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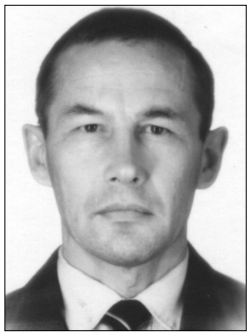

Energy Problems in Life Evolution

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Abstract—Evolutionary aspects of bioenergetics are considered. These include the origin of the first organisms, UV-protection and the beginnings of anoxygenic photosynthesis, the electron donor problem of life and the appearance of oxygenic photosynthesis, oxygen danger and strategies of defense, and the role of oxygen in programmed cell death.

Key words: evolution, bioenergetics, UV-protection, photosynthesis, oxygen, defense against oxygen, programmed cell death

Synthetic and decomposition processes occur simultaneously in the cell. They are balanced by constant energy supply. Evolution of life often faced energy problems and solved them, but evolution was also alternated with revolutionary changes.

At the beginning of life. There are two viewpoints on the predecessors of the modern cell. Oparin suggested that these were chemoorganoheterotrophs [1], whereas others believed that these were chemolithoautotrophs [2]. However, in either case life originated under anaerobic conditions.

According to the notion of heterotrophic origin of life, the primary atmosphere of the Earth contained CH_4, NH_3, H_2, and H_2O and a long period of chemical evolution of organic compounds synthesized due to light energy of the Sun and electric discharges preceded probiont appearance. HCN is the most important intermediate of synthesis of amino acids, nitrogenous bases, and other organic compounds. Early forms of life had fermentation reactions as energy supply. However, effective electron acceptors represented a crucial problem that had not been solved by fermenters [3].

According to the widely accepted alternative notions, the primary atmosphere had a different composition, and it contained CO_2, N_2, H_2O, and traces of H_2. Probionts appeared at sites of volcanic activity, in thermal reservoirs. They utilized CO or CO_2 as a source of carbon atoms. This involved acetyl-CoA synthase containing Ni-S-Fe-cluster in the active center: $CH_3{-}SH + CO + H_2O \rightarrow CH_3COOH + H_2S$. The major intermediate of this pathway, methyl mercaptan, is formed due to reduction of CO or CO_2 by FeS or H_2S. CO could be obtained by CO_2 reduction on Fe-catalyst. Accumulation of organic compounds in chemoautotrophs similar to modern aceto- and methanogens gave rise to subsequent development of chemoorganotrophic prokaryotes.

The first prokaryotes appeared about 3.5 billion years ago. Their life was poor. There was no oxygen and consequently ozone shield protecting our planet against UV-emission from the Sun. The probionts had to constantly escape light, and their life was in darkness. Absorption of UV light caused damages in the structure of proteins and nucleic acids. Subsequent development required protective mechanisms [4]. Reaction centers (RC) of the modern photosynthetic machinery exhibit UV protective properties; these transmembrane proteins contain clusters of UV-absorbing aromatic amino acids [4]. Such clusters decrease the lifetime of excited molecules and this property is used for photostability of industrial polymers. Some other amino acids (histidine, arginine, etc.) can serve as pigment ligands. High energy UV-excitement of the aromatic residues migrates to pigment molecules and loses harmful properties due to intramolecular emissionless transition (inner conversion occurs within 10^{-11} sec). Nature selected chlorophylls and bacteriochlorophylls as the most convenient pigments because their lower singlet excited level *in vivo* corresponds to the photochemically harmless spectral range of 650-1000 nm. Mulkidjanian and Junge [4] suggested that the photochemical machinery of photosynthesis was originally designed by Nature as a shield against UV-radiation. Subsequently this machinery was improved and adapted for energy conversion during photosynthesis.

This represented a giant evolutionary breakthrough: energy supply of cells with endless resources of light energy of the Sun. Other problems had also been solved.

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Within the chemoautotroph concept, these included obligatory dependence on certain environmental areas (volcanoes, hydrothermal springs) and constant mercaptan dependence. Within the chemoheterotroph concept, photosynthesis solved the electron acceptor problem: photoexcited chlorophyll (bacteriochlorophyll) and non-excited chlorophyll (bacteriochlorophyll) were electron donor and acceptor, respectively.

