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CHAPTER 14

BIOLOGICAL SIGNIFICANCE OF ACTIVE OXYGEN-DEPENDENT PROCESSES IN AQUEOUS SYSTEMS

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Abstract: Water actively participates in bioenergetics and bioregulation. It is essential for purposeful production of reactive oxygen species (ROS) in cells and extracellular matrix. Due to specific structuring of water itself, it may serve as the source of free radicals and initiate reactions with their participation. On the other hand, water structuring provides for its direct oxidation with oxygen. Processes going on in aqueous systems in which ROS participate are the sources of high-grade energy of electronic excitation which is not easily and uselessly dissipated in aqueous milieu of living systems but rather can be accumulated, concentrated, and used as energy of activation for the performance of biochemical reactions. Such processes spontaneously acquire oscillatory character and may serve as pacemakers for biochemical reactions dependent on them. Thus due to its unique structural-dynamic properties water may serve as a transformer of energy from low density to high density form, may accumulate the former and use it for organization and support of vital activity.

Keywords: Structured water; Reactive oxygen species; Free radicals; Electronic excitation; Photon emission; Oscillations; Self-organization

1. INTRODUCTION

Albert Szent-Gyorgyi has noted long ago ‘The cell is a machine driven by energy. It can thus be approached by studying matter, or by studying energy’ (Szent-Gyorgyi, 1968). From the chemical point of view more than 99% of all matter of which cells and intercellular matrix are built is water. Molarity of water is 55 M while molarities

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of even most abundant substances in the internal milieu of an animal, are 2-3 orders of magnitude less. Hence, water should play no less important role in all vital activities than all other biomolecules. Until recently water was neglected as a peer participant in studies of cellular mechanisms, but the situation is changing. More and more evidence appear in favor of the idea that water belonging to living things is an exceptional substance that in unison with low and high molecular weight solids which it embraces determines biological activity at all the levels of organization of living matter.

All the metabolic processes in which living matter participate imply consumption, transformation, or generation of energy. Albert Szent-Gyorgyi was probably the first to claim that ‘bioenergetics is but a special aspect of water chemistry’ and that ‘… water arranges an indivisible system with the structure elements (of a cell) making possible electronic excitations which otherwise are highly improbable… in structured water electronic excitation may be surprisingly long-living, and this may be of a paramount importance for the biological energy transfer’ (Szent-Gyorgi, 1957).

Energy may be characterized by quantity and by qualities (forms, levels, and orderliness). Levels of energy are subdivided into translational (energy associated with the motion of a molecule in space), rotational and vibrational energy of parts of di- and many-atomic molecules. The highest level of energy relevant to further discussion is energy of electronic excitation (EEE). Levels of energy differ significantly in their density and ‘quality’ – the higher is the energy level, the more different types of work may be performed by the same quantity of energy and the higher is the efficiency of its utilization. As Mae-Wan Ho noted: ‘Life uses the highest grade of energy, the packet or quantum size of which is sufficient to cause specific motion of electrons in the outer orbitals of molecules’ (Ho, 1993). However, the idea of the importance of EEE for bioenergetics besides specialized biological functions such as photosynthesis and vision is not still sufficiently absorbed by biological community.

According to the current concept of bioenergetics the overwhelming majority of living organisms gain energy from food burning by oxygen. In a simplified form of this concept specific dehydrogenases abstract ‘hot’ electrons (plus protons) from “fuel” (sugars and fats) and transfer them to NAD\(^+\) and NADP\(^+\). The reduced forms of these carriers donate electrons to the respiratory chain in mitochondria, where their energy is released stepwise and is used for the synthesis of ATP which supports energy requirements of an organism. Oxygen here is the final acceptor (a ‘trash box’) of electrons that had exhausted most part of their redox potential. As energy portions released in mitochondrial oxidation are equivalent to quanta of middle-far IR-photons (\(< 0.5 \text{ eV}, \text{rotational, at most, vibrational energy}\) this process is analogous to SMOLDERING COMBUSTION. An alternative form of energy gain from oxygen-dependent oxidation is genuine COMBUSTION when direct one-electron oxygen reduction occurs, and quanta of energy equivalent to energy of visible and even UV-photons (\(> 1 \text{ eV}\)) are generated. One of the classical examples of combustion is direct oxygenation of hydrogen resulting in water production and at which high density energy is released. Combustion, in particular combustion of
hydrogen has not been considered until now as relevant for bioenergetics. However, a lot of evidence argues that it should be taken into account as one of the most fundamental processes ensuring vital activity with high grade and well ordered energy. It turns out that water is essential for regular combustion flow in living cells and what is even more surprising, that water can be burnt itself. Here we consider this assertion in more details and try to discuss implication of these newly revealed properties of water for cell physiology.

