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Photoinduced Processes of Hydrogen Peroxide Formation and Decomposition and Their Role in Photosynthesis and Biosphere Origin

G. G. Komissarov and A. V. Lobanov

Semenov Institute of Chemical Physics, Russian Academy of Sciences, ul. Kosygina 4, Moscow, 119991 Russia
e-mail: gkomiss@yandex.ru

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Abstract—Processes of mutual photocatalytic transformation of O_2 and H_2O_2 in aqueous suspensions of chlorophyll and phthalocyanine immobilized on silica gel were investigated with application to the problem of the origin and evolution of the biosphere. It was demonstrated that chlorophyll affects the processes of H_2O_2 generation in water saturated in air oxygen and H_2O_2 decomposition in water upon illumination. The efficiency of these photocatalytic processes was shown to depend on pH of the medium and the amount of the pigment coating the substrate surface. The formation of organic products was detected at the irradiation of the suspensions of chlorophyll (phthalocyanine)/silica gel/water containing H_2O_2 and an inorganic carbon source.

Keywords: oxygen, hydrogen peroxide, chlorophyll, phthalocyanine, photoinduced processes

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INTRODUCTION

The problem of the origin of life and biosphere development can hardly be solved without the determination of the origin of natural photosynthesis, i.e., the formation of organic compounds under the influence of visible light from carbon dioxide and water accompanied by the simultaneous release of molecular oxygen. The modern paradigm that underlies approaches to the elucidation of photosynthesis mechanisms postulates the following fundamental reaction of this process, in which water is regarded as a source of photosynthetic oxygen (hydrogen):

$$CO_2 + H_2O \xrightarrow{\text{light}} \{CH_2O\} + O_2. \quad (1)$$

The aqueous origin of oxygen was established at the Vernadsky Institute of Geochemistry and Analytical Chemistry of the Academy of Science of the USSR more than 70 years ago by a research group led by academician A.P. Vinogradov. This was an outstanding scientific achievement, because atmospheric carbon dioxide had been considered previously as a source of photosynthetic oxygen. However, one “small” problem persisted. There has never been an exact coincidence between the isotopic compositions of photosynthetic oxygen and oxygen from water. As follows from the table presented in a lecture of Vinogradov and Kutyurin [1], the minimum isotope fractionation (1.0003) was observed in the reaction of hydrogen peroxide (H_2O_2) decomposition. This circumstance was emphasized by us, after a new equation of photosynthesis was proposed [2]:

$$\begin{gathered} CO_2(\text{air}) + H_2O_2(\text{water}) \\ \xrightarrow[\text{Thermal energy } (+/-)]{\text{Light energy}} \text{carbohydrates} + O_2. \end{gathered} \quad (2)$$

The reader can find its substantiation in the book of Komissarov [3]. Here, we note only the significant difference between the above two equations of photosynthesis. The source of photosynthetic oxygen is exogenous and endogenous H_2O_2. Natural water always contains H_2O_2. Its concentration increases during transpiration by several orders of magnitude. Endogenous H_2O_2 is formed owing to the functioning of mitochondria, by peroxisomes, etc. Water serves as a reaction medium in photosynthesis [3, 4].

It is possible that new components taking part in photosynthesis will be detected in the future in air and natural water. Because of this, we believe that it is reasonable to write the photosynthesis reaction including air, in addition to CO_2, and water, in addition to H_2O_2, and it is obvious that photosynthesis, as well as any other living process, is impossible without water.

How can the formation of the modern atmosphere be described in the light of the new concepts? Let us begin from a gas whose presence in air provides the possibility of the existence of all living organisms in our planet.

The appearance of oxygen in the terrestrial atmosphere is a key and still poorly understood aspect in the problem of the origin of the biosphere. During the whole time of the existence of our planet, bacterial and plant photosynthesis has produced (1.8–2.3) × 10^{22} g O_2 [5]. This estimate was obtained from the amount of organic carbon buried in sediments. The oxidation of rocks occurring on Earth would have required an order of magnitude more oxygen. Hence, oxygen in the Earth's atmosphere could only partly be produced by photosynthetic organisms. According to estimates, the radiolysis of groundwater has releases 1.9×10^{23} g O_2 into the atmosphere over 4×10^9 years [5].

Among the scenarios of the origin of the biosphere, the model proposed by E.M. Galimov is of special interest. Based on the investigation of the isotope composition of carbon, oxygen, and other elements, Galimov concluded that carbonaceous chondrites played a crucial role in the formation of the terrestrial biosphere; they fell on Earth during an early period of its history simultaneously with the meteorite bombardment of the Moon 4.2–3.9 Ga ago [6, 7]. It was estimated that carbonaceous chondrites, which are rich in water, could be the source of all the water on Earth. Moreover, they contain various organic compounds, including porphyrins, which could occur in primordial basins in fairly high concentrations. It is possible that these organic compounds could take part in the photochemical release of oxygen, mainly at the expense of H_2O_2 occurring in water. It is known that the concentration of H_2O_2 in natural water is 10^{-6}–10^{-5} M and even higher [8], and many metal porphyrin complexes show catalytic and photocatalytic activity in the reaction of H_2O_2 decomposition with the release of O_2 [3, 9]. Thus, according to Galimov, oxygen could appear in the Earth's atmosphere before the emergence of biologic structures.

This suggestion is consistent with the model of N.P. Semenenko, who argued that “the appearance of living matter did not lead to the appearance of oxygen in the atmosphere; but, vice versa, the presence of free oxygen related to the formation of the atmosphere and hydrosphere promoted the formation of the biosphere” [10].

The investigation of the isotope composition of atmospheric oxygen showed that it is composed of 2/3 of O_2 of geologic origin and 1/3 of O_2 generated by photosynthetic organisms [11]. It is supposed that primary oxygen was produced by the degassing of basaltic magmas (O_2 input from the Earth's interiors has continued up to the present day). Oceanic water served as a reservoir for this gas. Biogenic (photosynthetic) oxygen appeared later. Therefore, the present-day atmosphere includes both abiogenic and biogenic oxygen.

Thus, the role of plants among the factors responsible for the formation of atmospheric oxygen can be assessed. The ideas widely cited in the literature on the exceptional role of plants in the formation of the present-day oxygen atmosphere of the Earth seem to be misleading. The same conclusion can be drawn on the basis of the concept of relations between photosynthesis and photorespiration of plants and the participation of H_2O_2 of endogenous and exogenous origin in these processes [4].

Photosynthesis in green leaves is accompanied by the opposite process of photorespiration (carbon dioxide release and oxygen uptake), which is also stimulated by light. In addition, there is a process known as dark breathing accounting for up to 7% of photosynthetic gas exchange. The intensity of photorespiration is almost an order of magnitude higher than that of dark breathing and accounts for ~50% of photosynthesis gas exchange [12, 13]. Respiration produces endogenous H_2O_2, and photorespiration can therefore be described by the photosynthesis reaction written from right to left:

$$CO_2 + H_2O_2 \underset{\text{Photorespiration}}{\overset{\text{Photosynthesis}}{\rightleftarrows}} O_2 + \text{carbohydrates}. \quad (3)$$

It was supposed that the activity of metabolic processes in plant physiology is controlled by approximately equal degrees by the intensities of photosynthesis and photorespiration [3]. It is clear that photosynthesis cannot be regarded in such a case as the only process controlling the formation of the oxygen atmosphere of the Earth, because it appears that the transformation of H_2O_2 to O_2 and the opposite process are equally efficient in plants.

