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Photobiosynthesis of Isoprene and Excretory Function of Plant Leaves in the Light of Modern Thermodynamics

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ABSTRACT. Different aspects of synthesis and excretion of biogenic isoprene into the environment have been already studied well. Excretion of isoprene out of cell in essence is excretion of surplus energy which constantly arises in the process of metabolism, and it should be considered as a result of its excretory activity. Excretory activity of a cell is one of the most important functions of a living system. It completes the stable flow of a stationary condition of metabolism. A question arises as to the degree of the fundamental nature of constant loss of some part of free energy in the processes taking place in the cell.

In the given paper, these questions are considered from the standpoint of modern thermodynamics. The conclusion is made that the basis of excretory capacity of living cell is thermodynamic dissipation of entropy, excreting during irreversible processes, providing for the stability and ontogenetic steadiness of the living organism. © *2009 Bull. Georg. Natl. Acad. Sci.*

Key words*: entropy, isoprene effect, dissipative structures, excretory function.*

At present, i.e. in the period, when rapid accumulation of scientific information takes place and human mind is still inquisitive, the question of why this or that phenomenon occurs in nature, arises more and more frequently. Although the probability of getting a satisfactory answer is still low, the hope of getting a simple answer increases, and in the first place, as a result of emergence of numerous new sources of information. Secondly, thanks to the fact that mankind has actually entered the age of new rationality, in other words, the essence of science has somehow changed, it has ceased identifying with the definiteness of comprehending phenomenon on the whole, but it gained an important argument of getting probability in the form of real knowledge of its separate reasons of this phenomenon. The second law of thermodynamics is the fundamental beginning of comprehension of the role of irreversible processes in nature, and life, in essence, set of energy-plastic currents, running in the specific structures of cells (on biological matrices). Such comparison of peculiarities of vital activity with the laws of thermodynamics, as it takes place today, has become an indispensable scientific requirement.

As the living cell is the totality of energy-plastic currents, running on a specialized biological matrix, it is functionally of dissipative structure. This concept was first introduced into thermodynamics by the famous Belgian scientist I. Prigogine [1, 2]. He called them dissipative structural formations, which are in stationary condition, far from their equilibriums. If the whole living system can be divided conditionally into two types: first, nearest to the equilibrium – linear, and second – moved away from equilibrium – non-linear, then on the pathway of energy-plastic current, i.e. chain of irreversible reactions, dissipative structures must arise. As far as in the case of the living cells we undoubtedly deal with open systems where mainly irreversible reactions take place, we come across constant dissipation of entropy (surplus energy). In other words, living systems, owing to the presence of numerous dissipative structures in them, constantly produce and dissipate entropy into the environment. This means that excretion of some part of free energy is the compulsory result of the vital activity of a cell. The accumulation of surplus energy inside the cell leads to an inadmissible increase of entropy, on the other hand, in the same cell, depending on outer conditions; the danger of accumulation of surplus energy (ATP) appears in connection with the coming decompensation (discrepancy) between the amount of transforming substrate and surplus of ATP. Such a situation constantly takes place in nature because of natural changes of boundary conditions. For a thermodynamic description of the abovementioned, the formula on accretion of entropy can be used: $dS=d_eS+d_iS$ where dS – is accretion of entropy, d_eS is current of entropy, or in essence exchange of energy between cell and environment, and d_iS – are irreversible processes, taking place in a living cell, constantly supporting the process of production of entropy.

The relative stable equilibrium state of living cell is primarily determined by stationary parameters. The stationary state in which the cell is found at this period, is a more or less stable dynamic equilibrium and is called stationary. This state is characteristic of open systems where the speed of bringing in of substance and energy from the medium into the system is equal to the speed of its emission.

At the same time, the cell never achieves the state of complete equilibrium with the medium, but it firmly keeps its stationarity.

