Control of Triosephosphate Dehydrogenase in Photosynthesis[1, 2]

G. A. Hudock[3] and R. C. Fuller
Department of Microbiology, Dartmouth Medical School, Hanover, New Hampshire

The intracellular and phylogenetic distribution of the NAD and the NADP dependent triosephosphate dehydrogenases (TPD) in photosynthetic organisms has been examined in many laboratories (8, 11, 12, 20, 22). Studies by Fuller and Gibbs (6), and Smillie and Fuller (21) have indicated that the NADP enzyme occurs only in green tissues of higher plants and in O_2 evolving photosynthetic microorganisms. Brawerman and Konigsberg (4) have studied the kinetics of the formation of the NADP enzyme in *Euglena* and found that the rates of synthesis of chlorophyll and the increase in NADP activity are approximately equal. These results led to the suggestion that the NADP enzyme functions in photosynthetic metabolism while the NAD enzyme is operative in oxidative or glycolytic metabolism. This suggestion received further support from the demonstration by Heber, Pon and Heber (12) that the NAD linked TPD is confined to the chloroplast in several higher plant species. In contrast to these findings, however, no NADP dependent TPD activity has been observed in photosynthetic bacteria (6, 7).

To examine further the question of pyridine nucleotide specificity in photosynthesis, we have studied the kinetics of changes in NADP and NAD linked dependent TPD activities in bleaching and regreening cultures of *Chlamydomonas reinhardi* and *Euglena gracilis,* and the activity and properties of the NAD dependent TPD from *Chromatium* strain D grown under either photolithotrophic or photoorganotrophic conditions.

Materials and Methods

Organisms and Growth Conditions. The wild type strain (137c) of *Chlamydomonas reinhardi* and the mutant strain y-2 derived from it have been previously described (13). Cultures were grown in either a minimal medium containing only inorganic salts or in this medium supplemented with 0.2 % sodium acetate (13). *Chromatium* strain D was grown as previously described (7). The medium for photolithotrophic conditions contained CO_2 as the sole source of carbon and $Na_2S_2O_3$ as a source of reductant. The photoorganotrophic growth medium contained sodium malate as carbon source. All cultures were grown at an incident light intensity of 20,000 lux.

Euglena gracilis strain Z was grown in organotrophic medium at a light intensity of 1500 to 2000 lux or in the dark. Bleached cultures were allowed to regreen in a resting medium as previously described (22) at an incident light intensity of 1500 to 2000 lux.

Preparation of Enzyme Extracts. Crude enzyme preparations were prepared in the same way for all 3 organisms. Concentrated suspensions of cells were disrupted by sonic oscillation with a Mullard 20 kc ultrasonic disintegrator for 3 minutes at 0°. The suspensions were then centrifuged at 20,000 × *g* for 30 minutes and the supernatant fluid was used as a crude enzyme preparation.

TPD was extensively purified from *Chromatium.* Following disruption and initial centrifugation, the material precipitating between 0.5 and 0.8 saturation with $(NH_4)_2SO_4$ was collected. This fraction contained 70 to 80 % of the TPD activity. The precipitate was resuspended in a small volume of 0.1 M K phosphate buffer at pH 8.4 and centrifuged for 90 minutes at 144,000 × *g*. The supernatant fluid contained all of the remaining enzyme activity and was a clear, straw color. The enzyme was further purified by passage through a 150 ml (2.8 × 25 cm) column of Sephadex G-200 equilibrated with 0.1 M K phosphate buffer at pH 8.4 from which it eluted in about 1.5 times the void volume. In order to crystallize the enzyme, the Sephadex eluant was brought to turbidity with $(NH_4)_2SO_4$ and allowed to stand for 3 to 5 days. Twice recrystallized enzyme had no higher activity than the Sephadex eluant and the latter used for most studies. Purified enzyme was dialized against saturated $(NH_4)_2SO_4$ and stored in the cold in this manner until it was used.

Enzyme Assay. Triosephosphate dehydrogenase activity was assayed in both the oxidative and the reductive direction. In the oxidative reaction from glyceraldehyde-3-P to 1,3 diP-glycerate (DiPGA), the method of Fuller and Gibbs was used (6). In the reductive reaction from DiPGA to glyceraldehyde-3-P, the method of Heber, Pon and Heber (12) was used, except that PGA kinase was added to 2-fold excess in all cases. The OD change at 340 mμ was measured for at least 3 minutes and the rate of reaction was calculated from the linear portion of the curve. A unit of enzyme activity is defined as the amount that will oxidize 1 μmole of reduced pyridine nucleotide per minute.

