THE EFFECT OF NUCLEIC ACID SYNTHESIS INHIBITORS ON THE CHLOROPHYLL FORMATION BY ETIOLATED BEAN LEAVES

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Received April 26, 1966

The process of chloroplast development from proplastids is a very convenient model to study the functional role of nucleic acids in the chloroplast metabolism. Formation of the photosynthetic apparatus in the illuminated etiolated leaves is accompanied by profound morphological and biochemical changes of plastids (Sissakian and Kobiakowa, 1951; see also the review of Osipova, 1965).

The role of nucleic acids in this process as judged by chlorophyll formation and investigated by means of nucleic acid synthesis inhibitors has been described in several papers. The results obtained are however rather incompatible. In none of the papers has the necessity of DNA and RNA synthesis for chlorophyll formation been studied using the same system. It has been shown (Molotkovsky and Moriakova, 1963; Bogorad and Jacobson, 1964; McCalla and Allan, 1964; Aoki and Hase, 1965) that the DNA-dependent RNA synthesis is essential for chlorophyll formation in etiolated bean and maize leaves, Euglena cells and "glucose-bleached" Chlorella cells. On the other hand, it has

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* Deceased March 12, 1966.

been demonstrated (Smillie, 1963; Aoki and Hase, 1965) that the chlorophyll formation in etiolated Euglena and "glucose-bleached" Chlorella cells is independent on DNA synthesis. The latter, however, is obligatory for the chlorophyll formation in iron-deficient bush bean leaves (van Noort and Wallace, 1963).

In this investigation we studied the role of newly formed DNA and RNA in chlorophyll formation during the greening of etiolated bean leaves. The following compounds were used as agents that affect the nucleic acid synthesis: actinomycin D, mitomycin C, 8-azaguanine, 2-thiouracil, 5-fluorouracil (FU) and 5-fluorodeoxyuridine (FDU).

EXPERIMENTAL

Experiments were carried out according to the technique advanced by Margulies (1962). 8 to 10 day - old etiolated bean seedlings (Phaseolus vulgaris), devoid of one cotyledon and the major portion of hypocotyl, were incubated with 1.5 ml of inhibitor solution, or distilled water in the case of the control treatments, in 2.5 x 4 cm crystallizers for 24 hours in the dark. During the ensuing 12 to 14 hours they were illuminated by fluorescent lamps of the LB-40 type, the illumination intensity being 5000 lx. Chlorophyll content of the leaves was determined by the Arnon method (1949).

RESULTS

The results of our experiments on the effect of actinomycin D upon the chlorophyll formation are presented in Table I. As can be seen, actinomycin D at a concentration of 50 μgm/ml completely inhibits the chlorophyll synthe-

sis, this being in good agreement with the published results (Bogorad and Jacobson, 1964).

TABLE I

Effect of actinomycin D on chlorophyll formation by etiolated bean leaves

	µgm chlorophyll / gm fresh weight	per cent inhibition
Control*	406.8	
Actinomycin D:		
100 µgm/ml	-18.7	104.6
50 µgm/ml	-17.3	104.25
25 µgm/ml	131.8	67.6
10 µgm/ml	199.8	50.9

* In this and all other tables the figures are given with dark control subtracted.

In later experiments we studied the influence of 2-thiouracil and 8-azaguanine on chlorophyll formation, which are well known to be incorporated into the RNA of higher plants and other organisms.

As can be seen from Table II both analogues inhibit the chlorophyll formation significantly. The addition of uridine to the incubation mixture reduces the inhibitory action of 2-thiouracil whereas thymidine produces no effect. The data are indicative of the necessity of RNA synthesis for chlorophyll formation.

To find out whether the chlorophyll formation was dependent on DNA synthesis in subsequent experiments we used 5-FU and 5-FDU. 5-FDU is known to interfere with DNA and 5-FU with both RNA and DNA synthesis.

In our experiments these antimetabolites significantly inhibited chlorophyll formation (Table III). It is interesting to note that 5-FDU at a concentration of 10^{-3} M inhibited the process by 67%, further concentration increase causing no rise in the degree of inhibition. It is ve-

TABLE II

Effect of 2-thiouracil and 8-azaguanine on chlorophyll formation by etiolated bean leaves

	µgm chlorophyll / gm fresh weight	per cent inhibition
Control	545.8	
8-Azaguanine:		
5×10^{-3} M	17.2	96.85
10^{-3} M	194.8	64.4
Control	997.8	
2-Thiouracil:		
5×10^{-3} M	133.6	86.6
10^{-3} M	213.3	78.7
2-Thiouracil, 5×10^{-3} M + uridine, 5×10^{-3} M	780.1	20.8
2-Thiouracil, 5×10^{-3} M + thymidine, 5×10^{-3} M	163.6	83.6

TABLE III

Effect of 5-fluorouracil and 5-fluorodeoxyuridine on chlorophyll formation by etiolated bean leaves

	Experiment I		Experiment II	
	Chl*	Inh**	Chl*	Inh**
Control	432.8			
5-FU, 5×10^{-3} M	110.8	73.9		
5-FU, 10^{-3} M	349.8	17.7		
5-FU, 5×10^{-3} M + thymidine, 5×10^{-3} M	290.8	31.6		
5-FU, 5×10^{-3} M + uridine, 5×10^{-3} M	87.8	79.3		
Control	489.8		607.8	
5-FDU, 5×10^{-3} M	180.8	63.0	224.8	63.1
5-FDU, 10^{-3} M	161.8	67.0	191.8	68.5
5-FDU, 5×10^{-3} M + thymidine, 5×10^{-3} M	472.8	3.5	732.8	-20.7
5-FDU, 5×10^{-3} M + uridine, 5×10^{-3} M	237.8	51.5	99.8	83.6

* Chl = µgm chlorophyll/gm fresh weight
** Inh = per cent inhibition.

ry likely that under such conditions the concentration of 10^{-3} M is saturating for 5-FDU. The inhibitory effect of

5-FU and 5-FDU was reversed by an equimolar concentration of thymidine whereas that of uridine failed to reverse the inhibition. It should be noted that the inhibition by 5--FDU was reversed completely while that by 5-FU only partially. Uridine produces no effect upon the 5-FU induced inhibition.