The fundamental role of production of entropy ($p=ds/dt$) was first revealed in the middle of the 20th century. In natural processes production of entropy defines the character of entropy, but the law of conservation of energy defines the balance between inflow and outflow of energy in the process of dissipation. Thus, proceeding from a more general formula of thermodynamic $F=E–TS$, where F is free energy, which is the linear combination of energy E and entropy S at the constant temperature T. This means that entropy depends on completed work and is in direct dependence on the quantity of spent energy. As noted above, one of the main thermodynamic quantities is the production of entropy P, with which the theorem on minimal production of entropy proposed by I. Prigogine in 1945 is connected [3]. This theorem defines the condition of stationary state of system of maximum stability which is attained only at minimal value of P and invariability of boundary conditions, i.e. invariability of the parameters of external conditions. In this case it can be said that the system is in dynamic equilibrium with the medium, i.e. in "flowing equilibrium" where in a compulsory order minimal production of entropy, optimal effective expenditure of energy are executed and, according to boundary conditions, the state of dissipativity of structures is determined.

Spatial dissipative structures originating in nonhomogeneous thermodynamic currents are stable stationary systems. As diffusive currents in such nonhomogeneous structures do not stop constant dissipation of energy, it occurs in the system. Without going into details concerning the grounds of the proposed hypothesis, we come to the conclusion that dissipation of entropy (d_eS), in the end, can be considered as dissipation of energy. That is why increasing entropy, its dissipation and dissipation of energy must not be considered only as the metabolic losses of cell, because they are the result of a fundamental natural phenomenon characteristic of all open systems.

Isoprene effect or, in other words, biosynthesis or excretion of isoprene from green plant leaves in the light is a strikingly pronounced photobiological phenomenon.

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Energy spent on the accomplishment of conversion of carbon in this biosynthesis originates from the primary photochemical acts of photosynthesis, initiating the work of electron-transporting chains in I and II photosystems, ending in the formation of ATP and NADPH, subsequently being used in conversions of carbon in the course of a whole process of its metabolism. As is known, primary inclusion of carbon into cellular metabolism, occurs in the process of involving CO_2 in the reaction of reducing carboxylation.

Beginning with the reducing pentosoposphate cycle in the cell, subsequent conversion of carbon goes on right up to its partial excretion in the form of excreta. Seemingly, all the rest of known and yet unknown energy-plastic processes, combined or each of them separately, create irreversible currents ending in final products – metabolic deadlocks, possessing negative feedbacks which are most frequently used in the process of retroinhibition. On the other hand, such final products which, leaving the cells, are converted into excreta automatically, lose their negative function of feedback and acquire a positive one. This alteration of character of feedback (from negative to positive) seems to be the fundamental basis of metabolism, assisting in stable conservation of homeostasis of living cell. Thus, it becomes apparent that a living cell is an open thermodynamic system in which stationary state is preserved rather firmly only because of the presence of dissipative structures in it, constantly extracting that part of entropy which is left after attaining the minimum of its production. It is known that leaves are the most active and a varied organ of plant from the metabolic point of view. This means, that major currents of vital activity and generalized forces, imparting given speed to currents, are concentrated just in the cells of the leaf and so are the basic source of entropy. For lack of special excretory organs of plants, the bringing of metabolite cells out could be most effectively accomplished only in the case of volatility of these substances, which is actually observed. Moreover, in the case of isoprene special attention is attracted by the fact that effective bringing of substance out of cell in many respects should be dependant on the degree of its hydrophobity. In this connection, complete insolubility of isoprene in water should be specially underlined. It is known that isoprene-2-methyl-butadiene-1,3 is an unsaturated hydrocarbon of diene order. *This feature of isoprene has also a very important biological significance and it will be discussed below.*

In illuminated leaf of a main thermodynamic current can be conceptualized in the following way. It consists of two constituents: energy of ATP and $NADPH_2$, emerging as the result of absorption of quantum of light and assimilated carbondioxide (CO_2) by chloroplasts, i.e. limiting the photosynthesis factors of medium, called elements of boundary conditions in thermodynamics.

