde (0.67 mM) /Mn^{2+} (1.6 mM) /HRP (6.7 μM) /O_2 in 0.2 M acetate buffer (pH 4.8) determined with a filter spectrometer.

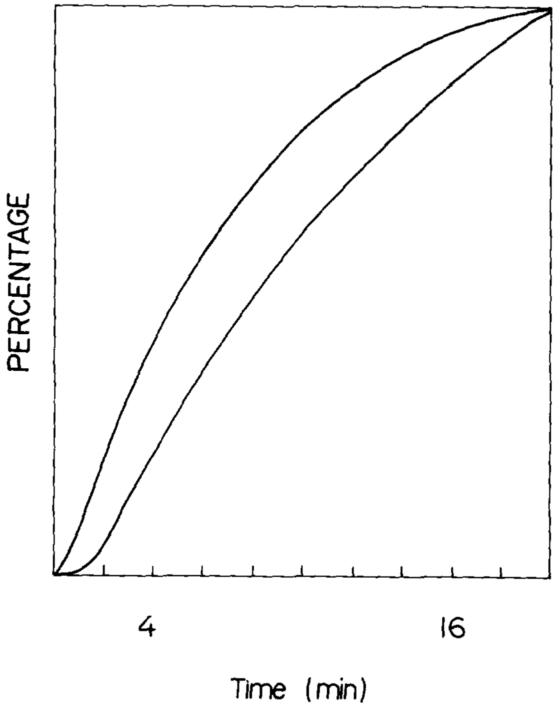

Fig.3. Normalized data for O_2 consumption (upper curve) and total light emission for the system malonaldehyde (0.67 mM) /Mn^{2+} (1.6 mM) /HRP (6.7 μM) in 0.2 M acetate buffer (pH 4.8).

ascertained because the reaction is inhibited. Although the quantum yield of this $^1O_2 \cdot {}^1O_2$ emission is only ~10^{-6} Einstein/mol, the chemiexcitation yield must be much higher (as much as 10^6-times greater [11]) and therefore almost quantitative.

The superoxide ion is not involved in the generation of $^1O_2$ because addition of superoxide dismutase does not affect the emission. The fact that O_2 uptake is faster than emission (fig.3) suggests that the latter arises from an intermediate which accumulates to some extent during the reaction. This intermediate should also be formed non-enzymically because older solutions of malonaldehyde give stronger emission with an identical spectral distribution. In principle the intermediate might be a dioxetane, which upon cleavage could give rise to a triplet species [12,13] capable of transfering energy to oxygen to form $^1O_2$ [14]. However, this route would only afford a poor yield of singlet oxygen [14] and is therefore excluded. Alternatively, the intermediate may be a hydroperoxide, which could decompose as reported for linoleic acid hydroperoxide [15,16], i.e., giving rise to $^1O_2$ via the disproportionation of peroxy radicals [17].

The question arises as to why the other 'dimol' emissions of $^1O_2$ are not observed. The lack of energy transfer to eosine would suggest that the $^1\Sigma_g^+$ state is either not populated or is immediately quenched [18]. The presence of the protein (HRP) may also alter the singlet oxygen emission spectrum [19]; indeed peroxidase is altered as a result of the reaction. The alteration is reminiscent of that observed in the acetoacetate/myoglobin/Mn^{2+}/O_2 [20,21] system.

In conclusion, the formation of singlet oxygen reported here provides the first example of the observation of a substantial $^1O_2 \cdot {}^1O_2$ emission from an enzymatic system. The ubiquitous generation of malonaldehyde and the presence of peroxidase and other species with peroxidase-like activity make feasible substantial formation of singlet oxygen 'in vivo'. Evidence is already available for the generation of $^1O_2$ as a transient by-product of the peroxidative decomposition of microsomal lipids [22–25].

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