Purple, green, and heliobacteria were the first representatives of the evolutionary series of photosynthetic organisms [5, 6]. They do not produce O_2 during photosynthesis; they are also heterogeneous in terms of: a) nature of RC components, especially their electron acceptors (special forms of chlorophyll *a* and some Fe_4S_4-centers in heliobacteria and green sulfur bacteria, i.e., RC type I; bacteriochlorophyll, bacteriopheophytins, and quinones in purple sulfur and non-sulfur and green non-sulfur bacteria, i.e., RC type II); b) structure of light-harvesting antenna; c) pathways of CO_2 assimilation including the ribuloso-*bis*-phosphate (Calvin) cycle, the reductive citric acid (Arnon) cycle, and the acetate–glyoxylate cycle (see [7]). Groups of phototrophic prokaryotes significantly differ in the amino acid sequence of reaction centers. Nevertheless, analysis of the homology of various types of RC suggests the existence of a common predecessor for anoxygenic and oxygenic phototrophs [8].

The pattern of the evolution of photosynthesis cannot be represented by a linear branching diagram. For example, green sulfur bacteria contain RC type I, whereas green non-sulfur bacteria contain RC type II. Both groups have chlorosomes, but the Arnon cycle is involved in the CO_2 fixation in the former, whereas CO_2 fixation occurs via acetate–glyoxylate cycle in the latter. In spite of primitivism of the photosynthetic machinery and its incapacity for phototrophic growth heliobacteria are grouped closer to cyanobacteria, whereas purple bacteria with their developed photosynthetic machinery and rather wide metabolic capacities are always considered as the most ancient branch of anoxygenic phototrophs [9]. It appears that photosynthesis was formed as a mosaic process lacking a well defined evolutionary origin with individual history of development of separate groups of phototrophs and even parts of the photosynthetic machinery inside separate groups [6].

The electron donor problem of living organisms and its solution: oxygenic photosynthesis. Synthesis of ATP from ADP and P_i is the most important function of bioenergetic systems. V. P. Skulachev suggests that adenine nucleotides could be selected for coupling of exergonic and endergonic reactions due to intermediate electron donor/acceptor properties of adenine among other nitrogenous bases [10] and its maximum resistance to UV-radiation [11]. It is also possible that in the initial period of life on the Earth ATP could be synthesized by phosphorylation of UV-excited ADP [10, 11]. The attachment of some parts of the ATP molecule (P_i, PP_i, ADP, AMP, or adenosine) to various compounds increases their amount of free energy, enhances their reactivity, and converts them into active participants in biochemical reactions. However, the role of bioenergetic systems is not restricted to ATP synthesis. Supply of reactions of reductive synthesis with effective electron donors, namely NAD(P)H, is the other problem which is especially important for autotrophs.

Although biogeochemical data suggest that the primitive Earth was rich in reduced iron and sulfur compounds [12] they could not provide substrate saturation of enzymatic reactions due to limited availability of these compounds for cells. For example, oxidation of Fe^{2+} to Fe^{3+} resulted in growth of purple bacteria, but the growth rate was low [13]. Iron is characterized by extremely low solubility. At pH 7.0, the concentration of Fe^{3+} in water is $1.6 \cdot 10^{-18}$ M [14]. Although the solubility of Fe^{2+} is two orders of magnitude higher than of Fe^{3+}, availability of Fe^{2+} is extremely low anyway. Within the physiological range of pH, alkaline metal sulfides are hardly soluble, whereas elemental sulfur and transition metal (Fe, Mo, Cr) sulfides are almost insoluble in water. Reduced nitrogen compounds are suitable electron donors, but the content of these substances obtained from N_2 as the initial substrate was low as well.

Progress in evolution of life required solution of the electron donor problem. And finally Nature "decided" to perform the following experiment. It combined the organizational principles typical of RC types II and I to design a new organism. About 3.2 billion years ago cyanobacteria appeared; they utilized the unlimited water resources as the electron donor and photomobilized it by two sequential photosystems.