Thus, thermodynamic currents of illuminated cells of a leaf are formed by a chain of metabolic conversions of carbondioxide-containing substances, using generalized force of ATP and NADPH2. All these represent the main energy-plastic current of vital activity of photosynthesizing cell and can be central in living nature.

We will try to describe shortly the metabolic situation in which the formation and excretion of isoprene in the cells of photosynthesizing leaf takes place. First, it is quite obvious that this process is light-dependent and its energetics is completely ensured by the activity of the electron-transporting systems of chloroplasts. For example, it is shown that the process of light formation and excretion of isoprene repeats the picture of intensifying photosynthesis (Emerson's effect). Moreover, the quantity of intensification of isoprene effect by its intensity somehow even exceeds that of photosynthesis measured both for absorption of CO_2 and for excretion of O_2 [4]. Second, manifestation of isoprene effect: its intensity is inversely proportional to CO_2 pressure in the medium. It must be stressed that in natural conditions plants use CO_2, concentration of which in the air usually amounts to about 300 ppm. This quantity of CO_2 concentration is nearly one order lower than potential possibilities of using CO_2 at photosynthesis. And indeed, increasing CO_2 concentration by an order relatively to the one existing in nature, i.e. about 3 000 ppm with attaining maximum photosynthesis, isoprene excretion sharply lowers and approaches the dark level of its excretion.

Thus, in natural atmospheric conditions the photosynthetic apparatus works actually at habitual deficiency of CO_2. Subsequent decrease of partial pressure of CO_2, which takes place in the closed chamber during the experiment, reaches stressed level.

At constant temperature and illumination the decrease of pressure of CO_2 in surrounding atmosphere of a leaf, i.e. in a closed chamber with a leaf, a carbondioxide compensating point characteristic of C_3 plants is attained, the quantity of which is usually close to 50 ppm CO_2 [5]. In the result of it, a sharp decrease of activity of the electron-transporting system occurs in chloroplasts. This, first of all, means that there is a well expressed correlation between the change of CO_2 pressure in the chamber and the action of electron-transporting chain, which resembles Onsager's model on "Correlation of reciprocity" (1931) [6], i.e. the interdependency of two thermodynamic currents. In our case, decrease of speed of carbon diffusion in chloroplast structure decreases the speed of current of energy (ATP and $NADPH_2$). Such interdependence between currents of energy (ATP, $NADPH_2$) and carbon inevitably results in the increasing the disbalance between the level of free energy and possibility of its use in the reaction of carbon conversion, which, in turn, causes the emergence of critical surplus of energy with regard to its use in metabolic conversions, on the whole. As in normal conditions the cell is in a stationary state, surplus of energy inevitably would result in breaching the stationary equilibrium. In order to avoid this situation, the self-regulatory essence of a living system reorganizes speeds of currents so that this surplus energy could be removed. Removal of surplus energy is attained first of all by means of dissipative structures and excreting entropy out of living cell (deS), i.e. in accordance with the above arguments, part of free energy which is not used in biochemical conversions dissipates. Thus, we come to the conclusion that excretion of entropy, in the end, is none but the deliverance of a system from surplus entropy, surplus of free energy and on the whole of metabolic wastes (excreta).

