MAGNESIUM PORPHYRINS AS POSSIBLE PHOTOSENSITIZERS OF MACROERGIC PHOSPHATE BONDS FORMATION DURING PREBIOTIC EVOLUTION

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Abstract. The paper presents experimental data on the light-induced ATP synthesis in the model systems containing chlorophyll adsorbates on aluminium or silicon oxides. The mechanism of phosphorylation observed in this system is based on the photosensitized electron transfer, where phosphate ion plays the role of an electron donor. Chlorophyll is a representative of magnesium porphyrins, which are known as photosensitizers. The formation of magnesium porphyrins in the prebiotic conditions seems to be quite probable, e.g. as a result of volcanic activity. During arising of life, magnesium porphyrins could participate in the formation of macroergic phosphate bonds of the dehydrating agents, which are necessary for the synthesis of biologically significant compounds.

1. Introduction

One of the prebiotic evolution problems is the formation of dehydrating agents in aqueous medium. These agents are necessary for the synthesis of polypeptides and polynucleotides from the corresponding monomers, and also for the formation of hypothetical more simple self-reproducing organic molecules, probably, arising at the earlier stages of chemical evolution (Allen, 1988). Carbamyl phosphate could be used as a dehydrating agent and as an intermediate for autocatalytic phosphorylation of AMP to ATP; the abiotic synthesis of carbamyl phosphate was realised by Saygin (1981). However, the dehydration reaction in biological systems most often involve only the pyrophosphate bonds. The formation of these bonds in aqueous medium during the chemical evolution, with the participation of some photosensitizers capable to use solar energy, is of special interest. Magnesium complexes of porphyrins could be such photosensitizers. It is believed that the presence of porphyrins in prebiotic conditions is quite possible (Calvin, 1969). The abiotic synthesis of porphyrins may occur in various simple reaction mixtures, for example, in the system methane-ammonia-water-hydrogen under the conditions of electric discharge (Hodgson and Ponnamperuma, 1968); in aqueous suspension of pyrrole and benzaldehyde on illumination with UV or visible light at room temperature (Szutka, 1964); and in the mixture of pyrrole and formaldehyde in methanol heated to 100 °C (Krasnovsky and Umrikhina, 1972). Porphyrins were also found among the products of volcanic eruption (Kolesnikov and Egorov, 1977; Markhinin and Podkletnov, 1978).

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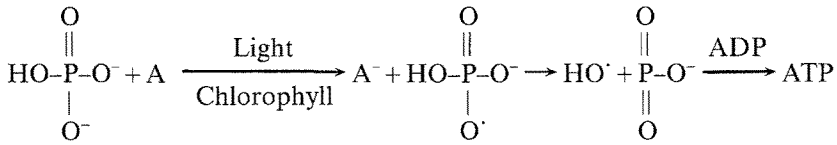
The formation of pyrophosphate bonds with the participation of magnesium porphyrins was shown first in the system contained chlorophyll adsorbates on aluminium oxide (Goncharova and Evstigneev, 1975a) and the following scheme of the process was proposed:



Here A is an electron acceptor. Chlorophyll photosensitizes electron transfer from phosphate ion to an electron acceptor. After electron loss phosphate ion through the intermediate formation of phosphate radical anion turns into methaphosphate which phosphorylates ADP to ATP.

In the subsequent works it was shown that as an oxidant to phosphate ion in a process completed by formation of pyrophosphate bonds, singlet oxygen (Goncharova and Evstigneev, 1975b) and also hydroxyl radicals (Goncharova *et al.*, 1980a; Masinovsky, 1984) may be used.