In the context of the problem of biosphere evolution and, especially, the origin of the primary stages of photosynthesis, we estimated the possibility of mutual photochemical transformations of O_2 and H_2O_2 under the influence of the main photosynthetic pigment, chlorophyll, and its synthetic analogue, phthalocyanine.

CHLOROPHYLL-PHOTOCATALYZED FORMATION OF HYDROGEN PEROXIDE IN WATER SATURATED IN AIR OXYGEN

It is known that some synthetic analogues of chlorophyll (Chl), metal complexes of porphyrins and phthalocyanines, take part in the formation of H_2O_2 in oxygen-saturated water solutions upon illumination [14, 15]. Porphyrin complexes of nontransition metals (Mg, Zn, Al, and Cd) compose a group of compounds with specific photophysical properties. The main advantage of these molecules is the ability to generate long-lived (up to 1 ms) triplet excited states with a high quantum yield of 60–90% [16], which is very important for photo-dependent processes involving pigments. Chlorophyll is also a member of this group of metal porphyrins, because its molecule contains an Mg ion. The similarity of the photophysical and photochemical properties of Chl, porphyrins, and phthalocyanines allows us to suggest that photoinduced H_2O_2 generation can also occur under the participation of Chl.

Our study demonstrated the activity of Chl with respect to H_2O_2 generation in water saturated in air oxygen at illumination under in vitro conditions. Since the solubility of photoactive molecules in water is low, they occurred in the primordial water basins of the Earth as diverse supramolecular complexes. Such systems could be represented by layers on inorganic substrates. In our experiments, Chl was deposited on silica gel. The immobilization of Chl allowed us to model processes in a heterogeneous regime, which is favorable for the occurrence of photocatalytic reactions and, moreover, is methodically convenient. Amorphous silica was used as a substrate; it is a chemically and photochemically inert and stable inorganic material. In our opinion, the suspension chlorophyll/silica gel/water is an adequate model for primary organic substances adsorbed on the particles of minerals, silica, and other natural materials in water basins. It was previously shown that Chl immobilized on silica gel is much more stable compared with Chl in solutions, micelles, soluble polymers, etc. [17].

Chlorophyll (a mixture of chlorophyll a and b) was extracted from dry nettle leaves [18]. Chlorophyll was purified from pheophetine and carotenoids using a 10 × 300 mm column packed with dry powdered sugar. For this purpose, powdered sugar was heated at 100°C for 4 h and pressed into the column in small portions. A pigment extract (~15 mg dry residue) was dissolved in a hexane–ether (3 : 7) mixture and introduced into the column. The same system was used for elution. Chlorophyll-colored zones were mechanically extracted, and Chl was washed with ether. The concentration and purity of Chl was controlled on the basis of its absorption spectra in ether ($\lambda_{max} = 663$ nm and $\varepsilon = 91500$ $(mol/L)^{-1}$ cm^{-1}) in 1-cm thick quartz vessels using a Beckman DU-8 spectrophotometer. Chlorophyll was immobilized on L 40/100 silica gel (Chemapol) using the following procedure. One gram of silica gel was mixed with 5 mL of Chl solution in ether with a concentration of 10^{-4} M. The mixture was kept in a dark place at room temperature until the complete evaporation of the solvent. The colored silica gel was repeatedly washed with distilled water and dried to constant weight in an evacuated desiccator. The material was stored in a dark place at +4°C. The samples prepared in such a way contained 0.5 μmol Chl per 1 g silica gel.

Kinetic experiments on H_2O_2 formation were performed in a glass reactor allowing simultaneous irradiation and stirring. The reactor was loaded with 10 mL of aqueous solution with a desired pH value (adjusted with NaOH or HCl) containing in some cases a reactant for the determination of intermediate products. Then, 200 mg of silica gel with immobilized Chl were added. The obtained chlorophyll/silica gel/water suspension was stirred for 5 min in darkness, after which an air flow and illumination were switched on under continued stirring. The source of visible light was a 150-W halogen lamp equipped with a condenser and lenses. All the experiments were carried out at 20°C.

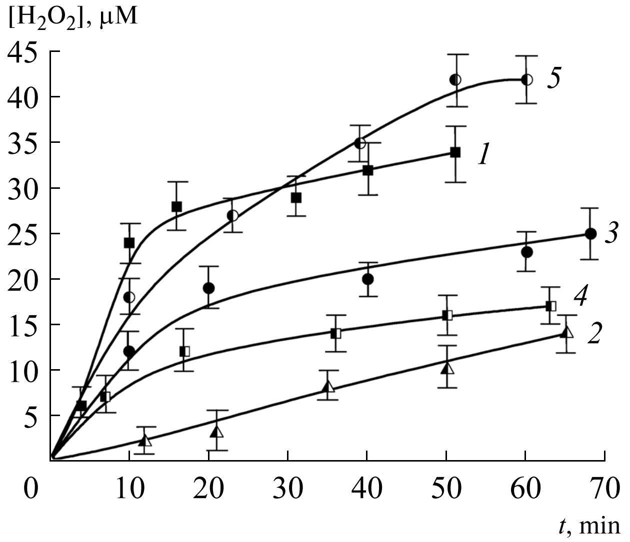

Fig. 1. Kinetics of H_2O_2 formation in the presence of Chl immobilized on silica gel (1×10^{-7} mol Chl per 10 mL of solution) under the influence of visible light: (*1*) pH 12.4; (*2*) pH 12.4, with addition of ethanol (0.1 M); (*3*) pH 3.8; (*4*) pH 3.8, with addition of DBB (1×10^{-5} mol DBB per 10 mL of solution); and (*5*) pH 3.8, 50% D_2O.

The formation of H_2O_2 was monitored using the iodometric titration method. For this purpose, 1-mL aliquots of the reaction solution were collected at certain time intervals and mixed with 1 mL H_2SO_4 (0.2 M); dissolved oxygen was replaced by carbon dioxide; and the solutions were mixed with 2 mL of oxygen-free 5% KI aqueous solution. The release of I, which formed the complex anion I_3^- with excess iodide, was detected by spectrophotometry. The parameters of the absorption band of I_3^- were the following: $\lambda_{max} = 351$ nm and $\varepsilon = 26400$ M^{-1} cm^{-1} [19].

In experiments without Chl, as well as in experiments without illumination, the formation of H_2O_2 was not observed. The kinetics of photoinduced H_2O_2 formation in the presence of adsorbed Chl is shown in Fig. 1. Thus, Chl adsorbed as a monomolecular layer on silica gel shows photoinduced activity in the process of H_2O_2 formation.