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Let us discuss issues regarding the supposed biochemical mechanism, dissipation of entropy (free energy) from photosynthesizing cell of the illuminated leaf. Since Molish [7], who discovered excretion of ethylene out of ripening fruits, then in the fifties of the last century the discovery of volatile phytogenous compounds, such as light hydrocarbons of limited order, and also isoprene [8], and afterwards alcohol and volatile terpenoids which, in the whole, constitute the totality of products of metabolism emitted by leaves. Isoprene takes special place among them, the intensity of excretion of which in the light is several times more than intensity of other excreted volatile combinations. Today it is well known that independent of light excretion of isoprene, i.e. “dark” excretion, is characteristic of most living beings, including microorganisms, mammals, human beings, lower and higher plants. It is known that the direct biochemical precursor of isoprene is dimethylallylpyrophosphate (DMAPP) [9], besides, two more biochemical mechanisms leading to the formation of this substance with isopentenylpyrophosphate (IPP) are known. First of them is called acetate-mevalonate, as biosynthesis of five-carbon compounds of IPP and of its isomer DMAPP originate from acetyl-KOA and acetoacetyl-KOA through mevalonic acid [10]. Correlation between IPP and DMAPP in reactive medium, is, as a rule, determined by means of enzyme IPP-isomerase. Formally, this isomerase regulates quantitative correlation between them and thus guarantees the necessary concentration of these substances for the synthesis of one or another biologically important compound. But, as it seems to us, the biological role of this enzyme is by no means exhausted only by this. It has more general implication for the metabolism of cell and, in particular, for regulation of the entire thermodynamic current of the main energy-plastic conversions in cells. Thus, for example, almost the whole pathway of carbon, beginning with initial compounds to these final five-carbon products, can be conceptualized in the form of a thermodynamic branch with a bifurcation point, the divarication of which leads to formation of DMAPP and IPP *and thus the precursor of isoprene emerges, the activation of which is accomplished by means of the abovementioned isomerase.*

In the case of methylerytritolphosphate pathway (MEP) five-carbon precursors of terpenoids IPP and DMAPP, and consequently, supposedly of isoprene, emerge by biosynthesis of xylulose-5-phosphate [11]. We must pay attention to the fact that in both cases the quantity of ATP spent on these syntheses exceeds those spent on hexose biosynthesis at photosynthesis, and in the case of mevalonate path is equal to 24 ATP, and in accordance with MEP path – 20 ATP [12], in both cases using 14 molecules of $NADPH_2$. Taking into account that at calculation for one carbon in case of five-carbon compounds, quantity of energy, spent on this synthesis increases approximately up to 20% in comparison with hexoses and is one and a half as much as in final products of photosynthesis. *From the point of view of dropping of surplus energy by the system, substances with double bonds and especially bivinyls, such as isoprene, seem economically effective, i.e. biologically profitable.* And really, the function of bringing of entropy out off cell boundaries (deS), the carrier of which is isoprene in the given case, is accomplished by means of a relatively small amount of carbon and hydrogen in comparison with any other substance, not having double bonds and particularly, with such typical compounds of photosynthetic conversions of leaf, as carbohydrates.

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General interest in studying the isoprene effect (IE) and generally in biological synthesis of isoprene arose in the last decade of the last century in the USA and some European countries, attracting great attention of scientists to it. Experiments of many years had preceded them, starting in the middle of the 1950s carried out first at the Institute of Botany in Tbilisi, then continued in the 60s at the Moscow Timiryazev Institute of the Physiology of Plants. From the end of 1968 to the present day these works have been carried on in photosynthesis laboratory of Tbilisi State University. When it was finally determined that among number of light hydrocarbons excreted by autotrophic cells of plants in the light, isoprene attracted the greatest interest, process of biosynthesis of which appeared to be light-dependent [13]. Later on it was found that isoprene also synthesizes and then excretes out of heterotrophic cells independently of light. As stated above, practically all living beings possess this feature. Unlike isoprene effect, i.e. light-dependent excretion of isoprene by photosynthesized autotrophic green cells of plants, the intensity of “dark” excretion of isoprene by heterotrophic cells is considerably weaker and about one to two orders lower.

Investigations showed that about 15-20% of species of plants existing at present on the earth possess the isoprene effect and most of them belong to arboreal forms.

