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The kinetics of photosensitized decomposition of ascorbic acid and the determination of hydrogen peroxide as a reaction product

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In this work, the kinetics of photosensitized decomposition of ascorbic acid in citrate buffer solution at 25°C, 35°C, 45°C under anaerobic conditions have been investigated. Riboflavin was used as a sensitizer. With increasing temperature it was observed that the initial rate of reaction also increased. However, with increasing concentration of citrate ions, the initial rate decreased. Reactions were studied at ascorbic acid concentrations of 10^{-3} M, 2×10^{-3} M and 4×10^{-3} M concentrations and in 0.1 M, 0.2 M and 0.4 M citrate ions at pH 4.5. A 100 W tungsten lamp was used as a light source. The amount of decomposed ascorbic acid was determined by a polarographic technique. The formation of hydrogen peroxide (H_2O_2) as a reaction product was followed quantitatively by recording the current of the H_2O_2 wave at a half-wave potential of about −0.8 V. This wave was the second polarographic wave of oxygen. The formation of H_2O_2 was confirmed by an infrared technique. An almost 1:1 molar relationship was found between decomposed ascorbic acid and H_2O_2 formed. In this reaction riboflavin is excited first by visible light and (through an excitation transfer) ascorbic acid is then oxidized. A reaction mechanism which includes H_2O_2 is proposed. © 1997 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Ascorbic acid is very important in human nutrition. It is also used as an additive in many kinds of food. Because the rapid decomposition of ascorbic acid affects food quality directly, its degradation has been a very important subject of research. There have been a number of investigations to determine the factors effective in its decomposition. Major factors include, pH, temperature, the presence of certain molecules, oxygen and light. The investigations on its decomposition can be divided into two groups. In the first group, synthetic ascorbic acid samples were used (Lin & Agalloco, 1979; Dekker & Dickinson, 1940; Kitagawa, 1968; Sattar *et al.*, 1977; Deschacht & Hendrics, 1964). In the second group, the decomposition of ascorbic acid was investigated in natural products (Mapson, 1967; Peterson & Walton, 1943). The effect of light is one of the least well known, although it has been investigated by some researchers (Kitagawa, 1968; Sattar *et al.*, 1977; Şahbaz & Somer, 1993*a*; Heelis *et al.*, 1979, 1981). Sattar *et al.* (1977) showed that light had no effect on the destruction of the vitamin in pure solution. They also confirmed the photosensitizing effect of riboflavin on ascorbic acid. The mechanism of photodecomposition of ascorbic acid by flavin mononucleotide has been studied by steady-state and flash photolysis techniques (Heelis *et al.*, 1981).

Light-induced degradation of ascorbic acid in the presence of riboflavin has not been clearly explained and no reaction mechanism has been suggested. We investigated the decomposition kinetics of the vitamin in the presence of riboflavin (Şahbaz & Somer, 1993*a*) as a photosensitizer and first observed formation of hydrogen peroxide (H_2O_2). However, because of problems in the determination of H_2O_2 the kinetics of the reaction could not be followed. The aim of this work was to determine the H_2O_2 produced during the reaction, to investigate the reaction under different conditions and to suggest a mechanism that would explain the experimental results, including H_2O_2 formation.

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MATERIALS AND METHODS

Reagents

Reagents such as ascorbic acid, citric acid, potassium hydroxide and riboflavin were of analytical reagent grade. Triple-distilled water was used for the preparation of the standard solutions and at all other stages of analysis.

Citrate buffers (pH 4.5)

The buffer solutions were prepared so that the analytical concentrations of citrate ion were approximately 0.1 M, 0.2 M and 0.4 M. The pH of the buffer solution was adjusted to the desired value by dropwise addition of 0.5, 1.0 and 2.0 M KOH solution to 0.5, 1.0 and 2.0 M citric acid, using a pH meter.

Preparation of the sample

The experiments were performed under anaerobic conditions. The reaction cell was prepared so that the photochemical reaction and the polarographic measurements were both performed in the same cell. The cell had a water jacket to hold the temperature constant and to afford protection from the infrared (IR) radiation during illumination.

