Biochimica et Biophysica Acta, 449 (1976) 209–223

BBA 47177

ALGAL GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASES

CONVERSION OF THE NADH-LINKED ENZYME OF *SCENEDESMUS OBLIQUUS* INTO A FORM WHICH PREFERENTIALLY USES NADPH AS COENZYME

MICHAEL J. O'BRIEN*, JOHN S. EASTERBY and ROY POWLS

Department of Biochemistry, University of Liverpool, Liverpool (U.K.)

(Received March 29th, 1976)

SUMMARY

Scenedesmus obliquus contains two glyceraldehyde-3-phosphate dehydrogenases (EC 1.2.1.–) one of which uses NADH as its preferred coenzyme (D-enzyme) and the other NADPH (T-enzyme). On incubation of the D-enzyme with cysteine and a 1,3-diphosphoglycerate-generating system the specific activity with NADH as coenzyme decreased whilst that with NADPH increased by a factor of 10. The components of the generating system had no effect on the D-enzyme individually and it is concluded that 1,3-diphosphoglycerate was probably responsible for the change in nucleotide specificity. The coenzyme specificity of the T-enzyme was not affected by such treatment. A similar type of activation occurred to a lesser extent on incubation of the D-enzyme with 2,3-diphosphoglycerate. The NADPH-dependent activity of the D-enzyme could also be promoted by incubation with NADPH. However, in this case the activation was less than that seen with either 1,3- or 2,3-diphosphoglycerate.

The change in coenzyme specificity of the D-enzyme occurred in parallel with changes in sedimentation behaviour. Initially, a single boundary of $S_{20,w} = 14.5$ S was present, but on conversion to NADPH-dependent activity by incubation with the 1,3-diphosphoglycerate-generating system, new boundaries of 7.5 S and 5.5 S appeared. The first of these corresponds in sedimentation coefficient to the native T-enzyme. On removal of 1,3-diphosphoglycerate the 7.5 S boundary disappeared accompanied by an increase in that of 14.5 S, whilst the 5.5 S boundary persisted. These changes are consistent with the reversible conversion of the D-enzyme into a form similar to the native T-enzyme in response to cysteine and 1,3-diphosphoglycerate. These effects may be explained if acylation of the active site of the D-enzyme by 1,3-diphosphoglycerate results in displacement of the bound nucleotide, thus promoting nucleotide exchange. These findings are consistent with the kinetic mechanism established for other glyceraldehyde-3-phosphate dehydrogenases.

Similar activation was seen in extracts of other species of the Chlorophyta but

* Present address: Department of Biochemistry, University of Leeds, Leeds, U.K.

not in other photosynthetic organisms. The significance of this type of activation of enzyme activity to the metabolism of these species of algae is discussed.

INTRODUCTION

In higher plants, two major forms of glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.–) are present, which differ in their coenzyme specificity. $NADP^+$-linked activity is found only in the chlorophyll-containing tissues whereas NAD^+-linked activity is ubiquitous [1]. Since the chloroplast enzyme (EC 1.2.1.13) shows mutual competition of its NAD^+-linked and $NADP^+$-linked activities, the two coenzymes are thought to occupy the same site on the enzyme [2]. In contrast the cytoplasmic enzyme (EC 1.2.1.12) is active only with NAD^+ or NADH as coenzyme.

A slow increase in $NADP^+$-linked glyceraldehyde-3-phosphate dehydrogenase activity is seen in etiolated seedlings on prolonged illumination with far red light. This process is mediated by phytochrome and is independent of photosynthesis. It is inhibited by inhibitors of protein synthesis and hence involves de novo synthesis of enzyme [3]. In contrast, Ziegler's group [4, 5] have reported a very rapid, reversible light-dependent activation of the $NADP^+$-linked glyceraldehyde-3-phosphate dehydrogenase of leaves, which is independent of protein synthesis. This stimulation of the $NADP^+$-linked enzyme activity was later shown to be accompanied by a decrease in the NAD^+-linked glyceraldehyde-3-phosphate dehydrogenase activity [6]. Illumination of broken chloroplasts [7] was also found to result in an increase in the $NADP^+$-linked enzyme activity at the expense of that linked to NAD^+. Interestingly, with these broken chloroplast preparations the same effect could be achieved in the dark by the addition of NADPH [8] or ATP [9]. These findings suggest that in vivo activation occurred as a result of the photosynthetic production of these two metabolites.

In algae, Hudock and Fuller [10] have reported light-dependent reciprocal activity changes of the NAD^+- and $NADP^+$-linked glyceraldehyde-3-phosphate dehydrogenase during the greening of the y-2 mutant strain of *Chlamydomonas rheinhardii*. They suggest that in this organism the NAD^+-linked enzyme might be converted into an $NADP^+$-linked enzyme during illumination. In contrast, their work with greening cultures of *Euglena gracilis* indicated no conversion of an NAD^+-linked enzyme into an $NADP^+$-linked form.

We have recently isolated two glyceraldehyde-3-phosphate dehydrogenases from the photosynthetic alga *Scenedesmus obliquus* [11], one of which had predominantly NADPH-linked activity (T-enzyme) whereas the other showed high NADH-linked activity (D-enzyme). Apart from their molecular weights and coenzyme specificities, these enzymes were very similar. It was found that whilst both enzymes could normally be isolated from extracts of *S. obliquus*, occasionally preparations were obtained which yielded only a single enzyme. In some cases, only the T-enzyme was obtained whereas in others only the D-enzyme was present. This suggested the possibility that the two enzymes might be interconvertible.

We now wish to report the conversion of the NADH-linked glyceraldehyde-3-phosphate dehydrogenase of *S. obliquus* into a NADPH-linked enzyme. A preliminary report of some of these findings has been published [12].