2. ROS GENERATION IS AN INTRINSIC PROPERTY OF WATER

There is growing understanding that water can not be regarded as some unstructured ‘liquid gas’. Many models of water structures are put forward (Bulionkov, 1988; Zenin, 1994; Chaplin, 2000; Maheshwary et al., 2001; see also Chaplin, http). In “real” water structuring is expressed much more than in ideal ultra-pure water because of contribution of multiple interfaces. They include interface between bulk water and walls of a vessel, water/air interface, interfaces with admixtures, etc. Vicinal water with special properties may extend far from the interface which it solvates (Ling, 2003). For example, many layers of structured water extend beyond the protein surface, and induced protein conformational change modifies the extent of non-ideally behaved water (Cameron et al., 1997). Several resilient water molecular layers close to the surface of a solid material immersed in water were detected using atomic force microscope (Jarvis et al., 2000). It was shown by subfemtosecond x-ray absorption spectroscopy that liquid water in a first coordination shell of ice consists of structures with two strong hydrogen bonds of each molecule to its neighbors, resulting in water chains and rings (Wernet et al., 2004). If water contains polymer-like associations, mechanochemical phenomena are expected to take place in it.

Polymers can undergo chemical transformations under the action of mechanical impacts, freezing-thawing and fast temperature variations, action of audible sound and ultrasound, and other low density energy forces that are too weak to induce chemical reactions in monomers or short oligomers. If macromolecules in polymers or their solutions are reluctant to shift along each other due to weak but multiple intermolecular bonds they may accumulate and concentrate mechanical energy to densities that comprise energy quanta enough to excite and break down internal covalent bonds in polymers. That means unpairing of electrons and appearance of a pair of free radicals followed with multiple chemical and physical consequences (Baramboim, 1971).

Basing on the presumption that liquid water contains quaizi-polymeric structures the team of Russian physicists headed by G.A. Domrachev started more then 10 years ago to investigate the effects of low density energy physical factors on homolytic water dissociation (H—O—H → HO• + •H, cf. ionic water dissociation: H—O—H → H⁺ + OH⁻). They estimated augmentation of hydrogen peroxide concentration in water because the most probable explanation for its appearance de novo is recombination of HO• radicals arising in homolytic water dissociation. It
was shown that water freezing-thawing, evaporation-condensation, sonication even with audible sound, filtration through narrow capillaries resulted in an increase of $\text{H}_2\text{O}_2$ even in ultra-pure and carefully degassed water. Efficiency of water splitting resulting from evaporation/condensation and freezing/thawing is $\sim 10$ times as effective, sonolysis $\sim 70$ times and water filtration through narrow capillaries – more than $100$ times as effective as its photodissociation with far UV-light (Domrachev et al., 1992, 1993). Yield of $\text{H}_2\text{O}_2$ in magnesium sulphate solution (a model of sea water) being in equilibrium with air was much higher than in pure degassed water. What is notable, $\text{H}_2\text{O}_2$ concentration continued to grow for some time after resumption of any treatment. About $3\%$ of all energy used for viscous flow of water through capillaries with diameter of $0.2$ nm was used for water splitting.