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[3] Present address: Department of Zoology, Indiana University, Bloomington, Indiana.

Protein Determination. Protein concentration was determined with the biuret reaction (9).

-SH Determination. Determinations of thiol groups were made with the nitroprusside reaction (10).

Results

Chlamydomonas. The activities of NAD and NADP dependent TPD were measured in cell-free extracts of wild type and mutant strain *y-2 C. reinhardi* following growth for 72 hours in the light on minimal medium, growth for 72 hours in the light on acetate supplemented medium and growth in the dark for 96 hours on acetate supplemented medium. The results given in table I for the 2 strains show that in no case was more NADP than NAD linked activity detected. Addition of sodium acetate to the growth medium in the light resulted in a 2-fold reduction in the NADP linked activity. Little change was detected in the NAD activity under these circumstances. Growth of both wild type and the *y-2* mutant strain in the dark resulted in an increase in NAD activity. In the case of *y-2*, dark growth was accompanied by a 15-fold reduction in chlorophyll content and a decrease in NADP activity comparable to that caused by the sodium acetate in the light. The NAD/NADP ratio varies from 1.7 to 5.0, depending upon environmental conditions (table I).

The chlorophyll content and NAD and NADP dependent activities were then examined during the entire process of bleaching and regreening in the mutant strain *y-2* (fig 1). During growth in the dark, chlorophyll synthesis is completely stopped. NADP linked TPD activity per cell begins to decrease at the same time but at a lower rate than the chlorophyll content. NAD linked activity increases almost 2-fold during this period. On return to the light, there is a rapid decrease in the NAD activity and increases in both chlorophyll content and NADP activity. A plot of units of enzyme per cell against chlorophyll per cell is given in figure 2. Changes in the NADP and NAD linked enzyme activities begin concomitantly with changes in chlorophyll content and the level of both enzymes characteristic of light grown cells is reestablished by the time the culture was fully regreened (table I). Thus, it is clear that

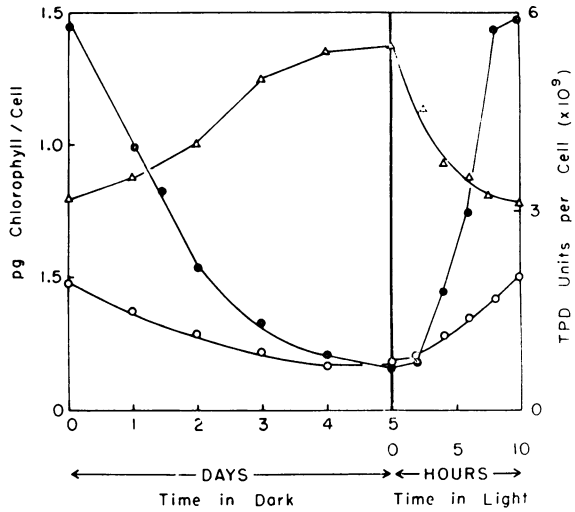

FIG. 1. Chlorophyll content, NAD dependent TPD activity and NADP dependent TPD activity during bleaching and regreening of the y-2 mutant strain of *Chlamydomonas*. ● = pg chlorophyll/cell; △ = units NAD dependent TPD per cell (× 10^9); ○ = units NADP dependent TPD per cell (× 10^9).

the kinetics of the formation of NADP linked activity and the disappearance of NAD activity do not follow those of other photosynthetic enzymes in *C. reinhardi* (13, 14). Both ribulose 1,5-diP carboxylase and photosynthetic pyridine nucleotide reductase (PPNR) activity show a lag in formation relative to chlorophyll synthesis that the TPD enzymes do not.

Chromatium. Enzyme was purified from cultures of *Chromatium* strain D grown either photolithotrophically or photoorganotrophically. Enzyme from both sources eluted from Sephadex G-200 (in which the enzyme was included) or G-100 columns (from which the enzyme was just excluded) in a volume corresponding to a molecular weight of 110,000 to 130,000 (1, 15, 16). The molecular weight of the purified enzyme was also determined by sedimentation velocity in the ultracentrifuge. For this purpose, purified enzyme was suspended in 0.1 M cysteine at pH 8.4. If this cysteine was omitted, the enzyme

Table I. *Triose Phosphate Dehydrogenase Levels in* Chlamydomonas

Measurements of enzyme activity and chlorophyll determinations were carried out as described in Materials and Methods.