MATERIALS AND METHODS

Culture of organisms. Rhodopseudomonas capsulata (ATCC. 23782) was cultured as described by Sistrom [13], *Anacystis nidulans* (Bloomington 625) as described by Kratz and Myers [14], *S. obliquus* (Cambridge 276/6a) and *Chlorella pyrenoidosa* (Cambridge 211/8h) as described by Kessler et al. [15], *E. gracilis* (Cambridge 1224/5z) as described by Hutner et al. [16], *C. rheinhardii* (Cambridge 11/32a) as described by Sueoka [17], *Porphyridium cruentum* (Cambridge 1380/1a) as described by Jones et al. [18].

Preparation of crude enzyme extracts. Algal or bacterial cells were broken by shaking at 4000 rev./min with glass beads in a Braun cell homogenizer, 20 ml glass beads : 20 ml of cell suspension in 0.1 I Tris · HCl buffer (0.12 M), pH 7.5, 5 mM mercaptoethanol. The total breaking time was 5 min, in 30 s bursts separated by 10 s cooling by means of liquid CO_2. After removal of the glass beads by filtration, the extract was centrifuged at 200 000 $\times g$ for 30 min and the supernatant used for enzyme assay.

50 g of spinach leaf was macerated for 3 min in a Waring blender, using the same buffer as used for the algal extracts. The large debris was removed by filtration through muslin; after which the 200 000 $\times g$ supernatant was used for enzyme assay.

Enzyme purification and assay. Glyceraldehyde-3-phosphate dehydrogenases of *S. obliquus* were purified and assayed as previously described [11].

Enzyme activation for kinetic measurements. 0.3 units of the purfied D-enzyme in 20 ml of 85 mM Tris · HCl, pH 7.5, was incubated at 30 °C with the compounds specified. At various times, 0.5-ml aliquots were removed and any remaining components necessary for assaying the enzyme were added. The enzyme activity was measured after starting the reaction with reduced pyridine nucleotide. The final concentrations in the assay mixture (1 ml) were ATP (1.6 mM), cysteine · HCl (3.3 mM), $MgCl_2$ (1.6 mM), phosphoglycerate kinase (10 units/ml), 3-phosphoglycerate (3.5 mM) and reduced pyridine nucleotide (0.25 mM).

Sedimentation velocity measurements in the presence of 1,3-diphosphoglycerate-generating system. The sedimentation velocity of the purified D-enzyme (4 mg/ml in 0.1 I (0.12 M) Tris, pH 7.5, 5 mM mercaptoethanol) was determined using a Beckman model E analytical ultracentrifuge at 60 000 rev./min and a rotor temperature of 10 °C. Double sector cells with Epon-aluminium centrepieces were used in an AN-D rotor.

The enzyme was incubated with a 1,3-diphosphoglycerate-generating system in the centrifuge cell. The final concentrations in the incubation mixture were D-enzyme (3.3 mg/ml), ATP (1.6 mM), cysteine · HCl (5.2 mM), $MgCl_2$ (2.5 mM), 3-phosphoglycerate (5.5 mM), phosphoglycerate kinase (16 units/ml), Tris · HCl, pH 7.5 (0.1 I) and mercaptoethanol (4 mM). After 30 min incubation at 30 °C, the determination of the sedimentation velocities of the components of the incubation mixture was commenced. The ultracentrifuge cell containing the incubation mixture was then allowed to stand overnight at 4 °C after which the sedimentation velocity of its components was redetermined. The NADH- and NADPH-linked glyceraldehyde-3-phosphate dehydrogenase was also measured at specified times in a parallel incubation.

Light-dependent greening of S. obliquus PG1. The PG*1* strain of *S. obliquus* (derived from the wild type by treatment with *N*-methyl-*N'*-nitroso-*N*-nitroguanidine [19] and selected for its reduced pigment content) was grown heterotrophically in the medium described above. Under these conditions, the strain is only able to synthesise

very small amounts of chloroplast pigment and consequently has a much reduced rate of photosynthesis compared with that of the wild type. After 3 days heterotrophic growth the alga was harvested, resuspended in autotrophic medium (heterotrophic medium without glucose and yeast extract) and shaken overnight in the dark. The following day the starved cells were diluted to a concentration of $4 \cdot 10^7$ cells/ml and illuminated in glass tubes with white light whilst being constantly gassed with a CO_2/air (5 : 95, v/v) mixture. On illumination, this strain is then able to synthesise chlorophylls and other chloroplast pigments; this is accompanied by an increase in the photosynthetic rate. At specified times before and during the illumination 500-ml aliquots were removed, the algal cells harvested, broken and the coenzyme dependence of the glyceraldehyde-3-phosphate dehydrogenase activity of the extracts was determined.

RESULTS

Ligand-induced changes of coenzyme specificity

The D-enzyme of *S. obliquus* had a very low level of NADPH-linked glyceraldehyde-3-phosphate dehydrogenase activity as well as its major NADH-linked activity. This NADPH-dependent activity, in contrast to the NADH-dependent activity, was not constant during the assay and increased with time. The possibility that the NADPH-dependent activity of the enzyme was being stimulated by a component of the assay was tested by incubation of the enzyme with the various components of the mixture prior to the measurement of the enzyme activities.