The rate of photocatalytic formation (decomposition) of H_2O_2 can be specified as

$$w = k_V[Chl]^n[H_2O_2]^m,$$

where k_V is the observed rate constant, and [Chl] is the number of moles of Chl per one liter of the reaction

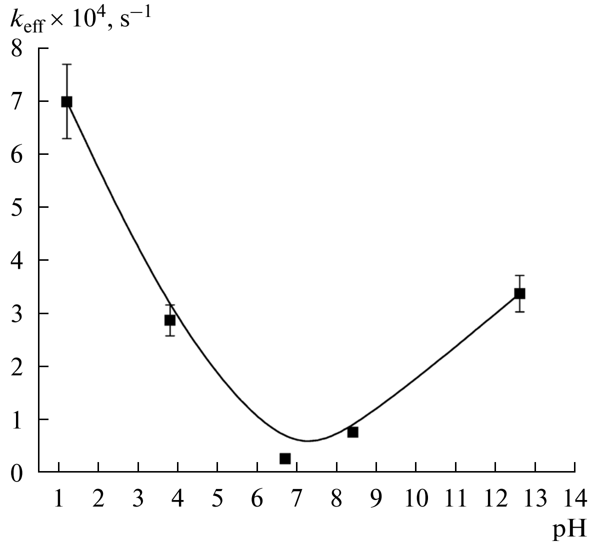

Fig. 2. Effective rate constant of H_2O_2 formation in the presence of Chl immobilized on silica gel under the influence of visible light as a function of the pH of the medium (other conditions are as above)

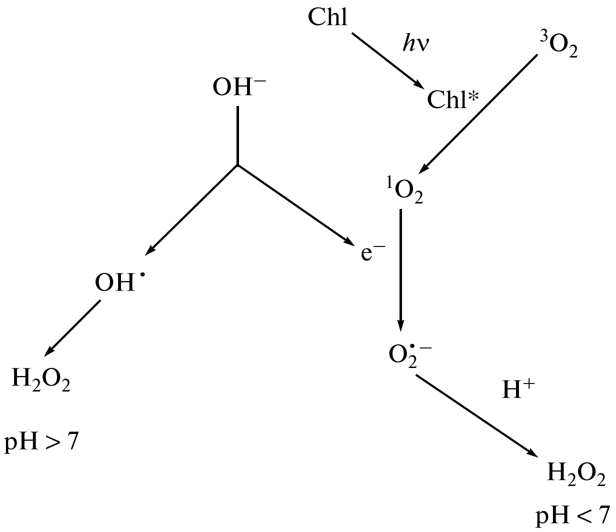

Fig. 3. Schematic reactions resulting in H_2O_2 generation in the presence of Chl depending on the pH of solution; Chl* is chlorophyll in a triplet excited state.

mixture. Taking into account that [Chl] = const and denoting $k_V[\text{Chl}]^n$ as k_{eff} (effective rate constant), we obtain

$$w = k_{\text{eff}}[H_2O_2]^m.$$

The initial segments of the kinetic curves in the $\ln[H_2O_2]$–t coordinates were approximated by straight lines, which allowed us to accept $m = 1$ for the calculation of k_{eff}.

The efficiency of H_2O_2 generation depended significantly on the pH value of solution (Fig. 2). For instance, k_{eff} decreased from 2.8×10^{-4} at pH 3.8 to 0.3×10^{-4} s^{-1} at pH 6.7. It is known that H_2O_2 is formed owing to the recombination of $^\bullet OH$ radicals in an alkaline environment and at the expense of $O_2^{\bullet-}$ and $HO_2^\bullet$ radicals in an acidic environment [20, 21]. The following reactions take place:

$$^\bullet OH + {}^\bullet OH \longrightarrow H_2O_2,\ k = 6 \times 10^9\ M^{-1}\ s^{-1}, \quad (4)$$

$$HO_2^\bullet + HO_2^\bullet \longrightarrow H_2O_2 + O_2,\ k = 2 \times 10^6\ M^{-1}\ s^{-1}, \quad (5)$$

$$HO_2^\bullet + O_2^{\bullet-}(+H^+) \longrightarrow H_2O_2 + O_2,\ k = 9 \times 10^7\ M^{-1}\ s^{-1}, \quad (6)$$

$$O_2^{\bullet-} + H^+ \longrightarrow HO_2^\bullet,\ k = 5 \times 10^{10}\ M^{-1}\ s^{-1}. \quad (7)$$

The addition of 1 M ethanol to the reaction mixture containing adsorbed Chl as a scavenger of hydroxyl groups [20] decelerated the formation of H_2O_2 in an alkaline solution with pH 12.4 (Fig. 1), which confirms the participation of these radicals in the process in accordance with Eq. (4). In an acidic medium with pH 3.8, the generation of H_2O_2 was decelerated by the addition of 1×10^{-5} mol of 1,2-dihydroxy-3,5-di(*tert*-butylbenzene) (DBB, Sigma) on silica gel, as a scavenger of superoxide radicals (Fig. 1). This supports the suggestion on the occurrence of reactions (5)–(7) under such conditions.

In the water suspensions of metal porphyrin complexes that can efficiently generate triplet excited states upon irradiation, the formation of singlet oxygen, $^1O_2$, is highly probable. It is known that the lifetime of $^1O_2$ increases in heavy water [21, 22]. Indeed, in an experiment with 50% D_2O, the rate of H_2O_2 accumulation showed an almost twofold increase (Fig. 1). It is very probable that the singlet oxygen generated by triplet excited Chl captures an electron from OH^-. The supposed scenario of H_2O_2 generation depending on pH of the medium is shown in Fig. 3.

In our opinion, one promising direction for the future investigation of H_2O_2 formation under the influence of photoexcited Chl is the estimation of the influence of the microscopic environment of the pigment. In the case of metal porphyrins, the role of the microscopic environment (protein macromolecule, lipid colloids, etc.) can be very important [3, 23]. Our reconnaissance experiments in this direction revealed the photocatalytic activity of Chl in a noncovalent complex with an albumin macromolecule, as well as Chl implanted in the micelles of cetyltrimethyl

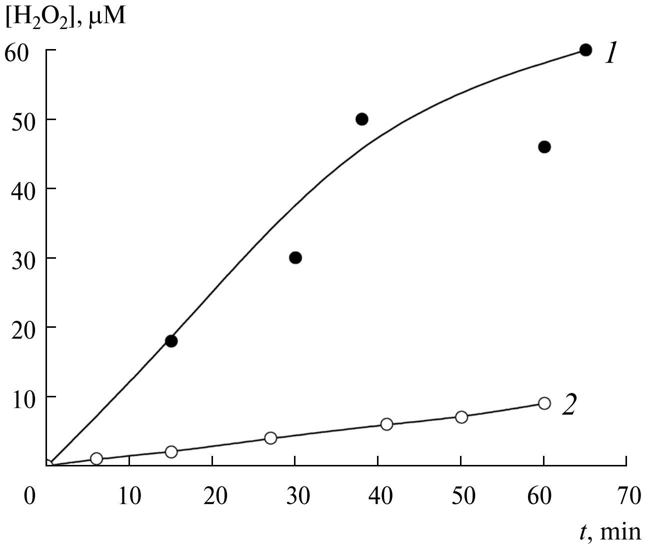

Fig. 4. Kinetics of H_2O_2 formation in the presence of AlPc immobilized on silica gel (1.1×10^{-5} mol AlPc per one liter of solution) and 0.1 M TEA under the influence of visible light at (*1*) pH 1.2 and (*2*) pH 6.7.

ammonium bromide, which is comparable with the activity of Chl immobilized on silica gel.