It seems that inorganic semiconductors such as ZnO, TiO_2, and CdS may act as photosensitizers of the phosphate ion oxidation. Their ability for light-induced electron transfer was demonstrated in a number of works (Krasnovsky and Brin, 1973; Krasnovsky *et al.*, 1979). Then it was shown that phosphorylation of adenosine nucleotides is possible in these conditions (Fan I-ji *et al.*, 1976, 1978). Hemoproteinoid which is a protein-like substance synthetized by thermal polycondensation of the mixture of amino acids and Fe III-protoporphyrin-IX and as it is assumed could arise under conditions of prebiotic Earth, is also able to photosensitize electron transfer (Kolesnikov *et al.*, 1981) and synthesizes ATP in the presence of inorganic phosphate and ADP (Kolesnikov *et al.*, 1979).

In this work we have used chlorophyll as a magnesium porphyrin photosensitiser. The synthesis of pyrophosphate bonds in aqueous medium with chlorophyll adsorbates on aluminium and silicon oxides simulates the conditions of prebiotic evolution. We also present some data on the pyrophosphate bond synthesis in alcohol solutions, which allows better to understand the reaction mechanism.

2. Methods and Materials

Chlorophyll *a* + *b* adsorbates on powder of aluminium oxide and silicon oxyde were prepared similarly (Goncharova *et al.*, 1980b). Aluminium oxide and silicon oxide are reagents used in chromatography, i.e. Aluminium-oxid, Brockmann II, neutr. (Reanal, Hungary) and Silochrom S-120 (Stavropol, USSR). In comparison with aluminium oxide, silicon oxide is a more neutral substance which more weakly adsorbs phosphates, more weakly affects on pH of the aqueous medium and that is particularly convenient for experiments with separation of light and dark stages.

Usually 1 mg of chlorophyll *a* + *b* microcrystal paste was dissolved in 0.5 mL of diethyl ether, then 0.1 g of Al_2O_3 or SiO_2 was added and diethyl ether was pumped out. Dry powder of adsorbate was used in experiments in the reaction medium containing phosphate solution and ADP or some other components. After light reaction 0.05 mL of 20% KOH for desorbtion of phosphate and adenosine phosphates from adsorbent was added to the reaction mixture. Then the reaction medium after vigorous shaking was separated from adsorbate by centrifugation, added 0.1 mL 2 N HCl for neutralizing of solution and used for determination of phosphorylation product. Similarly dark samples were prepared.

Chlorophylls *a* and *b* were obtained from dry nettle by the 80% acetone extraction and subsequent purification by dioxan according to Iriyama *et al.* (1974). Chlorophyll *a* from the *a* + *b* mixture was separated on a column of sucrose powder (Iriyama *et al.*, 1974).

For experiments in ethanol-water solutions, 1 mg of chlorophylls *a* and *b* was dissolved in 1 mL of ethanol, added 0.3 mL aqueous 0.1 M K_2HPO_4 solution, then added 1.6 mL of ethanol containing small amount of HCl for pH 7.6 and 0.1 mL of water.

Reaction vessels were illuminated in thermostating conditions usually at 20 °C by white light of incandescent lamp. Light intensity at vessel level was 30 000–40 000 lux.

In experiments presented in Figure 4, we used special quartz cell of 1 cm optical path with two branches and a stopcock for pumping out the air (Goncharova and Goldfeld, 1985, 1988). Before evacuation of the air, individual components of the reaction mixture were placed in different compartments, and after evacuation for 3 min, were poured together, adding the chlorophyll solution in a small volume of ethanol to an aqueous solution of K_2HPO_4 first and then the rest of the alcohol, containing HCl or KOH to create the necessary pH of the medium. A 750 W incandescent lamp with condenser, water filter and red light filter, $\lambda \geqslant 640$, were used in these experiments.

