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Respiration energetics of marine algae for total heat production and some features of photosynthesis[1]

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Abstract

A preliminary study of microplankton biothermodynamics in Sevastopol Bay showed an endothermic effect at the end of the first day of measuring the heat production. Measuring the heat dissipation of dark reactions in metabolism of some seaweeds, using a microcalorimeter, determined the analogous endothermic effect approximately within the same time period of measurements. One can observe that energy of consumption of O_2 exceeds that of heat-production energy in oxidative respiratory organisms and explain it on the ground that heat dissipation is the total index not only for biologic reactions in metabolism but also for physico-chemical reactions of hydration and dissolving metabolites for the cell-water phase. Thus, some substances (hydrocarbonates) reduce the solution temperature on dissolving and, consequently, reduce the total heat production. Experiments have shown that, when changing the oxygen respiration phase by an anoxidative one, the heat production can decrease temperature up to the conventional zero (20°C-experiment temperature) and even to a negative value. We think it can be due to sodium hydrocarbonate transportation as a final product of aerobic respiration out of membranes of intracellular organelles into the cell-water phase. By glycolysis, HCO_3 ion is not generated, and the heat production returns to its initial level in 2–3 h, but its nature changes. The obtained data show that W_s exceeds by several times the total energy of O_2 absorption and CO_2 (HCO_3) release. Rather intensive absorption of O_2, for the first hours of dark period, must be connected with the consequence of photorespiration, not with the aerobic (oxygenic) phase of respiration. As it has been shown in experiments, respiration energy of marine algae is contributed mainly by anoxic processes (glycolysis) even under sufficient (a few mg/l) O_2-supply. In the Black Sea macrophytes, photosynthesis is not followed by oxygen production in water photolysis. OH^- ions do not convert to O_2, but are transported to habitat that may result from primary (ancient) mechanisms of water photolysis (photolytic anoxic photosynthesis) preserved in the studied organisms.

Keywords: Glycolysis; Heat production; Marine algae; Respiration

1. Introduction

Seaweeds (especially red and brown) are known to be the most ancient eucaryotic photosynthetic organisms which may have retained some functions typical of photosynthesizing microorganisms and anaerobic bacteria [1,2]. The basis for such an assumption is not only the ancient origin of marine algae but also the ecological conditions of their habitat:

1. Water environment contains little oxygen in comparison with the atmosphere, and the rate of diffusion is much less;

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2. Seaweeds (especially the benthic ones) are often subjected to hypoxia, favoring preservation and functioning of respiratory anoxygenic mechanisms (glycolysis) and photosynthesis; and
3. Mitochondria have not as yet been found in some large taxons of marine algae (red, brown, *Ulotrix*). Mitochondria is a cell organelle in which oxidative phosphorylation takes place [3].

Contemporary aerobic organisms are known to have preserved anaerobic functions (glycolysis, cyclic phosphorylation, photosystem). One can assume that the transition from anoxybiotic bacterial photosynthesis to oxidative processes did not occur directly, but through some intermediate anoxybiotic mechanisms. In other words, as oxygen was a poison (strong toxicant) for the anaerobic photosynthetic organisms, transition to water photolysis could proceed through the transport of hydroxides into the environment but not through conversion of four hydroxides of O_2 and $2H_2O$. If such mechanisms, which can be called photolytic anoxybiotic photosynthesis, really had functioned earlier, then they would have been preserved in the contemporary water plants (especially in those of ancient origin) by analogy with glycolysis and cyclic phosphorylation.

The aim of this work is to study a specific character of respiration and photosynthesis in seaweeds and seagrass (e.g. in the Black Sea).

2. Experimental

Red (*Gracilaria verrucosa* (Huds.) Papenf. and *Phylophora nevrosa* (DS.) Grev.) and green (*Ulva rigida Ag.*) seaweeds and seagrass *Zostera marina* L., and (for comparison) freshwater flower plant *Elodea canadensis* R. were chosen as the objects of investigation. Changes in O_2 concentration, pH and temperature were determined by means of electrochemical methods in the closed vessel of 1.6 l volume with sensing elements connected with a computer. The observations were carried out for 24 h and recorded automatically after every hour with different light and dark periods. Change of O_2 concentration during photosynthesis was determined before saturation. When it was necessary, O_2 deficit was created at the expense of the seaweed dark respiration before the experiments on photosynthesis began. Increase in seaweed biomass was also determined for the duration of the experiment. The initial biomass was 1–2 g/l, and the illumination was 3000 lx. Three times the concentration of Goldberg nutrient medium was added to the experimental vessel of marine water. The rate and power of respiration were determined in thrice according to decrease in O_2 and pH of the environment and heat production. The heat flux was determined on a TAM-ThermoMetric device (Sweden) at 20°C. The temperature correction was made for the values of heat production and pH. When the temperature of the test vessel increased from 20° to 30°C, the correction was equal to two.

