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BARD

FINAL REPORT

PROJECT No. I-259-80

Chloroplast Development in Isolated Etiolated Leaf Cells Etioprotoplasts and Fusion Products

S. Klein (deceased), A. Kaplan, A.W. Galston

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BARD - קמח

I-259-80

*Due to the death of the principal investigator, Dr. S. Klein,
the final report of this project is composed of two interim reports
and the final report of Dr. A. Kaplan who replaced Dr. Klein as the
principal investigator.*

The contents are as follows:

First year report of the project

Dr. S. Klein

Second year (final) report of the U.S. part of the project

Dr. A.W. Galston

Final report of the Israeli part of the project

Dr. A. Kaplan

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Bet Dagan, ISRAEL

Date 6.3.83

BARD No. of Proposal 1-259-80

Title of Proposed Research

Chloroplast development in isolated etiolated leaf cells,
etioplasts and fusion products.

Name(s) of Investigator(s)

Principal Investigator: Professor Shimon Klein

Cooperating Investigator: Professor A. W. Galston

Name and Address of Affiliated Institution(s)

Grantee Institution: The Hebrew University of Jerusalem,
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Cooperating Institution: Yale University, New Haven, Conn., U.S.A.

Project's starting date October 1st, 1981

Type of Report: Initial ~~XXX~~ Comprehensive Final

Signature

Principal Investigator

Shimon Klein

Institution's Authorizing Official

Authorizing Official

DESCRIPTION OF RESEARCH RESULTS

As outlined in our proposal, during this first period attempts were made mainly

- A) to improve methods for preparation of etioprotoplasts from etiolated leaves;
- B) to continue the preliminary experiments on greening of maize etioprotoplasts;
- C) to adapt a suitable line of cultured cells for greening experiments.

A. *Etioprotoplast preparation:*

1) Yield of protoplasts from (green) leaves can be improved substantially when the leaf epidermis is removed prior to treatment with macerating enzymes. Yield of protoplasts from etiolated leaves is very low, among others because removal of the epidermis is, in the case of gramineae, practically impossible because the etiolated leaves are "rolled in". As a step towards improving the yield, experiments were carried out to cause unrolling of the leaves without causing their greening. (Leaf opening is controlled by phytochrome, and its action spectrum is very similar to that of Pchl-Chl conversion.) Two methods were tested: Treatment of the leaves in the dark with hormones (Wei-lun *et al.*, 1979, Acta Bot. Sinica, 21: 263-268) and short light exposure of the seedlings during early growth. Gibberellic acid did indeed cause partial unrolling of the leaves. Also, leaves after 8-10 days of growth in the dark were partially unrolled when given various light doses during their 3rd day of growth (in this case only the tip of the leaves contains Chl, while the other parts remain etiolated). The epidermis from such treated

leaves can be removed, but the removal is still a very time consuming process. Pretreatment of leaves resulting in their unrolling is useful when a short exposure to the macerating enzyme(s) is desired, and only a limited amount of protoplasts is needed. This method is useful for EM investigations and for fusion experiments. When larger amounts of etioplasts are needed, it is more economic to compensate for the low yield by using larger batches of leaves and a prolonged (9 hr) exposure of thin leaf slices to the macerating enzyme(s). Thus, after testing various incubation times, ways of collecting and partially purifying the protoplasts, such a method was adapted for preparation of *Z. mays* and *Avena* etioprotoplasts for routine work.

2) *Etioprotoplasts from Asparagus*: Photosynthetically active mesophyll cells from green *Asparagus* cladophylls can be rapidly isolated by simple mechanical grinding (Colman *et al.*, 1979, Can. J. Bot. 75: 1505-1510). When *Asparagus* plants were grown in the dark, stem growth was much promoted, while cladophyll development was severely inhibited. None of the various parts of the plant (stem tissue, stem-tips + cladophyll primordia) yielded intact cells after mechanical grinding. Treatment of the etiolated *Asparagus* with macerating enzymes was unsatisfactory.