Strain	Growth conditions: Light	Basic medium + or − Na acetate	Units enzyme cell (× 10^9) NAD	NADP	NAD/NADP	μg Chlorophyll per 10^6 cells
Wild Type	+	−	2.7	1.6	1.7	1.43
Wild Type	+	+	2.6	0.88	3.0	1.47
Wild Type	−	+	5.1	1.00	5.1	1.03
y-2	+	−	3.2	1.9	1.7	1.58
y-2	+	+	2.7	0.67	4.0	1.61
y-2	−	+	5.5	0.70	7.9	0.11

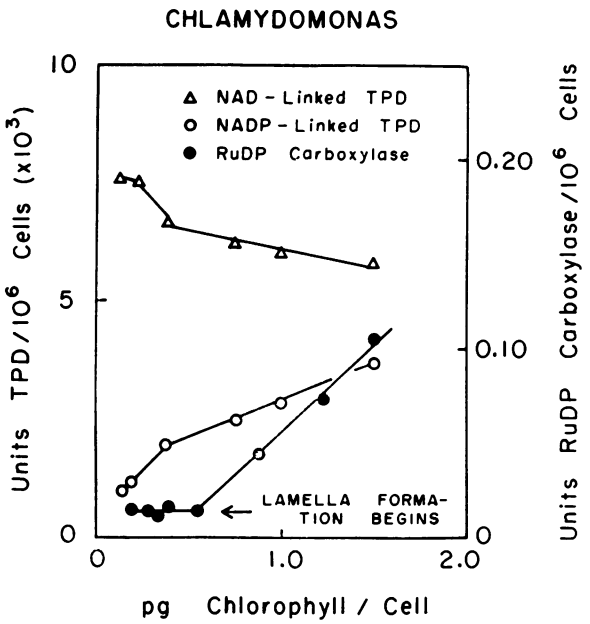

FIG. 2. Units of NAD and NADP dependent TPDs and of RuDP carboxylase per cell plotted as a function of chlorophyll content during regreening of y-2 *Chlamydomonas*. The arrow indicates the time at which paired lamellae are first seen in the developing chloroplast.

broke down completely during ultracentrifugation, and even the stabilized system shows a tendency to disassociate upon standing. However, an S_{20w} of 7.0 was obtained which is identical to that of the rabbit muscle TPD (23). If it is assumed that *Chromatium* TPD and rabbit muscle TPD have the same partial specific volumes and diffusion coefficients, the molecular weight of 118,000 can be calculated for *Chromatium* TPD, which is in agreement with the Sephadex method.

Whereas the enzymes prepared from cultures grown under these 2 different conditions were quite similar in terms of molecular weight, and both had the same pH optimum (pH 8.3–8.5), other properties were different. The Michaelis constants of the enzymes for DiPGA, glyceraldehyde-3-P, NAD and NADH were determined for both the purified enzyme preparations and the crude preparations from Lineweaver-Burk plots which were linear (table II). Measurements of both the purified and crude preparations were identical indicating that differences in K_m values were not purification artifacts. The results of these experiments are given in table II. Enzymes from cells grown under both sets of metabolic conditions had approximately the same K_m values for NAD and NADH but the K_m values for DiPGA and glyceraldehyde-3-P differed by a factor of 3. The enzyme prepared from photolithotrophically grown cells had a K_m for DiPGA of 3.0×10^{-3} M ($1.7–4.3 \times 10^{-3}$ M) and that prepared from photoorganotrophically grown cells had a K_m for DiPGA of 10^{-2} M ($0.9–1.2 \times 10^{-2}$ M).

