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Plant Physiology

Biosynthesis and Emission of Isoprene from the Standpoint of Non-Equilibrium Thermodynamics

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ABSTRACT. Studies of metabolic transformations in the living cells, applying non-equilibrium thermodynamics on the example of biosynthesis and emission of isoprene are discussed in the paper. It was proved that emission of isoprene from the living cells is the dissipation of the excessive energy and ensures sustainability of living system homeostasis by means of regulation of terpenoid biosynthesis. Herewith we answer the question, why the living cell emits isoprene. The process of isoprene emission from the cell is the necessity of dissipation of excessive energy. It is entropic phenomenon in its essence meaning fundamental law of sustainable existence of the cell. Besides this fundamental function, isoprene is accompanied by secondary concomitant functions: thermotolerance, antioxidation and regulatory activities of different types of energoplastic alteration. To study the biological role of this process thoroughly it is necessary to discuss it from an evolutionary perspective, because biosynthesis and emission of isoprene on light from the cell containing chlorophyll periodically was lost and reduced again in the process of evolution, as the ability of isoprene biosynthesis is kept in the genome of any vegetation cell in the form of information. New possibilities were brought to a number of genomic sequences available for photosynthetic organisms. This genome sequence data cover organisms starting with photosynthetic microbes and algae up to higher plants. Genome projects inform the variety of other molecular sequence databases including the terpenoid metabolism that analyze entire communities of organisms. This direction of scientific research aims towards a genome-level understanding of the structure, function and evolution of isoprene biosynthesis and its emission. *© 2014 Bull. Georg. Natl. Acad. Sci.*

Key words: *Biogenic isoprene, irreversible, thermodynamics, free energy, entropy.*

In the middle of the 50-s of the last century, a photobiological phenomenon – biosynthesis and emission of isoprene into the environment by photosynthesizing the cells – was discovered [1]. Later it was shown that emission of isoprene into the atmosphere takes place even without light. Discovery of this phenomenon attracted the attention of scientists of different fields. The thing is that, apart from the academic interest, the questions associated with this phenomenon affect acute problems, worrying the mankind, namely, changing of climate and energetics.

Isoprene is one of the mostly spread hydrocarbons in the nature. About 95 % of isoprene production is used for getting synthetic rubber. Isoprene is also used in pharmaceutical, energetic and other fields of chemical industry.

Aggravated problems in ecology, climate change, caused by antropogenic impact upon the nature, exhaustibility of reserves of natural hydrocarbons, rising of their price associated with political and social cataclysms have brought us to the necessity of mastering alternative methods of getting isoprene. One of the directions of development of new perspective technologies for bioisoprene production is the gene modification of algae and unicellular organisms.

It was found that ability of emission of isoprene is characteristic of all living organisms, including the man and the representatives of the ancient group of procariotic microorganisms – archebacteria. That is why it is necessary to investigate the phenomenon of isoprene emission in the evolutionary aspect on the basis of fundamental scientific laws. Large amount of works, dedicated to this question, has been already published.

Everything that surrounds us is the natural result of the chain of events occurring for billion of years on our planet, having taken place on the Earth since its formation. As a result, a rather perfect biological system was formed. We will try to examine essential principles of functioning of biological structures, their distinctive peculiarities, characteristic of only the living structures by means of basic thermodynamic laws. Why thermodynamics? The thing is that a living organism is, first of all, energetic system, where the same laws act as in inanimate nature. However, it should be taken into account that living organisms are characterized by the series of features, absent in physical objects. It is not surprising that energetic exchange of biological systems possesses qualitative originality and requires special analysis. The first law of thermodynamics – in its essence, the law of energy conservation – states that total energy of a system is constant and the energy can only be transformed from one form into another. As a fundamental law, it can be completely applied to the biological structures as well [2].

As applied to living organisms, the first law of thermodynamics can be formulated as follows: all kinds of works in the organism are fulfilled at the expense of equivalent amount of energy, excreted in the process of oxidization of nutrients. That is, if dQ is an amount of heat absorbed by the system from the environment, it goes on to increase the inner energy of the system to work against the forces of outer pressure P towards changing volume V of the system and fulfill useful work A, accompanied, for example, by chemical transformations:

$$dQ = dU+pdV+dA,$$

Initial source of energy in heterotrophic organism for conducting all kinds of works is the free energy of nutrients (proteins, fats, carbohydrates) excreting during their oxidization. For autotrophic organisms, energy of the sun eradiation, stored in the process of photosynthesis, is the initial source of energy. The same energy, in the end, passes on to animals. But neither the energy of sun eradiation, nor the energy excreting in the process of oxidization of nutrients is directly used for performing works in the organism. At the beginning the energy of the sun eradiation and nutrient is transformed into the energy of macroenergetic bonds of some substances, mostly of adenosine triphosphoric acid - ATP. Thus, the main source of energy for all processes of vital activity is the energy stored in high energetic compounds, first of all, in molecules of ATP and in potentials H^+ and Na^+.

