

METABOLITE REGULATION OF CHLOROPLAST GENOME EXPRESSION AND OF THE
ACTIVITY OF THE PHOTOSYNTHETIC APPARATUS

V. E. Semenenko

K. A. Timiryazev Institute of Plant Physiology, USSR Academy
of Sciences, Moscow

It is shown that stereochemical analogs of glucose (2-deoxy-D-glucose) completely suppresses RNA synthesis, synthesis of the main enzymes of the Calvin cycle and components of the chloroplast photochemical apparatus and inhibits photosynthetic evolution of oxygen in *Chlorella* cells. Chlorophyll is deaggregated and its synthesis inhibited. The activity of the reaction centres of photosystems I and II is impaired and the concentration of P700 reduced. The repression process is accompanied with the destruction of the chloroplast ultrastructure, the degradation and disappearance of the pyrenoid occurs. The glucose-induced effect of repression is light-dependent and completely reversible through washing out the analog. The synthesis of RNA is one of the first to be suppressed and restored when the analog is introduced and washed out, respectively. Phenomena similar to those evoked by the glucose analog could be observed in natural models in which hypertrophis accumulation of assimilates occurs in the *Chlorella* chloroplast. It is suggested that a molecular mechanism of metabolite regulation of chloroplast genome expression exists which underlies the regulation of the functional activity of the chloroplast.

The clarification of the molecular mechanisms of endogenous regulation of photosynthesis is a major problem which determines not only searches for new ways to optimize the photosynthetic productivity of plants, but also further studies of the elementary mechanisms underlying transformation of light energy in this process. The chloroplast is known to be not only the site of processes and reactions directly involved in the transformation of light energy and carbon reduction; it also possesses its own DNA and protein-synthesizing system which ensures synthesis of many important components of the photosynthetic apparatus. The integration and interaction of these two systems seem to underlie the action of the mechanisms of endogenous regulation of photosynthesis and determine the adaptive properties of the chloroplast [1].

It is known that the physico-chemical properties of the DNA and protein-synthesizing systems of the chloroplast are similar in many respects

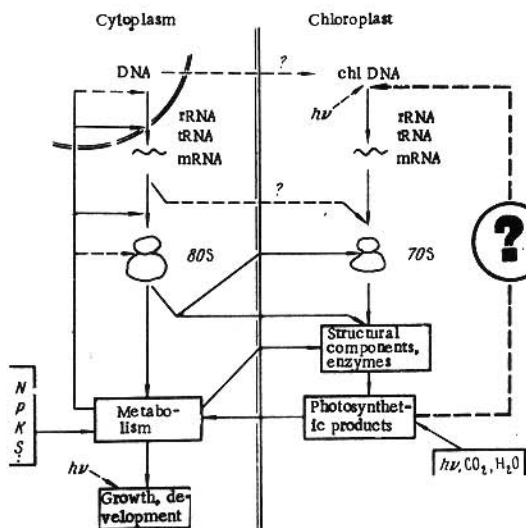


Fig. 1. Scheme of nucleo-chloroplast and chloroplast-cytoplasmic interrelationships in the intact cell.

to those of bacterial cells. It may be thought, therefore, that the system regulating chloroplast genome expression also employs principles characteristic of prokaryotic organisms and based on the mechanisms of metabolite induction-repression [1,2].

The present work is devoted to studying the problem of the existence of a chloroplast system of metabolite regulation of photosynthesis at a protein synthesis level with carbon reduction products used as effectors (Fig. 1).

1. Nature of the Effector Metabolite. Logical analysis leads to the conclusion [3,4] that the metabolite which performs functions of a corepressor must be found in the metabolic fork between synthesis of products transported from the chloroplast and of products deposited in storage. In this case, causing the repression of chloroplast protein synthesis, such a metabolite could accomplish this repression only after substrates are provided for cytoplasmic processes, and polysaccharides are stored in the chloroplast. These requirements are satisfied by ADPG, UDPG, glucose-1-phosphate, glucose-6-phosphate, and glucose which can appear in the chloroplast after the replenishment of the storage starch pool. In this connection, a high regulatory activity of glucose in coordinating the metabolism of microorganisms is noteworthy.