A 2.7 ml portion of citrate buffer, 1.0 ml of 60 ppm riboflavin, 0.1 ml of freshly prepared gelatin and 1.2 ml of water were added to the cell and the temperature was adjusted to the desired temperature. Nitrogen gas was passed for about 1 h until no oxygen wave was observed in the polarogram. The detection limit of oxygen is 10^{-4} M. During this time the cell was covered with carbon paper and aluminium foil to protect against daylight. Then 0.1 M ascorbic acid (0.05 ml, 0.1 ml or 0.2 ml) was added immediately to the reaction cell under nitrogen atmosphere. The ascorbic acid concentration was then 10^{-3} M, 2×10^{-3} M or 4×10^{-3} M. The riboflavin concentration was 3.2×10^{-5} M.

System for kinetic measurements

A home-made polarography system based on the diagram of a Heath EUW-198 and Heath-Schlumberger SR-255B type strip chart recorder was used. The polarographic cell was obtained from PAR and a saturated calomel electrode (SCE; Coleman) was used as the reference electrode. A dropping mercury electrode with a drop time of 3–4 s was used. The scan rate and chart speed were 0.2 V min^{-1} and 1 inch min^{-1}, respectively. The flow of nitrogen (40 ml min^{-1}) was adjusted by a flow-meter (Gilmont No. 11, from Cole-Parmer). The stream of nitrogen that passed through the cell during the reaction was presaturated with water vapour by streaming it through a wash-bottle. The temperature of the cell was controlled by means of a thermostat system (±0.5°C) and the light source was a 100 W tungsten filament lamp. The temperature of the cell was controlled continuously, particularly during illumination, and was determined to be constant.

Polarographic measurements

The ascorbic acid concentration in the cell was followed polarographically during the photoreaction (Şahbaz & Somer, 1992). Ascorbic acid has a well-defined anodic wave in citrate buffer. At the beginning of each experiment the anodic current of ascorbic acid, corresponding to the initial concentration, was measured and then