B. *Experiments with Z. mays and Avena etioprotoplasts*

1) Etioprotoplasts incubated under white light in a medium adapted from Kao *et al.* (1974, Planta, 120: 215-277) and Pelcher *et al.* (1975,

Can. J. Bot., 53: 427-431) remained structurally intact up to 12-24 hours. They do not show any spectrophotometrically detectable increase in Chl. content. So far we have made a preliminary survey of effect on Chl synthesis of the following conditions:

Preincubation of etioprotoplasts for various times in the cold prior to incubation at 25°C.

Incubation in presence of BAP and/or ALA.

Incubation in presence of BAP and/or Casein-hydrolysate at 2 concentrations.

Incubation with LA $\pm$ GA.

Incubation of protoplasts obtained from seedlings illuminated for up to 12 hrs.

2). Preliminary observations were made on the effect of blue and red light on fine structure and Chl content in detached *Z. mays* leaves, as a basis for further investigations into the effect of monochromatic light on etioprotoplast development.

3). Further investigations into the biosynthetic pathway in Chl synthesis in etioprotoplasts were carried out (in cooperation with Professor E. Harel).

In our proposal we reported that illuminated etioprotoplasts were able, in addition to converting Pchl(ide) to Chl(ide), to convert labelled glutamate into δ -ALA. In light, the accumulation of δ -ALA was more pronounced than in its absence, indicating that without LA the ALA may be

utilized further. Following this line of evidence, we investigated this possible further utilization of C^{14} -ALA and glutamate using TLC, fluorescence spectrophotometry and scintillation counting. In the *dark*, in *absence* of LA, radioactivity from these compounds was detected mainly in protoporphyrin IX, while in *illuminated* etioprotoplasts the label was found consistently predominantly in Pchl(ide) or Chl(ide), dependent on the light regime. These results will have to be verified by additional methods, but if indeed true, they would indicate that in etioprotoplasts there exists a block (control point?) for Pchl synthesis at the level of protoporphyrin IX, prior to insertion of Mg. Even a short light exposure appears to eliminate this block, and allows synthesis of Pchl(ide), when the protoplasts are returned to the dark, or of Chl, when they are kept in continuous light.

C. Cell cultures for greening experiments

Professor L. Reinhold from our department is using tobacco cells (*N. tabacum gossii*) for studies on adaption of cells to saline conditions. Preliminary observations indicate that salt adapted cells make more chlorophyll than non-adapted cells. With the permission of Professor Reinhold, which we gratefully acknowledge, we have made use of her stock and are now cultivating this cell line at various salt concentrations in the dark and under various light conditions. So far we have established the greening pattern in non-adapted cells as a basis for continued work.

EVALUATION AND PLANS FOR NEXT YEAR

The reported findings under B3 are the first on Chl synthesis in *Z. mays* etioprotoplasts, and the second on Chl synthesis in isolated protoplasts in general. We would like to substantiate these findings further, as they appear to us of importance by themselves. If Pchl synthesis can indeed be switched on by light in these etioprotoplasts, they would comprise a good system for many of the investigations suggested in our proposal, especially those involving effect of monochromatic light.

Work on greening in adapted and non-adapted tobacco cells in culture will be continued. Work on these cells was somewhat delayed because of my hospitalisation towards the end of last year. The work has now been resumed in full. Depending on the results obtained, protoplasts from these cells may be used in fusion experiments in cooperation with A. Galston.

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P.O. Box 6
Bet Dagan, ISRAEL

BARD Project No. I-259-80

Title of Research Project: Chloroplast development in isolated
etiolated leaf cells, etioprotoplasts and fusion products.