Experiments under similar conditions were also performed with spectrally pure chlorinated aluminum phthalocyanine (AlPc, Kodak) as an analogue of Chl. On the one hand, the photophysical and photochemical properties of phthalocyanine are similar to those of Chl, but its resistance to destructive impacts is much higher, which motivated the choice of AlPc for our experiment [3]. On the other hand, phthalocyanines can be considered as more primitive and evolutionally earlier precursors of Chl.

Figures 4 and 5 show the kinetics of photoinduced H_2O_2 formation in the presence of AlPc. As can be seen, AlPc adsorbed on silica gel can serve as a photocatalyst for H_2O_2 generation. The kinetic curves of H_2O_2 formation in the presence of AlPc are mostly near exponential.

Figure 4 shows that the yield of the H_2O_2-forming reaction is increased by the addition of 0.1 M triethylamine (TEA) to the reaction medium. The addition of this nitrous Lewis base also affects the AlPc-catalyzed formation of H_2O_2, and the identity of the base is of particular importance. For instance, in an acidic medium, TEA co-catalyzes H_2O_2 generation more efficiently than morpholine (Fig. 5) in the presence of immobilized AlPc. The nitrous base shows a co-catalytic activity serving as an electron donor, polarizing chemical bonds, etc. This result is of interest in view of the reducing character of the primordial terrestrial atmosphere.

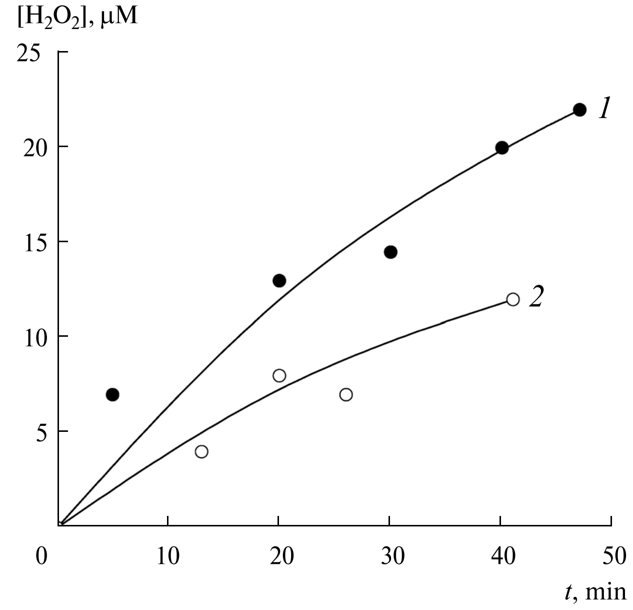

Fig. 5. Kinetics of H_2O_2 formation in the presence of AlPc immobilized on silica gel (1.1×10^{-5} mol AlPc per one liter of solution) and 0.1 M morpholine under the influence of visible light at (*1*) pH 1.2 and (*2*) pH 12.6.

Similar to the experiments with Chl, the efficiency of H_2O_2 generation is strongly dependent on solution pH (Figs. 4, 5). The pH dependence of the rate of H_2O_2 generation in the presence of AlPc indicates that the process occurs in highly alkaline and acidic media (effective rate constant, $k_{\text{eff}} = (4–8) \times 10^{-4}\ \text{s}^{-1}$) but is strongly depressed at near neutral pH ($k_{\text{eff}} = 5 \times 10^{-5}\ \text{s}^{-1}$).

Thus, Chl and its analogues show a photo-depending activity in the process of O_2 reduction to H_2O_2. The formation of H_2O_2 involves $^1O_2$, $^{\bullet}OH$, and $O_2^{\bullet -}$. The activity of Chl depends on the pH value of the system. We believe that H_2O_2 can be formed in green leaves under the influence of photoexcited Chl. This reaction could contribute, therefore. to the formation of endogenous H_2O_2 under the participation of atmospheric oxygen. In the context of problems of the evolution of photobiological processes, this reaction could probably be considered as a simple proxy for plant photorespiration (3).

CHLOROPHYLL-PHOTOCATALYZED DECOMPOSITION OF HYDROGEN PEROXIDE IN WATER

As can be seen in Figs. 1, 4, and 5, the accumulation of H_2O_2 is described in all cases by kinetic curves with a saturation segment; therefore, a steady-state H_2O_2 level was established and maintained at the illu-

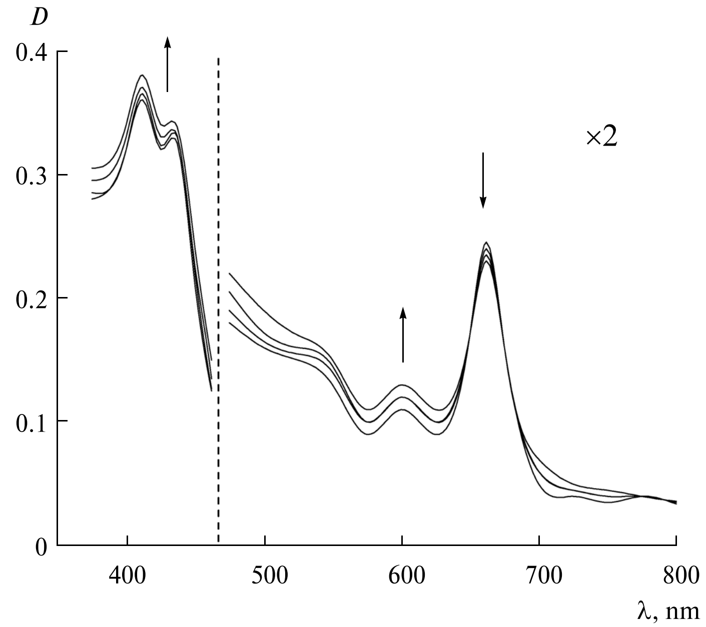

Fig. 6. Variations in the absorption spectrum of Chl in 50% ethanol (~10^{-5} M) at the addition of 10^{-7} to 10^{-5} M H_2O_2. The upward- and downward-pointing arrows show an increase and a decrease, respectively, in the intensity of absorption bands in the Chl spectrum at the addition of H_2O_2.

mination of chlorophyll-bearing aqueous systems. As a certain amount of H_2O_2 is accumulated, H_2O_2 decomposition begins competing with its generation. Chlorophyll can be considered as a photocatalyst for both H_2O_2 formation and decomposition with the release of oxygen and water. The latter reaction is of interest as a model for the prebiotic release of oxygen in the presence of primary photoactive compounds.