Structurally, RC complexes of photosystem II are close to purple bacteria RC. Oxygen-evolving complex (OEC) was an important "purchase" for the photosynthetic machinery. The E_0' values for O_2/H_2O, photosystem II RC, and purple and green bacteria RC are 0.82, 1-1.2, and 0.24-0.515 V, respectively. So RC required the adjustment of their redox properties. This was achieved by changes in the protein environment of the RC. For example, gene engineering manipulations causing substitutions of amino acid residues near RC of the purple bacteria *Rhodobacter sphaeroides* resulted in an increase in the number of hydrogen bonds between bacteriochlorophyll and protein; under these conditions, P870 E_0' value increased to 800 mV and even higher [15]. Carbon dioxide may act as a catalyst during H_2O oxidation (see [16] and references given there); hence, bicarbonate can be an electron donor in oxygenic photosynthesis.

Oxygen danger: defense lines. Oxygenic photosynthesis was a progressive stage in the evolution of life. It supplied our planet with organic compounds. However, oxygenic photosynthesis also produced oxygen, a strong oxidant, and products of its one, two, and three electron reduction: $O_2^{\cdot-}$, H_2O_2, and $OH^{\cdot}$, respectively. The latter,

hydroxyl radical ($E_0' = 2.31$ V), is the most dangerous; it can oxidize any organic compound of living cells. The other form of reactive oxygen species, singlet oxygen ($^1O_2$), which may be formed from ordinary triplet O_2 during its interaction with triplet chlorophyll, is also very dangerous. Singlet oxygen represents an effective weapon employed by neutrophils for killing pathogenic microorganisms. It is formed from H_2O_2 and HOCl, the product of the myeloperoxidase reaction: $H_2O_2 + HOCl \rightarrow {}^1O_2 + HCl + H_2O$. Antibodies can catalyze H_2O oxidation (a new function of immunoglobulins) by short-lived $^1O_2$ ($t_{1/2}$ in water of ~1 μsec) followed by formation of long-lived hydrogen trioxide H_2O_3 ($t_{1/2}$ in water of ~20 msec) and ozone O_3 (see [17, 18] and references given there).

Formation of reactive oxygen species (ROS) in cells sharply increases when O_2 concentration increases. This means that the evolution of photosynthesis had to be accompanied by the development of protective mechanisms against O_2. Time resources for the development of such mechanisms were very great. At least during the first billion years after the appearance of primitive oxygenic photosynthesis, O_2 was not accumulated in the atmosphere of the Earth; as evidenced by biogeochemical data, there were just traces of oxygen. The modern level of O_2 in the atmosphere was achieved more than 2 billion years later [12]. Oxidation of reduced compounds, primarily Fe^{2+}, was accompanied by O_2 consumption. The development of photosynthetic organisms and generation of photosynthetic O_2 was restricted by a deficit of orthophosphate, the crucial nutrient of living cells: P_i bound Fe_2O_3 and thus transformed into a form inaccessible for organisms [19].

Of course, the history of oxygen in the atmosphere of the Earth did not begin with the appearance of primitive oxygenic photosynthesis. As evidenced by geochemical data, oxygen and other gases (nitrogen, inert gases, carbon dioxide) were initially evolved abiogenously, from the bowels of the Earth during basalt degassing [20]. Abiogenic O_2 is also generated in modern times. In the modern atmosphere, oxygen is 2.3% heavier by isotope composition than photosynthetic oxygen; atmospheric oxygen represents a mixture of photosynthetic (light) and abiogenic (heavy) O_2 [20]. This also increased time resources for the development of protection against oxygen.

Clayton [21] suggested that carotenoids were the first protective tools against damaging effects of oxygen. They deactivate long-lived triplet chlorophyll (bacteriochlorophyll) and thus prevent its interaction with $^3O_2$, leading to formation of $^1O_2^*$. Moreover carotenoids deactivate $^1O_2^*$ and convert it into $^3O_2$. Under intense illumination, excessive energy of excited chlorophyll is dissipated as heat; this non-photochemical deactivation of excitements involves special carotenoids, xanthophylls [22]. There is another form of non-photochemical quenching: quinones quench photoexcited chlorophyll (bacteriochlorophyll) in natural and artificial systems [23, 24].