Names of Investigators: Prof. Shimon Klein

Principal Investigator: Prof. Shimon Klein

Cooperating Investigator(s): Prof. Arthur W. Galston

Name and Address of Affiliated Institutions:

Principal Institution: Institute of Life Sciences, Hebrew University
of Jerusalem

Cooperating Institution(s): Department of Biology, Yale University

Project's starting date: October 1, 1981

Type of Report: 1st Annual 2nd Annual X Final

Signatures:

Principal Investigator

Arthur W. Galston

Institution's Authorizing Official

W. D. Bonney

SUMMARY OF WORK ACCOMPLISHED AT YALE UNIVERSITY
DURING 1983-4 ON BARD GRANT I-259-80.

1. Development of a competent *Vigna* protoplast system.

Last year's report described our efforts to produce a system capable of regenerating an entire leguminous plant from a single isolated mesophyll protoplast. This system seemed especially attractive for our project in that darkness, or at the very most dim light, was a necessary prelude for the development of protoplasts, first into cells with walls, and later into microcalli. Later steps were either independent of light or were, in fact, facilitated by light. This work has now been published and a reprint of the article is herewith enclosed. Shekhawat, N.S. and A.W. Galston. 1983. Isolation, culture, and regeneration of moth bean *Vigna aconitifolia* leaf protoplasts. Plant Science Letters 32: 43-51.

2. Successful regeneration of another legume, *Trigonella foenumgraecum* from mesophyll protoplasts.

In order to provide greater flexibility for our manipulations, and to have alternative systems for plastid developmental studies, we proceeded with the development of a parallel legume regeneration system, starting from leaf mesophyll protoplasts. *Trigonella foenumgraecum* is an important forage legume in hot, dry countries. The plant is also used as a vegetable, and has the additional potential of oleo-resin and steroid production for oral contraceptives.

First leaves were washed with tap water, surfaced sterilized with 5.25% sodium hypochlorite in 0.2% Tween-20. After rinsing with sterile water, the lower epidermis of leaves was peeled off with a fine forceps, and floated, peeled side down, on a mixture of 1% desalted Onozuka R-10 cellulase, mannitol, salts and pH 5.8 MES-KOH buffer. The digestion was allowed to proceed for 4 hours at 30° C. The debris was then removed by filtering through a 55 µm size nylon filter, and the protoplasts pelleted by centrifugation at a 100 x g for five minutes. The protoplasts were resuspended and washed twice with culture medium, then purified by flotation over 21% sucrose, containing calcium chloride. The protoplasts were removed by gentle aspiration with a broad Pasteur pipette, and after another rewashing and repelleting they were suspended in culture medium at a density of 10⁵ protoplasts/ml.

The nutrient solution contained Murashige-Skoog macro- and micro-nutrients, Kao-Michayluk organic acids, an osmoticum and some sugar. The hormones included 0.5 mg/l of auxin, cytokinin and gibberellins.

The cultured protoplasts increased in size and became obviously vacuolated after 24 hours. The chloroplasts were then dispersed throughout the protoplasts and became a light green under

dim light. After about 48 hours of culture the protoplasts had regenerated cell walls and changed in shape from spherical to elongated or oval. Cell plate formation and first cell divisions were observed after about 60-80 hours. The second cell division was observed on the 7th day of culture, after the addition of fresh and slightly altered medium. From 20-70% of the cells produced microcolonies in 2 to 3 weeks in various experiments. For this step it was important to keep the cultures in diffuse light, at least for the initial 10 days, since high light intensity caused brown precipitates to form in the culture drops, and proved deleterious to the protoplasts. Serial and gradual addition of fresh medium with reduced mannitol and glucose levels facilitated a high frequency of divisions.

When cultured on agar under high light intensity, the calli became hard, compact and deep green with globular nodular structures on the surface and periphery. When these were transferred to media without auxin, but containing cytokinin, the nodular bodies formed green buds, which in turn produced leafy shoots. Both chloroplast development and morphogenesis was diminished under lower light intensities and were absent in dark cultures. Glutamine and asparagine greatly aided the growth of callus, and the avoidance of marginal necrosis. These results have now also been published; the reference is the following: Shekhawat, N.S. and A.W. Galston. 1983. Mesophyll protoplasts of fenugreek (*Trigonella foenumgraecum*): Isolation, culture and shoot regeneration. Plant Cell Reports 2: 119-121.