The second law of thermodynamics can be formulated as follows: entropy of isolated thermodynamic system either increases, or stays constant $dS/dt = dQ/T$, reaching maximum at thermodynamic equilibrium (in which system can stay as long as possible, and at the same time the thermodynamic parameters retain their constant values).

Notion of entropy was introduced in 1865 by Rudolf Clausius [3] and since then this function has attracted the attention of physicists, chemists and biologists. The second law of thermodynamics is a fundamental law of nature, it defines that in isolated system, energy can be spontaneously transformed only from higher level to lower and not vice versa. Clausius postulated that heat cannot pass by itself from colder body to warmer. Boltzmann gave entropy molecular-statistic interpretation connecting entropy with the order of a system by means of formula:

$$S=k\ln W \text{ (Plank-Boltzmann's formula) [4]}$$

where S is entropy, k – Boltzmann's constant, W – thermodynamic probability, i.e., number of methods, by means of which given state is achieved. Entropy reflects that part of the system energy, which degraded, i.e., dissipated as heat. Thus, the lower is the order in a system, i.e., the smaller are the gradients of the energy, the greater is its entropy.

Entropy is the function of a system state. It characterizes dissipation measure of energy at irreversible processes. The greater the increase of entropy in any process the greater is dissipation of energy.

Living organism exchanges energy and substance with surrounding and therefore it is an open thermodynamic system. The second law of thermodynamics for open systems was first formulated by Belgian scientist Ilia Prigogine [5]. If we designate the velocity of entropy change of the open system as dS/dt, the velocity of entropy formation in the system at the expense of inner irreversible processes as diS/dt, the velocity of entropy exchange with surroundings as deS/dt, then I. Prigogine's equation assumes the following form [6]:

$$dS/dt = diS/dt + deS/dt.$$

Exchange of substance and energy with surroundings occurs continuously: the organism gets energy from outside and stores it, as a rule, in the form of compounds rich with energies. This energy is used for performing useful work – growth, adaptation, reproduction. Part of energy is excreted into surroundings in the form of heat or final products of metabolism. It causes increase of entropy out of organism, and at the same time the living system itself acquires negative entropy. The notion of negentropy was introduced by E. Shrödinger in his brilliant book "What is Life - the Physical Aspect of the Living Cell" [7]. He put the following question: "What is that valuable SOMETHING, contained in our food, that protects us from death?". The living organism increases entropy $S \rightarrow S_{max}$ and approximates it to the state of thermodynamic equilibrium – death. Escape from this state is possible at the big outflow of entropy into surroundings or, in other words, by extraction of negative entropy from surroundings, which is called "negentropy". Negative entropy is just that valuable SOMETHING, by which our organism is fed. In metabolism, organism release from entropy, produced by it, is essential.

Owing to negentropy the living system preserves and develops its structure, reserves stability. During the vital activity of an organism, continuous destructive processes, the processes of demolition of structure, manifested in permanent emission of heat and degrading substances – slag – from the organism (entopic phenomenon) take place. So, stopping or breaching of the working processes at the temperature of existing living system entails loss of structurality – death. Working processes in the living system are continuously taking place chemical reactions of structure formation, and it is continuous removal of chaotically occurring structural defects, associated with thermal destruction of a living system. All working processes in the living system are catalytic and the catalysts are structural formations of the living system themselves – enzymes.

It is peculiarity of biosystems, that they are not just open systems, rather the systems of stationary state. Why do biosystems need stationary state? The answer is obvious. Owing to it, at the expense of continuous exchange of energy and substance with environments, the biosystems are far from thermodynamic equilibrium (when entropy is maximal and constant - death) and, moreover, they retain their workability and support constancy of all the parameters. It is also of no small importance that biosystems possess autoregulatory ability in stationary state.

At least, two main features are characteristic of the stationary state of biosystems. First of all it is its energetic level, which shows, how far a system is from thermodynamic equilibrium. This is the living organism which is the system of established stationary states. Their levels are not accidental. They originated in the process of evolution and provide the organism with advantageous energetic exchange. Thus, processes proceeding in biological structures are at considerable distance from thermodynamic equilibrium. In these conditions, at intensive exchange of entropy with the surroundings, the possibility of proceeding of processes of self-organization and originating of specific dynamic structures in it is ensured [8]. Systems, being far from equilibrium, in which intensive dissipation of energy takes place and their regulation is guaranteed, are called dissipative structures.