As for the second mechanism of protection against toxic O_2, Clayton [21] suggested respiration, i.e., electron transfer from organic and inorganic substrates to O_2 followed by water formation. According to Skulachev [25], reduction of intracellular concentration of O_2 is a special function of respiratory systems.

Purple and green non-sulfur and cyanobacteria maintain both photosynthetic and respiratory electron transfer. Green sulfur bacteria have not invented a reliable protection against O_2; they lack a respiratory chain [26, 27] and are confined to O_2-free zones. After the emergence of respiration, some photosynthetic organisms completely lost their photosynthetic capacity. Others partially lost photosynthetic facilities, but they can still use photosynthesis under certain conditions. These include quasi-phototrophs or para-photosynthesizing, aerobic bacteria [7]. The latter became predecessors of mitochondria, and cyanobacteria became predecessors of chloroplasts [28]. New obligatory O_2-dependent organisms appeared. It is difficult to estimate the extent to which oxygenic photosynthesis was responsible for the emergence of aerobic organisms. The discovery of obligate anaerobic organisms similar to intestinal *Bacteroides fragilis*, which contains *bd* type genes encoding cytochrome oxidase and which are stimulated by nanomolar concentrations of O_2 [29] suggests appearance of oxygen respiration as the energy transducing mechanism using resources of abiogenic O_2 long before oxygenic photosynthesis.

Phototrophs can reduce partial O_2 pressure via a light-dependent pathway: under $NAD(P)^+$ deficient condition oxygen oxidizes a secondary quinone (photooxidase activity) and this yields O_2^- [26, 27]. Photooxidase activity is typical of non-sulfur and purple and green sulfur bacteria. Pseudocyclic electron transfer also known as "water-water cycle" exists in chloroplasts and cyanobacteria; at one end H_2O is oxidized and at the other end O_2 is reduced with O_2^- formation [30]. The latter (as in the case of photooxidase activity) is further metabolized to water by superoxide dismutases, catalases, and peroxidases. The physiological importance of photooxidase activity and pseudocyclic electron transfer consists in the reduction of partial O_2 pressure in cells and prevention of photoinhibition of photosynthesis at excessive illumination.

Accumulation of O_2 in the atmosphere was accompanied by formation of the ozone shield in the stratosphere, which absorbed light with $\lambda < 290$ nm. This ozone shield was formed from O_2 by UV-emission of the Sun. The absorption spectrum of O_3 overlaps with the optical absorption bands of DNA and protein.

Thus, oxygenic photosynthesis (i) provided sustainable formation of organic compounds and oxygen on the Earth, (ii) formed the protective ozone shield around our planet, and (iii) gave a powerful impetus for the development of aerobic organisms.

Oxygen and programmed cell death. Nature did not leave "unattended" the oxidant properties of oxygen and used oxygen as a tool for realization of the final stage of a cell's life-cycle, genetically programmed cell death. Death is the natural final stage of a cell's life-cycle. It can be programmed (programmed cell death) and non-programmed (necrosis). Programmed cell death is a physiological variant of cell death, whereas necrosis represents a pathological variant of cell death [31]. Programmed cell death is "peaceful" death, which does not disturb neighboring cells (because it does not damage them). Necrosis is an "explosion" when components of a dying cell in an avalanche-like manner are released into intercellular space and uncontrolled enzymes and other biologically active substances exert harmful action on surrounding healthy cells. Programmed cell death is accomplished via the mechanisms of apoptosis, autophagy, and autolysis. Recent data suggest that the various kingdoms of life share common mechanisms of programmed cell death.