3. Effects of polyamine depletion and supplementation on the greening process.

Last year we reported on the use of inhibitors of ornithine decarboxylase, arginine decarboxylase, S-adenosylmethionine decarboxylase, and spermidine synthase in studies on polyamines and development in tissue cultures. These inhibitors are respectively α -difluoromethylornithine (DFMO), α -difluoromethylarginine (DFMA), methylglyoxal bis- (guanyl hydrazone), and dicyclohexylamine (DCHA). In experiments with tissue cultures and individual cells of *Vigna*, oat, petunia and tobacco, we have found some paradoxical results. These compounds, which we had expected to inhibit the development of chloroplasts, instead paradoxically stimulated greening and differentiation in some cell cultures. Our hypothesis at the moment is that we have selected cells which overproduced polyamines, and that this is related to the high rates of greening. We deduced this because we know that chloroplasts can synthesize large quantities of spermidine, which in in vitro experiments we find facilitate the greening process.

We are pursuing this lead by the application of these inhibitors, we well as the reversing polyamines individually and in groups to single protoplasts, single cells and small groups of cells. So far, we are noting effects on chlorophyll synthesis, as well as on cell division and development.

Publication: Galston, A.W. 1983. Polyamines as modulators of plant development. BioScience 33, 382-388.

4. Attempted greening of embryonic protoplasts of Phaseolus.

We have now concluded that our early results (see last year's report), which purported to show greening-up in these cultures, represented a false lead. We have not been able to reproduce our earlier results, which indicated a continued chlorophyll increase over a two-week period of small groups of etiolated protoplasts, cultivated in 100 μ l droplets. We have thus abandoned this approach.

EVALUATION OF THE RESEARCH RESULTS

While we have not succeeded in our original aim of facilitating the development of chloroplasts in isolated etioprotoplasts, we have been able, through this study, to accomplish the successful development of individual semi-etiolated leguminous protoplasts all the way to entire plants. Since the isolated protoplasts had a mandatory optimum development cycle that included some darkness or restricted dim light, which caused some etiolation of the isolated green protoplasts, we feel we have partially and indirectly accomplished what we set out to do. The polyamine lead seems to warrant further investigation, and is being carried on under the aegis of other granting agencies.

DESCRIPTION AND RESULTS OF COOPERATION

Prof. Klein had recently spent a sabbatical year in my laboratory, and we had planned our respective roles in general outline during that time. I was to concentrate on etioprotoplast culture, visualization of plastid development and promotion of greening by polyamines. He was to concentrate on pigment quantification and fine structural studies. During the past year, we exchanged about four rounds of correspondence and three telephone calls to report our results and to develop new suggestions for each other. Finally, I visited him during August 1983. We had conversations at home, where he was recovering, and at the laboratory, together with his technical assistant. I would say that within the limitations of our great geographical separation, our collaboration worked very well.

CONCLUSIONS AND PLANS FOR NEXT YEAR

I knew of Prof. Klein's serious illness and impending death about a year ago. To take advantage of his presence for however long he would be around, I doubled the rate of expenditure for remaining BARD money by having an extra researcher. Thus, the funds are now essentially gone. I plan to continue to carry along some of this work for a while, using other grant support available to me. The polyamine-promoted greening phenomenon will be studied in etioprotoplasts of Vigna, Trigonella, Petunia and Avina.