IE was studied in conditions of closed chamber, at constant parameters, boundary conditions of temperature, intensity of illumination and CO_2 concentration. First two factors limiting photosynthesis were naturally regulated and stabilized by means of using common technical methods: ultrathermostatic and special illuminating devices. *The concentration of CO_2 in the atmosphere of the chamber was self-regulated by the leaf to carbon-dioxide compensating point, quantity of which, as said above, was within the limits of 50 ppm of CO_2.* In standard conditions of experiment temperature was 25-30 C, intensity of illumination was within the limits sufficient for saturating photosynthesis. In these conditions energy-plastic currents arise in the leaf, these currents due to stability of boundary conditions, bring the metabolism of the leaf into stationary state, firmly kept for the period of some hours. By varying boundary conditions, using different metabolites, exogenously putting them into the cell and specific inhibitors of metabolism, it was found, that the isoprene effect arises as the result of regulatory reorganization of metabolism, caused by changing the CO_2 concentration in the atmosphere surrounding the leaf. As noted above, as the result of strengthening CO_2 deficiency in chloroplasts surplus energy of ATP accumulates, which starts to exceed significantly the possibility of its realization, which in its turn results in increasing the production of entropy in cell, which in accordance with the basic law of thermodynamics must be finely excreted from the system. In the case of isoprene effect, this release of entropy occurs by means of biosynthesis and dissipation of isoprene itself.

The present success of thermodynamics of open systems has resulted in determining the presence of dissipative structures in them, responsible for bringing surplus of entropy out of the system in to the medium. This fundamental feature of non-equilibrium open systems permanently producing entropy permits to make the conclusion that part of free unused energy dissipates with entropy, which is also a fundamental sign of the vital activity of cells.

The property of living cells to excrete part of metabolites into the medium is a special function of living organism and is called excretory. If one imagines a whole living cell as a non-equilibrium thermodynamic system dissipating entropy, then it follows that the sum of energy excreted out of this system will correspond to the sum of deS which is produced by the totality of dissipative structures of cell. Therefore, it is natural that considerable part of free energy spent on fulfilling the function of dissipative structures will be summarized in that part of metabolites which is excreted out of the cell in the form of excreta. It is clear from the above, that the excretory function of a living cell is a direct result of the dissipativity of open thermodynamic systems and is a permanent constituent of cellular metabolism. As the amount of isoprene considerably exceeds that of all other biogenic volatile substances, it is clear that the share of its participation in this process is rather large. Moreover, isoprene can be considered as a universal, widespread product of metabolism in its own way, characteristic, as it seems, of all living kingdom.

The isoprene effect or light-dependent emission of isoprene should be estimated as the use of widely spread biochemical reactions by chloroplasts, leading to the formation of dimethylallylpyrophosphate – precursor of many terpenes, including isoprene, excreted out of cell in the form of excrete.

That is why the answer to the question why plants excrete isoprene is: because excretion of isoprene is the result of excretory activity of cell. In its turn, a cell, being a dissipative structure, by means of excretory activity guarantees the stability of processes of vital activity running in it and, as a whole, of the living organism.

Over the last decades, our knowledge in the sphere of biological synthesis of isoprene and in particular of the isoprene effect has considerably widened. A specific protein catalyzing conversion of dimethylallylpyrophosphate into isoprene, named isoprene synthesis has been identified [14]. Compartmentation of this protein in cell and chloroplasts has been determined. Membrane connected isoprene synthesis activity has been found in thylakoids [15, 16]. In 2001 a report was published on isolation of isoprene synthesis gene from poplar [16, 17], which was successfully expressed in *E. Coli*. Subsequently this gene was excreted from poplar by other scientists too, and from kudzu [18-20].

Some information has recently appeared on producing transgenic unicellular water plants and photosynthesizing bacteria, in which isoprene synthesis activity is expressed [21]. The above-indicated transgenic microorganisms were placed in a specially constructed photobioreactor, in which sufficient amount of isoprene was produced and later used as a fuel gas. It is safe to say that prototype of a biotechnological method of obtaining isoprene in considerable amount has been already created. Certainty has arisen that this method will be soon improved and mankind will receive an alternative source of isoprene, i.e. a new source of strategic raw material.

As is known, at present the basic source of this raw material is oil, the reserves of which, according to geologists, is extremely restricted and should run low in the nearest future.

