Chemiluminescence in the Coupled Oxidation of Lecithin and Ascorbate

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Chemiluminescence (CL) that appears during oxidation of lecithin and ascorbate has been studied. A simple system consisting only of purified lecithin, which has one double bond, and ascorbate as a physiological reductant with a low redox potential, was used. The CL spectrum of lecithin contain a strong band lying in the near infrared, and three bands at 20 900 cm^{-1}, 17 700 cm^{-1} and 15 800 cm^{-1}, being characteristic of singlet molecular oxygen ($^1O_2$). The effect of $^1O_2$ quenchers on both autooxidation processes has also been investigated. The obtained results indicate that the main emitter is the $^1O_2$.

An addition of ascorbate to the system lecithin plus buffer causes a decrease of CL intensity. That is a result of stronger quenching properties of ascorbate and not due to efficiency of the generation of $^1O_2$.

Introduction

Many authors have attributed the main part of the low level chemiluminescence (CL) from various tissue homogenates, mitochondria and muscle preparations to the nonenzymatic oxidation of tissue lipids by oxygen [1, 2]. It has also been suggested that during enzymatic as well as nonenzymatic oxidation of lipids the excited molecular oxygen, so-called singlet oxygen ($^1O_2$), is responsible for CL emission [3, 4]. Our earlier manometric investigation concerning coupled oxidation of lecithin and ascorbate [5] has encouraged us to define the conditions for CL generation during autooxidation of lecithin and also to study the effect of ascorbate on this process.

Materials and Methods

L-ascorbic acid of a tested grade [6] from "Polfa" and lecithin from BDH, prepurified by means of thin-layer chromatography, were used in the study. Compounds employed as quenchers of $^1O_2$ were obtained from Merck. The solutions were prepared just before tests in quartz redestilled water. The stock lecithin solution was prepared in methanol. Universal buffer according to Britton and aqueous NaOH were used to determine the dependence of CL on pH.

CL kinetics as well as the effects of pH, of reagent concentrations and of quenchers, on the CL intensity were measured by means of a M12FQC51 photomultiplier with S20 cathode operating jointly with a K-200 recorder (G.D.R.). Measurements of CL spectra were taken by employing a set of calibrated cut-off filters GOST9411-66 and an EMI 9558QB photomultiplier, cooled to 203 K by the method described by Vassilev first [7] and widely used by other authors [8 – 10]. The width of each rectangle is equal to the half spectral width of the difference in transmission of each pair of filters providing one experimental point. The single photoelectron counting method and a flow system of solvent were applied. Fluorescence spectra were measured with a spectrofluorimeter consisting of a monochromator SPM-2, M12FQC51 photomultiplier and K-200 recorder. A flow system of solvent was used here too. Fluorescence was excited of 365 nm. The quantum yield of CL was determined by the method of Stauff and Schmidkunz [11].

Abbreviations: CL, chemiluminescence.

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Results

Addition of lecithin to the buffer or NaOH solution results in fast luminescence increase during approx. 0.3 s period. Then the luminescence slowly decreases within some minutes. In order to establish the optimal conditions of the reaction the CL dependence on lecithin concentration as well as dependence of CL intensity on pH were examined. The results are presented in Figs. 1 and 2 respectively. The curve depicted in Fig. 1 shows that the CL rise accompanies lecithin concentration increase.

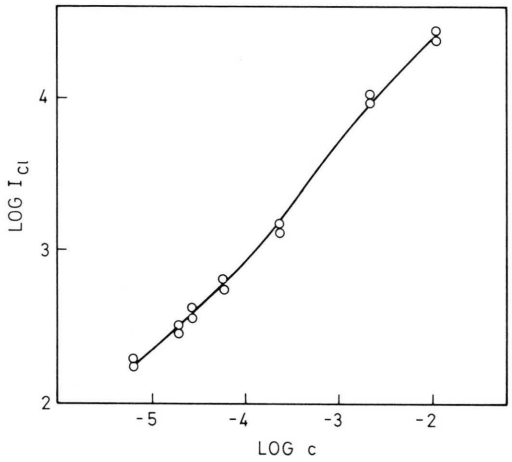

Fig. 1. The plot log $I_{CL}=f(\log c)$ where c is lecithin concentration in mol/l; pH = 11.92.