The activity of glyceraldehyde-3-phosphate dehydrogenase from other sources has been shown to be stimulated by sulphydryl-containing reagents [8]; incubating the D-enzyme of *S. obliquus* with cysteine did cause a slight stimulation of the NADPH-dependent activity although the NADH-dependent activity was unaffected (Fig. 1A). This activation with cysteine was complete after 40 min but did not account for the total activation observed when the enzyme was incubated with the assay mixture. If the enzyme was incubated with the 1,3-diphosphoglycerate-generating system of the assay after preincubation with cysteine, further activity changes were seen to have occurred when the reaction was started by the addition of reduced nucleotide (Fig. 1A). A large increase in the NADPH-dependent enzyme activity was seen, accompanied by a considerable reduction in the NADH-dependent activity. In general a 10-fold increase in the NADPH-dependent rate (relative to that after preincubation with cysteine) was observed; whilst the NADH-linked activity fell to half its original value (Fig. 1A). This degree of stimulation of the NADPH-dependent activity could only be achieved after preincubation with both cysteine and the 1,3-diphosphoglycerate-generating system. When cysteine was left out of the preincubation system, the activated NADPH-dependent enzyme activity was less than half that seen previously. However, this rate could be stimulated by incubation with cysteine (Fig. 1B). Thus, for complete activation of the NADPH-dependent enzyme activity, both cysteine and some component of the 1,3-diphosphoglycerate-generating system were required.

The NADPH-dependent activity of spinach [9] and bean [20] glyceraldehyde-3-phosphate dehydrogenases is stimulated by ATP; however, this was not the case for the enzyme from *S. obliquus* which was not stimulated by either ATP or phospho-

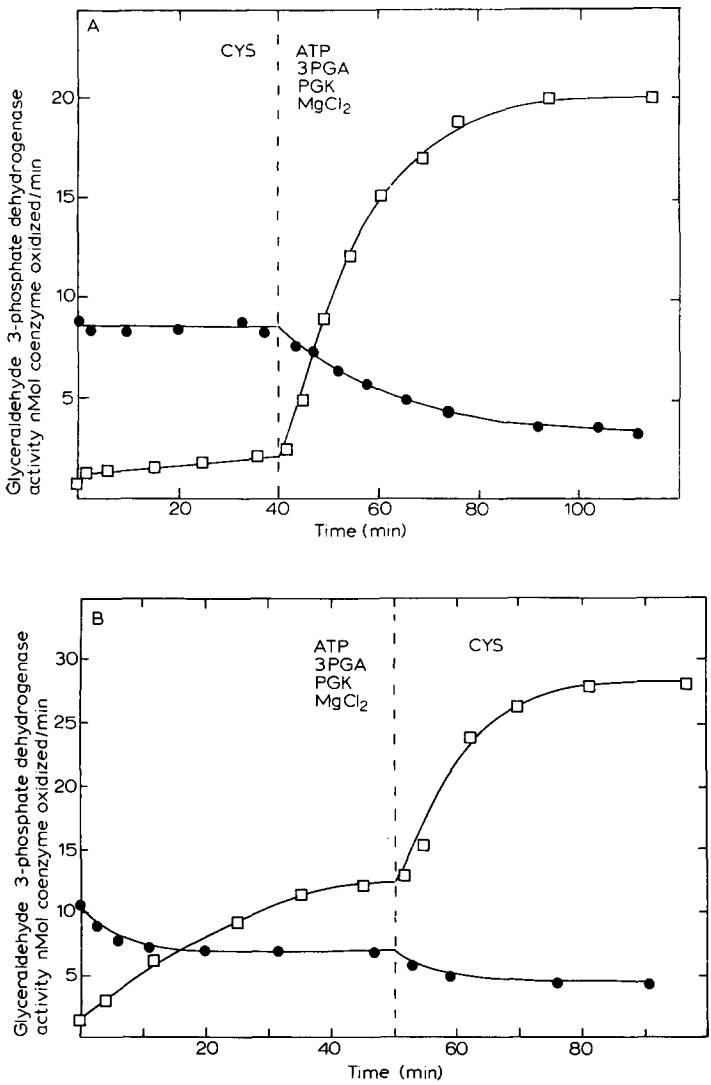

Fig. 1. Variation in the coenzyme specificity of glyceraldehyde-3-phosphate dehydrogenase (*S. obliquus* D-enzyme). The enzyme was preincubated with either (A) cysteine · HCl (3.3 mM) or (B) a 1,3-diphosphoglyceric acid synthetic mixture (1.6 mM ATP, 3.5 mM 3-phosphoglyceric acid, 1.6 mM $MgCl_2$, and 10 units/ml of phosphoglycerate kinase), until no further increase in NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase activity occurred; at this time either (A) a 1,3-diphosphoglyceric acid synthetic mixture or (B) cysteine · HCl were added for a further period of incubation. □—□, NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase activity; ●—●, NADH-dependent glyceraldehyde-3-phosphate dehydrogenase activity.

glycerate kinase. Indeed the only component of the 1,3-diphosphoglycerate-generating system which had any effect on the NADPH-dependent activity of the enzyme was 3-phosphoglycerate. This compound promoted a slight increase in the NADPH-dependent activity of the enzyme, accompanied by a very slight decrease in the NADH-linked activity.

The large stimulation of the NADPH-dependent activity resulting from the incubation of the cysteine-preincubated enzyme with the 1,3-diphosphoglycerate system does not occur when the enzyme is incubated with each of the components of the generating system separately. Hence it would appear that the compound promoting this effect is likely to be one of the products of the phosphoglycerate kinase reaction. Since ADP did not stimulate the enzyme, 1,3-diphosphoglycerate is most likely to be responsible for the effect observed.

It has been difficult to test the effect of 1,3-diphosphoglycerate directly because of its very high lability. The related compound, 2,3-diphosphoglycerate, which is not a substrate for the enzyme, was incubated with the D-enzyme. This treatment also resulted in a large increase in the NADPH-dependent activity accompanied by a corresponding decrease in the NADH-dependent activity (Fig. 2). However, only on further incubation with the 1,3-diphosphoglycerate-generating system was the full activation seen.

