

**Physiological and genetical investigations of the photosynthesis intracellular regulation in algae as objects of biotechnology**

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Photosynthesizing microorganisms, in particular microalgae, are new non-traditional objects in biotechnology. For the development of this field of biotechnology the following steps are necessary:

- 1 - to design new, mainly fermenter-type photobioreactors which will provide maximal effective transformation of light energy into bioproducts;
- 2 - to carry out a broad biochemical screening of natural forms and collected strains of microalgae as producers of unique proteins, enzymes, polyunsaturated fatty acids, different toxins and antibiotics, carotenoids, phytohormones and other growth regulators, polysaccharides, etc.;
- 3 - to study physiological conditions and intracellular regulatory mechanisms, provoking the synthesis of the required substances, and, on this basis, to formulate the regulations controlling qualitative ways of biosynthesis in phototrophic cells;
- 4 - to carry out genetic and gene engineering investigations, creating new strains, i.e. superproducers of components of practical importance.

Some results, obtained according to points 3 and 4, are given below.

Formerly, our laboratory has discovered that stereochemical, weakly metabolizable glucose analogues (2-desoxy-D-glucose, 3-O-methylglucose) in chloroplast will initiate a molecular mechanism of the negative metabolic regulation of photosynthesis at the genetic level, at which glucose as the end product in photosynthetic carbon reduction plays the role of a negative effector [1, 2].

Table 1 shows that 2-desoxy-D-glucose (2dDG) is causing complex reversible repression (repression/derepression) of many key chloroplast genes, to control protein synthesis of reaction centres of photochemical systems as well as electron carriers in chloroplast EFC, enzymes in Calvin cycle, ribosomal and messenger RNA. The glucose effect of the regulation of the matric activity of chloroplast genome apparently is a typical example of a biological regulatory system with significant negative, reversible action ("feed-back" mechanism), limiting the supersynthesis of structural proteins, enzymes, and assimilates in a chloroplast, as well as fulfilling the function of the final executing mechanism of donor accepted connexions in the photosynthetic mechanism with substralconsuming processes in cells.

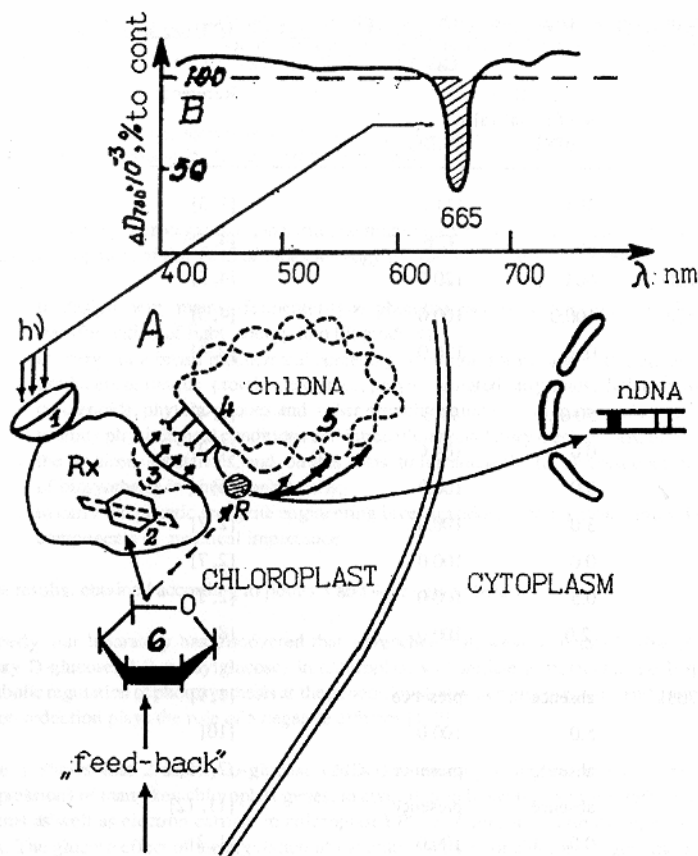
We think that the regulatory effect of glucose in chloroplast is based on its reversible interaction with Rx regulatory macromolecules (fig. 1) to control the light-dependent activity of Chl-DNA genes, responsible for the synthesis of R repressors in chloroplasts [4, 14].