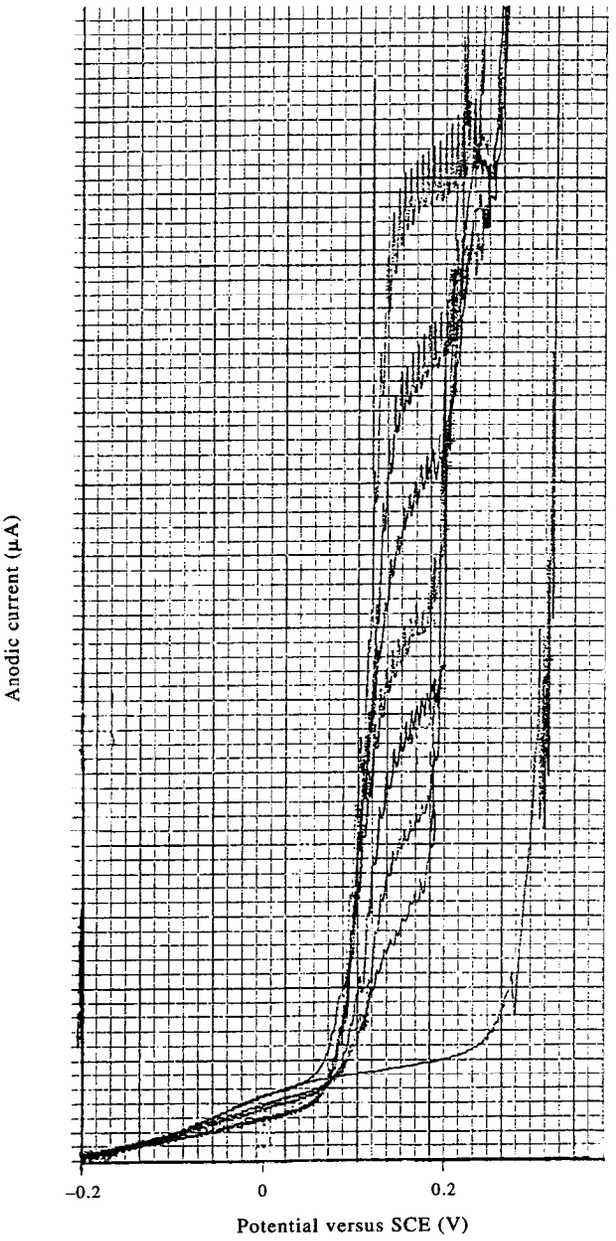

Fig. 1. Photosensitized decompostion of 2×10^{-3} M ascorbic acid in the presence of 3.2×10^{-5} M riboflavin at 25°C. Polarograms were taken after 5 min irradiation.

anodic waves were recorded at 5 min or 10 min intervals during the photoreaction. Thus ascorbic acid content was determined by the comparison of wave heights measured at the beginning and during the run. Riboflavin had a cathodic wave with a half-wave potential at −1.32 V vs SCE. Oxygen had two cathodic waves which occurred at about 0 V and −0.8 V in the citrate buffer. The presence of oxygen was controlled during the reaction with these waves. The formation of H_2O_2 as a reaction product was followed quantitatively (Somer &