The relationship between the extent of activation of the NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase activity and the concentration of the various ligands was investigated and is shown in Fig. 3. The effects of 3-phosphoglycerate and 2,3-diphosphoglycerate were exhibited in the millimolar concentration region. When the concentration of 1,3-diphosphoglycerate present in the generating

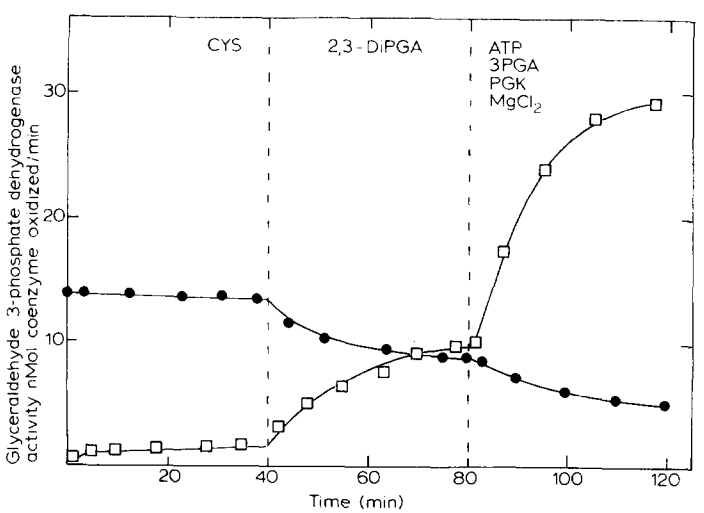

Fig. 2. The effect of 2,3-diphosphoglycerate on the nucleotide specificity of the D-enzyme. D-enzyme was pre-incubated for 40 min with cysteine · HCl (3.3 mM) and the activity with NADH (●—●) and NADPH (□—□) as cofactors was measured. 2,3-Diphosphoglycerate (7.5 mM) was then added and after a further period of incubation of 40 min, the 1,3-diphosphoglycerate-generating system was also included. Stimulation of the NADPH-dependent enzyme activity was seen on addition of 2,3-diphosphoglycerate but complete activation of the enzyme was only observed in the presence of the 1,3-diphosphoglycerate-generating system.

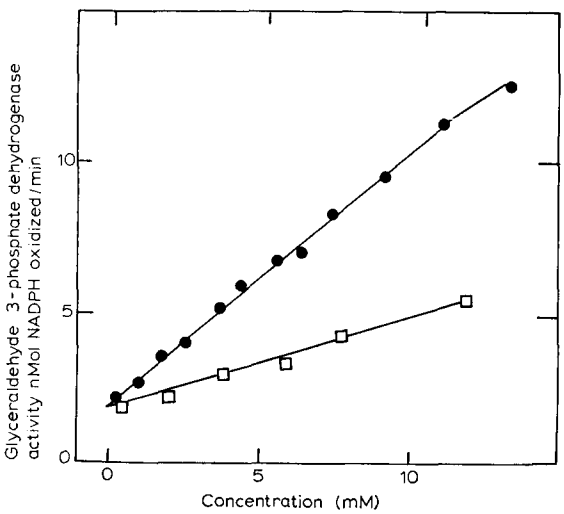

Fig. 3. Effect of incubation of the D-enzyme with varying concentrations of 3-phosphoglycerate and 2,3-diphosphoglycerate. The NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase activity (V) was determined (as described in Materials and Methods) after incubation of the D-enzyme with 3-phosphoglycerate (□—□) and 2,3-diphosphoglycerate (●—●) of varying concentrations. The NADPH-dependent activity (V_0) after 40 min pre-incubation with cysteine · HCl (3.3 mM) was taken as the zero level.

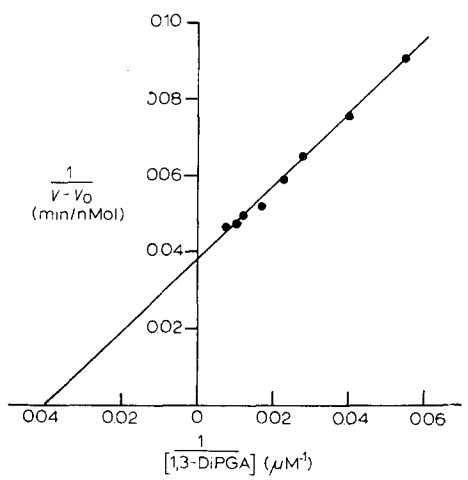

Fig. 4. Relationship between the NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase activity of the D-enzyme and the 1,3-diphosphoglycerate concentration. D-enzyme was incubated with the 1,3-diphosphoglycerate-generating system, as described in Materials and Methods, until maximum activity with NADPH as coenzyme was attained (in practice incubation for 40 min sufficed). The initial concentration of 3-phosphoglycerate in the generating system was adjusted to give the required 1,3-diphosphoglycerate concentration which was calculated from the equilibrium constant of the phosphoglycerate kinase reaction [21]. The final NADPH-dependent rate (V) was corrected for the initial activity of the D-enzyme with this cofactor (V_0). The linearity of the reciprocal plot demonstrates the hyperbolic nature of the binding curve for 1,3-diphosphoglycerate and linear regression analysis of the data gives a value of 25 μM for the dissociation constant of the enzyme-1,3-diphosphoglycerate complex.