At the same time numerous studies of the effect of exogenous glucose on photosynthesis fail to reveal depression of photosynthesis. This, however, may be due to the compartmentation of a cellular structure and the presence of many metabolite barriers (Fig. 2) on the path of exogenous glu-

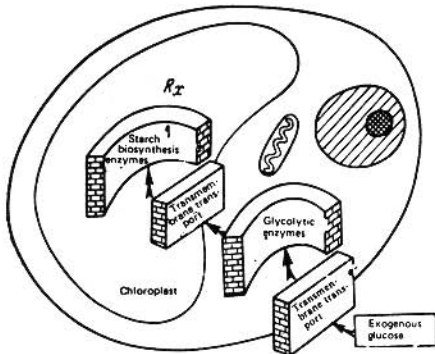


Fig. 2. Metabolic barriers on the path of exogenous glucose to the site (R_x) of its possible regulatory action in the chloroplast.

...cose to the centres of its possible regulatory action in chloroplast [1,3].

In the research on the mechanisms of endogenous regulation of photosynthesis conducted in our laboratory to study the regulatory role of glucose in the chloroplast, new approaches [3] have been employed based on utilization of stereochemical analogs of glucose (2-deoxy-D-glucose, 3-O-methylglucose, xylose) that are poorly metabolizable (or non-metabolizable), but are readily transported into the cell and are "biologically full-valued".

2. Inhibition of Photosynthetic Oxygen Evolution, Chlorophyll Synthesis, and Synthesis of Fraction I Protein under the Effect of 2-Deoxy-D-Glucose (2dDG) and Reversibility of Such Inhibition. Adding 2dDG to algal cultures under optimal cultivation conditions (on complete nutrient media with illumination) leads (Fig. 3) to rapid (setting in after 2-3 hours) depression of photosynthesis, and

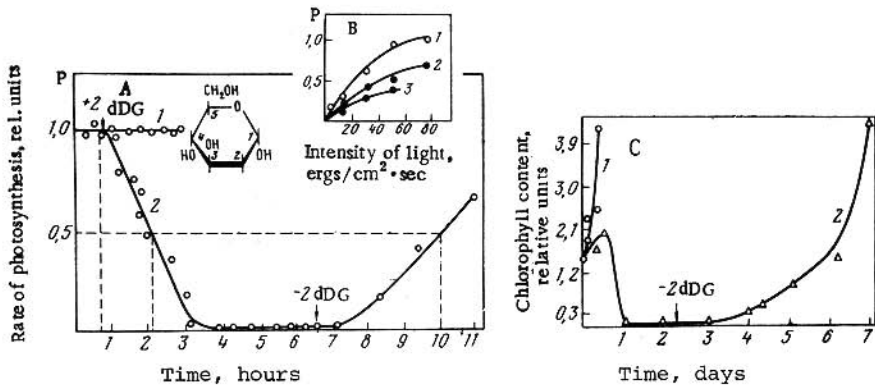


Fig. 3. Influence of 2-deoxy-D-glucose on the rate of photosynthesis (A), configuration of the light curves of oxygen evolution (B) and chlorophyll synthesis (C) in *Chlorella sp. K*, and reversibility of its action. A: 1 - control, 2 - experiment; B: 1 - control, 2,3 - after 1 and 2 h of 2dDG action, respectively; C: 1 - control, 2 - experiment. Final concentration of 2dDG, 0.5%.