Japanese authors who were looking for a new way to produce hydrogen by water splitting have shown that powders of NiO, Cu$_2$O, Fe$_3$O$_4$ suspended in distilled water by magnetic stirring, catalytically decompose it into $\text{H}_2$ and $\text{O}_2$. Efficiency of the mechanical-to-chemical energy conversion under these very mild conditions exceeded $4\%$ (Ikeda et al., 1999). Here water splits to the final products because presumably metal oxides instantaneously decompose intermediate peroxides.

In case if a water molecule has dissociated as a mechanically excited polymeric entity:

\[ (\text{H}_2\text{O})_n(\text{H}_2\text{O} \ldots \text{H} - | - \text{OH})(\text{H}_2\text{O})_m + E \rightarrow (\text{H}_2\text{O})_{n+1}(\text{H}) + (\uparrow\text{OH})(\text{H}_2\text{O})_m, \]

the initial products of water splitting are free radicals $\text{H}_\downarrow$ and $\uparrow\text{OH}$ (here we symbolize a given electron as $\uparrow$ or $\downarrow$ to stress their alternative spin states). Indeed, if water is in apparent rest this singlet pair of radicals readily recombine back to water:

\[ \text{H}_\downarrow + \uparrow\text{OH} \rightarrow \text{H}_2\text{O} \]

However even in such a case this is not just a reverse, equilibrium reaction because water splitting has been achieved under the action of mechanical forces while back recombination of radicals gains an energy quantum of $5.2$ eV. In an aqueous system, condensed and organized medium ‘electronic excitation may be surprisingly long-living’ as A. Szent-Gyorgyi stressed. In fact, long-range energy transfer of electronic and vibrational excitation in water has been demonstrated already in 1930ies-1940ies by J. Perrin, S. Vavilov, Th. Foerster, and others. This phenomenon was confirmed with new techniques recently (Woutersen and Bakker, 1999).

The probability of radicals to move away of each other significantly increases in ‘real’ water, in which dissolved gases and other molecules and particles are present, especially in cases when multiple layers of water are organized by surfaces which it hydrates and when these layers move along each other with different rates (consider a vortex as an example). This is proved by aforecited data on of the appearance of
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H$_2$O$_2$ in water filtered through narrow capillaries and H$_2$ and O$_2$ in water stirred in the presence of metal oxides. Here the following reactions may proceed:

(3) \( \text{HO}^\uparrow + \text{HO}^\downarrow \rightarrow \text{H}_2\text{O}_2 \)

(4) \( \text{H}^\uparrow + \downarrow \text{H} \rightarrow \text{H}_2 \)

(5) \( \text{H} \cdot + \text{O}_2 \rightarrow \text{HO}_2 \cdot \)

(6) \( \text{HO}_2^\uparrow + \text{HO}_2^\downarrow \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \)

(7) \( 2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \)

The most important of them are the reactions in which oxygen molecules are released ([6] and [7]). It should be reminded that O$_2$ is unique among other molecules because in its ground state its two electrons are unpaired \([\text{O}_2(\uparrow\downarrow), \uparrow\uparrow \text{or}\text{O}_2(\uparrow\downarrow), \downarrow\downarrow]\) (besides, an oxygen atom also has two unpaired electrons). Thus, oxygen molecule is a bi-radical (in fact it is a tetra-radical) and it represents a vast store of energy. But it is stable because the laws of quantum physics forbid direct reactions of bi-radicals (they are called also particles in a triplet state) with molecules in which all electrons are paired (singlet state particles). That is why to release its energy reserve oxygen needs to be initially activated.