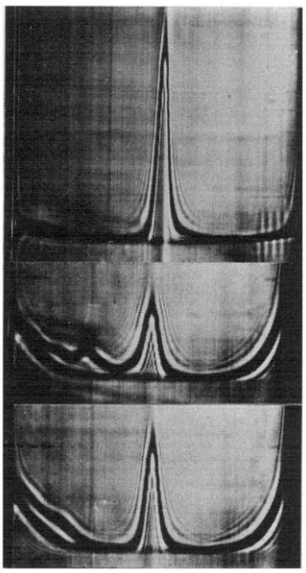

Fig. 5. Sedimentation characteristics of the D-enzyme. Top: Native D-enzyme. Centre: After incubation with cysteine · HCl and 1,3-diphosphoglyceric acid-generating system (as described in Materials and Methods). Bottom: as centre but after storage overnight. Buffer used was 0.1 I (0.12 M) Tris · HCl, pH 7.5. Photographs were taken 37 min after reaching speed of 60 000 rev./min at 10 °C, using a Schlieren plate angle of 60 °.

system was calculated from the equilibrium constant of the phosphoglycerate kinase reaction [21], it was found to be effective in stimulating the NADPH-dependent activity at much lower concentrations than those of the other activators. The dissociation constant of the enzyme 1,3-diphosphoglycerate complex was determined from a reciprocal plot (Fig. 4) and found to be 25 μM.

The stimulation of the NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase activity by 1,3-diphosphoglycerate was blocked by both NAD^+ (1 mM) and inorganic phosphate (50 mM).

The NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase activity of the spinach chloroplast enzyme is known to be stimulated by incubation with NADPH [8]. However, whilst incubation of the D-enzyme of *S. obliquus* with cysteine and NADPH did cause a stimulation of the NADPH-dependent enzyme activity, the extent of this stimulation was even less than that seen after incubation with 2,3-diphosphoglycerate.

The D-enzyme sedimented as a single, symmetrical boundary of $s_{20,w}$= 14.5 S (Fig. 5, top) but on incubation with cysteine and the 1,3-diphosphoglycerate-generating system, the Schlieren pattern demonstrated the presence of three distinct sedimenting boundaries with $s_{20,w}$ values of 14.5 S, 7.5 S and 5.5 S (Fig. 5, centre). The sedimentation coefficients of the faster two boundaries correspond to the native D- and T-enzymes, suggesting that the D-enzyme might have been converted to a form similar to the native T-enzyme. If this sample was allowed to stand overnight at 4 °C, subsequent centrifugation resulted in the distribution of components shown in the lower photograph of Fig. 5. The boundary corresponding to the sedimentation

TABLE I

ACTIVATION OF THE NADPH-DEPENDENT GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE ACTIVITY OF THE D-ENZYME OF *S. OBLIQUUS* ACCOMPANYING SEDIMENTATION VELOCITY CHANGES (Fig. 5)

D-Enzyme (4 mg/ml) was incubated with the 1,3-diphosphoglycerate-generating system as described in Materials and Methods. Aliquots were removed at specified stages of the procedure and the coenzyme dependence of the glyceraldehyde-3-phosphate dehydrogenase activity determined.

Stage	Glyceraldehyde-3-phosphate dehydrogenase activity (units/ml)		Ratio of NADH : NADPH-dependent activity
	NADH-linked	NADPH-linked	
Pre-incubation in the absence of the 1,3-diphosphoglycerate-generating system (Fig. 5, top)	112	10	11.2
After 30 min incubation with the 1,3-diphosphoglycerate-generating system (Fig. 5, centre)	50	35	1.4
After overnight standing, following incubation with the 1,3-diphosphoglycerate-generating system (Fig. 5, bottom)	61	6	10.2

coefficient of the D-enzyme (14.5 S) was enlarged whilst that corresponding to the T-enzyme (7.5 S) was no longer evident. Analysis of the sample at this stage demonstrated that neither ATP nor 1,3-diphosphoglycerate were present and this may be readily accounted for by the lability of the 1,3-diphosphoglycerate. The removal of the 1,3-diphosphoglycerate by overnight standing was accompanied by reversal of the major sedimentation changes promoted by the 1,3-diphosphoglycerate-generating system. However, the slow sedimenting boundary of $s_{20,w} = 5.5$ S remained even after the removal of the 1,3-diphosphoglycerate, suggesting that the generation of this species is not readily reversible (Fig. 5, bottom). These observed changes in sedimentation are consistent with a reversible D- to T′-enzyme (activated D-enzyme with activity similar to the T-enzyme) conversion in the presence of cysteine and the 1,3-diphosphoglycerate-generating system. However, this conversion is not complete by the criterion of ultracentrifugation, a substantial proportion of the D-enzyme (14.5 S) remaining in the presence of 1,3-diphosphoglycerate (Fig. 5, centre). The conversion was also incomplete by the kinetic criteria already described (Table I). The data in Table I demonstrate that following overnight storage of the 1,3-diphosphoglycerate-treated D-enzyme, the coenzyme specificity had returned to that of the untreated enzyme. This is shown by the decline in NADPH-dependent activity and the increase in the ratio of activity with NADH and NADPH, respectively, as cofactors. The overall decrease in enzyme activity can probably be attributed to the irreversible generation of the 5.5-S species, if this species is enzymically inactive.

The dissociation of the enzyme when incubated with the 1,3-diphosphoglycerate-generating system was blocked by either NAD^+ or inorganic phosphate, as was the kinetic activation of the NADPH-dependent enzyme activity.

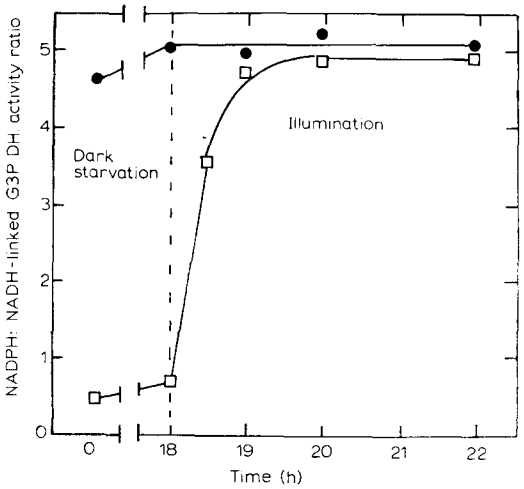

Fig. 6. Effect of illumination on the NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase activity of heterotrophically grown *S. obliquus* PGl. Heterotrophically grown *S. obliquus* PGl was harvested; the cells were washed and starved by overnight shaking in autotrophic medium. The starved cells were illuminated with white light. At various times aliquots were taken and extracts made from the harvested cells. The extracts were assayed for their glyceraldehyde-3-phosphate dehydrogenase activity. The ratio of the NADPH : NADH-dependent enzyme activity of the extracts (□—□) was compared with that following incubation of the extracts with the 1,3-diphosphoglycerate-generating system for 30 min (●—●).