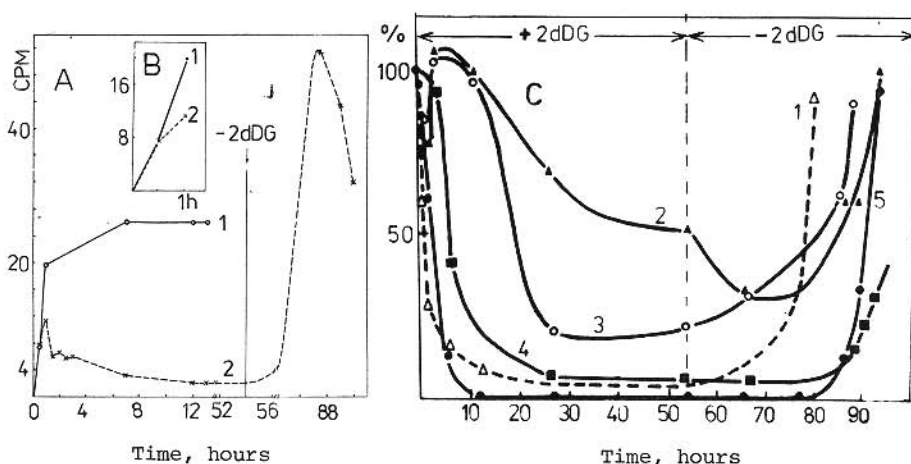


Fig. 4. Kinetics of depression of RNA (A,B) and enzymes of Calvin cycle (C) synthesis in cells of *Chlorella sp.K* under the effect of 2-deoxy-D-glucose. A: Incorporation of 2-¹⁴C-uracyl into total nucleic acids. 1 - control, 2 - with 2dDG; B: Fragment of Fig. A; C: 1 - rate of RNA synthesis, 2 - activity of ribose-5-phosphate isomerase, 3 - phosphoribulokinase, 4 - ribulosebiphosphate carboxylase, 5 - carbonic anhydrase. Values are given in per cent of the starting value per unit of culture volume.

inhibition of chlorophyll synthesis with the appearance of colourless (white) cells after 24-48 h. This effect is observed in various green and blue-green algae and is completely reversible after washing out of 2dDG from the cells [1]. 3-O-methylglucose and xylose exert a similar action, in particular, on chlorophyll synthesis in *Chlorella*. 2dDG causes also specific and reversible depression of the synthesis of fraction I protein, contrary to the sum of other cellular proteins whose content not only fails to decline under the influence of 2dDG, but even rises slightly [5].

3. Repression of the Synthesis of RNA and Certain Enzymes of the Calvin Cycle under the Effect of 2dDG. The stereochemical analog of glucose not only depresses the synthesis of fraction I protein in photosynthesizing cells, but also inhibits incorporation of ¹⁴C-uracil into RNA and the synthesis of ribose-5-phosphate isomerase, phosphoribulokinase, ribulose-1,5-bisphosphate carboxylase and carbonic anhydrase (Fig. 4). 2dDG did not affect the activity of the above enzymes in the measuring mixtures. Therefore, blocking of the synthesis of these enzymes evidently occurs in the intact cells under the influence of the analog. RNA synthesis is switched off most rapidly of all under the effect of 2dDG after which the amounts of the studied enzymes start to decrease.