**Table 1:** Effect of 2-desoxy-D-glucose (2dDG) on synthesis of chloroplast components, its structural organization and functional activity

| Parameters  | Effect 2dDG                 |          | Reference |
|---|-----------------------------|----------|-----------|
|   | % of initial value<br>+2dDG | -2dDG    |           |
| Protein of fraction I                             | 29.0                        | 111.8    | [1, 3]    |
| RuBisCO   | 26.3                        | 97.6     | [4, 5]    |
| Large subunit of RuBisCO                          | 60.0                        | 120.0    | [4, 5]    |
| Small subunit of RuBisCo                          | 100.0                       | 100.0    | [4, 5]    |
| Phosphoribulokinase                               | 0.0                         | 130.0    | [2, 4]    |
| Ribose-5-phosphate isomerase                      | 50.0                        | 94.3     | [2, 4]    |
| Carbonic anhydrase                                | 0.0                         | 104.6    | [1, 2]    |
| Protein 32 kD                                     | 0.0                         | 100.0    | [6]       |
| Activity of RC PSI                                | 3.0                         | 100.0    | [2, 7]    |
| Activity of RC PSII                               | 0.0                         | 100.0    | [2, 7]    |
| P700  | 0.5                         | 100.0    | [2, 7]    |
| Chlorophyll                                       | 2.0                         | 100.0    | [8]       |
| Aggregated chlorophyll forms (686, 692, 697, 703) | absence                     | presence | [1, 9]    |
| Cytochrome f                                      | 5.0                         | 100.0    | [10]      |
| Pyrenoid  | absence                     | presence | [2, 11]   |
| Stack of thylakoids                               | absence                     | presence | [11, 12]  |
| Oxygen evolution                                  | 0.0                         | 100.0    | [1, 2]    |
| rRNA of 70S ribosome (1.1)                        | 32.5                        | 100.0    | [7]       |
| rRNA of 70S ribosome (0.56)                       | 28.2                        | 100.0    | [7]       |
| mRNA of psbD gene                                 | repression                  |          | [13]      |
| mRNA of desA gene                                 | induction                   |          | [13]      |

To clear out the molecular organisation of glucose effect on the regulation of expression of chloroplast genome and physico-chemical features of the regulatory macromolecules, taking part in this process, genetic methods mainly should be used as well as ways to obtain and to study first of all features of regulatory mutants which have defects in the system of the negative metabolic regulation of genomes in chloroplasts [15, 16].

The green unicellular alga, i.e. *Chlorella vulgaris* Beijer. var. *vulgaris* IPPAS C-1 (Kosikov strain, known as *Chlorella* sp. K) from the collection of microalgae cultures at the Plant Physiology Institute of Russian Academy of Sciences (IPPAS), is used as test object [17].



**Figure 1:** Diagram of possible molecular interactions in the process of light-dependent metabolic regulation of expression of the chloroplast genome with glucose

A. Diagram of molecular interaction. Rx-regulatory conformationally mobile macromolecule of protein nature. 1 - site of photoreceptor binding; 2 - site of glucose molecule binding (the allosteric centre); 3 - site of binding with regulatory elements of the gene to code the repressor in ChlDNA; 4 - regulatory sequences of repressor gene; 5 - structural genes in ChlDNA; 6 - glucose molecule R-repressor.

B. Spectrum of glucose effect of repression/derepression in ChlDNA. In the dark, Rx is at the conformational state when site 3 is not active, repressor gene (4) is constitutively expressed and repressor molecules (R) suppress structural genes of photosynthesis (5). At the illumination as the result of photoconformational translocation, Rx is activated by site 3, suppressing repressor synthesis (R) which results in expression of photosynthesis genes (5). The binding of glucose molecule with the allosteric centre 2 results in the reverse translocation of Rx conformation, the active centre 3 is inactivated which results in renewing repressor synthesis and in repressing photosynthesis genes, correspondingly.