The difference in the properties of TPDs prepared from cultures grown under 2 different growth conditions leads to the question of whether there are 2 separate proteins, as in higher plants, or a single protein, the properties of which vary with growth conditions. The major differences between the growth conditions are source of carbon (inorganic vs. organic) and the redox potential of the growth medium. Thiosulfate or H_2S as reductant have lower redox potentials than does an organic source of hydrogen. Since Bose and Gest (3) have shown that photosynthetic bacteria will carry out photosynthetic phosphorylation within only a very narrow range of redox potential, the effect of mild oxidation or reduction on the properties of the enzyme was studied. Reduction of the enzyme prepared from cells grown with malate as a source of carbon and reductant was reduced by treating the enzyme with 0.1 M Na ascorbate (pH 8.5). Treatments for periods of 1 to 15 hours were examined and all produced the same result. Mild oxidation of the enzyme prepared from cells grown with CO_2 as the sole source of carbon was also carried out. Since the purified enzyme was stored in the presence of cysteine, the cysteine was removed from a standard preparation by dialysis against 0.1 M K phosphate buffer at pH 8.4 for 12 hours. The

Table II. *K_m Values (M) for Purified Triose Phosphate Dehydrogenase from Chromatium*

Reaction mixtures: A, glyceraldehyde 3-P → DiPGA. The reaction mixture contained in a total of 3.0 ml: 100 μmoles of Tris (hydroxy methyl)-aminomethane, pH 8.4; 17 μmoles of Na arsenate; 12 μmoles cysteine, pH 8.4; 0.18 μmole of NAD in standard reaction mixtures in determination of K_m for G-3-P (varied from 0.002 μmole-0.36 μmole for determination of K_m for NAD) and 0.1 ml enzyme preparation. The reaction mixture was incubated at 25° for 7 minutes and 1.0 μmole glyceraldehyde 3-P was added (varied from 0.10–2.0 μmoles in determination of K_m for G-3-P). After 30 seconds, the OD change at 340 mμ was followed for 3 minutes. B, DiPGA → glyceraldehyde-3-P. The reaction mixture contained in a total of 3.0 ml: 100 μmoles Tris, pH 8.4; 20 μmoles of $MgSO_4 \cdot 7H_2O$; 12.0 μmoles cysteine, pH 8.4; 45 μmoles of PGA in standard assay (3.0–67.5 μmoles in determination of K_m for DiPGA); 2-fold excess of PGA-kinase (Sigma) in 0.10 ml; 5.0 μmoles of ATP, 0.80 μmole of NADH in standard assay (0.01–1.0 μmole in determination of K_m for NADH); 0.10 ml enzyme preparation. The OD change at 340 mμ was followed for 3 minutes.

	Cells grown on: H_2S and CO_2	Malate
Glycerate 1, 3-DiP	$3.0 (\pm 1.3) \times 10^{-3}$*	$1.0 (\pm 0.2) \times 10^{-2}$
NADH	$5.0 (\pm 1.2) \times 10^{-5}$	$6.5 (\pm 0.8) \times 10^{-5}$
Glyceraldehyde-3-P	$1.7 (\pm 0.5) \times 10^{-4}$	$5.0 (\pm 1.0) \times 10^{-4}$
NAD	$3.0 (\pm 0.9) \times 10^{-6}$	$4.0 (\pm 1.2) \times 10^{-6}$

* K_m values, range of at least 5 separate determinations is given.

results are given in table III. Mild oxidation of the enzyme prepared from CO_2 grown material altered the K_m of the enzyme for DiPGA from 3×10^{-3} M to 9×10^{-3} M ($8–11 \times 10^{-3}$ M), that is, approximately equal to enzyme prepared from malate grown material. Conversely, mild reduction of enzyme freshly prepared from malate grown cells changed the K_m for DiPGA from 10^{-2} M to 4×10^{-3} M ($2.6–4.4 \times 10^{-3}$ M) approximating the enzyme from CO_2 grown material. Both changes were reversible.

The number of reactive SH groups per mole of protein was measured in highly purified enzyme prepared from both sources immediately after elution from Sephadex G-200 (table III). Determinations of thiol groups, therefore, were unaffected by added cysteine. The native enzyme prepared from CO_2 grown material contained 4 (3.8–4.3) available -SH groups per protein molecule. The enzyme prepared from malate grown material contained about 2.4 (1.9–2.7). Oxidation of the former reduced the number of reactive -SH groups to 2.4 (2.1-2.6) and the reduction of the latter increased the number to 3.3 (2.9–3.4). The change in the K_m values were accompanied by a change in the number of reactive -SH groups per protein molecule and all changes were reversible. Thus, these in vitro changes in both the available -SH groups and K_m parallel the in vivo differences, suggesting the existence of 1 alterable protein in the 2 metabolic states.