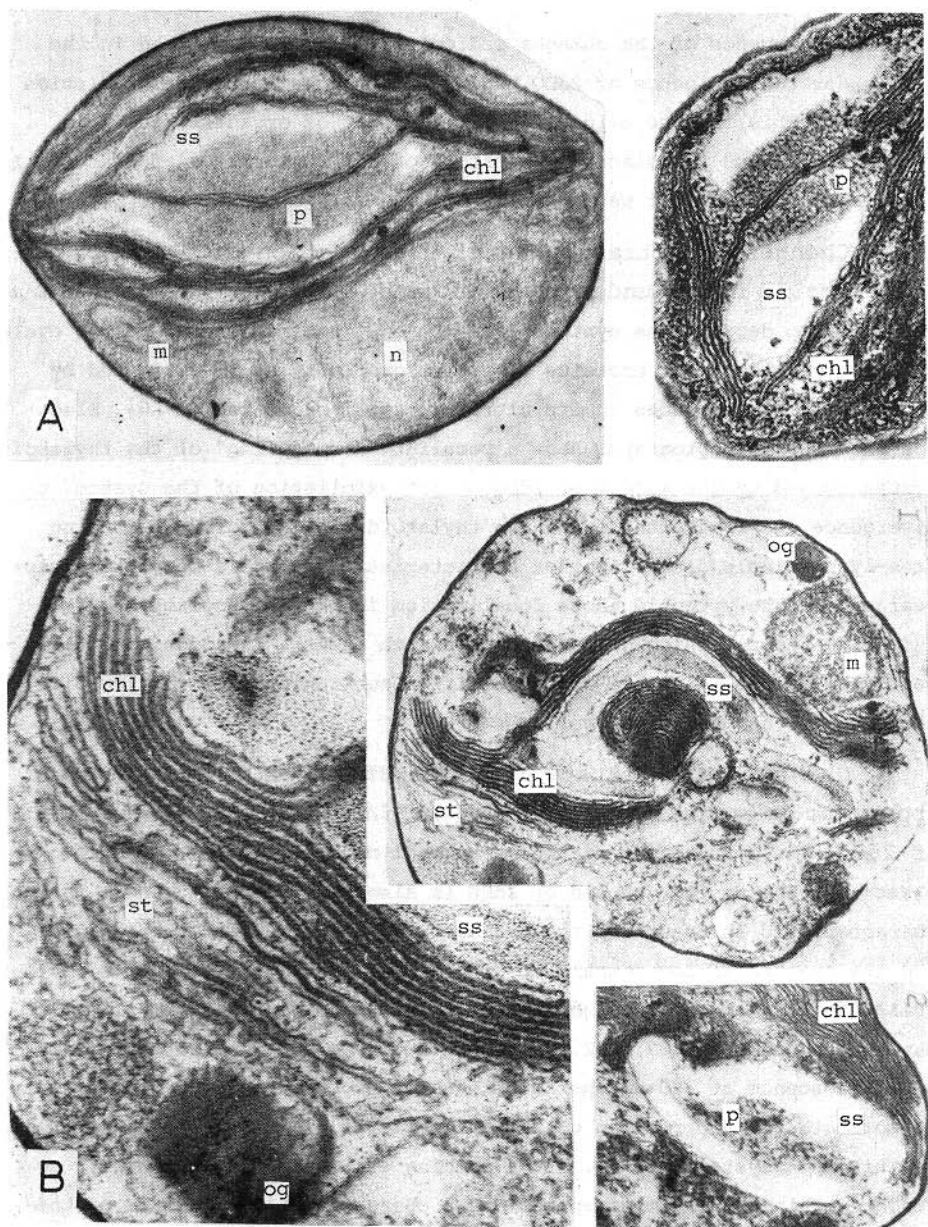


Plate I.

Changes in ultrastructural organization of the cell of *Chlorella sp. K* under the effect of 2-deoxy-D-glucose. A: control cell; B: cell after 48 hours of 2dDG action.

chl - chloroplast, *m* - mitochondrion, *n* - nucleus, *p* - pyrenoid, *ss* - grains of pyrenoid starch sheath, *st* - single thylakoids, *og* - osmiophilic globules.

This time sequence of the changes indicates that events induced in the cell under the influence of 2dDG begin with changes in the transcription processes. This is also evidenced by the effect of organelle-specific transcription and translation inhibitors on the restoration of fraction I protein synthesis after washing 2dDG off the *Chlorella* cells [1,5].

4. Changes in Ultrastructural Organization of Chloroplast in *Chlorella* Cells under the Influence of 2dDG. The glucose analog seems to depress the synthesis of not only enzymes of the Calvin cycle, but also of structural proteins of the chloroplast, as is evidenced by significant disturbances in its ultrastructural organization [6]. Electron-microscopic photographs show a peculiar "dismantling" of the thylakoid system caused by the effect of 2dDG: first exfoliation of the system, then divergence and fragmentation of the thylakoids to the point of forming free-lying single disks. Another characteristic change is complete disappearance of the pyrenoid whose localization in the chloroplast can be judged by the remaining grains of the starch sheath. In addition, multivesicular bodies (globules of unidentified substances of a varying electron density) appear in the cell (Plate I).