Proceeding from the hypothesis mentioned above (fig. 1), the regulatory mutants, to have a defect in the "feed-back" mechanism of Chl-DNA regulation, must differ in the following marker features:

- (1) the ability for the growth on medium with glucose analogues;
- (2) the ability for the active translocation into cells of exogenous glucose and its analogues;
- (3) the ability for the heterotrophic growth on glucose in the dark, which is typical for the initial strain of *Chlorella vulgaris* IPPAS C-1 (WT);
- (4) the resistance of photosynthetic oxygen evolution, synthesis of pigments and chloroplast proteins to the repressive effect of glucose analogues;
- (5) the ability for storing up super-large quantities of assimilates in chloroplasts.

Taking into mind these considerations, we have worked out the method [15] of two-steps selection of regulatory 2dDG resistant mutants to have a defect in the system of metabolic regulation in the expression of chloroplast genome, wherein glucose is playing the role of a negative effector, i.e. CRS-mutants (chloroplast regulatory system mutants). Figure 2 contains the diagram of such a selection.

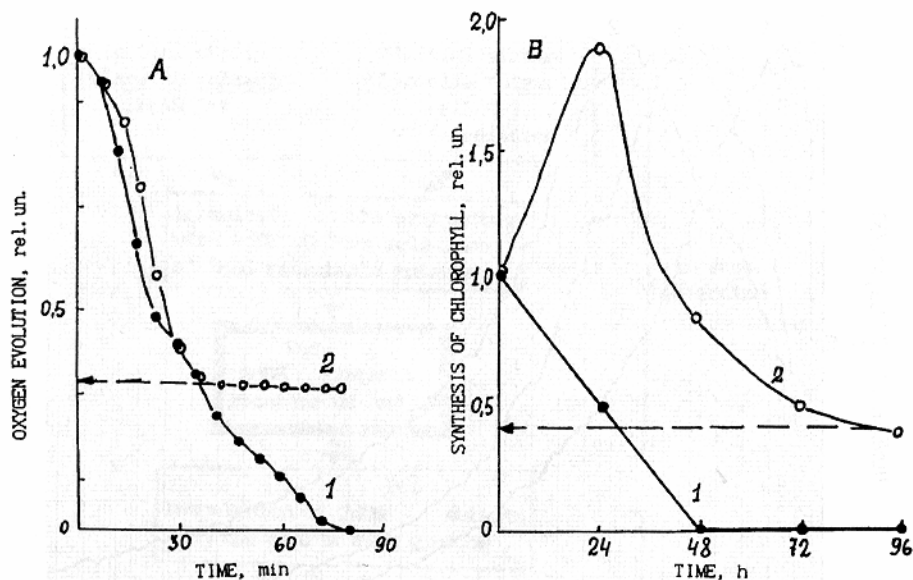
As a result, 2dDG-Res mutants were selected, differing in their ability to grow on media with 2dDG. The property of 2dDG resistance at least may have two causes, i.e. the disturbance in the system of translocation of exogenous glucose (as well as its analogues) into cells and the disturbance of glucose effect of metabolic regulation of photosynthetic genes in chloroplasts. The 2dDG-Res mutants selected were subjected to further selection (in the second step), according to the diagram in figure 2.

As a result, two groups of mutants were obtained (tab. 2). The first group was related to the mutants with disturbed system of glucose translocation into cells, i.e. GT-mutants (glucose translocation mutants) and the second one - to the mutants with the disturbance of glucose mechanism of metabolic regulation in expression of chloroplast genome, i.e. CRS-mutants (chloroplast regulatory systems mutants).