Effect of growth conditions on the coenzyme dependence of S. obliquus glyceraldehyde-3-phosphate dehydrogenase activity

The ratio of NADPH : NADH glyceraldehyde-3-phosphate dehydrogenase activity in extracts of the heterotrophically grown alga was 0.3, whereas the extracts from autotrophically grown alga showed a ratio of 6.0.

In heterotrophically grown PGl strain of *S. obliquus* the glyceraldehyde-3-phosphate dehydrogenase activity was predominantly linked to NADH (Fig. 6). After overnight starvation, there was only a slight increase in the NADPH : NADH dependency of the enzyme activity. However, on illumination of the suspension of these starved cells in autotrophic medium a marked increase in this ratio occurred, the enzyme activity becoming predominantly linked to NADPH. Even after only 1 h of illumination the NADPH : NADH ratio of enzyme activity of the extracts had become the same as that when the extracts had been incubated with cysteine and the 1,3-diphosphoglycerate-generating system for 30 min.

Distribution of similarly activatable glyceraldehyde-3-phosphate dehydrogenases

The activation of the NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase activity of the D-enzyme of *S. obliquus* by cysteine and the 1,3-diphosphoglycerate-generating system is so pronounced that it can easily be seen in crude extracts of this alga. The effect of such an incubation on the glyceraldehyde-3-phosphate dehydrogenase activity of crude extracts of other photosynthetic organisms is shown in Table II. A similarly activatable enzyme has been detected in *Chlamydomonas* and *Chlorella*; however, it has not been detected in extracts of higher plants nor in extracts of algae from groups other than the Chlorophyta.

TABLE II

GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE ACTIVITY OF EXTRACTS OF PHOTOSYNTHETIC ORGANISMS

Results are expressed as μmol coenzyme oxidized/min per ml.

Organism	Initial NADH-dependent activity	Activated NADH-dependent activity	Initial NADPH-dependent activity	Activated NADPH-dependent activity
Athiorhodaceae				
Rhodopseudomonas capsulata	4.2	4.1	0	0
Cyanophyta				
Anacystis nidulans	0.3	0.3	2.0	1.9
Chlorophyta				
Scenedesmus obliquus	4.0	1.4	0.9	3.0
Chlamydomonas rheinhardii	7.5	1.5	3.5	6.0
Chlorella pyrenoidosa	3.3	1.9	0.8	3.5
Euglenaphyta				
Euglena gracilis	21.0	20.0	3.0	3.0
Rhodophyta				
Porphyridium cruentum	4.0	4.0	1.5	1.5
Euphyta				
Spinach leaf	3.0	3.0	5.7	5.1

DISCUSSION

Incubation of the D-enzyme with a 1,3-diphosphoglycerate-generating system results in an increase in the NADPH-dependent specific activity accompanied by a decrease in NADH-dependent specific activity. The data presented suggest that 1,3-diphosphoglycerate itself is responsible for the changes in nucleotide dependence and it thus acts as both substrate and activating ligand. The dissociation constant determined for the enzyme-1,3-diphosphoglycerate complex in the activation process is the same as the K_m for 1,3-diphosphoglycerate and implies that 1,3-diphosphoglycerate binding to the active centre is responsible for the changes in nucleotide dependence. It also implies that the observed changes could be elicited at physiological concentrations of the ligand. The changes in kinetic behaviour are accompanied by alteration in the sedimentation behaviour of the enzyme. Prior to activation, the D-enzyme exists as a discrete 14.5-S species. Following activation, boundaries of 7.5 S and 5.5 S are generated. The former corresponds in value to that of the native T-enzyme. It is likely therefore that the changes in nucleotide specificity are accompanied by depolymerization of the D-enzyme (550 000 mol. wt.) to a species resembling the T-enzyme (140 000 mol. wt.). However, under the conditions of ultracentrifugation, the transition from the D to the T′ form of the enzyme is incomplete as determined both kinetically and by the ultracentrifugation. This is most probably due to the higher protein concentration employed in the ultracentrifuge and implies that dissociation of the enzyme is a prerequisite for the change in nucleotide specificity. The long-term loss in activity of the enzyme is paralleled by an increase in the 5.5-S species which probably represents an inactive form of the enzyme.

The mechanism of activation by 1,3-diphosphoglycerate can best be explained in terms of the compulsory order mechanism proposed for the reduction of this substrate by glyceraldehyde-3-phosphate dehydrogenase in a number of species [22, 23] (Fig. 7). According to this mechanism, acylation of the active centre sulphydryl group by 1,3-diphosphoglycerate is followed by the release of phosphate and NAD^+. Hence inhibition of the D to T′ transition by these ligands in the present investigation can be explained if the dissociation of the enzyme and the change in nucleotide specificity arises from a conformational change in the acyl-enzyme intermediate ($_DE_{SCOR}$ Fig. 7). The ability of 2,3-diphosphoglycerate to elicit a moderate stimulation of the NADPH-dependent activity could be achieved through the formation of an abortive complex similar to the acyl-enzyme. The slight stimulation of activity by incubation with NADPH may result from a preference of this nucleotide for the T′ configuration of the enzyme. It should be noted, however, that all ligand-mediated stimulation of the NADPH-dependent activity is dependent on the presence of cysteine or other mercaptans. It is likely that reduction of inter- or intrasubunit disulphide bonds is an integral factor in the activation mechanism.