LIST OF PUBLICATIONS

- (1) Dumortier, Françoise M., Hector E. Flores, Narpal S. Shekhawat and Arthur W. Galston. 1983. Gradients of polyamines and their biosynthetic enzymes in coleoptiles and roots of corn. *Plant Physiol.* 72, 915-918.
- (2) Young, Nevin D. and Arthur W. Galston. 1983. Are polyamines transported in etiolated peas? *Plant Physiol.* 73, 912-914.
- (3) Galston, Arthur W. 1983. Polyamines as modulators of plant development. *BioScience* 33, 382-388.
- (4) Shekhawat, N.S. and A.W. Galston. 1983. Mesophyll protoplasts of Fenugreek (Trigonella foenumgraecum): Isolation, culture and shoot regeneration. *Plant Cell Reports* 2, 119-121.
- (5) Shekhawat, N.S. and A.W. Galston. 1983. Isolation, culture, and regeneration of moth bean Vigna aconitifolia leaf protoplasts. *Plant Science Letters* 32, 43-51.

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Bet Dagan, 50250 ISRAEL

BARD Research Proposal No. 1-259-80

Title of Proposed Research

Chloroplast development in isolated etiolated leaf cells,
etioplasts and fusion products,

Name and Address of Investigators

Principal Investigator: . . . Dr. Aaron Kaplan

Cooperating Investigator(s) . . . Professor A. W. Galston

Name and Address of Affiliated Institutions

Principal Institution: . . . The Hebrew University of Jerusalem

Cooperating Institution(s): . . . Yale University, New Haven, Conn., U.S.A.
.

Project's Commencement date . . . October 1st, 1981

Type of Report: Initial Comprehensive . ✓ Final ✓

Signature

Principal Investigator

Institution's Authorized Official

(signature and official stamp)

. . . A. Kaplan
Dr. Aaron Kaplan

SCIENTIFIC REPORT

Introduction

Photoautotrophic cell cultures derived from crop plants are highly desirable for a number of important uses in agricultural practices and research. This includes development of cell lines resistant to photosynthetic herbicides, heat shock and photoinhibition. Photoautotrophic growth has been obtained in only a limited number of cases (1, 2, 3). Last year we reported on the development of a line of tobacco cells grown in suspension culture capable of high photosynthetic performance. These cultures, however, exhibited high photosynthetic performance only when the cells were rather old and were not capable of dividing. This year our studies focused on two major aspects.

- (1) Experiments in which we attempted at optimising the growth conditions in order to obtain high photosynthetic rate while the cells are still capable of dividing.
- (2) Studies on the factors rate limiting the photosynthetic performance at different stages of growth.

(1) Optimisation of growth conditions

Since the number of possible interesting combinations of growth conditions is very large, we had to limit ourselves to those which seem to have the greatest potential for success.

(a) Enrichment with CO₂ -- Cultures growing photoautotrophically appear to require an elevated concentration of CO₂ in the environment (1). While the rate of photosynthesis, measured in the intact leaves, saturates at 0.1 - 0.2% CO₂ (v/v), concentrations as high as 1% are required for an as yet unknown reason to sustain photoautotrophic growth. We studied the effect of CO₂ enrichment on cell growth in the presence of various levels of sucrose in the medium. Figure 1 demonstrates the ratio of chlorophyll accumulated per cell protein (µg/mg respectively) in the presence or absence of added CO₂. Data depicted in Fig. 1 are those obtained 10 days after the initiation of the experiments. The levels of sucrose depicted are those initially provided to the cells. Sucrose concentration decreased with time at a rate depending on the growth rate (not shown, but see Figure 1 in the last report). In the absence of added CO₂ the chlorophyll/protein ratios were much lower than in its presence over the entire range of sucrose concentration. Further, the chlorophyll/protein ratio decreased in response to elevated concentrations of sucrose. This reflects the faster growth of the cells (as determined by the amount of protein accumulated) at 2% sucrose while chlorophyll accumulation was hardly affected. In the presence of CO₂, on the other hand, the maximal chlorophyll/protein ratio was reached at 0.5% sucrose and was 6-7 times larger than in the absence of CO₂. This is mainly due to faster accumulation of chlorophyll in the presence of CO₂

(not shown). Protein accumulation was only slightly affected being somewhat larger in the presence of CO_2 than in its absence (not shown). Figure 1 also presents data on the rate of CO_2 fixation in the case of CO_2 supplementation during growth. The capacity for CO_2 fixation decreased considerably as the concentration of sucrose experienced by the cells was raised.