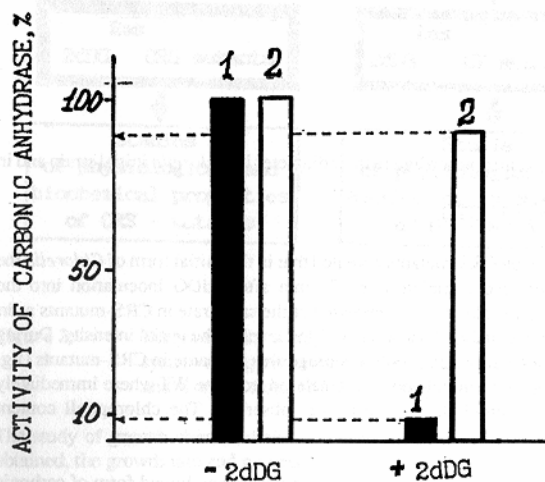
**Table 2:** Comparative characteristics of initial strain (WT) and 2-desoxy-D-glucose resistant mutants of *Chlorella vulgaris*

| Strains | Autotrophic growth |       | Heterotrophic growth on glucose in the dark | Number of strain in the collection IPPAS* |
|---------|--------------------|-------|---|---|
|         | -2dDG              | +2dDG |   |   |
| WT      | +                  | -     | +   | IPPAS C-1                                 |
| CRS 03  | +                  | +     | +   | IPPAS C-75                                |
| CRS 2   | +                  | +     | +   | IPPAS C-78                                |
| CRS 3   | +                  | +     | +   | IPPAS C-79                                |
| CRS 7   | +                  | +     | +   | IPPAS C-81                                |
| CRS 8   | +                  | +     | +   | IPPAS C-82                                |
| CRS 10  | +                  | +     | +   | IPPAS C-83                                |
| GT 1    | +                  | +     | -   | IPPAS C-76                                |
| GT 2    | +                  | +     | -   | IPPAS C-77                                |

\* IPPAS - Collection of unicellular algal culture of Plant Physiology Institute of the Russian Academy of Sciences [17].



**Figure 4:** Effect of 2-deoxy-D-glucose on photosynthetic oxygen evolution (a) and chlorophyll synthesis (b) in *Chlorella* initial strain and CRS-03 mutants.  
1 - initial strain; 2 - CRS-03 mutants



**Figure 5:** Effect of 2-deoxy-D-glucose on the synthesis of chloroplast membrane-bound carbonic anhydrase in *Chlorella* initial strain and CRS-03 mutant.  
1 - initial strain; 2 - CRS-03 mutant

Proceeding from the predictions to result from the hypothesis of the molecular organisation of mechanisms of glucose regulatory effect in chloroplasts (fig. 1), it is to be supposed that CRS-mutants with the disturbed system of metabolic regulation in chloroplast genome, could differ in feature of superproducers and store up superlarge quantities of assimilates (starch) in chloroplasts. The model experiments to separate cell functions of cell division and photosynthesis (we could obtain this by isolating nitrogen from the nutrient medium) have shown that the cells store up approx. 30-40 % more carbohydrates in CRS-mutants than in the WT cultures (fig. 6).

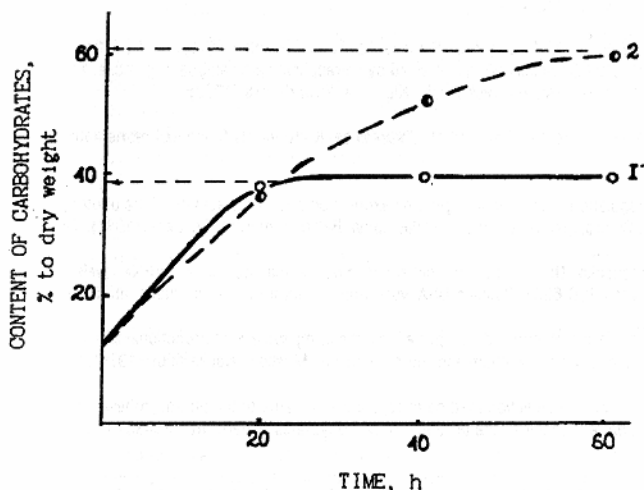


Figure 6: Dynamics of carbohydrate storage in cells of *Chlorella* initial strain and in CRS-03 mutant under conditions of nitrogen starvation.  
1 - initial strain; 2 - CRS-03 mutant