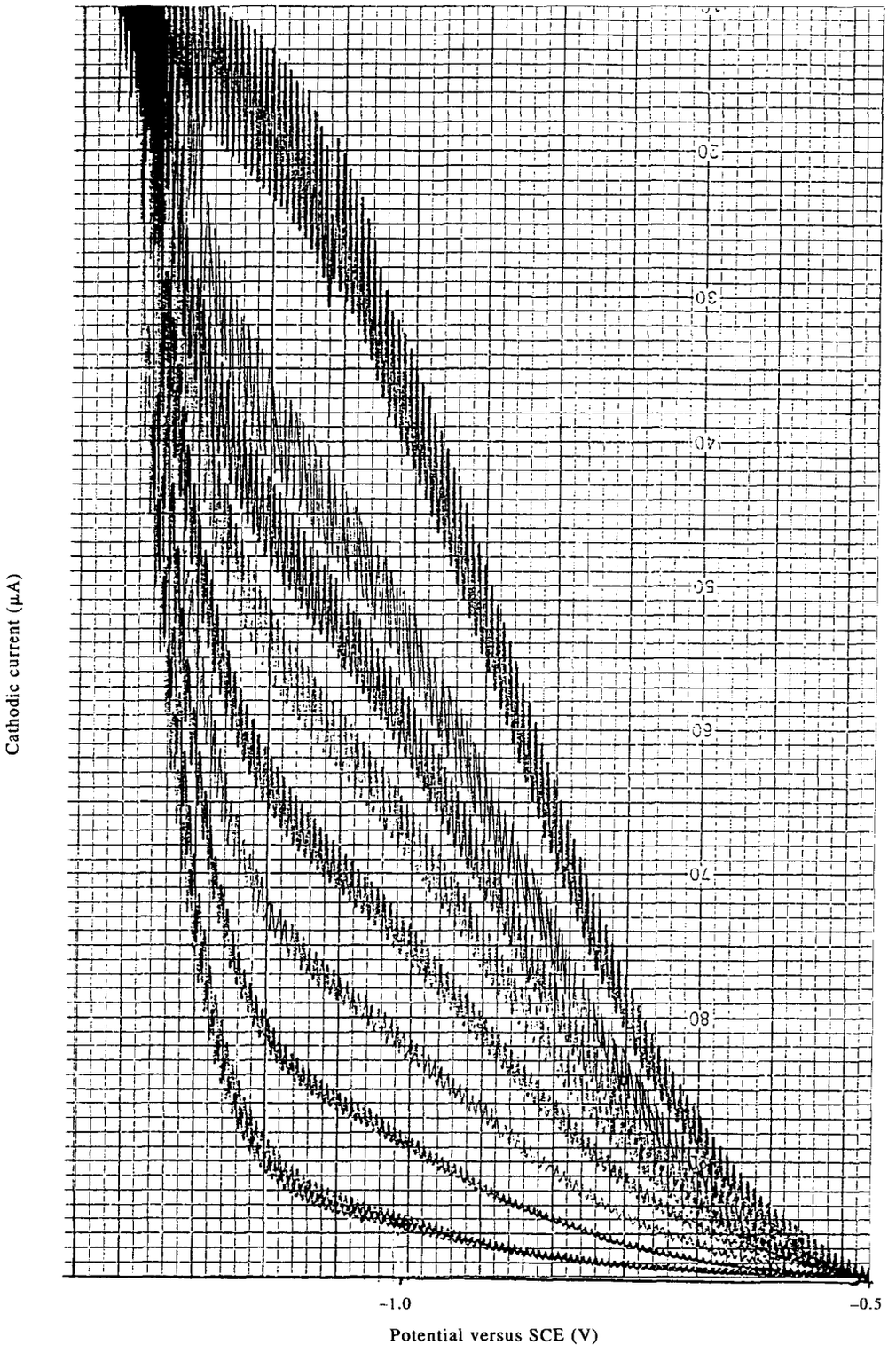

Fig. 2. Formation of H_2O_2 during the photosensitized decompostion of 2×10^{-3} M ascorbic acid in the presence of 3.2×10^{-5} M riboflavin at 25°C. Polarograms were taken after 5 min irradiation.

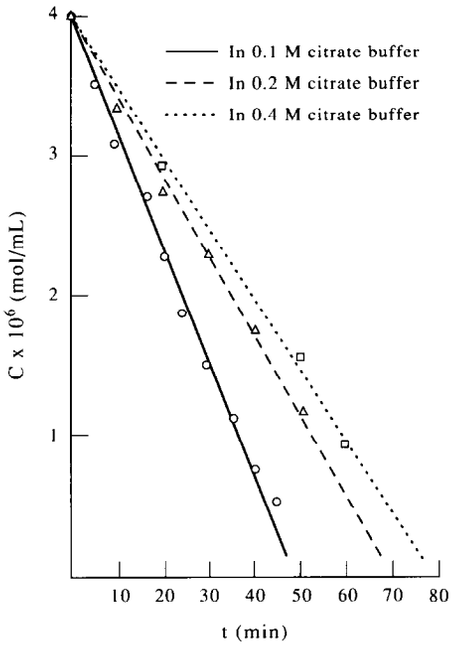

Fig. 3. The rate of decomposition of ascorbic acid in different buffer concentrations. Ascorbic acid = 4×10^{-3} M; riboflavin = 3.2×10^{-5} M; T = 35°C; pH = 4.5; nitrogen flow rate = 40 ml/min; light source = 100 W tungsten filamented lamp.

Green, 1973; Şahbaz & Somer, 1993*a*) by recording the current of the H_2O_2 wave with a half-wave potential of about −0.8 V. This wave was the secondary wave of oxygen. The quantity of H_2O_2 was measured by addition of standard H_2O_2 solution.

To follow the kinetics of the reaction polarographically, first the oxidation wave of ascorbic acid was obtained by a potential sweep from −0.2 V in the positive direction. Then, the direction of the potential sweep was reversed, starting from −0.5 V in the negative direction, and thus H_2O_2 was followed.

IR measurements

A Mattson 1000 FTIR instrument was used. A solution containing ascorbic acid, buffer and riboflavin was prepared; the IR spectrum in the 500–400 cm^{-1} range was taken using a KBr window. The solution was illuminated for 20 min and further IR spectrum was taken. A peak at 418.48 cm^{-1} appeared. This peak increased with the addition of H_2O_2. In a separate experiment under the same conditions H_2O_2 showed a peak at 418.48 cm^{-1}. Thus the formation of H_2O_2 during the photoreaction could be followed using this IR peak.

RESULTS

It was observed that, in the presence of riboflavin, ascorbic acid was photo-oxidized with visible light and H_2O_2 was formed as a reaction product. Here riboflavin acted as a photosensitizer since ascorbic acid does not absorb in the visible region.