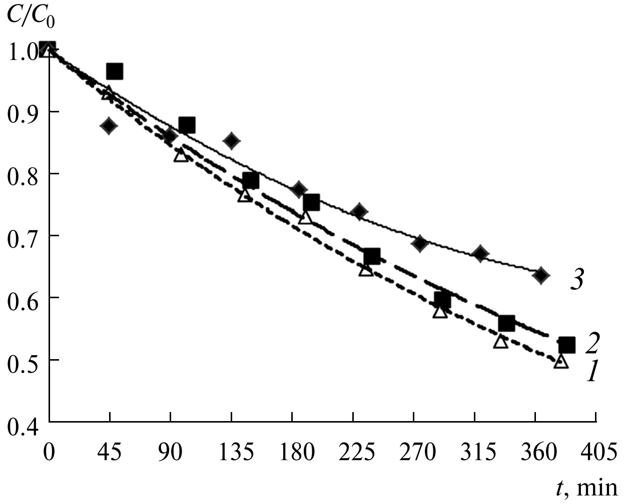

Fig. 7. Kinetics of H_2O_2 decomposition under the influence of light in the presence of immobilized Chl; the degree of Chl deposition is (*1*) 0.23, (*2*) 0.57, and (*3*) 1.92 μmol/g; and the initial concentration of H_2O_2 is $C_0 = 0.03$ M.

The coordination of H_2O_2 by Chl as extra ligands was confirmed by the method of electron absorption spectroscopy (Fig. 6). The sequential addition of from 10^{-7} to 10^{-3} M H_2O_2 to 10^{-5} M Chl solutions results in changes in the intensity of bands in the absorption spectra with a distinct isobestic point.

In order to evaluate the photocatalytic activity of Chl in the H_2O_2 decomposition reaction, Chl immobilized on silica gel was used. The procedure of sample preparation was similar to that described above, but the concentration of Chl in ether solutions before evaporation was varied in these experiments for obtaining photocatalysts with different degrees of Chl deposition.

Kinetic experiments on H_2O_2 decomposition were carried out using the photochemical reactor described above. The reaction vessel contained 10 mL of H_2O_2 solution in water with a concentration of 0.03 mol/L (pH 6.7) and 200 mg of Chl-coated silica gel. The suspension was stirred for 5 min in darkness and, then, illuminated under continuous stirring. The experiments were carried out at 20°C. The consumption of H_2O_2 was monitored by the permanganatometry.

The obtained samples of immobilized Chl have a photocatalytic effect on H_2O_2 decomposition (Fig. 7). The kinetic curves of H_2O_2 consumption were successfully linearized in the $\ln[H_2O_2] - t$ coordinates (Fig. 8), which allowed us to assume a (pseudo)-first order reaction with respect to H_2O_2. For samples with the degree of Chl deposition of 0.57 μmol/g, $k_{eff} = 2.8 \times$

10^{-5} s^{-1}, which is consistent with the suggestion on the competing decomposition and formation (increase in H_2O_2 concentration) of H_2O_2.

It can be seen from the results reported above that the photocatalytic activity of Chl immobilized on silica gel in the reaction of H_2O_2 decomposition decreases with increasing degree of Chl deposition on the substrate surface. Perhaps, as the specific content of Chl increases, the amount of its surface aggregates also increases, which either causes steric hindrances to H_2O_2 coordination or produces channels for the deactivation of the excited states of Chl molecules.

Thus, chlorophyll can serve as a photocatalyst for H_2O_2 decomposition. This is in line with the concept of photocatalyzed oxygen formation from primary organic molecules at the early stages of evolution.

REDUCTION OF INORGANIC CARBON CONJUGATED WITH CHLOROPHYLL-ASSISTED PHOTODECOMPOSITION OF HYDROGEN PEROXIDE

Currently, of special interest is the problem of the role of H_2O_2 as an electron donor in natural photosynthesis and the evolutionary abiogenic reduction of inorganic carbon. The results of such investigations may appear helpful for the development of artificial photosynthetic systems and the evaluation of such fundamental problems as the origin of living matter.

Note that, in the light of the photosynthesis concept and the assumption that H_2O_2 is a source of photosynthetic oxygen [2–4], H_2O_2 is considered as a donor of electrons migrating along the electron transfer chain, which results in the accumulation of NADPH. Up to now, extensive experimental data have been accumulated confirming the stimulating influence of H_2O_2 on the intensity of photosynthesis [24, 25]. In this regard, an important question arises on the possibility of electron transfer in the $H_2O_2 \longrightarrow$ chlorophyll $\longrightarrow$ acceptor chain. Of special interest is the possibility of inorganic carbon reduction under such conditions.

The main feature of tetrapyrrol pigments is their ability to change the sign of the redox potential $\varphi°$ upon transforming into an excited state under the influence of light. In particular, in the ground electron state, the pigment molecule can oxidize an electron donor; whereas in the excited state, it can transfer an electron to an available acceptor suitable with respect to the reducing level. It is essential that the oxidation of H_2O_2 and HO_2^- is thermodynamically advantageous compared with water oxidation.

The essence of pigment photocatalysis is schematically illustrated by Fig. 9 [26].

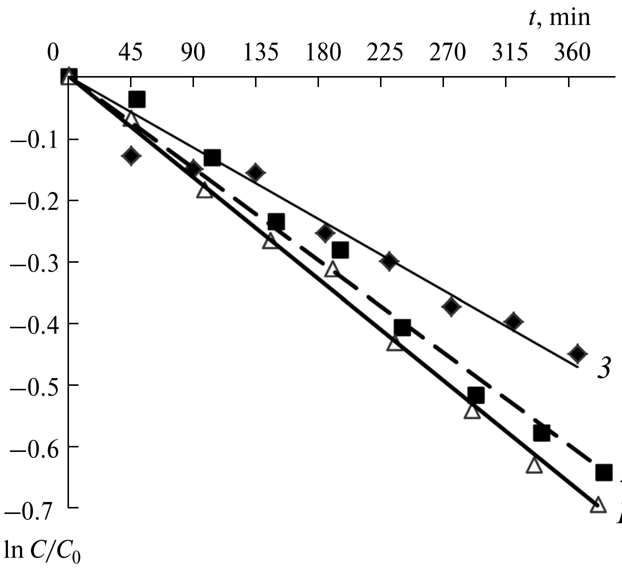

Fig. 8. Linear anamorphoses of the kinetic curves presented in Fig. 7. The line numbers correspond to the numbers of curves in Fig. 7.

In accordance with the scheme, CO_2 can be reduced to organic products. With this in mind, we calculated and compared the thermodynamic parameters of formation of various reduced carbon species taking water and hydrogen peroxide as electron donors (Table 1).

A comparison of the thermodynamic characteristics presented in Table 1 indicates that the reactions with H_2O_2 require much smaller energies than similar reactions with H_2O.