Another interesting peculiarity of stationary state of biological structures is a certain degree of their stability. If stationary state is sufficiently stable, then after not a very strong deviation caused by some exciting influence the system again returns to its initial state. The reason of stability of stationary states was found by Prigogine [6]. He proved that biosystems possess a very interesting feature in the stationary state. If the system is not very far from the state of thermodynamic equilibrium, the term *diS/dt* (*entropy production*) retains its positive value under the stationary state, but strives to minimum, i.e.,

$$dis/dt > 0 \rightarrow \text{min.}$$

The system being in an extremum, corresponding to the minimum entropy production, ensures it with the most stable state.

Dissipative processes take place simultaneously with transformations producing energy. They must be locally subordinated to Prigogine's principle on minimum entropy production, i.e., dissipation produced in energetic cycles of energy is always minimally possible. Exceess of dissipation over the energy production is incompatible with life. That is why in the living systems energy production exceeds its dissipation, and the surplus energy is accumulated as "stocks" (starch, fats, cellulose and etc.), or is used for functional activity of the cell, or is excreted by metabolites into surroundings.

Let us consider this question on the example of biosynthesis and emission of isoprene from the cells. "Isoprene effect" or light-dependant formation and isoprene emission from photosynthesizing cells occurs by using the energy of photons, absorbed by photosystems I and II, i.e., as a result of work of Z-scheme photosynthesis, the main energy-supplier link for the photosynthesis processes. Production of free energy, which is the important part of general energy incoming the living cell, is the first stage of formation of the living matter. The final stage of utilization of energy incoming into the cell is its emission in the form of waste "bound" energy, which, as it was marked above, generally dissipates (disperse) in the form of heat, other electromagnetic radiations, and catabolism products, i.e., in the form of different waste materials containing bound energy, which is useless for conducting useful work and is easily brought out of a cell [8]. This model completely corresponds to modern ideas of thermodynamics, where an open non-equilibrium system – a living cell can function steadily only under the condition, when its excretory function works effectively, i.e., waste energy, which in fact is a summary result of action of dissipative structures, dissipates from the cell.

Being the result of metabolic transformations, isoprene emitted from the cell contains waste energy, which in the process of vital activity of a cell leaves its limits just as entropy. Thus, isoprene emission can be considered as a necessary natural process, which is the component part of metabolism in whole.

Further, isoprene belongs to the group of substances, named terpenoids, key precursor of which is dimethylallyldiphosphate (DMADP) together with its isomere, isopentenylpyrophosphate (IPP)[10]. They form two isolate pools of final products. These substances are interconverted by means of enzyme isopentenylpyrophosphate isomerase. It is one of the key positions of terpenoid metabolism strictly regulated by the retroinhibitory mechanism. IPP is used for steroid synthesis, fat acids and etc., DMADP – for synthesis of terpenoid (polyisoprenoids), essential (volatile) oils and etc.

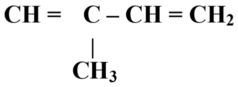
Isoprene biosynthesis is associated with those transformations of hydrocarbon, which originate in the initial processes of photosynthesis and lead through DOXP/MEP to the formation of DMADP [11]. Origin of an individual isoprene molecule in these transformations could be imagined as a regulation of the volume of DMADP pool, i.e. as an action of trigger mechanism, ensuring though not very large but constantly acting way of taking aside DMADP off its overfilled pool, so that to permanently control the degree of action of negative feedback, checking velocity of hydrocarbon flow from assimilated at photosynthesis CO_2 up to DMADP.

The important feature of isoprene effect, i.e. light isoprene emission, is its dependence on CO_2 concentration in the medium surrounding the leaf [12]. Experiments in hermetic glass chambers show that this dependence of synthesis and emission of isoprene is rather complicated. At CO_2 concentrations, saturating photosynthesis, velocity of isoprene emission falls up to the level, which corresponds to the amount of its emission in the dark. It increases with the decrease of CO_2 concentration in the air space of chamber and reaches maximum when CO_2 concentration becomes close to the carbon-dioxide compensating point (CCP). This dependence gave us possibility to conclude, that the increase of the isoprene emission velocity is connected with considerable decrease of the velocity of carbon transformations in terpenoid metabolic flow leading to the disbalance between the amount of free energy and possibility of its utilization in those transformations.

Surplus energy, appearing in this process as, useless for accomplishing work, as it was said above, should be dissipated from the cell without fail as an entropic phenomenon.