In experiments presented in Tables I–III, the amount of formed phosphorylation products was measured by the radioactivity incorporated from inorganic ^{32}P into the organic phosphate fraction according to Avron (1960). To obtain reliable results the amount of counts/min in experimental samples must exceed the amount of counts/min in dark control more than several thousand units. Therefore, the reaction mixture contained together with 50 mM K_2HPO_4, pH 8.0, $Na_2H^{32}PO_4$ equivalent to 10^6 counts min^{-1}. To measure reaction yields with accuracy to $10^{-4} - 10^{-8}$ M we used amounts of ^{32}P equivalent to 5×10^7 counts min^{-1}. Measurements were made with counter Mark-2 ('Nuclear Chicago', U.S.A.). Pyrophosphate and arginine phosphate were identified by the position of a spot with ^{32}P on a paper chromatogram. Pyrophosphate was analyzed by ascending paper chromatography in solvent containing isopropyl alcohol, an aqueous solution of trichloroacetic acid and ammonia (Karl-Kroupa, 1956). A chromatogram was sprayed by a solution of ammonium molybdate containing perchloric acid and hydrochloric acid and then placed under

the ultraviolet lamp and irradiated until blue spots of ortho- and pyrophosphate appear. The spots corresponding to pyrophosphate from samples after illumination contained radioactivity.

Arginine and arginine phosphate were separated by ascending paper chromatography in solvent used for separation of adenosine phosphates (Cohn and Carter, 1950) and containing isoamyl alcohol and 5% solution of Na_2HPO_4, 1:1 v/v. A chromatogram was sprayed by 0.2% acetone solution of ninhydrin. In comparison with dark control an arginine spot from the samples after illumination divided into two ones and radioactivity was in the zone of arginine phosphate.

For determination of the amount of adenosine phosphate (Figure 1) the spots of ATP, ADP and AMP, located by ultraviolet fluorescence, were cut from the paper chromatogram, eluted with 0.01 N hydrochloric acid and the solutions were examined spectrophotometrically at 260 nm (Cohn and Carter, 1950). Together with the phosphorylation of ADP to ATP, the phosphorylation of AMP to ADP also occured. Because in both cases macroergic phosphate bonds were synthesized, all reaction yield was attributed to the ATP formation.

In some experiments the phosphorylation was measured by the inorganic phosphate (P_i) decrease using the colorimetric method of Fiske and Subbarow in Allen's modification (Allen, 1940).

In experiment presented in Table IV, determination of ATP formation was made by the firefly luciferase assay according to Strehler and Totter (1954) with Chemiluminometer medical 01 (Kiev, U.S.S.R.).

3. Results and Discussion

The chlorophyll adsorbated on aluminium and silicon oxides are a model system for the possible adsorption of magnesium porphyrins on the surface of various clays and clay particles which, apparently, were a favourable medium for the synthetic

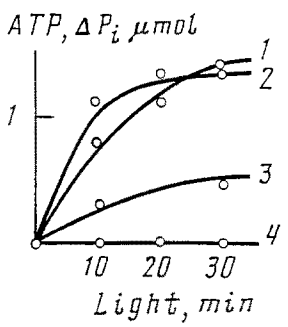


Fig. 1. ATP formation photosensitized by chlorophyll *a* + *b* adsorbate on aluminium oxide (pH 7.8). (1) K_2HPO_4 decrease; (2) ATP formation, chromatography data; (3) ATP formation without oxygen; (4) in the absence of pigment no decrease of P_i and ATP formation. 3 mL reaction mixture contained 0.2 g Al_2O_3 with 4.5 mg chlorophyll *a* + *b*, 30 μmol K_2HPO_4, and 10 μmol ADP.

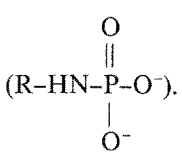
processes in the period of prebiotic evolution (Cairns-Smith, 1986; Goldfeld and Goncharova, 1989).

As it was earlier shown, chlorophyll in the adsorbed state may photosensitize electron transfer from donor (ascorbic acid) to acceptor (methyl red) in aqueous medium (Evstigneev and Gavrilova, 1960). It was found that the same mechanism of photosensitized electron transfer is the basis of phosphorylation of adenosine phosphate occuring in the experiments described in the present paper.