Table 1. Thermodynamic parameters of CO_2 reduction to CO and organic products

Product	Electron donor	ΔH^0, kJ/mol	ΔG^0, kJ/mol
CH_2O	H_2O	563	521
	H_2O_2	465	405
HCOOH	H_2O	270	286
	H_2O_2	172	166
CH_3OH	H_2O	727	703
	H_2O_2	530	464
CO	H_2O	569	494
	H_2O_2	377	316
CH_4	H_2O	890	818
	H_2O_2	694	580

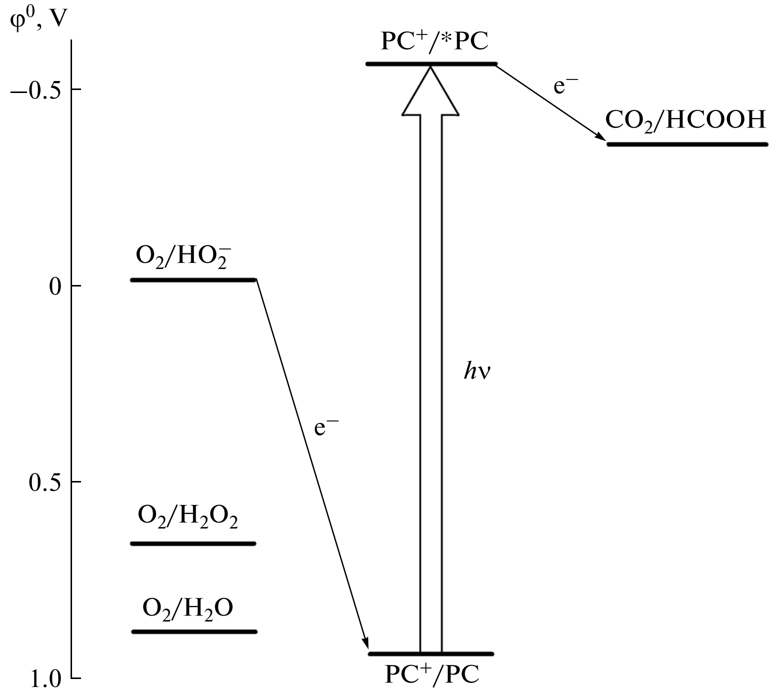

Fig. 9. Schematic mechanism of the photocatalytic activity of pigments (PC is a porphyrin photocatalyst, for instance, chlorophyll or phthalocyanine).

An experiment with the addition of sodium bicarbonate into the reaction system was carried out using a method similar to that employed in the experiment on the consumption of H_2O_2. The initial H_2O_2 and $NaHCO_3$ concentrations were 0.08 and 0.2 mol/L, respectively. Photocolorimetric analysis [27] revealed the formation of formaldehyde in this system upon irradiation (Table 2). The concentration of formaldehyde increased with increasing degree of Chl deposition. This implies that $NaHCO_3$ was reduced in the system by H_2O_2 under the influence of immobilized Chl.

In another experimental series, Zn- and Al-phthalocyanine complexes were used instead of chlorophyll as photocatalysts for the synthesis of organic substances from HCO_3^- and H_2O_2. The metal complexes were preliminarily adsorbed on silica gel. The silica gel samples contained 5.4×10^{-7} mol of pigment per 1 g of the substrate.

Table 2. Concentration of CH_2O produced by the irradiation for 6 h of chlorophyll/silica gel suspensions in water in the presence of hydrogen peroxide and bicarbonate anions

Degree of Chl immobilization, μmol/g	$[CH_2O] \times 10^4$, M
1.92	1.25
0.57	0.16
0.23	0.087

Samples of the reaction mixture collected after 11–22 h of illumination yielded a pinkish violet color with fuchsine sulfurous acid; this is indicative of the presence of carbonyl-bearing organic compounds, among which formaldehyde was identified. The results of the quantitative determination of formaldehyde depending on the duration of the reaction in the case of the use of AlPc as a photocatalyst are shown in Fig. 10. It can be seen that the CH_2O concentration reached 6.5×10^{-5} M after illumination for 22 h. By that moment, more than 70% of the initial H_2O_2 were consumed (0.2 M), mostly owing to (photo)catalytic disproportionation. When ZnPc was used as a photocatalyst, the CH_2O concentration increased up to 5×10^{-5} M after illumination for 24 h under the same conditions.

The concentration of produced CH_2O is approximately six times higher than the concentration of the photocatalyst in the reaction system. In these experiments, we did not observe the destruction or poisoning of the photocatalysts, which retained their activity after the use at least three times for 25 h. The conclusion on the stability of immobilized metal complexes is also supported by the results of control experiments without $NaHCO_3$, the products of which did not con-

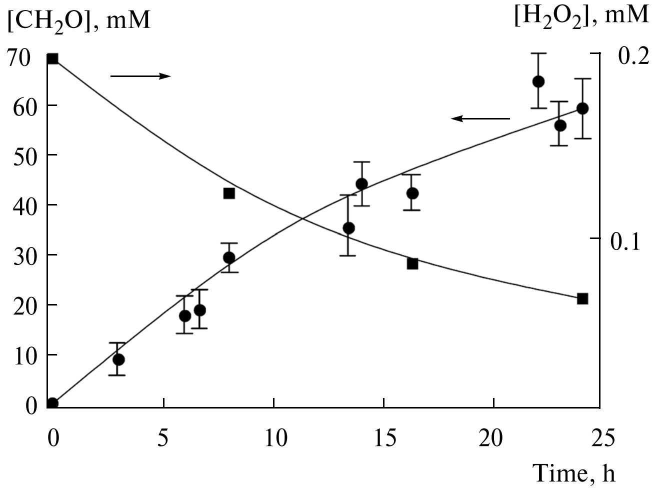

Fig. 10. Kinetics of formaldehyde accumulation and hydrogen peroxide consumption by the reaction of the photocatalytic reduction of bicarbonate ($[NaHCO_3]_0$ = 0.4 M) at 20°C.

tain formaldehyde. This allows us to conclude that CO_2 (HCO_3^-) is the only source of CH_2O. It should be emphasized that the formation of formaldehyde was not detected in an H_2O_2-free system even at experiment durations of 30–40 h.

Thus, the obtained results demonstrate that H_2O_2 is the main and necessary electron donor during the abiogenic photocatalytic reduction of inorganic carbon in an aqueous suspension of AlPc. Using analytical methods, we showed that formaldehyde (CH_2O) is formed from CO_2 and H_2O_2, and H_2O_2 plays an essential role in this process.