Thus biosynthesis and emission of isoprene from photosynthesizing cells is the manifestation of fundamental feature of metabolic activity of flow of carbon transformation in the reactions of terpenoid biosynthesis and these processes should be considered as a necessary condition for retaining steadiness and stable vital activity of the living cell.

Let us dwell on the originality of structure features of isoprene molecule. Its empiric formula C_5H_8 shows that carbon atoms are not reduced completely and that is why isoprene is strongly reactive. From its structural formula



$$\mathbf{CH = \underset{\substack{|\\ CH_3}}{C} - CH = CH_2}$$

it is seen that isoprene contains two double bonds and has got branched system unlike other pentadienes.

Relative amount of energy per carbon atom in isoprene is approximately 1.3 times bigger than in carbohydrates. It follows that for taking out the energy, useless for accomplishing work, from a cell in the process of isoprene emission, less material resources (carbon and hydrogen) are spent, than it could be in the case of carbohydrates. Isoprene as a typical excretes does not possess negative feedback and assists in retaining uninterrupted work of energyplastic flows of terpenoids. Isoprene does not almost dissolve in water medium of a cell and after its origin is immediately thrown out from a cell by hydrophobic forces.

So, emission of isoprene is the result of releasing a cell from surplus free energy and entropy, which constantly originate in it. This surplus must be permanently taken out of borders of a cell by all means – in the form of heat, other kinds of radiation these or those compounds – excretes.

Thus, there is a direct answer to the question why isoprene emits: because origination and emission of isoprene – the representative of terpenoid branch of metabolism – performs taking out of surplus energy (entropy) and by this stability of vital activity of a cell is ensured.

Side by side with the above-mentioned fundamental function ensuring stationary vital activity of a cell, biosynthesis and emission of isoprene perform series of adaptive functions, such as thermotolerance [13], antioxidation [14], regulation of metabolism in a cell and many other regulatory activities, including hypothesis of "Metabolic relief valve" [15].

The following studies of light-dependent biosynthesis and isoprene emission in different domains of the living nature will be undoubtedly carried out in phylogenetic aspect. These studies will give us possibility to reveal the reason of periodic loss and acquiring of this phenomenon in the process of evolution, retaining their potential abilities, informationally consolidated in the genome of a living cell.

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მცენარეთა ფიზიოლოგია

იზოპრენის ბიოსინთეზი და გამოყოფა არაწონასწორული თერმოდინამიკის თვალსაზრისით

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ნაშრომში განხილულია ცოცხალ უჯრედებში მიმდინარე მეტაბოლური გარდაქმნების შესწავლის შესაძლებლობა არაწონასწორული თერმოდინამიკის გამოყენებით იზოპრენის ბიოსინთეზისა და გამოყოფის მაგალითზე. დადასტურდა, რომ იზოპრენის გამოყოფა ცოცხალი უჯრედიდან არის ჭარბი ენერგიის დისიპაცია და იგი უზრუნველყოფს ცოცხალი სისტემის ჰომეოსტაზის მდგრადობას ტერპენოიდების ბიოსინთეზის რეგულირების მეშვეობით. გაცემულია პასუხი კითხვაზე, თუ რატომ გამოყოფს ცოცხალი უჯრედი ოზოპრენს. უჯრედიდან იზოპრენის გამოყოფის პროცესი წარმოადგენს ჭარბი ენერგიის დისიპაციის აუცილებლობას, რაც ნიშნავს იმას, რომ იგი თავისი არსით ენტროპიული ფენომენია ე.ი. უჯრედის მდგრადი არსებობის ფუნდამენტური კანონი. იზოპრენის გამოყოფას, გარდა ამ ფუნდამენტური ფუნქციისა თან ახლავს მეორადი თანმდევი ფუნქციები: თერმოტოლერანტობა, ანტიოქსიდანტობა და ენერგოპლასტიკური ცვლის სხვადასხვა ხასიათის რეგულატორული აქტივობები. ამ პროცესის ბიოლოგიური როლის უფრო ღრმად შესწავლისათვის აუცილებელია მის ევოლუციურ ჭრილში განხილვა, ვინაიდან სინათლეზე იზოპრენის ბიოსინთეზი და გამოყოფა ქლოროფილის შემცველ უჯრედიდან ევოლუციის პროცესში პერიოდულად იკარგებოდა და კვლავ აღდგებოდა ხოლმე, რადგან იზოპრენის ბიოსინთეზის უნარი ინფორმაციის სახით ინახება ნებისმიერი მცენარეული უჯრედის გენომში.

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