Figure 1 demonstrates that the decrease in P_i and the simultaneous formation of ATP depend both on the presence of chlorophyll on aluminium oxide and on the presence of final electron acceptor (oxygen from air) in the system. The removal of oxygen from the reaction vessel by evacuation of the air using a vacuum pump decreased the amount of ATP formed. However, the reaction did not cease completely, because the molecular oxygen bound by sorbent was not fully removed by this way. It is known that for its complete removal evacuation of the air must be accompanied by heating of samples.

Table I presents the results of experiments with chlorophyll adsorbates on silicon oxide. From these data, it follows that the phosphorylation of adenosine nucleotides is inhibited by a more active electron donor, sodium ascorbate, which apparently replaces phosphate ion as an electron donor in the reaction of photosensitized electron transfer.

As it is demonstrated by the data given in Table II, an intermediate reaction product, apparently, is the methaphosphate ion. In the reaction mixture containing arginine, arginine phosphate is formed. The phosphoryl group (in the free state a shortliving methaphosphate ion) in arginine phosphate is attached to the nitrogen atom of guanidine group with the formation of N–P bond



It appears that in the conditions studied the phosphorylation may occur only using

TABLE I

Inhibition of phosphorylation by sodium ascorbate. Illumination time 1 min; temperature 20 °C; reaction mixture (3 mL) contained 1 mg chlorophyll $a + b$ per 0.1 g SiO_2, 50 mM K_2HPO_4, $Na_2H^{32}PO_4$ at a concentration corresponding to the activity of 10^6 counts/min, and 3.3 mM ADP, pH 7.7

Sodium ascorbate, (mM)	Phosphorylation yield, (nmol ATP)	Degree of inhibition, (%)
0	20.8	0
5	3.1	85.1
50	0.2	99.1
163	0	100

TABLE II

Phosphorylation in the presence of various acceptors of phosphate. Reaction conditions as in Table I

Phosphate acceptor	Reaction product	Reaction yield, nmol
ADP, 3.3 mM	ATP	24.2
P_i, 50 mM	Pyrophosphate	2.3
50 mM	Pyrophosphate	0.1
50 mM	Pyrophosphate	0.9
Arginine, mM		
3.3	Arginine phosphate	1.4
13.3	Arginine phosphate	3.7
26.6	Arginine phosphate	8.5
40	Arginine phosphate	15.3
40	Arginine phosphate	17.5
Leucine, 40 mM	Pyrophosphate	0.1
40 mM	Pyrophosphate	0.3

the nitrogen atom of guanidine group. In the control experiments with the other amino acid, leucine, no such a high level of phosphorylation was observed and the reaction product was rather represented by pyrophosphate.

Owing to high reactivity a metaphosphate monomer does not observed at the free state in an aqueous medium. However, its appearance in chemical reaction may be confirmed, according to Kosower (1962), by the formation of compounds with macroergic phosphate bonds.

Table III presents the data on the separation of dark and light steps of the process. It was found that the chlorophyl adsorbate could be illuminated in the absence of the reaction mixture components, with the subsequent addition of P_i and ADP in the dark. It seems that a cation radical of chlorophyll is formed during the light step, because the succesful realization of the dark step depends on the presence

TABLE III

Separation of light and dark reaction steps. Illumination time 20 s; temperature 0–5 °C; other conditions as in Table I.

Composition of illumiated reaction mixture	Components added in the dark	Reaction yield (nmol)
Chl/SiO_2, dry	P_i,ADP	0.50
Chl/SiO_2, dry	P_i	0
Chl/SiO_2, H_2O	P_i,ADP	0.24
Chl/Sio_2, H_2O, $K_3Fe(CN)_6$	P_i,ADP	0.42
SiO_2, H_2O, $K_3Fe(CN)_6$	P_i,ADP	0
Chl/SiO_2, dry, without O_2	P_i,ADP	0.08
Chl/SiO_2, H_2O, without O_2	P_i,ADP	0.14
Chl/Sio_2, H_2O, P_i, ADP, $K_3Fe(CN)_6$	–	6.0; 4.3

TABLE IV

ATP formation with chlorophyll adsorbates as determined using the firefly luciferase assay.