There are few ways for O$_2$ activation. It may be excited by an appropriate energy quantum (\( \geq 1\) eV) and turn into a highly reactive singlet oxygen \([\text{O}_2(\uparrow\downarrow), \uparrow\downarrow \text{or}\text{O}_2(\uparrow\downarrow), \downarrow\downarrow]\), its another symbol, $^1\text{O}_2$). A peculiar feature of O$_2$ is that singlet oxygen may exist only in an electronically excited state from which it may relax only to triplet state. As soon as singlet-triplet transition is “forbidden” by quantum physics laws lifetime of excited singlet oxygen is usually much longer than that of any other molecules in an excited singlet state. Triplet O$_2$ is also activated by transition metals because in their field its spin state is changed. Finally, triplet oxygen easily reacts with free radicals – atoms and molecular particles possessing an odd number of electrons on their valence orbital. In these reactions oxygen gains or loses an electron, turns into a free radical which can easily take new electrons releasing large portions of energy at each consecutive step of one-electron reduction. Another peculiar feature of free radical reactions in which oxygen participates is that they may easily turn into branching (or runaway) process (Voeikov and Naletov, 1998a), and concentration of free radicals in a reaction mixture grows up exponentially until the rates of their production and annihilation equalize. That is why elevation of H$_2$O$_2$ yield in water equilibrated with air in course of its splitting occurs faster, continues for a long time after initial perturbation, and reaches higher levels than in degassed water (Domrachev et al., 1993).

Thereupon it is interesting to speculate that an outcome of water splitting may be significantly influenced by external magnetic fields. There are a lot of reports on the long lasting effects of even a brief treatment of water with magnetic fields, though these effects are not easily reproduced. In principle, magnetic fields may modulate the outcome of free radical reactions. Initial radicals, as mentioned, emerge in a singlet form \((\text{H}^\uparrow + \downarrow \text{OH})\) and they may easily recombine back into water. Under the action of a magnetic field singlet-triplet transition \((\text{H}^\uparrow + \downarrow \text{OH} \rightarrow \text{H}^\downarrow + \downarrow \text{OH})\)
may occur. This prohibits recombination of the radicals favoring the development of the array of reactions 3-7 and others. If water system contains oxygen and some other admixtures development of branching chain reactions in it significantly changes its properties, but as soon as free radical reactions, especially branching chain reactions are highly non-linear, the overall effect should depend drastically upon slight variations of initial conditions.

As it is mentioned above, singlet oxygen belongs to the family of ROS. Recently it was discovered that besides being a source of O$_2$, water may be directly oxidized with it. This reaction is readily catalyzed \textit{in vitro} by antibodies (immunoglobulins) provided that energy of activation for excitation of molecular oxygen to its singlet state was supplied by dim light illumination of an antibody solution (Wentworth et al., 2000). In other words, antibodies promote water ‘burning’. Catalysts do not ‘invent’ reactions that can not go without them. They organize the reactants in space (and time) so that thermodynamically favorable processes go much faster. Quantum chemical calculations has shown that if two or more water molecules are arranged in space in particular disposition in relation to singlet oxygen and to each other, energy of activation for oxidation of a water molecule with singlet oxygen diminishes to reasonable values and such exotic peroxides as HOOOH, HOOOOH, HOO-HOOO may be produced under mild conditions as intermediates on the way to a more stable H$_2$O$_2$ (Xu et al., 2002). Water oxidation goes on very fast in a solution of antibody because its active center provides for the optimal arrangement of water molecules for the process. However, if water is organized in a favorable way by some other means, if singlet oxygen is supplied, for example by the reactions [6] and [7], water oxidation may proceed in aqueous solutions in which water splitting had been initiated. We observed that in the course of branching chain reaction of slow oxidation of amino acids in aqueous solutions initiated with H$_2$O$_2$, concentration of H$_2$O$_2$ increases to the levels that can be explained only by water oxidation with O$_2$ (Voeikov et al., 1996). Recently it has been shown that in water containing carbonates and phosphates (Bruskov et al., 2003) or noble gases, such as argon (Voeikov and Khimich, 2002) concentration of H$_2$O$_2$ spontaneously increases and its augmentation goes on faster in case of water stirring. Using chemiluminescent methods we also found that such process goes on in aerated mineral waters from natural sources (Voeikov et al., 2003b).

Thus, water – the most abundant substance in any living system, may regularly produce oxygen free radical and another forms of ROS under mild physiological conditions. The fact that a substantial part of organismal water is more or less structured increases the probability of its splitting and oxidation with all the above-listed consequences.