It is thus concluded that the presence of high sucrose concentration (2%), which is commonly used in cell cultures inhibits the photosynthetic performance of the cells. This system can thus be used as a model system in which the effect of sucrose and starch on the photosynthetic performance of leaves is being studied. These studies are currently of great interest as they bear on the important question of partition of assimilates. The mechanism(s) by which CO_2 affected the accumulation of chlorophyll, to the extent it did (Fig. 1) are not yet understood. While CO_2 may act as an electron acceptor in the photosynthetic machinery, its effect on the pathway of chlorophyll synthesis and degradation is not yet clear. This effect of CO_2 is of great importance in view of the current increasing interest in the assembly of proteins to form functional complexes in the biological membranes. In my opinion this is an ideal system to study the assembly and degradation of chlorophyll-protein complexes in the membrane.

(2) Photosynthetic performance studies

The rate of photosynthesis depicted in Fig. 1 is expressed on a chlorophyll basis. It is possible that some of the changes observed are due to changes in the organisation and functionality of the chlorophyll. One way to study this possibility is by an analysis of the fluorescence kinetics under various conditions. We compared the fluorescence rise kinetics in the presence or absence of DCMU. This substance which was originally used as an herbicide blocks electron transport at the accepting side of PS II thus leading to fast increase of the fluorescence yield to its maximum (F_m). The fluorescence rise kinetics in the presence of DCMU depends among other things on the light intensity and photosynthetic unit size (i.e. chlorophyll per reaction center). The larger they are the faster the fluorescence will reach maximum. The fluorescence yield at steady state (F_s) is determined among other things by the ability of the photosynthetic machinery to dissipate the light energy into biochemical energy. The F_0 is a measure of the chlorophyll molecules which fluoresce immediately since they are unable to transfer energy, i.e. they are not organised properly within the light harvesting complex. Thus the term $T = (F_m - F_s)/F_0$ is being used as a criteria for the functionality of PS II. Fluorescence rise kinetics was determined as a function of sucrose concentration, supplementation with CO_2 and culture age.

Figure 2 presents some data obtained with cells grown in the absence of sucrose but in the presence of CO_2 . T increased with age and reached maximum at 15-20 days. The same pattern was also observed with cultures grown in the presence of sucrose with or without CO_2 supplementation. We should emphasize, however, that a large variability was obtained between different experiments and

thus we cannot draw any conclusions as yet. The variability most probably reflected the different capacities to perform CO_2 fixation obtained in different cultures (see below).

The fluorescence spectra at 77°K reflects the distribution of energy between PS II and PS I (fluorescence maxima at 685 and 735 nm respectively). A clear difference was observed between cells grown at 2% sucrose without additional CO_2 compared to 0.2% sucrose supplemented with CO_2 . In the latter case the ratio of fluorescence at 685/735 nm was larger than in the former. These data indicate that relatively larger amounts of energy reach PS II center in the case of cells grown at 0.2% sucrose and supplemented with CO_2 compared with cells grown at 2% sucrose without CO_2 . These data are consistent with the larger chlorophyll/protein ratio observed in cells supplemented with CO_2 (Fig. 1) as well as with the larger T observed in cells grown at 0.2% sucrose plus CO_2 at early stages of growth (Fig. 2).

We determined the activity of several component reactions of photosynthesis in an attempt to specify those rate limiting the photosynthetic rate at early stages of development of the cells. Reactions studied were: CO_2 dependent O_2 evolution, CO_2 fixation measured by ^{14}C , O_2 uptake in the presence of methylviologen, O_2 evolution in the presence of DCPIP, activity of RuP_2 carboxylase (Rubisco), and the pool size of ribulose bisphosphate (RuP_2). These studies were performed on the tobacco cells grown at 2% sucrose or 0.2% sucrose plus 5% (v/v) CO_2 in air. Figure 1 presents data showing the strong effect of sucrose concentration on CO_2 dependent O_2 evolution, discussed above. Whole chain photosynthetic electron transport from water to methylviologen showed very little activity and was very variable between different experiments as were most

of the parameters determined. Though the exact reaction which limits electron transport has not yet been defined, we conclude that the capacity for electron transport most probably limits the photosynthetic rate.