Euglena. A culture of *E. gracilis* was grown in the dark on heterotrophic medium at 25°. After total bleaching the cells were harvested and returned to the light in resting medium. Samples were taken at daily intervals and the chlorophyll content and NADP and NAD linked TPD activities were measured (fig 3). The results agree quite well with those of Brawerman and Konigsberg (4), who found that during regreening, NAD linked activity decreased about 20 % and NADP linked activity increased about 10-fold. We suggest that the decrease in NAD linked activity may be more significant than relative constancy. Quantitatively, there is a decrease in NAD linked activity of about 0.03 unit per 10^6 cells and a net increase in NADP linked activity of 0.06 unit per 10^6 cells. In the absence of information

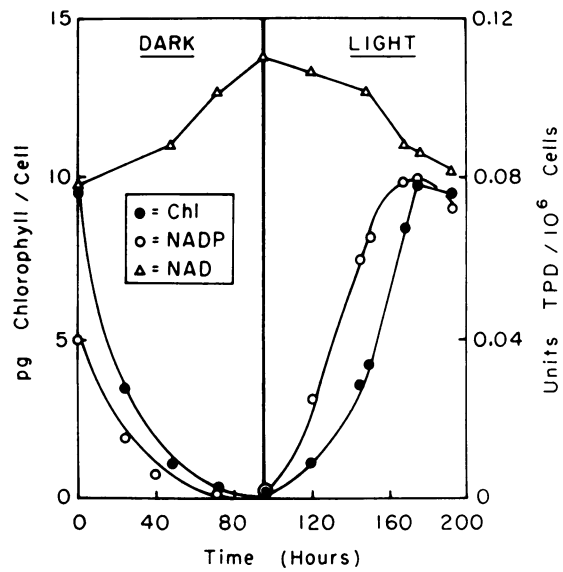

FIG. 3. Chlorophyll content, NAD dependent TPD activity and NADP dependent TPD activity during bleaching and regreening of *Euglena*.

concerning the turnover number of the 2 enzymes, it can still be suggested that a conversion of NAD linked activity to NADP linked activity might take place during regreening. This hypothesis was tested by analyzing the first 25 hours of regreening of a bleached culture of *Euglena* in detail. A dark grown culture was returned to the light in resting medium and aliquots were taken every hour for 7 hours and then roughly every 5 hours. Chlorophyll content and NAD and NADP linked activity were then measured. These results are given in figure 4. During the first 25 hours of regreening, there was a 5 to 6-fold increase in NADP linked activity but no decrease in NAD linked activity.

Discussion

The results obtained in bleaching and regreening cultures of *Euglena* (fig 3) agree well with those obtained in other laboratories (4). Chlorophyll syn-

Table III. *Effect of Oxidation and Reduction on Purified Triose Phosphate Dehydrogenase Purified From Chromatium*

Reduction of oxidized TPD was effected by adding one-tenth volume of 0.10 M Na ascorbate to the enzyme preparation. Oxidation of reduced TPD was carried out by overnight dialysis against 0.10 M K-phosphate, pH 8.4, to remove cysteine and other possible reductants. In the former treatment, there was no loss of activity; in the latter treatment no more than 20 % of the activity was lost. Reactions carried out as described in table II. Thiol groups were measured as described.

	Enzymes from CO_2 cells	Enzymes from malate cells	Enzymes from CO_2 cells oxidized (O_2)	Enzymes from malate cells reduced (ascorbate)
Available -SH Groups	4.2	2.4	2.4	3.3
K_m for DiPGA	3×10^{-3}	10×10^{-3}	9×10^{-3}	4.0×10^{-3}

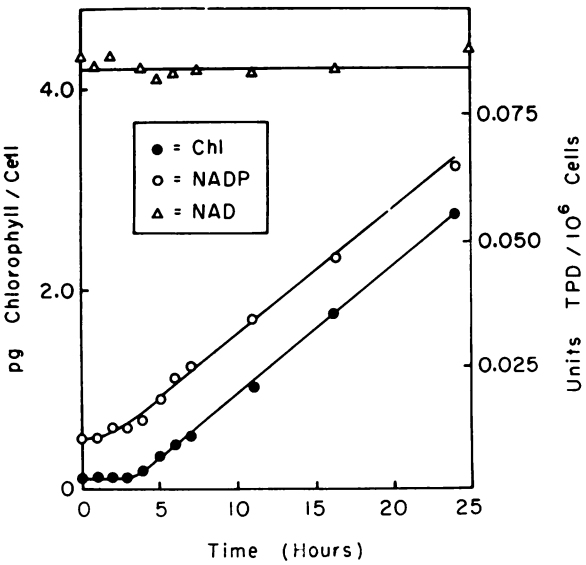

FIG. 4. Chlorophyll content NAD dependent TPD activity and NADP dependent TPD activity during the first 24 hours of regreening of a dark grown culture of *Euglena*.