3. COMBUSTION IN A LIVING MATTER

Reactions in which ROS participate has been considered for a long time to be mostly deleterious for cells and tissues, as they may propagate in living matter as chain reactions in which a lot of important bioorganic molecules are corrupted.
They are blamed as a cause of many diseases and as the major cause of aging (Beckman and Ames, 1998). Current studies of oxygen free radicals and other ROS are still to a large extent based on the old idea that they arise as side products of biochemical processes in which oxygen is involved. According to this concept antioxidant enzymes (superoxide dismutase (SOD), catalase, peroxidases) and low molecular weight antioxidants such as ascorbic acid and α-tocopherol should efficiently combat sporadically produced ROS. But when adverse factors induce ‘oxidative stress’ – excessive ROS generation, antioxidant system can not manage them, and different pathologies arise (Fridovich, 1999).

Recently this seemingly neat theory started to face serious problems. Evidence is accumulating that ROS are purposefully produced in all living organisms. ROS production by cells of immune system in the course of their immune reaction has been known since 1970s (Babior et al., 1973). But only now it became clear, that practically all the cells – animal, plant, cells of unicellular organisms are equipped with the enzymes belonging to NADPH-oxidase family, that directly reduce oxygen. One-electron oxygen reduction is naturally accomplished by many other enzymatic and non-enzymatic mechanisms (Voeikov, 2001).

It turned out that a share of oxygen that undergoes one-electron reduction (actually participates in combustion) is surprisingly high. Any new life begins with egg fertilization, and just after a spermatozoid merges with an ovum oxygen consumption drastically increases. At the cleavage-stage embryos non-mitochondrial respiration accounts for 70% of all oxygen consumption. Only by the stage of blastocysts this share decreases to 30%, however, not at the expense of diminution of direct O₂ reduction, but due to increase in mitochondrial respiration (Trimarchi et al., 2000). Many adult animal organs and tissues use at least 10-15% of all oxygen for generation of ROS (Shoaf et al., 1991), while in intact segments of rat aorta up to 26% of oxygen is directly reduced to superoxide (Souza et al., 2002). White blood cells as well as platelets are actively respiring, and superoxide generation continuously proceeds in whole blood of healthy donors (Voeikov and Novikov, 1997). It is noteworthy that practically all oxygen consumed by neutrophils and eosinophils is one-electronically reduced (Peachman et al., 2001). As mentioned, oxygen is consumed in plasma where immunoglobulins catalyze oxidation of water by it (Wentworth et al., 2000).

What is the purpose of the directional ROS production in living systems? By 1990ies it was already shown that ROS (H₂O₂ and O₂•−) regulate carbohydrate and lipid metabolism, poly-ADP-ribosylation, release of calcium from mitochondria, protein kinase and phosphatase activities (Ramasarma, 1990). Now it is known that ROS regulate practically all manifestations of vital processes on molecular, cellular, and tissue levels in all living things (Droge, 2002; Thannickal, and Fanburg, 2000). ROS are shown to have wholesome effects: they promote differentiation of cultured malignant cells into their benign counterparts (Sauer et al., 2001), improve properties of taken out blood (Bocci, 1994), and exercise significant therapeutic effects (Nathan and Cohn, 1981).
Despite the fact that a substantial part of inhaled oxygen is used for ROS generation, stationary levels of $\text{O}_2\cdot$ in cells and tissues do not exceed $10^{-10} - 10^{-11}$ M (Niviere and Fontecave, 1995), while that of $\text{H}_2\text{O}_2$ in a cell cytoplasm is estimated as $10^{-7} - 10^{-9}$ M (Tyler, 1975). ROS are kept at such low levels due to their nearly immediate elimination by the powerful antioxidant system. Thus, the probability that ROS may bind specifically to alleged macromolecular ‘receptors’ except for the enzymes or low molecular weight antioxidants that degrade them is very low. This seems to be puzzling: an organism converts a substantial share of oxygen into ROS and immediately eliminates these particles. How to explain such apparent squandering? And how can these particles, which are so short-lived and practically devoid of chemical specificity, exercise specific bioregulatory actions?