The rapid in vivo light-dependent stimulation of NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase activity has been observed in all plants and algae with the exception of *E. gracilis* [5]. Inhibition by 3(3,4-dichlorophenyl)-1,1-dimethylurea suggests that non-cyclic photosynthetic electron flow is responsible for this light-dependent stimulation of enzyme activity [5]. The activation occurs even in broken spinach chloroplast preparation [7], and in this preparation addition of ATP [9] or NADPH [8] in the dark results in activation of the NADPH-dependent enzyme activity. Dark activation is also found in partially purified spinach chloroplast glyceraldehyde-3-phosphate dehydrogenase [8]. In vivo illumination results in the production of NADPH and ATP, both of which are capable of stimulating the NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase activity of the chloroplast enzyme, so fitting the enzyme for its role in the Calvin cycle [8]. In the case of the D-enzyme of *S. obliquus*, ATP has no activating effect on the NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase activity. Whilst NADPH itself does stimulate the NADPH-dependent activity of the enzyme 3-fold; this is much less than the 10-fold stimulation produced by the 1,3-diphosphoglyceric acid-generating system.

The properties of the D-enzyme of *S. obliquus* reported here are consistent with an in vivo light activation of the NADPH-dependent glyceraldehyde-3-phosphate

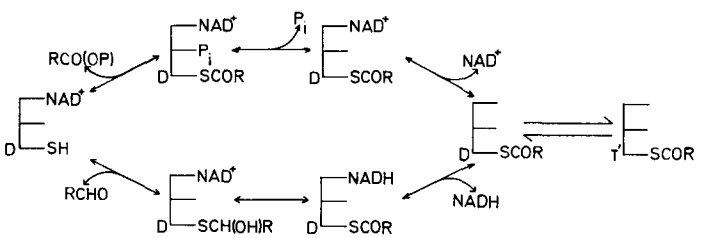

Fig. 7. Scheme for the interconversion of the D- and T′-enzyme forms in the presence of 1,3-diphosphoglycerate. The model is based on the kinetic mechanism demonstrated for the muscle and pea enzymes [22, 23] and accounts for the inhibition of D- to T′-enzyme conversion by inorganic phosphate and NAD^+.

dehydrogenase activity similar to that proposed for higher plants [8]. However, in the case of the alga we believe 1,3-diphosphoglycerate to be directly responsible for this activation. Although photosynthetically produced ATP will have no direct action on the enzyme, its availability will directly determine the level of 1,3-diphosphoglycerate in the chloroplast. This would account for the interconversion of nucleotide dependence of the glyceraldehyde-3-phosphate dehydrogenase activity observed during the light-dependent greening of the y-2 mutant of *C. rheinhardii* [10] and also in the illuminated cultures of dark grown *S. obliquus* PGl.

The differences seen in the modes of activation of the NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase activity of *S. obliquus* and spinach chloroplasts [8, 9] probably reflect the differences in the metabolism of the two species. Whilst spinach is an obligate autotroph, the alga is capable of both autotrophic and heterotrophic growth. In the heterotrophic alga, the chloroplast glyceraldehyde-3-phosphate dehydrogenase would be expected to be in the D-configuration, since under these conditions the concentration of chloroplast 1,3-diphosphoglycerate would be insignificant and the D to T′ transition driven by 1,3-diphosphoglycerate is reversible (Fig. 5, Table I). Extracts of heterotrophic alga do indeed exhibit a preponderance of NADH (NAD^+)-dependent enzyme activity whereas in those from the autotrophically grown alga the activity is predominantly linked to NADPH ($NADP^+$). The heterotrophically grown alga metabolizes glucose by glycolysis and the products are imported into the chloroplast, the site of the synthesis and storage of starch. The import of carbon compounds from the cytoplasm occurs at the level of triose phosphates [24] and these are able to act both as precursors of starch and as sources of chloroplast ATP by oxidation to 3-phosphoglycerate. This ATP must be provided to drive the later stages of starch synthesis [25] and in the dark can no longer be produced by photosynthetic phosphorylation. This indirect import of ATP is necessary because the chloroplast membranes are virtually impermeable to ATP [26]. The oxidation of the imported triose phosphate is likely to be catalysed by glyceraldehyde-3-phosphate dehydrogenase in the D-configuration. The NADH generated in this oxidation could then be exported to the cytoplasm by operation of the oxaloacetate/malate shuttle [27]. This type of oxidative function of the chloroplast enzyme (summarised in Fig. 8) would clearly be required only in organisms capable of substantial heterotrophic nutrition. The inability of ATP to activate the NADPH-dependent enzyme activity of the D-enzyme of *S. obliquus* may merely be a consequence of the need to retain the enzyme in the D-configuration to generate chloroplast ATP in the dark. In contrast, the spinach chloroplast enzyme would not usually be required to function in this way since the autotrophic metabolism of the leaf results in export of carbon coumponds from the chloroplast rather than their import.