5. Changes in the State (Deaggregation) of Photosynthetic Apparatus Pigments in Intact *Chlorella* Cells under the Effect of 2dDG. The disturbance of the structural organization of the chloroplast membrane systems caused by 2dDG is also evidenced by changes in the characteristic structure of the red absorption maximum for chlorophyll *a* and the spectra of low-temperature fluorescence of *Chlorella* cells. A peculiar feature of these changes (Fig. 5) is the disappearance of long-wavelength peaks at 687, 692, 697 and 702-703 nm and gradual extinction of fluorescence at 726 nm characteristic of photosystem I [7] which seems to point to the suppression of the synthesis of pigment-protein components in this photosystem by 2dDG. After washing off of the analog, highly aggregated chlorophyll forms appear and a characteristic structure of the low-temperature fluorescence spectrum is restored.

6. Effect of 2dDG on the Activity of Reaction Centres in Photosystem I (P700 Concentration). Another indication of the upset functional activity of the reaction centres in photosystem I caused by 2dDG is a gradual decrease in light-induced absorption at 700 nm (Fig. 6A) which is associated with photooxidation of P700 [8]. Interestingly, the magnitude of the signal associated with the photooxidation of

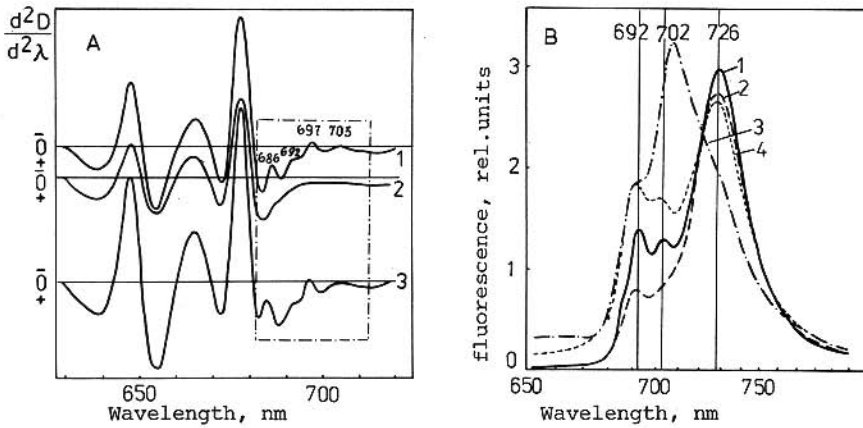


Fig. 5. Effect of 2dDG on absorption spectra at -196°C (A) and low-temperature (-196°C) fluorescence spectra (B) of *Chlorella sp.K* cells. A: 1 - control, 2 - 31 hours of 2dDG action, 3 - 47 hours after washing out of 2dDG; B: 1 - control, 2,3 - 8,44 hours, respectively, of 2dDG action, 4 - 47 hours after washing out of 2dDG.

P700 is reduced, whereas its kinetics undergoes no significant changes (P700 is rapidly oxidized and then quickly reduced in darkness). In this connection, it would seem that a gradual decrease in ΔD is not due to a lower activity of the reaction centres, but due to a decrease in the concentration of P700 and, hence, in the number of photochemically active centres of photosystem I.

Indeed, the calculation of the number of P700 molecules and the total number of chlorophyll molecules has shown [9] that the starting culture (control) contains 600 chlorophyll molecules per P700 molecule. Under the effect of 2dDG these ratios change quickly: there will be already 900 chlorophyll molecules per P700 molecule in 3.5 h, 1800 in 8 h, and as many as 20,000 chlorophyll molecules in 24 h. After the analog is washed off, the initial concentration of P700 capable of reversible phototransformation, is restored.

7. Reversible Repression of Reaction Centres of Photosystem II under the Effect of 2dDG. The glucose analog causes also a reversible repression of photosystem II as was demonstrated in the studies of photo-induced changes in the chlorophyll fluorescence yield (ΔF) of this photosystem in intact *Chlorella* cells. It can be seen from Fig. 6B that under the action of 2dDG in light the magnitude of positive ΔF declines rapidly to the level of about 30% of control ΔF after 4-5 hours. After 20-22 hours of 2dDG action, ΔF practically vanished which points to upset functioning of the reaction centres of photosystem II [9].