4. BIOENERGETIC FUNCTIONS OF ELECTRON EXCITED STATES

We suppose that difficulties in comprehension of the real role of ROS in vital activity are related to the attitude to them only as to chemical particles, while they should be considered as participants of the continuous flux of oxygen reduction to water: $\text{O}_2 + 2\text{H}_2 \rightarrow 2\text{H}_2\text{O}$ (Voeikov, 2001). This reaction consists of several steps:

\begin{align}
(8) & \quad 4(\text{O}_2 + e^- + \text{H}^+) \rightarrow 4\text{HO}_2\cdot \\
(9) & \quad 2(\text{HO}_2\cdot + \text{HO}_2\cdot) \rightarrow 2\text{H}_2\text{O}_2 + 2\text{O}_2 \\
(10) & \quad \text{H}_2\text{O}_2 + \text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \\
(11) & \quad 4\text{O}_2 + 4e^- + 4\text{H}^+ \rightarrow 2\text{H}_2\text{O} + 3\text{O}_2
\end{align}

From such a notation of oxygen reduction (though we could not find similar notation in available literature) several important conclusions follow. First, if oxygen excess over the electrons that reduce it is less than 4-fold, combustion does not go to a final point, and intermediate ROS accumulate, which may initiate chain reactions with bioorganic molecules. Thus, an adequate supply of oxygen is necessary for maintaining low stationary level of ROS and other free radical particles. Second, all these reactions imply recombination of unpaired electrons. This applies also to reaction [10] where one $\text{H}_2\text{O}_2$ molecule may be considered as an electron donor and another as an electron acceptor. Third, all these reactions are sources of energy quanta equivalent to electronic excitation energy. Energy yield in the reaction of dismutation of two superoxide radicals is $\sim 22 \text{kcal/mol}$, equal to the energy gap between triplet and excited singlet states of oxygen and equivalent to a near IR-photon ($\lambda \sim 1269 \text{ nm}$). When two singlet oxygen particles transit to triplet state simultaneously, EEE may be ‘pooled’ and a doubled quantum of energy (equivalent to $\lambda \sim 635 \text{ nm}$, red light) is released (Cadenas and Sies, 1984). Decomposition of two molecules of $\text{H}_2\text{O}_2$ donates an equivalent of 2 eV or $\lambda < 610 \text{ nm}$. When dismutation of $\text{HO}_2\cdot$ (reaction [9]) is catalyzed by SOD or decomposition of $\text{H}_2\text{O}_2$ [10] is
performed by catalase, quanta of high density energy should be generated with some megahertz frequencies due to very high turnover numbers of these two enzymes. This prevents energy from its immediate dissipation into heat and is favorable for energy pooling to even higher quanta.

A key role of EEE and related photon emission in the regulation of vital processes was discovered 80 years ago by A.G. Gurwitsch in the form of the so-called ‘mitogenic radiation’ – ultra-weak photon emission in the UV-range of EM-spectrum responsible for triggering cell division (Gurwitsch and Gurwitsch, 1943). This radiation is emitted not only by living cells and tissues, but also by enzymatic (hydrolytic and glycolytic) and chemical reactions including gel-sol transitions in aqueous media. Water splitting and accessibility of active oxygen is a prerequisite condition for the emergence of this radiation (Voeikov, 2003). Ultra-weak photon emission in the range from UV- to near IR of electromagnetic spectrum from living cells and chemical reactions in aqueous media (Slawinski, 1988) affect activity of enzymes (Cilento, 1988), activity and morphology of cells and tissues (Galantsev et al., 1993), regulate locomotion and mutual orientation of cultured cells (Albrect-Buehler, 1995). Back reflected photons emitted during respiratory burst in human blood affect the intensity of this immune reaction by a feed-back mechanism (Voeikov et al., 2003a).