In view of the activation properties of the D-enzyme of *S. obliquus*, it is likely that the alga only synthesises a single glyceraldehyde-3-phosphate dehydrogenase and this protein adopts a particular configuration dependent upon the ligands it encounters. Thus in the autotrophic chloroplast, in the presence of 1,3-diphosphoglycerate a form of the enzyme with predominantly NADPH-dependent activity would be favoured; whereas in the chloroplast under heterotrophic conditions and also in the cytoplasm, the D-form of the enzyme would be adopted. Indeed the use of selective inhibitors of protein biosynthesis in *C. rheinhardii* has shown that the synthesis of the chloroplast NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase does

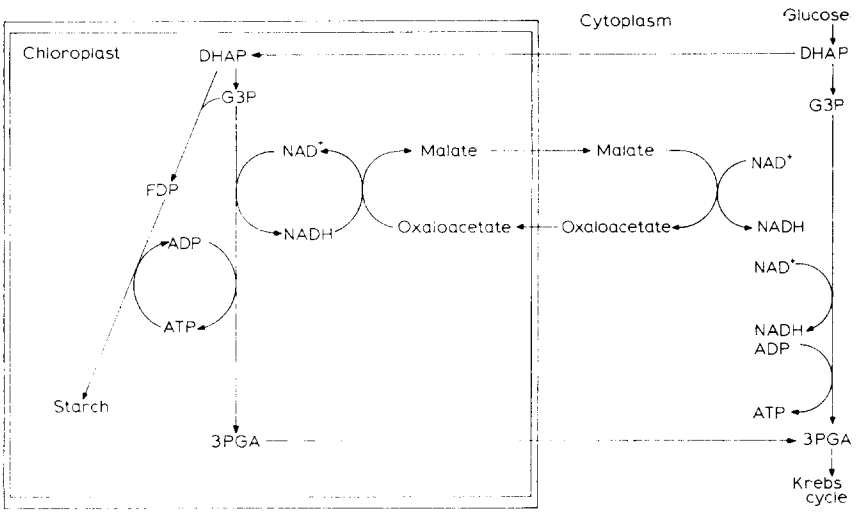

Fig. 8. Possible role of chloroplast glyceraldehyde-3-phosphate dehydrogenase in the heterotrophic metabolism of *S. obliquus*. In this situation both the chloroplast and cytoplasmic glyceraldehyde-3-phosphate dehydrogenases would be expected to be in the D-configuration whereas in the autotrophic alga the chloroplast enzyme would be in the T′-form.

not involve the 70-S chloroplast ribosomes [28] suggesting that the enzyme is transported into the chloroplast from its cytoplasmic site of synthesis. This contrasts with the case in pea plants, where an immunological study indicated that the chloroplast enzyme differs appreciably from the cytoplasmic enzymes in respect to primary structure and is likely to be transcribed from a different structural gene [1].

ACKNOWLEDGEMENTS

We thank the Science Research Council for the award of a Research Studentship to M. J. O'Brien; and Professor D. A. Walker for helpful discussions.

REFERENCES

1 McGowan, R. E. and Gibbs, M. (1974) Plant Physiol. 54, 312–319
2 Melandri, B. A., Baccarini, A. and Pupillo, P. (1968) Biochem. Biophys. Res. Commun. 33, 160–164
3 Cerff, R. and Quail, P. H. (1974) Plant Physiol. 54, 100–104
4 Ziegler, H. and Ziegler, I. (1965) Planta 65, 369–380
5 Ziegler, H., Ziegler, I., Schmidt-Clausen, H. J., Müller, B. and Dorr, I. (1969) Prog. Photosynth. Res. 3, 1636–1645
6 Ziegler, H., Ziegler, I. and Schmidt-Clausen, H. J. (1968) Planta 81, 181–192
7 Müller, B. and Ziegler, H. (1969) Planta 85, 96–104
8 Müller, B., Ziegler, I. and Ziegler, H. (1969) Eur. J. Biochem. 9, 101–106
9 Müller, B. (1970) Biochim. Biophys. Acta 205, 102–109
10 Hudock, G. A. and Fuller, R. C. (1965) Plant Physiol. 43, 1205–1211
11 O'Brien, M. J. and Powls, R. (1976) Eur. J. Biochem. 63, 155–161
12 O'Brien, M. J. and Powls, R. (1974) Proc. 3rd Int. Congr. Photosynth. Res. pp. 1431–1439, Elsevier, Amsterdam

13 Sistrom, W. R. (1960) J. Gen. Microbiol. 22, 778–787
14 Kratz, W. A. and Myers, J. (1955) Am. J. Bot. 42, 282–287
15 Kessler, E., Arthur, W. and Brugger, J. E. (1957) Arch. Biochem. Biophys. 71, 326–335
16 Hutner, S. H., Bach, M. K. and Ross, G. I. M. (1956) J. Protozool. 3, 101–112
17 Sueoka, N. (1960) Proc. Natl. Acad. Sci. U.S. 46, 83–89
18 Jones, R. F., Speer, H. L. and Kury, W. (1963) Physiol. Plant. 16, 636–643
19 Bishop, N. I. (1971) in Methods in Enzymology (San Pietro, A., ed.), Vol. 23, pp. 130–143, Academic Press, London
20 Bradbeer, J. W. (1973) in Biosynthesis and its control in plants (Milborrow, R. V., ed.), pp. 279–302, Academic Press, London
21 Cori, C. F., Velick, S. F. and Cori, G. T. (1950) Biochim. Biophys. Acta 4, 160–169
22 Moras, D., Olsen, K. W., Sabeson, M. N., Buchner, M., Ford, G. C. and Rossman, M. G. (1975) J. Biol. Chem. 250, 9137–9162
23 Harrigan, P. J. and Trentham, D. R. (1973) Biochem. J. 135, 695–703
24 Heber, U. (1974) Annu. Rev. Plant. Physiol. 25, 393–421
25 Recondo, E. and Leloir, L. F. (1961) Biochem. Biophys. Res. Commun. 6, 85–88
26 Heldt, H. W. (1969) FEBS Lett. 5, 11–14
27 Heber, U. and Krause, G. H. (1971) Proc. 2nd Int. Congr. Photosynth. Res., Stresa, Vol. 2, 1023–1033
28 Surzycki, S. J., Goodenough, U. W., Levine, R. P. and Armstrong, J. J. (1970) Symp. Soc. Exp. Biol. 24, 13–37