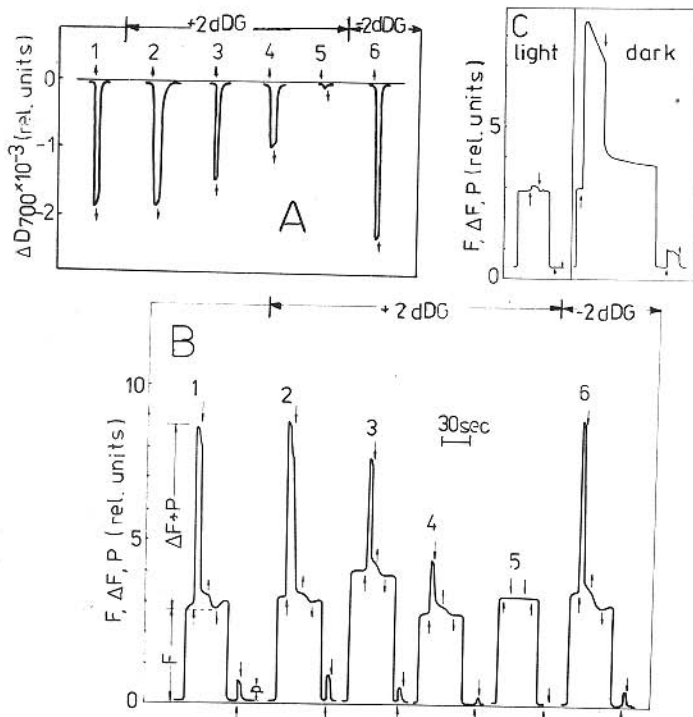


Fig. 6. Effect of 2dDG on the activity of photosystem I (A) and photosystem II (B,C) and on their interaction in intact cells of *Chlorella sp.K.* A: Kinetics of light-induced decrease of absorption at 700 nm (ΔD_{700}); B: Kinetics of light-induced changes in chlorophyll fluorescence yield of photosystem II (ΔF); "dark" fluorescences (F). A and B: 1 - control, 2,3,4,5 - after 1,2,6,30 hours of 2dDG action, respectively, 6 - 71 hours after washing out of 2dDG. C: ΔF 22 hours after 2dDG addition under light (1) and in the dark (2). Upward arrows - switching on of effective light absorbed by photosystem II (\uparrow); downward arrows - switching off of light.

The above data on disturbances in the chloroplast photochemical reactions under the effect of 2dDG seem to show that the glucose analog represses the synthesis of photosynthetic membrane components, in particular, proteins of the pigment-protein complexes which are specifically responsible for the native forms of chlorophyll in the photochemical systems of the chloroplast. It is important to note that in all the experiments repression of the photochemical apparatus activity was preceded by blocking of RNA synthesis similar to the case of inhibiting the enzymes of the Calvin cycle.

8. Light-Dependence of 2dDG Effect on Repression of Biosynthesis and Functional Activity of Chloroplast. An important factor is that the glucose analog suppresses the functional activity of the

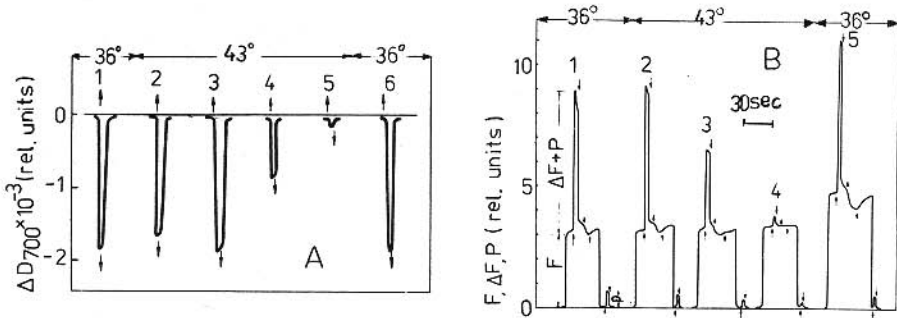


Fig. 7. Kinetics of light-induced ΔD_{700} (A) and ΔF (B) in intact cells of *Chlorella sp.K* in the conditions of accumulation of assimilates in the chloroplast at extreme temperature. A: 1 - control, 2,3,4,5 - after 1,6,30,70 h of extreme temperature action (43°C), respectively; 6 - 24 h after changing temperature to optimal (36°C); B: 1 - control, 2,3,4 - after 1,23,55 h of extreme temperature action, respectively, 5 - 24 h after changing to 36°C . Arrows - as in Fig. 6.