In our opinion regulatory role of ROS is provided by the unique feature of reactions with their participation – generation of electronic excitation energy (EEE) that continuously pumps biophotonic fields of living systems. But if reactions with ROS participation play such a versatile role, they should proceed in all living things including those that are considered to be anaerobic. Indeed, even obligate anaerobic bacteria are equipped with SOD (Hewitt and Morris, 1975) indicating that ROS appear even when molecular \( O_2 \) concentration in water is negligibly low. However, the intrinsic property of water to produce oxygen radicals due to its splitting makes their appearance in liquid water practically inevitable.

5. OSCILLATORY NATURE OF ACTIVE OXYGEN DEPENDENT REACTIONS IN AQUEOUS SYSTEMS

Besides serving a role of a source of ‘the highest grade of energy, the packet or quantum size of which is sufficient to cause specific motion of electrons in the outer orbitals of molecules’ (Ho, 1993), processes in which EEE is generated going on in aqueous systems may automatically acquire oscillatory character and may serve as pacemakers for biochemical reactions dependent on them.

Arousal of ROS in reactions going by in water and generation of EEE provides for the involving of other substances such as nitrogen and carbon dioxide into the process. They may beget amine and carbonyl compounds, and when concentrations of the latter exceed certain thresholds amino-carbonyl (Maillard) reaction develops. In this reaction biologically significant heterocyclic, aromatic, polymeric substances appear (Namiki et al., 1973). Some of them activate oxygen resulting in ROS production and generation of EEE (Voeikov and Naletov, 1998b). We found that
profound oscillations of photon emission (Voeikov et al., 2001a) and redox potential (Voeikov et al., 2001b) emerge in Maillard reaction. Oscillations last for many hours and even days and their periods extend from fractions of minutes to tens of minutes. Amplitudes of redox potential variations may reach 0.3 V (from −0.2 V to −0.5 V).

Intensity of photon emission and amplitude of their oscillations and intensity of oscillations of redox potential as well as periods and phases of oscillations differ in different parts of the reaction system. Mean values of redox potential near the bottom are much more negative and amplitudes of their oscillations are higher than near the air/water interface. On the other hand, the most intense photon emission modulated with profound oscillations comes from the part of the reaction system close to the water/air interface. Here oscillations of photon emission and redox potential are highly correlated. Thus, the bottom part of a reaction system is the source of electrons that reduce oxygen incoming from the air.

High redox potential differences between different parts of the system can not be explained only from uneven distribution of reduced and oxidized forms of organic components because of their low concentrations (few tens of millimolar). It is interesting to speculate that these differences reflect gross changes in reduction and oxidation state of aqueous medium itself.

What is the primary cause of the development of oscillations of ROS production and oscillations of EEE generation? Our experimental data indicates that generation of EEE in reactions with ROS participation is prerequisite for self-organization observed as these processes develop. Initial building up of EEE fosters oxidation and oxygenation of available substrates resulting in an exhaust of dissolved oxygen and accumulation of reducing (easily oxidizable) equivalents. Oxygen continues to diffuse into the system from the air and when its concentration and concentration of reducers reach optimal ratio, a new wave of burning appear followed with the next oxygen depletion until the concentration of diffusing oxygen reaches a threshold value again. Thus, oscillatory behavior naturally emerges in such systems.

It is notable, that oxygen consumption in single neutrophils and other cells that reduce it to ROS using NADPH-oxidase exhibits multimode oscillatory patterns of ROS generation (Kindzelskii and Petty, 2002). Some hormones influence the amplitude of these oscillations, other affect their frequency. In other words, both deepness of respiration of single cells and its rate are related to their functional activity. Respiration rate and deepness (especially in case when oxygen consumption is realized through it one-electron reduction) define in their turn downstream regulatory processes.

Oscillatory behavior is characteristic not only of single cells, but of their populations as well. We observed pronounced oscillations of photon emission from neutrophil suspensions containing hundreds of thousands of cells and even in whole blood, indication of a collective behavior of these big groups of cells related to metabolism of ROS in them (Voeikov et al., 2003a).