#### Literature

- [1] Semenenko, V. E. Endogen regulation mechanisms of photosynthesis and adaptive properties of chloroplasts. In: Physiology of Photosynthesis. Moscow, Nauka (1982), 164.
- [2] Semenenko, V. E., Zvereva, M. G., Kuptsova, E. S. et al. Metabolic regulation of the chloroplast genome expression and the chloroplast-cytoplasm regulatory relationships. Compartment in algal cells and their interaction. Berlin, Springer-Verl. (1984), 128
- [3] Semenenko, V. E., Kasatkina, T. I. and T. S. Rudova. Reversible suppresion of ... fraction I protein synthesis by 2-desoxy-D-glucose. Sov. Plant Physiol. 23 (1976) 6, 1225
- [4] Semenenko, V. E. Genetic control and cell mechanisms of photosynthesis regulation. Photosynthesis and productional process. Moscow (1988), 69
- [5] Kasatkina, T. I., Vedeneev, A. N. and V. E. Semenenko. Synthesis of ribulose 1.5-biphosphate carboxylase and its subunits during adaptive rearrangements in *Chlorella* sp. K. cells. Sov. Plant Physiol. (1989) 6, 1080
- [6] Setlik, I., Setlikova, E., Masojidek, J. et al. The effect of translation and transscrition inhibitors on the development of the photosynthetic apparatus in *Scenedesmus*. Photosynthesis. V. Chloroplast development. Philadelphia, Balaban Intern. Sci Serv. (1981), 807
- [7] Zvereva, M. G., Limova, L. A. and V. E. Semenenko. Repression of RNA synthesis and disturbances in the activity of photochemical systems of the chloroplast as affected by 2- desoxy-D-glucose and hypertrophic accumulation of assimilates in *Chlorella* cells. Sov. Plant Physiol. (1980) 7, 1218

- [8] Semenenko, V. E. and V. P. Afanasieva. Studies on the mechanisms of autoregulation of photosynthesis. A reversible 2-deoxy-D-glucose effect of repression of the photosynthetic apparatus in *Chlorella* cells. *Sov. Plant Physiol.* (1972) 19, 1074
- [9] Zvereva, M. G., Shubin, L. M., Klimova, L. A. and V. E. Semenenko. Reversible change in low-temperature fluorescence spectra of *Chlorella* sp. K. intact cells, due to the repression of photosynthetic apparatus by 2-deoxy-D-glucose. *Dokl. Akad. Nauk* (1979) 244, 1244
- [10] Klimova, L. A., Roshina, V. V., Zvereva, M. G. and V. E. Semenenko. The reversible destroying of electron transport chain of the photosynthesis of intact *Chlorella* cells as affected by stereochemical analogue of glucose. XI Vsesoyuzn. rab. sov. po voprosam krugovorota veshchestv. Kiev, Naukova dumka (1983)
- [11] Vladimirova, M. G. Changes in ultrastructure of the cell of *Chlorella* sp. K. during its functional reorientations. *Plant Physiol* (1976) 23, 1180
- [12] Semenenko, V. E. Metabolic regulation of chloroplast genome expression and of the activity of the photosynthetic apparatus. *Photosynthesis V. Chloroplast development*, Philadelphia, Balaban Internat. Sci Serv. (1981), 767
- [13] Lebedeva, N. B. and V. E. Semenenko. The effect of stereochemical glucose analogue on the mRNA levels of *psbD* and *desA* genes in *Synechocystis* PCC 6803. Russian-USA workshop on photosynthesis, Pushchino (1992), 20
- [14] Semenenko, V. E. Metabolic regulation of light-induced protein synthesizing system and functional activity of chloroplast. In: *Photoregulation of plant metabolism and morphogenesis*, Moscow, Nauka publ. (1975), 135
- [15] Semenenko, V. E. and L. A. Shitova. The selection method of regulatory mutants of the photosynthesizing microalgae and the strain of *Chlorella* species - the producer of carbohydrates. Patent No. 1654337 (1991) bul. No. 21
- [16] Semenenko, V. E. and L. A. Shitova. 2-d-D-glucose resistant mutant of *Chlorella* with the destroyed system of the metabolic regulation of the chloroplast genome expression. XVI meeting FEBS, 253
- [17] Catalogue of microalgal cultures in the collections of the USSR. Semenenko, V. E. (ed.), Moscow (1991)