In later experiments we used mitomycin C. This antibiotic is able to block the DNA synthesis in E.coli cells, leaving unaffected the RNA and protein synthesis (Shiba et al., 1959). In our experiments mitomycin C at a concentration of 200 μgm/ml inhibited chlorophyll synthesis by 70%. The degree of inhibition did not rise upon an increase of the antibiotic concentration up to 300 μgm/ml (Table IV).

TABLE IV

Effect of mitomycin C on chlorophyll formation by etiolated bean leaves

	μgm chlorophyll / gm fresh weight	per cent inhibition
Control	402.8	
Mitomycin C:		
300 μgm/ml	172.8	57.4
250 μgm/ml	131.8	67.3
200 μgm/ml	139.8	65.3
100 μgm/ml	257.8	36.0
50 μgm/ml	305.8	24.1

Discussion

The data obtained in this study suggest some conclusions with respect to the participation of nucleic acids in chlorophyll synthesis. Experiments carried out in relation to the effect of some inhibitors of RNA synthesis - actinomycin D, 2-thiouracil, 8-azaguanine - have demonstrated that the synthesis of light-induced RNA is obligatory for chlo-

rophyll formation in cells of etiolated bean leaves, which lends support to the data previously obtained (Pogo et al., 1962, Molotkovsky and Moriakova, 1963). The study of the effect of 5-FDU and mitomycin C shows that blocking of DNA synthesis does not lead to a complete inhibition of chlorophyll formation. The above results can be accounted for by the fact that proplastids as well as chloroplasts of different age are capable of division (Green, 1964; Strugger, 1964). Cytological studies have shown that the amount of chloroplasts in mature cells exceeds by three or four times that of proplastids in meristematic cells (Granick, 1961). The assumption that a similar increase of the chloroplast amount in the cell occurs upon the greening of etiolated cells can help understand why inhibition of DNA synthesis suppresses chlorophyll formation partially while that of RNA synthesis blocks it completely. The conversion of proplastids of etiolated cells of higher plants to chloroplasts can be conventionally divided into

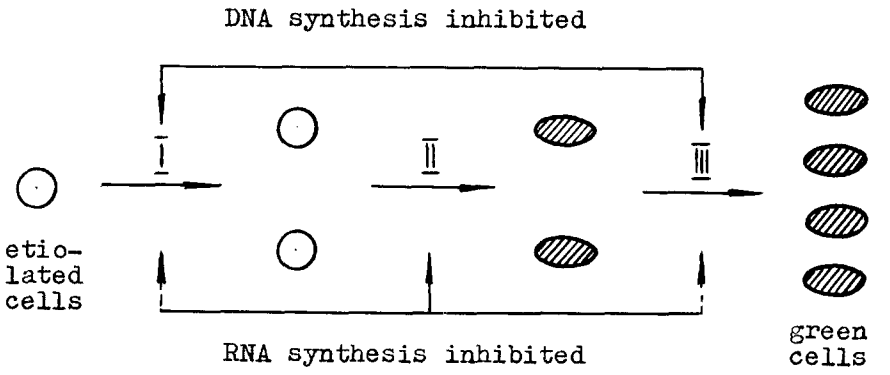

Fig.1. Scheme of plastid differentiation and division during the greening of etiolated leaves

○ - proplastids; ⬭ - chloroplasts; I,II,III - see the text.

three stages (Fig.1): I - division of proplastids, II - proplastid differentiation and chloroplast formation, and III - division of mature chloroplasts.

The inhibitors affecting RNA synthesis block the 2nd stage, thus arresting proplastid conversion to chloroplasts. It is here that plastid division also seems to stop. Inhibition of DNA synthesis blocks the 1st stage; proplastids, escaping the stage of division, are directly converted to chloroplasts which are, similarly to those formed in the first case, unable of division, the amount of chloroplasts being equal to that of proplastids in etiolated cells.

The data obtained in this study allow the conclusion that it is DNA that controls proplastid differentiation, chloroplast formation and plastid division. Further studies are called for to reveal the specific role of chloroplast DNA in these processes.

REFERENCES

Aoki, S. and Hase, E., Plant and Cell Physiol., 6, 347 (1965).
Arnon, D.J., Plant Physiol., 24, 1 (1949).
Bogorad, L. and Jacobson, A.B., Biochem. Biophys. Res. Communs., 14, 113 (1964).
Granick, S., in: "The Cell", ed. by J.Brachet and A.E. Mirsky, N.Y.-London, 2, 489 (1961).
Green, P.B., Amer. Journ. of Botany, 51, 334 (1964).
Margulies, M.M., Plant Physiol., 37, 473 (1962).
McCalla, D.R. and Allan, R.K., Nature, 201, 504 (1964).
Molotkovsky, J.G. and Moriakova, V.F., Izv. Akad. Nauk SSSR, ser. biol., 5, 719 (1963).
van Noort, D. and Wallace, A., Biochem. Biophys. Res. Communs., 10, 109 (1963).
Osipova, O.P., in: "Biochemistry and Biophysics of Photosynthesis" (in Russian), ed. by A.Krasnovsky, Moscow, 146 (1965).