Scientific researches, which contributed to discovery of the isoprene effect and the appearance of first photobioreactor, i.e. the first practical application of

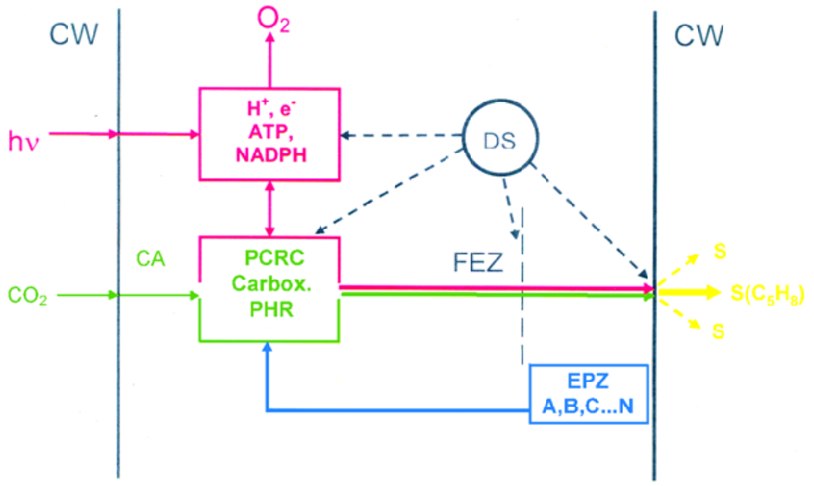

Fig. 1. Simple scheme of supposed thermodynamical currents in the isoprene photobiosynthesis process.

hν - light; CA - carbonic anhydrase; CW - cell wall; PCRC - photosynthethic carbon reduction cycle; PR - photorespitation; DS - dissipative structures; Carbox. - carboxilation; FEZ - fluctuations enhancement zone; EPZ - ending products zone; Colors: **CREEN** - carbon metabolism current; **RED** - energy current; **YELLOW** - entropy (S); **BLUE** - negative feedback.

scientific knowledge in the sphere of biogenic isoprene, will greatly expand, in future. On the grounds of the foregoing, one can be sure that by the time when the source of fossil photosynthesis will dissapesr on the earth and it will have to be replaced by modern photosynthesis, mankind will be quite ready for resolving this task.

ბიოფიზიკა

იზოპრენის ბიოსინთეზი და მცენარეთა ფოთლების ექსკრეტორული ფუნქცია თანამედროვე თერმოდინამიკის თვალთახედვით

გ. სანაძე*, ს. ფხაჭიაშვილი**

** აკადემიკოსი, საქართველოს მეცნიერებათა ეროვნული აკადემია*
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მეცნიერებაში კარგად არის შესწავლილი ბიოგენური იზოპრენის სინთეზისა და მისი გარემოში გამოყოფის სხვადასხვა ასპექტები. იზოპრენის გამოყოფა უჯრედიდან არსებითად წარმოადგენს ჭარბი ენერგიის განბნევას გარემოში, რომელიც მუდმივად წარმოიქმნება მეტაბოლიზმის პროცესში და განიხილება როგორც უჯრედის ექსკრეტორული ფუნქცია. აღნიშნული ფუნქცია კი ცოცხალი სისტემის უმნიშვნელოვანეს ნიშან-თვისებას წარმოადგენს. იგი მეტაბოლიზმის სტაციონარული მდგომარეობის მდგრად მიმდინარეობას განაპირობებს. ისმება კითხვა უჯრედის მიერ თავისუფალი ენერგიის ნაწილის მუდმივი კარგვის ფუნდამენტური მნიშვნელობის შესახებ.

წარმოდგენილ ნაშრომში ეს საკითხები თანამედროვე თერმოდინამიკის თვალსაზრისით განიხილება. კეთდება დასკვნა, რომ შეუქცევად პროცესებში ცოცხალი უჯრედის მიერ ნივთიერებათა გამოყოფა არის ენტროპიის თერმოდინამიკული დისიპაციის შედეგი, რომელიც უზრუნველყოფს ცოცხალი ორგანიზმის სტაბილურობასა და ონტოგენეზურ მდგრადობას.

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