Table 1. The initial rates (10^5 M min^{-1}) of photosensitized oxidation of ascorbic acid as a function of ascorbic acid concentration and temperature

Ascorbic acid concentration (M)	Temperature	
	25°C	35°C
10^{-3}	4.3 ± 1.1	5.6 ± 1.4
2×10^{-3}	4.5 ± 0.6	6.7 ± 0.8
4×10^{-3}	5.6 ± 0.3	8.6 ± 0.5

Conditions: pH 4.5; nitrogen flow rate, 40 ml min^{-1}; riboflavin, 3.2×10^{-5} M; buffer, 0.1 M; light source, 100 W tungsten filament lamp.

The ascorbic acid solution in the cell was illuminated for 5 or 10 min intervals. After each illumination a polarogram was taken in both the anodic and cathodic directions, to follow the concentration changes of ascorbic acid and H_2O_2. The polarograms for ascorbic acid are given in Fig. 1. Each was taken after 5 min of irradiation. As expected, while the ascorbic acid concentration was decreasing, the H_2O_2 concentration was increasing. The polarograms for H_2O_2 are given in Fig. 2 for the same experiment. At the end of each experiment a known amount of H_2O_2 was added and a further polarogram was taken. The increase in the wave was used for the calculation of H_2O_2 concentration formed during the photoreduction. Calculations showed that, during the photoreaction, when 1 mole of ascorbic acid was decomposed 0.65–1.00 mole of H_2O_2 was formed.

This photoreaction was followed under different conditions. The effects of factors such as ascorbic acid concentration, temperature and citrate ion concentration on reaction rate were also investigated.

The initial ascorbic acid concentrations were taken as 10^{-3}, 2×10^{-3} and 4×10^{-3} M. For each initial ascorbic acid concentration, polarograms were taken during the photoreaction and ascorbic acid concentrations were calculated at 5 or 10 min intervals. The initial rates were measured from time versus concentration plots. The effect of temperature was also investigated for each ascorbic acid concentration at constant buffer concentration. It was observed that the decomposition rate increased with temperature. The same experiment was repeated at 35°C and 45°C. However, at 45°C the reaction rate was too fast to follow.

For medium concentrations, 0.1, 0.2 and 0.4 M citrate buffer concentrations were investigated. For each ascorbic acid concentration three different buffer concentrations and three different temperatures were used. For each condition, ascorbic acid concentration versus time plots were obtained. Such a plot is shown in Fig. 3. As expected, the rate of reaction decreased with buffer concentration because of quenching. Table 1 summarizes the effect of ascorbic acid concentration and temperature on photoreaction rate. The reaction rate

Table 2. H_2O_2 formation (mol mol^{-1} ascorbic acid) after 20 min of irradiation

Initial concentration of ascorbic acid (M)	Citrate buffer concentration (M)		
	0.1	0.2	0.4
10^{-3}	1.2	0.5	0.5
2×10^{-3}	0.6	0.6	0.6
4×10^{-3}	0.7	0.8	0.7

Conditions: temperature, 25°C; riboflavin concentration, 3.2×10^{-5} M; light source, 100 W tungsten filament lamp; illumination time, 20 min; pH 4.5; nitrogen flow rate, 40 ml min^{-1}.

increased with temperature. The photo-oxidation rate increased with ascorbic acid concentration which is in accordance with our earlier work (Şahbaz & Somer, 1993*a*). For each condition, the formation of H_2O_2 was also followed. After each illumination, ascorbic acid and H_2O_2 concentrations were calculated. Table 2 summarizes the results obtained after 20 min of irradiation at different conditions. For each mole of ascorbic acid 0.5–1.0 mole of H_2O_2 was formed.

In our earlier work (Şahbaz & Somer, 1993*b*), the rate was shown to be dependent on the concentration of the monoascorbate ion HA^-. However, it was also shown that, although HA^- was the main species responsible for the photochemical reaction, the neutral form of ascorbic acid may also take part in the reaction. Since ascorbic acid does not absorb in the visible region, riboflavin must act as a photosensitizer. Flavins are well known to associate with a wide range of compounds (Slifkin, 1971). It may therefore be possible for it to associate with ascorbic acid. The formation of a triplet of this kind was shown by Heelis *et al.* (1981) for FMN and the ascorbic acid molecule.