Quantitative ratios of the studied parameters are given below.

$1\ \text{mg dry weight h}^{-1}{=}2150\ \mu\text{W}$

$1\ \text{mg O}_2\ \text{h}^{-1}{=}31\ \mu\text{Mol h}^{-1}{=}3900\ \mu\text{W}$

$1\ \mu\text{Mol O}_2\ \text{h}^{-1}{=}125\ \mu\text{W}$

$1\ \mu\text{Mol h}^{-1}61\ \mu\text{g HCO}_3^-\ \text{h}^{-1}{=}32\ \mu\text{g O}_2$

$0.01\ \Delta\text{pH h}^{-1}{=}10\ \mu\text{Mol}\ \ \Sigma\text{CO}_2\ \ \text{h}^{-1}{=}1250\ \mu\text{W}$ (per liter)

The value of 1250 μW (when pH is 8.0±0.4) is an empirical one, calculated from the average value of *Gracilaria* calorie content [4]. The calculated value of $\Delta\Sigma O_2$, according to the formula used in marine hydrochemistry, is 1.5–2 times less than the experimental ones used in this work.

3. Discussion

Table 1 shows that the intensive aerobic (oxidative) respiration was observed only during the first hours of the dark period. Respiration rate decreased approximately exponentially, becoming two times less every 1-2 h. Table 1 gives the average (for 1 h) values for different respiration phases. The rate and specific rate of O_2 consumption reduced during 3–5 h by several times. But, a decreasing O_2 consumption rate is not accompanied with a reduction in the metabolic rate because $\Delta\Sigma CO_2$ and heat flux do not decrease. Oxidative mechanisms of respiration are replaced by anaerobic (anoxidative) processes in several hours, though the content of O_2 (several mg/l) still remains rather high in the environment.

The glycolysis inhibitor, monoiodoacetate, inhibits the heat production almost completely. Thus, the

Table 1
Matter and energy fluxes during respiration of seaweeds studied (averaged for 1 h)

Time from the beginning of dark period (h)	O_2 uptake rate [a]		CO_2 production rate [a]		Heat-production rate [a]
	(µMol)	(µW)	(µMol)	(µW)	(µW)
Phyllophora nervosa					
1–4	1.60	220	0.6	80	1000
5–8	0.2	30	0.4	40	600
9–18	0.1	9.0	0.3	40	260
Gracilaria verrucosa					
1–7	0.8	≈100	1.0	125	400
8–19	7×10^{-3}	0.4	0.7	64	260
Ulva rigida					
1–2	4.0	530	2.8	350	2000
3–20	0.14	18	1.4	180	1800
Zostera marina					
1–2	2.3	280	2.8	360	1380
3–4	0.5	60	2.0	260	1350
5–19	1.5	18	0.7	90	690
Elodea canadensis					
1–2	0.9	115	4.3	530	750
3–4	0.25	32	4.3	530	760
5–19	7×10^{-3}	0.9	2.1	260	370

[a] Per gram of seaweed wet weight.

mechanisms of substrate phosphorilations, occurring in cytoplasm functional in water plants (irrespective of their origin) mainly show that the process of glycolysis is unlikely to proceed to the final product CO_2, but rather to some intermediate products, which can be included again into the photosynthesis cycle for the light stage. The principal (from the viewpoint of ecological physiology) difference between seaweed glycolysis and that of the anaerobic bacteria lies in that the seaweed glycolysis increases the metabolism efficiency (consumption of the respiration substrates) instead of decreasing it, because the products of glycolysis in seaweeds can be re-synthesized again by photosynthesis, rather than release them into the environment (as bacteria or yeast do).

The difference between heat production and ΣCO_2 yield will be greater, if we take into consideration the efficiency of respiration which is ≈50% (toal for the substrate and oxidative phosphorilations [5]). The calculated values of ΣCO_2 in sea water also reduce (in comparison with the biomass increase) the value of CO_2 transport from cells and, correspondingly, increase the fraction of heat production in respiration. One can account for such a high difference between heat production and gas–ion-exchange energy by the energy of solvation (hydration) of the intermediate products of metabolism when non-electrolytes (lactose) transform into electrolytes (phosphorylated products of glycolysis). This energy is quite comparable with the energy of the oxidative–reductive reactions [6], i.e. in glycolysis the water in the cell is likely to obey the same function (from energetic point of view) as does oxygen for the anaerobic (oxidative) respiration.