Figure 3 presents some representative data on the Rubisco activity per chlorophyll as a function of culture age and growth conditions. Taking into account that the amount of chlorophyll per protein is far larger in cells grown at 0.2% sucrose plus CO_2 (Fig. 1), these data clearly suggest larger Rubisco activity per cell in cells supplied with CO_2 . Rubisco activity was highest at early stages of growth and decreased later when the overall photosynthetic activity increased. Thus, Rubisco activity could limit photosynthetic activity at the later stages of growth but not at an early stage. We determined also the Rubisco activity in protoplasts isolated from pea mesophyll cells lysed into Rubisco assay medium. Here the Rubisco activity was 10 times higher than in the tobacco cells, and most probably did not limit the photosynthetic rate (which was 25% of the maximal Rubisco activity).

The pool size of RuP_2 the substrate of Rubisco, which reflects the rate of its synthesis and dissipation was 90 nmole.mg^{-1} chl in cells grown at low sucrose and only 20-35 nmole.mg^{-1} chl in cells grown at 2% sucrose. This indicates a rather large difference in the amount of RuP_2 per cell, taking also into account the different chlorophyll/protein ratios (Fig. 1). To determine whether RuP_2 pool limits photosynthesis it is essential to plot the photosynthetic rate as a function of RuP_2 pool (see 4). However when such a plot was made we obtained the largest photosynthetic rates at the lowest RuP_2 level and vice versa, contrary to the expected behaviour (see 4). This data strongly suggests that the rate of formation of RuP_2 limits the rate of

photosynthesis thus leading to a lower level of RuP_2 at increasing photosynthetic rates. This conclusion is consistent with our findings that the photosynthetic electron transport most probably limits photosynthetic rate. It is also possible that the activity of several enzymes involved in the synthetic pathway of RuP_2 such as phosphoribulose kinase, fructose biphosphatase or the photophosphorylation capacity (all not determined here) may limit the rate of formation of RuP_2 .

Conclusions

In this study we focused on two major aspects of photosynthetic performance of cells grown in culture: (a) Interrelation between sucrose and CO_2 as carbon source; and (b) factors rate limiting photosynthesis at early stages while the cells are still capable of dividing. Our major findings are:

(1) Feeding the cells with sucrose inhibits the accumulation of chlorophyll and various photosynthetic component reactions. This system may be used as a model system for studies relating to: (a) assembly of protein complexes in the membrane (CO_2 affects chlorophyll/protein ratio); (b) regulation of photosynthetic rate and partition of assimilates by sucrose and starch (sucrose inhibits photosynthesis).

(2) Several component reactions limit the photosynthetic rate including capacity for electron transport carboxylation capacity and reactions leading to the formation of RuP_2 .

Results obtained in this section were very variable and experiments are required to define the rate limiting steps at an early stage of growth. This will enable manipulation of growth conditions and in the future also genetic manipulations to enable fast photoautotrophic growth of cells in culture.

Acknowledgments

I wish to thank Mrs. Emma Ne'eman for conducting the research with me and Professor I. Ohad for many helpful discussions.

References

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- (2) Yamada Y, Sato F 1978 Plant Cell Physiol. 19, 691-699.
- (3) Dalton CC 1980 J Exp Bot 31, 791-804.
- (4) Farquar GD, S von Caemmerer, JA Berry 1980 Planta 149, 78-90.

Fig 1

Fluorescence, Relative Units.

Rubisco activity, $\mu\text{mol CO}_2 \cdot \text{mg}^{-1} \text{chl h}^{-1}$