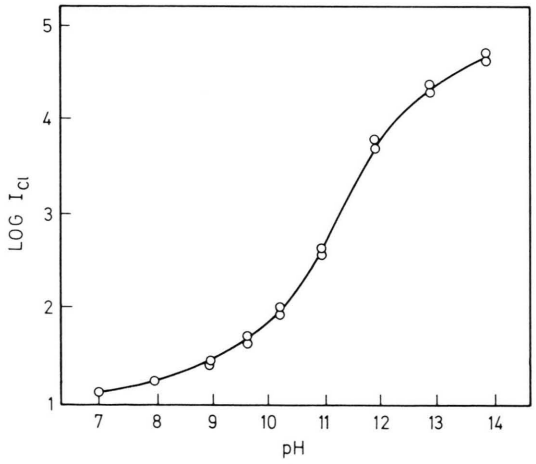

Fig. 2. The plot log $I_{CL}=f(\text{pH})$. Lecithin concentration 2.6×10^{-4} M.

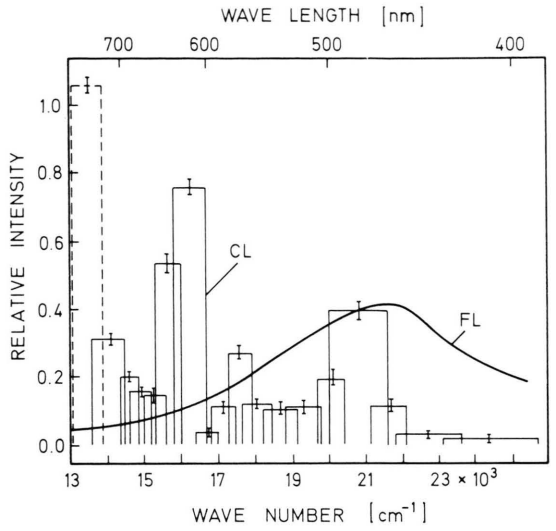

Fig. 3. Chemiluminescence (CL) and fluorescence (FL) spectra of system lecithin-buffer measured 25 s after the beginning of the reaction on pH 11.92.

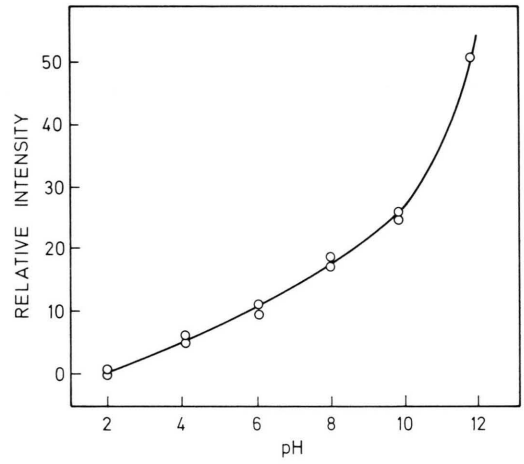

Fig. 4. Maximum light intensity as function of pH. Ascorbate concentration 10^{-2} M.

The estimated CL reaction order in relation to lecithin was ⅔ in the examined range. Relatively low CL intensity in weakly basic solutions went up rapidly for higher values of pH (Fig. 2). Under optimal conditions *i.e.* pH = 11.92 and lecithin concentration 2.6×10^{-4} M CL quantum efficiency was approx. 10^{-10} photons/lecithin mol. Temperature in all the measurements was 293 K.