There is a small difference between the rate of O_2 consumption and heat production in land plants and animal organisms having aerobic respiration [7,8]. Some of the excess of O_2 consumption over the heat production, which is observed in organisms with aerobic (oxidative) consumption [8] can be accounted for by the fact that the heat is not only produced by reactions of metabolism but also by reactions of hydration and dissolution of metabolites in the cell water phase. Some substances are known to decrease the solution temperature by dissolving (e.g. hydrogencarbonate ion) and, consequently, decrease the total heat production. Our experimental data show that heat-production rate can decrease to zero and even to negative values when the oxidative phase of respiration is replaced by the anoxidative one. In our opinion, this can be due to the transport of sodium hydrogencarbonate as a final product of aerobic respiration through the membranes of intracellular organelles to the water phase of a cell. HCO_3^- ion is not formed in glycolysis.

Using the obtained data, the general formula which takes into consideration the solvation energy contribution (W_s) to the process of respiration can be written as follows:

$$W_s = [W_t - (W\Delta\Sigma CO_2 + W\Delta O_2)]C,$$

where C is the respiration efficiency and is ≈ 0.5 [5] and W_t the heat production energy.

Data given in Table 1, calculated according to the formulas, show that W_s exceeds several times the total energy of O_2 consumption and CO_2 (HCO_3) release.

A somewhat intensive consumption of O_2 for the first hours of the dark period is likely to result from photorespiration but not from the aerobic (oxygenic) phase of respiration.

Data describing the processes of photosynthesis (Table 2) show the difference between the values of rate O_2 production, on the one hand, and $CO_2(HCO_3)$ absorption, on the other. The difference between the rates of CO_2 absorption ($W\Delta\Sigma CO_2$) or biomass increase ($W\Delta\delta$) and the rate of O_2 production ($W\Delta O_2$) is rather considerable, and repeats from one experiment to another. In our opinion, this describes photolytic anoxygenic photosynthesis (PAP), which takes the intermediate position between bacterial photosynthesis and classical oxygenic photosynthesis (OP). The predominance of anoxygenic photosynthesis in water plants over the oxygenic one is from 113 to 860%.

Physiologic importance of anoxygenic photosynthesis in water plants is likely to lie in the fact that when the O_2-production rate decreases, the losses of substance and energy caused by photorespiration also decrease, whereas the rate of CO_2 uptake can be the same. Moreover, a reduction in the rate of O_2 production will, in turn, increase the rate of O_2 assimilation, because CO_2 and O_2 are competitors in carboanhydrase–oxygenase enzyme [9]. Land plants are likely to be deprived of a similar photosynthesis-regulating mechanism. Their photosynthesis coefficient (PC) is $\cong 1$, but only for a short period of time (1.5 h of exposure, [10]). The PC of the species, under investigation, is also close to one, and even changes sign. By 24-h illumination, one can see the rotation of photosynthesis and respiration. Increase of O_2 concentration and pH now changes into their reduction. Sometimes, O_2 concentration increases whereas pH decreases. As to the latter case, there is an isolation of H^+ and CO_2 in the environment, while water photolysis and O_2 generation take place. It is probable that O_2 production and synthesis of organic substances can proceed independently of one another.

So, why do sea algae need O_2 at all? A small amount of O_2 is necessary for photorespiration, but is inhibited with increasing O_2 concentration (Varburg's effect). Photorespiration may have appeared in ancient algae as a protection mechanism from O_2, but then became part of photosynthesis. Production of OH^- and H^+ in ionic cells by light can be considered as one of the

Table 2
Energetic power (μW) of oxidative ($W\Delta O_2$) and anoxidative ($W\Delta\Sigma CO_2 - W\Delta O_2$) characteristic of photosynthesis or biomass increase ($W\Delta\delta - W\Delta O_2$)

Date	Object	$W\Delta O_2$	$W\Delta\Sigma CO_2$	$W\Delta\delta$	$W\Delta\delta - W\Delta O_2$
04.11.91	*G. verrucosa*	460	2120	~1000	~540
18.05.91	*G. verrucosa*	~560	~2000	1390	890
25.05.92	*G. verrucosa*	420	2100	2900	2480
23.06.92	*G. verrucosa*	500	—	4800	4300
29.06.02	*G. verrucosa*	700	2500	2600	1900
20.07.92	*Ulva rigida*	360	2500	3400	3040
08.09.02	*Ulva rigida*	830	2370	1770	940

consequences of the chemiosmotic theory of Mitchell [11]. According to this theory, these ions are necessary for the synthesis of ATP by phosphorylation processes. By introducing H^+ and OH^-, a cell regulates its metabolism, including the reproductory processes.