Adsorbate	Reaction yield, nmol 3 mL^{-1} reaction mixture, light-minus-dark
Chl *a* + *b* on SiO_2	12.7 ± 4.5
Chl *a* + *b* on Al_2O_3	11.9 ± 4.2

Reaction mixture (3 mL) contained 0.5 mg chlorophylls *a* + *b* on 0.1 g SiO_2 or Al_2O_3, 0.01 M K_2HPO_4 and 0.33 mM ADP; pH 7.6. Illumination time 10 min; temperature 20 °C.

of final electron acceptor during illumination. Thus, the addition of potassium ferricyanide during illumination increases the ATP formation on the dark step and the removal of oxygen by pumping out the air from the reaction vessel decreases the reaction yield.

Table IV demonstrates the ATP formation as a result of determination by means of the firefly luciferase assay. The reaction yields with chlorophyll adsorbates on aluminium and silicon oxides are approximately equal.

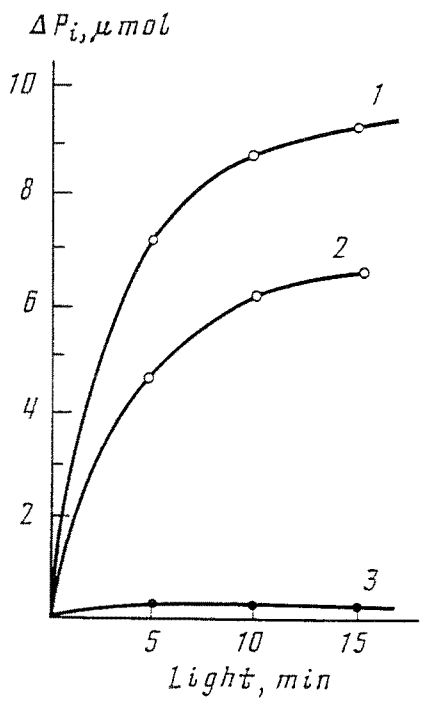


Fig. 2. P_i consumption on light in aqueous ethanol solutions of chlorophyll. (1) 14% water; (2) 13% water; (3) 13% water without chlorophyll. 3 mL reaction mixture contained 1 mg chlorophyll *a* + *b* and 30 μmol K_2HPO_4, pH 7.6.

The utilization of chlorophyll in the absorbed state permits to carry out experiments in the aqueous medium. Chlorophyll retains its ability to photosensitize electron transfer with all used adsorbents both water insoluble as aluminium oxide, calcium phosphate, ferrous oxide, silica gel, diphenyl, etc. (Evstigneev, Gavrilova, 1960) and water soluble, e.g., sucrose (Vernon, 1961), detergent (Krasnovsky *et al.*, 1974). For the photosensitization of phosphorylation such investigations were not made. However, it appears reasonable to assume that for this reaction it is necessary the magnesium atom of chlorophyll to remain in free state for the formation of a complex with an oxygen atom of phosphate.

Some additional data on the phosphorylation mechanism photosensitized by chlorophyll were obtained in experiments with aqueous ethanol solutions of chlorophyll. The amount of water in the aqueous ethanol solution depends on the solubilities of chlorophyll and phosphate: chlorophyll is insoluble in water, and phosphate is insoluble in ethanol. Therefore phosphorylation occurs in the narrow range of water concentration (2–27%) in alcohol. The most suitable concentration is 16% (Goncharova, 1981). Apparently, this region of water concentration is the most favourable for the stabilization of chlorophyll-phosphate