On the basis of our experimental results, we proposed a mechanism under anaerobic conditions:

$$(\text{Rib}\ldots\text{HA}^-) + h\nu \rightarrow (\text{Rib}\ldots\text{HA}^-)^1 \quad (1)$$

$$(\text{Rib}\ldots\text{HA}^-)^1 \rightarrow (\text{Rib}\ldots\text{HA}^-)^3 \quad (2)$$

$$(\text{Rib}\ldots\text{HA}^-)^3 \rightarrow \text{Rib}^{\cdot} + \text{HA}^{\cdot} \quad (3)$$

$$\text{Rib} + h\nu \rightarrow \text{Rib}^1 \quad (4)$$

$$\text{Rib}^1 \rightarrow \text{Rib}^3 \quad (5)$$

$$\text{Rib}^3 + \text{H}_2\text{A} \rightarrow \text{RibH}_2 + \text{A} \quad (6)$$

$$\text{HA}^{\cdot} + \text{HA}^{\cdot} \rightarrow \text{H}_2\text{A} + \text{A} \quad (7)$$

$$\text{RibH}^{\cdot} + \text{RibH}^{\cdot} \rightarrow \text{Rib} + \text{RibH}_2 \quad (8)$$

$$\text{RibH}^{\cdot} + \text{HA}^{\cdot} \rightarrow \text{Rib} + \text{H}_2\text{A} \quad (9)$$

$$\text{RibH}_2 + \text{A} \rightarrow \text{Rib} + \text{H}_2\text{A} \quad (10)$$

$$\text{RibH}^{\cdot} + \text{H}_2\text{A} \rightarrow \text{Rib} + \text{H}_3\text{A}^{\cdot} \quad (11)$$

$$(\text{Rib}\ldots\text{HA}^-)^3 + 2\text{H}_2\text{O} \rightarrow 2\text{OH}^{\cdot} + \text{RibH}_2 + \text{HA}^{\cdot} \quad (12)$$

where H_2A is ascorbic acid, HA^- is monoascorbic acid and A is dehydroascorbic acid.

The reactions in eqns 1–11 are mostly in accordance with former work. However, the formation of H_2O_2 (eqn 12) has not been observed in any previous work except ours (Şahbaz & Somer, 1992). Although we observed the formation of H_2O_2 polarographically in our earlier work (Şahbaz & Somer, 1992), it could not be followed quantitatively during the experiment. It is known that H_2O_2 is not easily determined. In this work, its existence is shown both polarographically and spectrometrically (infrared). For the IR spectrometric determination, we illuminated a solution of 5×10^{-4} M ascorbic acid, 1.6×10^{-5} M riboflavin and pH 4.5 citrate buffer for 20 min and the IR spectrum was taken using an FTIR instrument. A peak at 418 cm^{-1} could be attributed to H_2O_2. The quantitative determination of H_2O_2 was done polarographically.

Plausibly, the formation of H_2O_2 could occur by reaction between excited riboflavin and H_2O. When aqueous riboflavin solutions were illuminated with the same light, H_2O_2 formation did not occur. H_2O_2 was formed only when ascorbic acid was also present in the solution.

According to the proposed mechanism, for 1 mole of ascorbic acid 1 mole of H_2O_2 should be formed; usually, however, it is less than 1. To try to explain this, the reaction between riboflavin and H_2O_2 was also studied. First, a solution containing 4×10^{-4} M H_2O_2 was prepared and a polarogram taken. Then riboflavin was added so that its final concentration was 3×10^{-5} M. A further polarogram was taken and there was no change in H_2O_2 concentration. Then, the same solution was illuminated for a period and a polarogram taken. The H_2O_2 concentration was nearly halved. The yellow colour of riboflavin was also diminished. This explains why, for each mole of ascorbic acid decomposed, less than 1 mole of H_2O_2 was formed.

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