Thus, for example, pH decrease in the light can be explained by a temporary excess of the rate of anoxygenic respiration over the rate of biosynthetic reactions, if the intermediate products of photosynthesis are not utilized in dark reactions [12]. Transport of H^+, OH^- or organic substances into the environment can be considered a peculiar valve which lets excess energy out, if the rate of production, e.g. of carbon, exceeds the rate of biosynthesis of nucleic acids, albumins etc., which are regulatory reactions.

If a cell needs to limit its photorespiration rate, then the four hydroxyl ions do not convert into O_2 and $2H_2O$ but are transported partially (or completely) into the water environment. Such a mechanism of photosynthesis is likely to have existed in the ancient bacteria [1]. The rotation of photosynthesis mechanisms, observed by long light period, can be considered as a system of intracellular mechanisms which regulates metabolism.

It follows from the results obtained that the rate of photosynthesis described CO_2 assimilation rate and not the rate of O_2 production. This should be taken into consideration when studying quantum expenditures of photosynthesis, production process, etc.

Measuring the heat production of the principal-size categories in microplankton (demto- pico-, nano- and not phyto- and zooplankton) showed the endothermic effect in the beginning of the folowing experimental day [13]. This effect is probably due to the features of photorespiration and rotation of the photosynthesis mechanisms in the unicellular planktonic algae (particularly picoplankton), which must be closest to the Precambrian forms of life. This problem should be studied individually within the program of origin and development of the organic world on our Earth [14].

4. Conclusions

1. Glycolytic mechanisms of respiration predominate in the studied sea algae and water flower plants, oxidizing mechanisms of respiration function only for the initial hours of the dark period.
2. Glycolytic processes in the cells of the studied sea algae are unlikely to go on to the final product – CO_2, but they do lead to intermediate products, which can be reduced again by photosynthesis.
3. The experiments show that sea algae have rather powerful photolytic anoxygenic mechanisms. The OH^- ion, which is produced by water photolysis, can convert to O_2 only incompletely: redundancy of OH^- is transported into the water environment and H^+ joins in the restoration cycles of carbon (carbonic acid).
4. Respiratory and photosynthetic features found during the experiment can be considered as the sign of phytogenetic ancient origin of sea algae, on the one hand, and as the physiologic–bioenergetic mechanism for regulating the rate of metabolism and adaptation of sea algae (and water flower plants) to the conditions of relatively (and completely) low content of O_2 in water, on the other.
5. Study of algae production, according to the values ΔpH ($\Delta\Sigma CO_2$), is much more correct compared with that of ΔO_2.

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References

[1] E. Broda, Evolution of Bioenergetic Processes (in Russian), Moscow, Mir, (1978), p. 304.
[2] M.G. Rutten, The Origin of Life (by natural causes), Elsevier, 1971, p. 411.
[3] S.P. Vasser, N.V. Kondratyeva, N.P. Masuk, G.N. Palamar-Mordvinova, Z.I. Vetrova, E.L. Kordum, N.A. Moshkova, L.P. Prikhodykova, O.V. Kovalenko, V.V. Stupina, P.M. Zarenko, V.P. Unger, M.I. Radtschenko, O.N. Vinogradova, L.N. Bukhtiarova, L.F. Razumna, Algae: Reference book, Naukova Dumka, Kiev, 1989, p. 605.
[4] M.D. Hanisak, M.M. Littler, D.S. Littler, Marine Biology 99 (1988) 157.

[5] A. Leninger, Fundamentals of Biochemistry, Vol. 2 (in Russian), Mir, Moscow, 1985, p. 368.

[6] G.V. Barinov, Izv. Acad. Nauk USSR 3 (1989) 408.

[7] V.N. Zholkevich, Energetics of Respiration in Higher Plants under the Water Deficiency Conditions (in Russian), Nauka, Moscow, 1968, p. 230.

[8] L.I. Zotin, Thermodynamic Basis of Organisms Reactions on the Outside and Inside Factors (in Russian), Nauka, Moscow, 1988, p. 272.

[9] G. Edwards, D. Worker, C_3 and C_4 Photosynthesis of Plants: Mechanisms and Regulation (in Russian), Mir, Moscow, 1986, p. 98.

[10] S.N. Chmora, V.P. Egorov, V.I. Alekhin, Plants Physiology 39 (1992) 775.

[11] D.G. Nicols, Bioenergetic. Introduction to the Chemiosmotic Theory (in Russian), Mir, Moscow, 1985, p. 190.

[12] R.P. Trenckenshu, Molecular Biology 22 (1988) 1464.

[13] A. Lopukhin, Yu. Kamenir, Thermochimica Acta 252 (1995) 53.

[14] A.S. Lopukhin, Geologiska Foreningens i Stockholm Forhandlingar [GFF (Stockholm)] V. 98 (in English), 1976, pp. 297–315.