Amino-carbonyl reaction proceeding in aqueous systems in which oscillations and waves spontaneously emerge is, in our opinion, the simplest model of arousal
and performance of the respiratory process. Such conditions for the emergence of oscillations of EEE are common to all cells. A steep oxygen gradient between a metabolizing cell and its environment exists. Oxygen is poorly soluble in water, and what is more important, its delivery to a cell may be regulated by interfacial water at a cell-environment boundary. Due to cellular metabolic activity reducing equivalents (e.g., NAD(P)H) accumulate in it. When the ratio of these equivalents to incoming O\textsubscript{2} reaches threshold values energy discharge primarily in the form of EEE occurs. Oxygen is rapidly exhausted, and released energy is directed for metabolic needs. That oxygen in fact taken by single cells in an oscillatory mode has been indeed recently experimentally demonstrated (Porterfield et al., 2000). Oscillations of EEE may play the role of pacemakers for the processes going on on different levels of biological organization. On the other hand oscillatory nature of all these processes provides them the properties of sensible receptors for external electromagnetic and other physical fields.

6. RESPIRATION CYCLE OF WATER: A HYPOTHESIS

It seems trivial that respiration as we know it is a cyclic process. Though it is not so obvious that respiration at a level of a single cell should also be cyclic, experimental evidence supports this conclusion. It can be suggested that cyclic nature of respiration emerges on the one hand from the spatial relationship of oxygen consuming system and its environment and on the other from the orderliness of energy fluxes and high density of energy (EEE) that is generated in the course of oxygen-dependent processes in which ROS participate. Taking into consideration that all the aforementioned phenomena occurred in aqueous systems and that ROS generation is the intrinsic property of water we suggest a hypothesis of the existence of the ‘respiratory cycle of water’. Splitting of water molecules under the action of low density energy (mechano-chemical or mechano-catalytic water decomposition) results in the appearance of oxygen and hydrogen in aqueous systems:

\begin{equation}
8\text{H}_2\text{O} \rightarrow 4\text{O}_2 + 16\text{H}^\bullet
\end{equation}

Four hydrogen atoms (H\textbullet) are needed for complete reduction of one oxygen molecule, the rest hydrogen atoms recombine to H\textsubscript{2} molecules: 12 H\textbullet \rightarrow 6\text{H}_2 \uparrow + n(h\nu). EEE released may be used, for example, for excitation of oxygen with the appearance of singlet oxygen, for sustaining of an aqueous system in a non-equilibrium, excited state, etc. This sequence of events may be by convention defined as an ‘exhale’ stage because water splitting is accompanied with gas (hydrogen) release.

What may follow afterwards is analogous to an ‘inhale’ stage, as here oxygen is consumed. We remind that for the complete reduction of oxygen molecule a 4-fold excess of oxygen is needed:

\begin{equation}
4\text{O}_2 + 4\text{H}^\bullet \rightarrow 2\text{H}_2\text{O} + 3\text{O}_2 + m(h\nu)
\end{equation}
Energy released in the course of the reactions [12] and [13] is enough to excite oxygen to a singlet state, and under appropriate conditions $^1\text{O}_2$ may go on water oxidation:

$$3\text{O}_2^* + 6\text{H}_2\text{O} \rightarrow 6\text{H}_2\text{O}_2$$

‘Respiration cycle of water’ allows to transform low density energy (freezing-thawing, evaporation-condensation, energy of sound, energy of shearing forces of water filtration or its vortexing) into a high density one; at least some part of the latter may accumulate in water in the form of metastable substances such as $\text{H}_2\text{O}_2$ and other peroxides as well as in long-living water excitation making it an active physical medium.

As other gases and substances that are present in ‘real’ water should get involved in the process, respiration cycle should be considered not as a closed loop, but rather as a single convolution of an untwisting helix. Real processes proceeding in water should significantly depend upon the presence of positive and negative catalysts of particular reactions, of substances affecting water structure, upon the nature of interfaces that it solvates, upon the action of external physical factors and fields. Studies of phenomena related to water may help in solving many practical problems of medicine, agriculture, environmental problems, in providing people with healthy drinking water, in optimization of technologies in which water is important.

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