The promising results of experiments producing significant amounts of formaldehyde after irradiation of suspensions with adsorbed AlPc and ZnPc containing bicarbonate and H_2O_2 do not rule out the possible formation of more complex compounds in these systems. Such a suggestion was supported by Fourier transform infrared (FTIR) spectroscopy. Figures 11 and 12 show the IR spectra of products ether-extracted from irradiated reaction mixtures. The initial H_2O_2 and $NaHCO_3$ concentrations were 0.1 and 0.2 M, respectively. The IR spectra display signals at 1120 and 1600–1750 cm^{-1} corresponding to carbonyl groups. Bands at 1280–1470 cm^{-1} and 2800–3050 cm^{-1} correspond to the bending and stretching vibrations of CH_2 and CH_3 groups, respectively. These signals were not observed in the IR spectra of phthalocyanines (ZnPc). Hence, it is reasonable to conclude that the bands of IR spectra shown in Figs. 11 and 12 are due to the products of HCO_3 reduction. This conclusion is corroborated by the investigation of a control system without $NaHCO_3$. The formation of organic products was not detected in the control system. Furthermore, organic substances were not observed in samples free of H_2O_2 or stirred for 27 h in darkness. Therefore, H_2O_2 and light are essential under the given conditions for the synthesis of organic substances from the bicarbonate anion.

The products formed in laboratory photosynthetic systems containing H_2O_2 were explored in detail by chromatography–mass spectrometry. The organic substances were photogenerated in an aqueous suspension of adsorbed AlPc, H_2O, and HCO_3^-, as well as in a system containing H_2O_2 and CO_2, which is considered by us as a new step toward artificial photosynthesis [28].

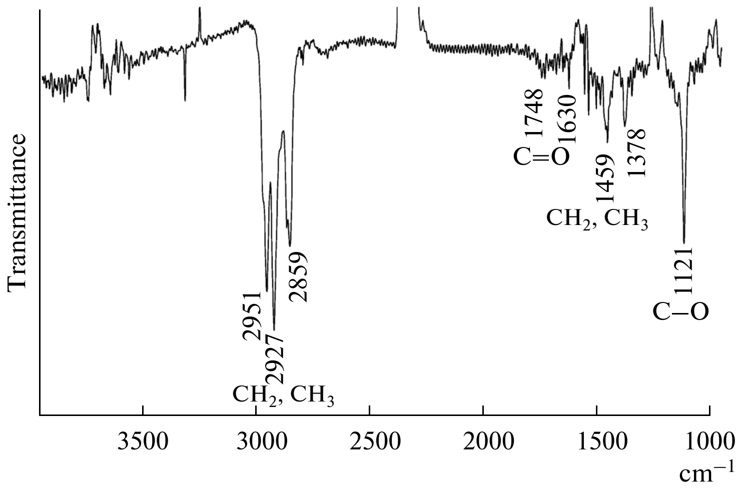

Fig. 11. IR spectra of compounds produced from H_2O_2 and CO_2 under photocatalysis with Al-phthalocyanine for 23 h.

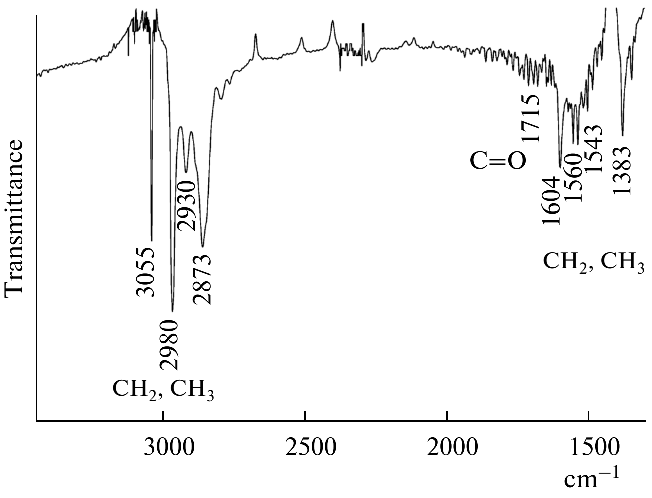

Fig. 12. IR spectra of compounds produced from H_2O_2 and CO_2 under photocatalysis with Zn-phthalocyanine for 27 h.

Chromatography–mass spectrometry revealed the presence of organic substances of the alcohol and ketone classes in the reaction mixture obtained under conditions identical to those in the photochemical experiment described above with $NaHCO_3$ (at the initial concentrations 0.2 M H_2O_2 and 0.4 M $NaHCO_3$) (Fig. 13). The elimination of any component (hydrogen peroxide, bicarbonate, or phthalocyanine) from the reaction system results in that organic products are not detected.

In another experiment, CO_2 gas was used instead of $NaHCO_3$; it was passed continuously through the suspension during the whole irradiation procedure (24 h). Other conditions were the same as in the previous experiment. The chromatography–mass spectrometry of the irradiated reaction mixture indicated the presence of formic acid (Fig. 14). In the same system without either H_2O_2 or CO_2, the formation of organic substances was not detected by chromatography–mass spectrometry.

Another important circumstance should be noted. The higher reducing ability of H_2O_2 compared with H_2O was unequivocally established in enzymatic photosynthesis reactions, for instance, in the reaction of $NADPH^+$ reduction [29]. If water is used as a reducer, reaction does not occur, whereas the use of 0.01 M H_2O_2 results in the significant accumulation of NADPH after the illumination of the reaction mixture for 25 min (Fig. 15).

It is interesting that the addition of H_2O_2 increases the rate of ATP formation from ADP and inorganic phosphate during in vitro experiments by a factor of 2–3 compared with a control experiment [3].

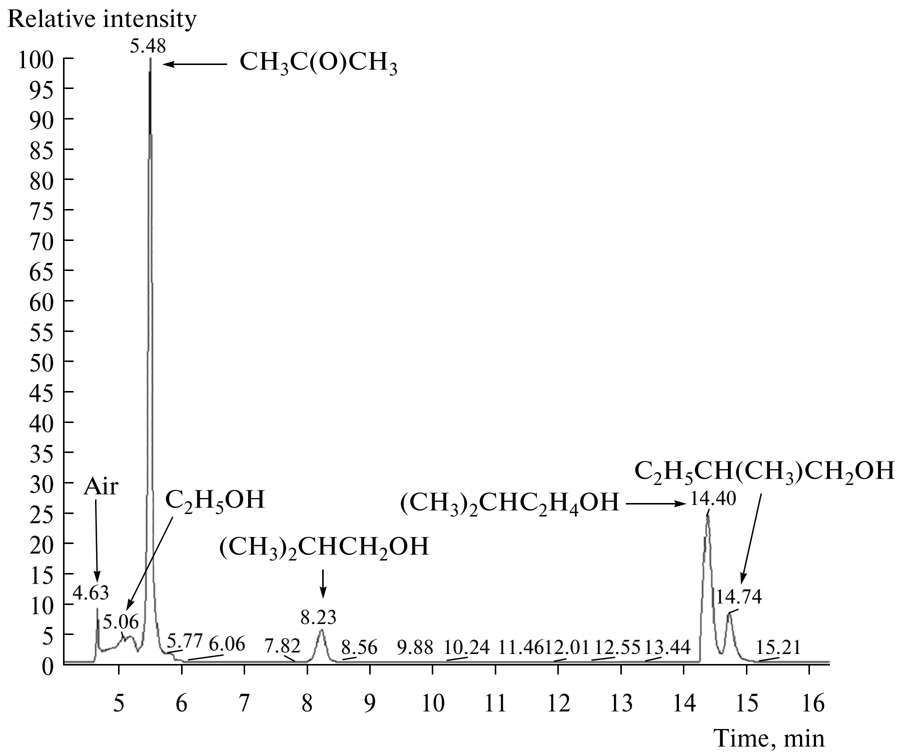

Fig. 13. Composition of organic products in a reaction mixture containing H_2O_2, $NaHCO_3$, and immobilized Al-phthalocyanine after irradiation for 24 h according to the results of chromatography–mass spectrometry.