thesis and increase in NADP linked TPD activity have been separated in higher plants by Marcus (17) and by Mego and Jagendorf (18). It was shown that an increase in enzyme activity could be induced in etiolated seedlings by irradiation with red light absorbed by phytochrome but not light absorbed by protochlorophyll. Such irradiation initiated an increase in leaf area and in NADP enzyme activity but not in chlorophyll content. Although these results indicate that chlorophyll synthesis and the increase in the activity of NADP dependent TPD are separable, a well-integrated control over the development of the overall photosynthetic activity is still suggested. An increase in NADP linked TPD in the dark has not been demonstrated.

The kinetics of the formation of TPD obtained with the y-2 strain *Chlamydomonas* are different from those obtained by Hudock and Levine (13) for changes in the activity of both ribulose 1, 5-diP carboxylase and photosynthetic pyridine nucleotide reductase. A 2-fold decrease in NADP linked activity was caused by the addition of sodium acetate to the growth medium. Growth of the wild type strain of *C. reinhardi* in the dark produced no further decrease in NADP linked activity and little decrease in chlorophyll content. But, growth of the mutant strain y-2 in the dark resulted in a 15-fold reduction in chlorophyll content and an overall 3-fold reduction of NADP linked enzyme activity. The effect of acetate in the growth medium alone was almost as effective as decrease in chlorophyll content in reducing NADP linked TPD activity.

There are thus, major quantitative differences between the control of ribulose 1,5-diP carboxylase and NADP linked TPD in *C. reinhardi* since bleaching resulted in a large decrease in the activity of the former enzyme. Changes in dehydrogenase activity are considerably less well correlated with changes in chlorophyll content in *Chlamydomonas* than in *Euglena*. However, variation of the ratio of NAD to NADP dependent activity under various growth conditions (table I) still indicate a primarily photosynthetic function of the NADP dehydrogenase, and a primarily glycolytic function for the NAD dehydrogenase in *C. reinhardi*.

The presence of acetate in the growth medium in the light, under conditions where photosynthetic activity is still high (13), resulted in a 2-fold decrease in NADP linked TPD activity. Organotrophic growth in the dark resulted in a 2-fold decrease in DPN linked dehydrogenase activity. Thus, in *C. reinhardi*, the type of metabolism, i.e. phototrophic, myxotrophic or organotrophic, seems to control a balance between the 2 TPD activities rather than the synthesis of one or the other.

Control of NADP linked dehydrogenase in *C. reinhardi* can be contrasted further with control of ribulose 1,5-diP carboxylase activity. Increase in ribulose 1,5-diP carboxylase and PPNR activities, the rate of photosynthetic O_2 evolution, and increases in the value of enhancement of photosynthesis do not begin until a chlorophyll content of 0.5 pg/cell is obtained during regreening (13). This chlorophyll content can be correlated with the appearance of paired lamellae within the chloroplast (14). In contrast to this, NADP dependent dehydrogenase activity began to increase and NAD dependent activity began to decrease simultaneously with the onset of chlorophyll synthesis, indicating a different type of control mechanism for these enzymes.

The metabolic situation in the photosynthetic bacteria in the regulation of TPD activity is considerably different than that in green plants. In the bacteria, the photosynthetic apparatus does not contain the enzymes of the Calvin cycle (2). In this procaryotic cell there is no separation of the function of TPD as a consequence of the isolation of 1 enzyme within the photosynthetic organelle. In addition, whereas the green plants must support high TPD in both the glycolytic and synthetic direction, the primary direction of reaction in *Chromatium* is biosynthetic, regardless of carbon source (7).