chloroplast only in light. Very low light intensities [4] are sufficient for 2dDG to manifest its repressing effect. The light dependence of the 2dDG repression effect can be clearly seen in Fig. 6C.

9. Changes in State of Pigments, Activity of Photochemical Systems and RNA Synthesis when Hypertrophic Accumulation of Assimilates Occurs in Chloroplast of Non-Dividing *Chlorella* Cell. Hypertrophic accumulation of assimilates (starch) in the chloroplast of *Chlorella* cells was achieved through dissociating the cellular functions with the aid of extreme temperature (43°C) which suppresses cell division, but does not affect photosynthesis [10]. As can be seen from Fig. 7, the activity of the reaction centres of photosystems I and II is depressed in these conditions similar to what occurs under the effect of 2dDG. Identical changes in the state of pigments and repression of RNA

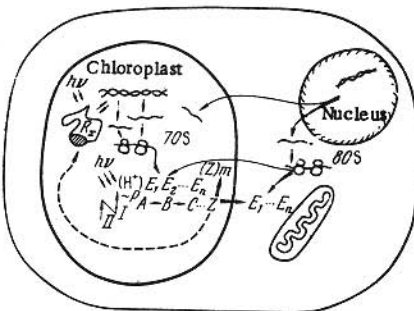


Fig. 8. Theoretical scheme of dynamic metabolite regulation of photosynthesis at the level of chloroplast protein (RNA) synthesis. The dashed line indicates the connection between the effector metabolite (Z) and the allosteric centre of the hypothetical light-sensitive regulator macromolecule (R_x).

synthesis take place [9]. The only difference is that the process has a time lag which is probably associated with the period during which assimilates are stored in the chloroplast.

The data obtained using glucose analogs and in the conditions of hypertrophic accumulation of assimilates in the chloroplast suggest that the chloroplast (Fig. 8) possesses a system of metabolite regulation of photosynthesis at a level of the synthesis of photosynthetic apparatus components involving glucose as an effector. It would be interesting to conduct further studies of the phenomenon in *in vitro* systems using methods of molecular biology and genetics.

References

- 1 Semenenko, V.E. (1978) Soviet Plant Physiol. 25, 715-731
- 2 Kung, S. (1977) Ann. Rev. Plant Physiol. 28, 401-437
- 3 Semenenko, V.E. and Afanasyeva, V.P. (1972) Soviet Plant Physiol. 19, 918-923
- 4 Semenenko, V.E. (1975) in Photoregulation of Metabolism and Morphogenesis of Plants (Kursanov, A.L. and Voskresenskaya, N.P., eds.), pp. 135-157 (in Russian), Nauka Publ., Moscow.
- 5 Semenenko, V.E., Kasatkina, T.I. and Rudova, T.S. (1976) Soviet Plant Physiol. 23, 1036-1041
- 6 Vladimirova, M.G. (1976) Soviet Plant Physiol. 23, 847-853
- 7 Zvereva, M.G., Shubin, L.M., Klimova, L.A. and Semenenko, V.E. (1979) Dokl. Akad. Nauk SSSR, 244, 1244-1247
- 8 Kok, B. (1956) Biochim. Biophys. Acta 22, 399-401
- 9 Zvereva, M.G., Klimova, L.A. and Semenenko, V.E. (1980) Soviet Plant Physiol. 27, in the press
- 10 Semenenko, V.E., Vladimirova, M.G., Orleanskaya, O.B., Raikov, N.I. and Kovanova, E.S. (1969) Soviet Plant Physiol. 16, 172-181