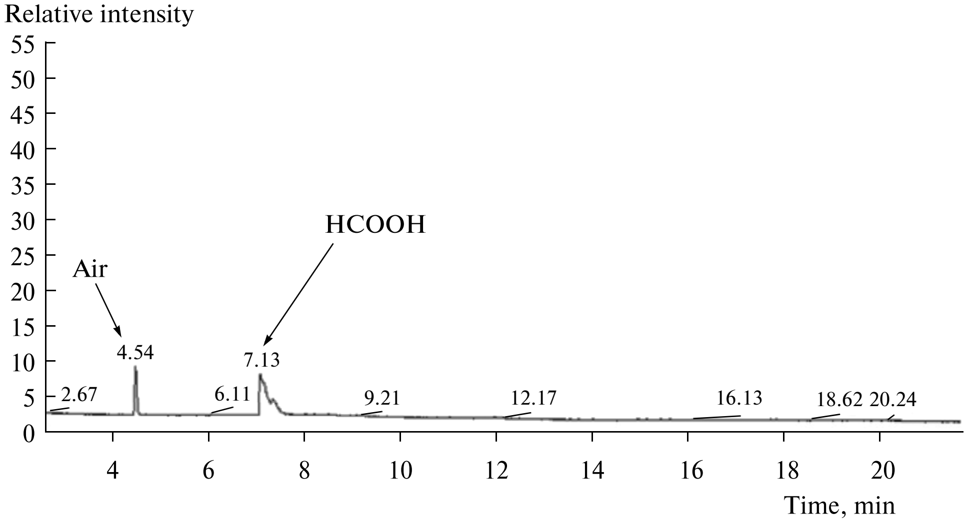

Fig. 14. Formation of formic acid in a reaction mixture containing H_2O_2 and immobilized Al-phthalocyanine after irradiation for 24 h under continuous passage of CO_2 according to the results of chromatography–mass spectrometry.

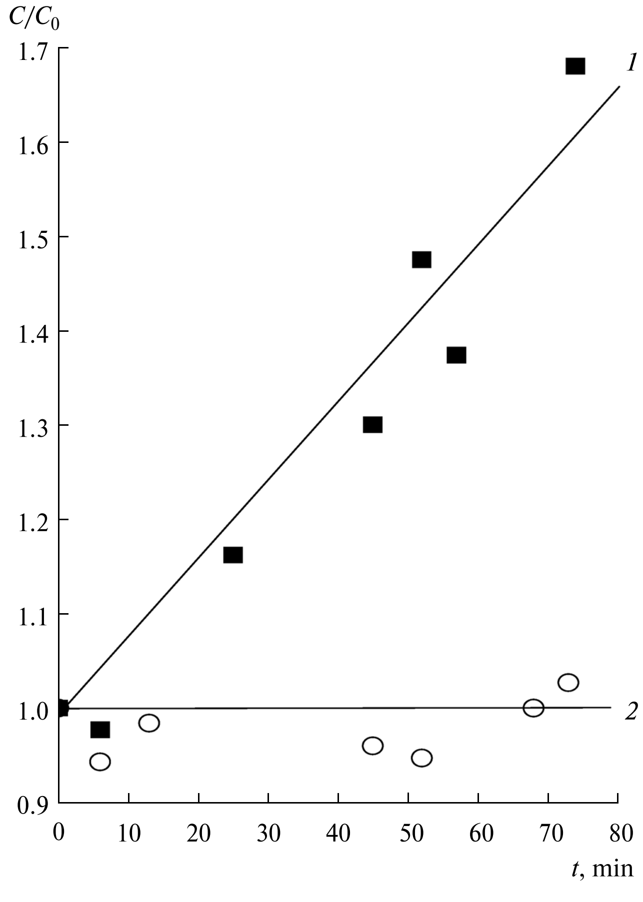

Fig. 15. Photoreduction of $NADPH^+$ in the presence of chlorophyll (1×10^{-5} M) and (*1*) with and (*2*) without hydrogen peroxide; the initial $NADPH^+$ concentration is $C_0 = 0.7 \times 10^{-4}$ M.

The results described above provide additional arguments for the new concept of photosynthesis. In addition, they convincingly demonstrate that photosynthesis can be adequately reproduced under laboratory conditions in systems containing photosynthetic enzymes or their synthetic analogues and H_2O_2 [3].

CONCLUSIONS

Chlorophyll and its chemical analogue phthalocyanine show photoinduced activity in the reduction of O_2 to H_2O_2. The formation of H_2O_2 involves active oxygen species, $^1O_2$, $^{\bullet}OH$, and $O_2^{\bullet -}$. The activity of a pigment depends on its microscopic environment and pH of the medium. The yield of the H_2O_2-forming reaction increases at the addition of nitrous bases to the reaction medium, which is intriguing in view of the reducing character of the primordial atmosphere. After the accumulation of a certain amount of H_2O_2, its decomposition begins to compete with the H_2O_2-forming reaction. As a result, a certain steady-state level of H_2O_2 is established in aqueous systems. Chlorophyll is therefore a photocatalyst for both the formation and decomposition of H_2O_2. The activity of Chl immobilized on silica gel in the H_2O_2 decomposition reaction declines with increasing degree of Chl deposition, which is probably related to an increase in the surface abundance of Chl aggregates. The decomposition of H_2O_2 can be considered as a model for prebiotic oxygen release in the presence of primary photoactive substances. This is consistent with the suggestion of oxygen formation at the early stages of biosphere evolution before the appearance of plants.

In chlorophyll/silica gel suspensions in water containing H_2O_2, inorganic carbon is reduced under the influence of light, and formaldehyde, alcohols, and other compounds are produced. Control experiments showed that organic products are not formed in the absence of H_2O_2 or bicarbonate, as well as in darkness. In addition to oxygen release, simplest organic molecules could be formed in primary water basins at the early stages of biosphere development under the influence of photochemically active compounds delivered from the outer space or formed on Earth.

The reaction of CO_2 reduction, which is a basis of modern photosynthesis, is probably one of the main processes during prebiotic evolution [30, 31]. In our opinion, H_2O_2 could actively contribute to the synthesis of primary organic substances not only on Earth but probably also in the space [3]. The presence of H_2O_2 in cosmic object was established by recent studies [3, 32–35].

The results obtained in this study and the analysis of published data suggest that H_2O_2 plays a crucial role in photosynthesis and is essential for the solution of the problem of biosphere origin [3].

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