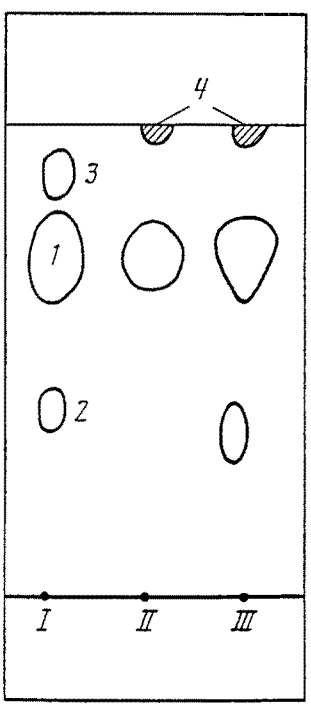


Fig. 3. Chromatogram of phosphorylation products. (I) labels; (II) initial reaction mixture containing chlorophyll *a* + *b* ethanol solution, 18% water, 10 mM K_2HPO_4, pH ~ 8; (III) reactioin mixture after illumination for 20 min in air; (1) orthophosphate, (2) pyrophosphate, (3) monoethyl phosphate, (4) chlorophyll residues.

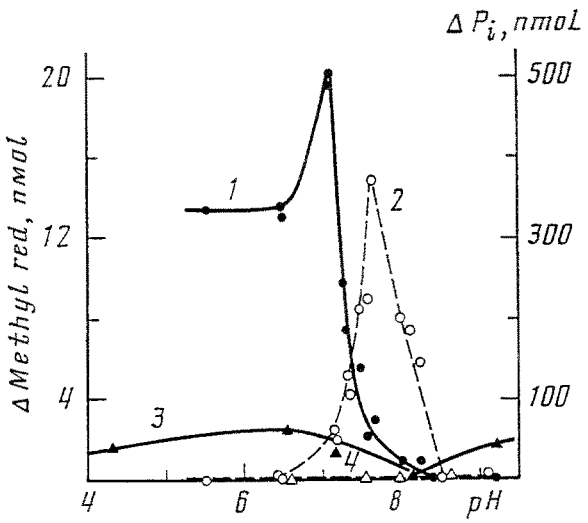


Fig. 4. pH dependence of the reduction rate of methyl red and the decrease of phosphate in aqueous ethanol solutions of chlorophyll *a* on illumination. (1) reduction of methyl red in the presence of K_2HPO_4, (2) decrease of phosphate in the presence of methyl red, (3) reduction of methyl red in the absence of K_2HPO_4, (4) decrease of phosphate in the absence of methyl red. Reaction mixture contained 10^{-5} M chlorophyll *a*, 6.5×10^{-3} M K_2HPO_4, 2.2×10^{-5} M methyl red, and 2.5% water.

complex, allowing to obtain higher yields of phosphorylated products (pyrophosphate, Figures 2 and 3) in comparison with the adsorbate system (Figure 1). It seems that the use of the ethanol medium reduces the loss of methaphosphate ions due to the reaction with water regenerating orthophosphate.

Figure 4 illustrates the phosphorylation in ethanol solutions of chlorophyll *a* with 2.5% water by the phosphate decrease and the reduction of methyl red, an electron acceptor in the absence of oxygen in the system, in dependence on the pH. Although the optimum pH values for the phosphorylation (pH 7.6) differ from those for the reduction of methyl red (pH 7.1), it is essential that the methyl red reduction occurs only in the presence of phosphate and the phosphate decrease occurs only in the presence of methyl red. It is possible that under pH values unfavourable for any of these two reactions the yield of either the phosphorylation or the methyl red reduction decreases owing to a higher rate of reverse reactions.

the magnesium atom of chlorophyll and inorganic phosphate. Figure 5 presents the absorption spectra of chlorophyll *a* in diethyl ether (spectrum 1), in 96% ethanol, and in the same alcohol with an addition of 2.5% by volume water (the spectra coincide, spectrum 2). Spectrum 3 belonged to chlorophyll in ethanol with 2.5% water in the presence of K_2HPO_4. Noteworthy is the fact that in the blue region the height of the longer wave of the two absorption bands of chlorophyll *a* decreases in the sequence from diethyl ether to ethanol and to aqueous alcohol solution and decreases further when K_2HPO_4 is added into the aqueous alcohol solution. The effect of phosphate is rather small but it has the same direction as the effects of alcohols, H_2O, O_2 and many other molecules possessing atoms with unshared