In order to obtain further information concerning the nature of the excited species, CL spectra were measured (Fig. 3). The CL spectrum was found to

have three emission bands with maxima at 21000 cm^{-1}, 17 700 cm^{-1} and 15 800 cm^{-1} and the fourth strong one situated in the near infrared below 14 000 cm^{-1}. The influence of the $^1O_2$-quenchers on the CL has been investigated and the results are summarized in the Table I. Quenching constants were calculated from Stern-Volmer graph for quencher concentrations $10^{-6} - 10^{-4}$ mol/l. For higher pH values the increase of CL intensity accompanying ascorbate autooxidation was observed (Fig. 4). The increase was particularly rapid for pH approx. 12. However the observed luminescence was insufficient to determine reliable CL spectrum. Quenchers, added to the system ascorbic acid-buffer, reduced CL intensity according to Stern-Volmer equation which is also evidence for $^1O_2$ involvement in this emission (Table I). Addition of ascorbic acid to the system lecithin-buffer resulted in CL intensity decrease dependent upon ascorbic acid concentration (Fig. 5).

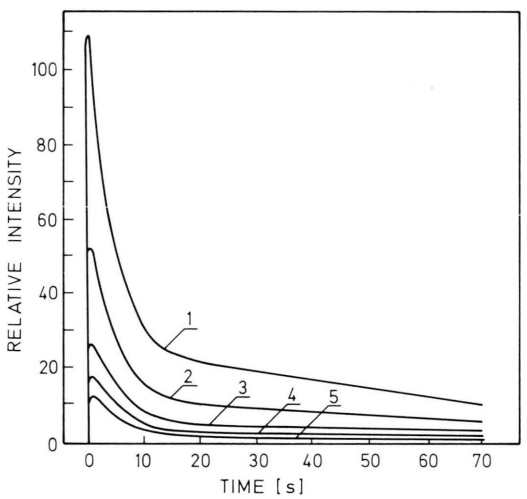

Fig. 5. Influence of ascorbate on chemiluminescence during autooxidation of lecithin. Ascorbate concentration in mol/l: $1 - 0$; $2 - 2\times10^{-2}$; $3 - 3\times10^{-2}$; $4 - 5\times10^{-2}$; $5 - 4.5\times10^{-1}$.

Table I. Effect of singlet oxygen quenchers on the intensity of CL.

Quencher	Quenching constant k_q [l/mol · s]	
	pH 11.92	pH 7.96
A for 5×10^{-2} M of ascorbate in buffer according to Britton		
Methionine	2.5×10^8	1.4×10^8
Histidine	4.1×10^8	2.1×10^8
Menadione	3.1×10^8	–
5.5'-dimethylcyclo-hexandione-1.3	–	5×10^7
B for 2×10^{-3} M of lecithin in the 10^{-2} M NaOH solution		
Methionine	1.4×10^8	–
Histidine	5.5×10^7	–

Discussions

Taking into account earlier reported data [12] the CL spectra bands at 15 800 cm^{-1}, 17 700 cm^{-1} and 21 000 cm^{-1} should be ascribed to the vibrational components (0.0) and (1.0) of the electronic transition in $O_2/^1\Delta_g$/dimole. The fourth strong band in the infrared, the exact shape of which could not be measured due to low sensitivity of the photomultiplier in this range, is analogues to the observed one during formaldehyde oxidation [13]. It may be the result of the dismutation of peroxy-radicals, as suggested by Stauff and Rümler [14].

The quenching process consists in transferring the energy from the excited molecule to the quencher molecule. The process is specific for both molecules involved in this reaction. The characteristic quenching constant value for the interaction between the quencher and singlet oxygen may be regarded as one of the identification criteria of the singlet oxygen. That was the guideline of this paper. $^1O_2$-quenchers decrease the CL intensity remarkably. The obtained quenching rates (Table I) are comparable with the values reported in earlier papers [15, 16].

Joint lecithin and ascorbate oxidation leads to lower CL intensity than oxidation of lecithin by itself. Ascorbate oxidation can be a singlet oxygen source indeed (Fig. 4) but the ascorbate is the investigated system that acted mainly as $^1O_2$ scavenger. According to Brogan [17] it is possible that for higher ascorbate concentrations its autooxidation properties can reduce lipids peroxidation. It seems that $^1O_2$ produced during lipids oxidation plays a more essential role in the mechanism of this reaction than it is done by oxygen in its basic form $^3O_2$. The ascorbate added in this study has always claimed the function of singlet oxygen scavenger in lecithin peroxidation and quenched CL intensity. Therefore the elucidation of catalytic as well as antioxidative properties of ascorbate requires further research that is carried on.

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