Some authors believe that necrosis may be programmed. However, as a rule necrosis represents accidental cell death. It is hard to believe that such rough treatments of cells as exposure to boiling water, strong alkaline, or mechanical disintegration of cells (or pickling of mushrooms or cucumbers) can trigger a coordinate response. It is hard to believe that death of disintegrated fragmented cells involves concerted action of programmed mechanisms. Impairments of plasma membrane followed by a loss of enzyme regulation are the first signs of necrosis. Disrupting the death program which prevents apoptosis (due to defects in one of the links of this process) may also result in necrosis. The opposite situation is also possible: the necrotic cell death scenario may be blocked because hidden resources have been discovered and the dying cell enters apoptosis.

Mitochondria are the main bioenergetic structure of the cell. They are involved in programmed cell death in animals, plants, and fungi as suppliers of ROS and a number of apoptotic factors, such as cytochrome *c*, AIF flavoprotein, procaspases, and protein regulators of programmed cell death. What about chloroplasts? Do chloroplasts play any role in programmed cell death in plants?

We investigated epidermal films from plant leaves [32-34]. The epidermis is a monolayer of guard (phototrophic) and epidermal (chemotrophic) cells. Guard cells contain chloroplasts and mitochondria, whereas epidermal cell contain only mitochondria. Illumination significantly facilitated the apoptosis of guard but not epidermal cells. In both cell types programmed cell death was prevented by anaerobiosis and antioxidants. Additionally, programmed cell death of guard cells was also prevented by electron acceptors in the Hill reaction, diuron, and competitive inhibitors of plastoquinone oxidation at the *o*-site of chloroplast b_6f-cytochrome complex. Thus, chloroplasts are involved in programmed cell death in plants. Light, oxygen, and their mediator, the chloroplast, drive plant cell death to the "right stream", harmless for its environment.

Programmed cell death is an important constituent of developmental processes in many bacteria [31]. Spore formation in bacilli is accompanied by death of the vegetative cell inside which the spore grows and maturates. Differentiation processes in eukaryotes could inflict significant damage on (e.g., mammalian erythrocytes lacking nucleus, plant phloem cells). A similar situation occurs during differentiation of bacteria that is accomplished for the benefit of the whole population. For example, heterocysts of cyanobacteria lacking photosystem II play a specialized role: they fix nitrogen from the atmosphere, but they die when nitrogen fixation is not needed any more. Programmed cell death in bacteria is observed during phage infection.

A part of a starving population of *Bacillus subtilis* cells enters a "cannibal pathway": they avoid spore formation or postpone it to later stages. The cannibal cells produce a protein triggering programmed cell death in sister cells and utilize products of the sister cell lysis [35]. Programmed cell death in cyanobacteria (as well as in eukaryotes) is an active process [36]. In cyanobacteria, programmed cell death induced by salt stress is accompanied by DNA fragmentation, vacuolization of cytoplasm, increased activity of proteases, impairment of integrity of plasma membrane, and fragmentation followed by subsequent autolysis of the cells.

This scenario of programmed cell death in bacterial cells is consistent with the results of earlier observations of death of bacterial cells under physiological conditions. Studying the process of cell death in growing cultures of cyanobacteria, Fedorov [37] concluded that "continual death of a certain proportion of offspring of each cell represents the basis of this phenomenon". Consequently, in spite of the circular structure of bacterial DNA, inevitable cell death has been encoded within this circular DNA, i.e., death has been genetically programmed. It appears that death of cyanobacteria described by Fedorov is related to the restricted capacity of prokaryotic cells for division and aging. For example, when normal human embryonal cells are grown under the most favorable conditions, aging and programmed cell death inevitably occur after ~50 duplications (see for review [38]). Aging and programmed cell death of *Saccharomyces cerevisiae* yeasts occurs after ~30 generations (see for review [39]). One of the first signs of triggering of the cell death program is the formation of vacuolar structures in cyanobacteria [40]. Based on the data of electron microscopy, the vacuoles obviously derive from thylakoid membranes; these vacu-oles represent vesicles filled with electron clear material, possibly hydrolytic enzymes. Functionally bacterial vacu-oles share some common features with autophagosomes during programmed cell death in

eukaryotic organisms. This suggests that autophagy in eukaryotes and autolysis in prokaryotes may have a common evolutionary background.

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