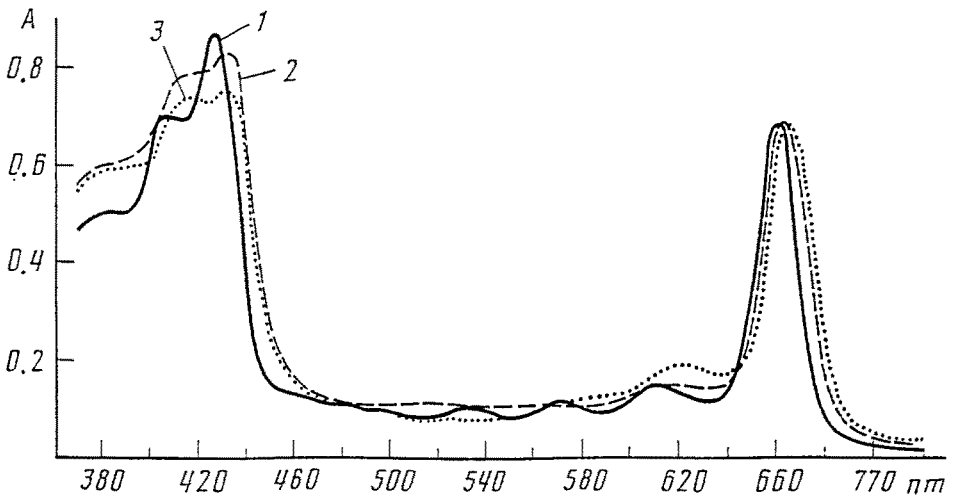


Fig. 5. Influence of solvent and inorganic phosphate on the absorption spectrum of chlorophyll *a*. (1) in diethyl ether; (2) 96% ethanol and 96% ethanol + 2.5% water (by volume), two spectra coincide; (3) 96% ethanol + 2.5% water + 6 mM K_2HPO_4, pH 7.6.

electron pairs (Evstigneev *et al.*, 1950; Rabinowitch, 1951). These effects are absent with the chlorophyll analogues not containing magnesium, e.g., pheophytin and phthalocyanin (Evstigneev *et al.*, 1950).

4. Conclusion

Phosphorylation resulting from photosensitized electron transfer with the participation of magnesium porphyrins particular in the adsorbed atate in contact with aqueous medium represents a possible pathway for the conversion of solar energy into the energy of pyrophosphate bonds. This mechanism could be used for the production of dehydrating agents required for the synthesis of various biologically significant compounds including polypeptides and polynucleotides in the conditions of prebiotic evolution. It is also possible that such mechanism of the pyrophosphate bonds formation could be employed by nature during arising of primary photosynthetising organisms.

In model systems we used oxygen of the air as an electron acceptor. Could molecular oxygen serve as an electron acceptor in prebiotic conditions? Earlier it was supposed that the atmosphere of the primary Earth was only of reductive nature (Oparin, 1957). However, later the conception about the composition of the primary atmosphere slightly changed. It was assumed that as soon as the Earth got cold and liquid water appeared, molecular oxygen in a small amount could be formed as a result of the water photolysis by ultraviolet light (Bütner, 1961; Hart, 1978; Mukhin, 1980). Oxygen could be adsorbed on clays created there its high enough concentrations and could take a part in various chemical reactions.

Magnesium porphyrins tend to irreversible bleaching under illumination in the

presence of molecular oxygen. However, at low concentrations of oxygen, as it might be during early evolution of Earth, the rate of this process was slow and under such conditions these compounds could act for a long time.

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