During the photolithotrophic growth, *Chromatium* contains high ribulose 1,5-diP carboxylase activity and actively reduces CO_2 via the Calvin cycle (7). TPD activity is needed both for maintenance of the Calvin cycle and for biosynthesis. Under photoorganotrophic conditions, ribulose 1,5-diP carboxylase activity is reduced 10-fold and there is little Calvin cycle activity (7). The main function of TPD under such conditions would be the biosynthesis of 6 carbon compounds and glycolysis.

From the results obtained, it can be suggested that *Chromatium* may have developed a control mechanism which combines constitutive synthesis of TPD with the control of its activity. Extracts of

Chromatium grown either photolithotrophically or photoorganotrophically contain the same amount of TPD activity. Control of activity is indicated by the variation of properties of the enzyme prepared from *Chromatium* grown under the 2 conditions studied. Studies of carbon metabolism in *Chromatium* have failed to detect any sizable pool of DiPGA or triosephosphate within the cell (6), implying low intracellular concentrations. With a large amount of enzyme, minor changes in K_m values under the 2 metabolic conditions could alter strikingly rates (or direction) of TPD reactions.

In view of the results obtained with *Chromatium*, one might ask if pyridine nucleotide specificity of green plants is related to an altered form of 1 protein rather than the induction of a new protein for the specific enzyme function. The results presented in figure 4 provide a tentative answer to this question in *Euglena*. While there is decrease in NAD linked activity per cell during the total regreening process (fig 3) (4), during the first 25 hours of regreening, there is a 6-fold increase in NADP linked dehydrogenase activity but no decrease in NAD linked activity.

In *Chlamydomonas* however, a different situation exists. The entire regreening process under the conditions studied requires only 8 to 10 hours. During the first 2 to 3 hours, both an increase in NADP linked activity and a decrease in the NAD linked activity are observed and these changes do not follow the kinetics of formation of other photosynthetic enzymes (fig 2). This result is necessary for the hypothesis that 1 enzyme is converted to the other but clearly is not sufficient to prove it. It is particularly interesting in view of the recent findings of Ogren and Krogmann that there is a light dependent conversion of NAD to NADP in several O_2 evolving photosynthetic organisms (19).

With the exception of the blue-green algae (5), all organisms that show a NADP linked TPD activity are true eucaryotic photosynthetic cells. Their carbon metabolism, along with their other photosynthetic reactions are contained in the chloroplast compartment. The bacteria, however, do not show this evolved compartmentalization. The 2 alternate forms of TPD in *Chromatium* might provide for a rapid direct metabolic control or biochemical compartmentalization of the Calvin photosynthetic cycle in a procaryotic cell. It can be suggested that, as the photosynthetic apparatus evolved from the light absorbing, ATP producing particle to the level of the chloroplast of higher plants, numerous functions were included until all of the enzyme of photosynthesis were included in the photosynthetic organelle. The isolation of these functions within the chloroplast clearly would constitute a strong selective agent. A primarily photosynthetic TPD would seem to be a logical development in such an organelle. Furthermore, the occurrence of a photosynthetic TPD which is NADP specific and the light dependent conversion of NAD to NADP would seem to be natural concomitants, although primacy cannot be ascribed to either development.

Summary

The kinetics of changes in chlorophyll content and in NAD and NADP dependent triosephosphate dehydrogenases were studied in *Euglena gracilis* and in *Chlamydomonas reinhardi* during bleaching and regreening. In *Euglena*, the kinetics of changes in NADP dependent enzyme activity closely paralleled changes in chlorophyll content under all conditions. Changes in NADP linked activity were less marked in *Chlamydomonas* and the presence of a reduced carbon source in the growth medium was as effective in causing a decrease in activity as was bleaching during growth in the dark. The kinetics of changes in the activities of the 2 enzymes during regreening of chlorotic *Euglena* indicate no conversion of NAD to NADP dependent enzyme. The kinetics of changes in activity observed in *Chlamydomonas* are compatible with such a conversion.

The NAD dependent triosephosphate dehydrogenase of the obligately phototrophic bacterium *Chromatium* was purified extensively. While enzyme from photoorganotrophically and photolithotrophically grown cells were identical in some respects, the affinities of the 2 enzymes for triose substrates differed. It was found that these K_m values and the reactive -SH contents of one form of the enzyme could be varied in vitro by mild oxidation and reduction to approximate those of the other form. It was suggested that *Chromatium* contains a single NAD dependent enzyme, the properties of which vary with growth conditions, and may play a role in the regulation of photosynthesis and glycolytic carbon metabolism.

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