FURTHER OBSERVATIONS ON GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASES IN PLANTS AND PHOTOSYNTHETIC BACTERIA[1]

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Three different dehydrogenases acting on glyceraldehyde 3-phosphate have been found in plants. Gibbs (1952) and Arnon (1952) have shown that green leaves contained two separate GPD[2] enzymes, one requiring DPN, the other TPN. The TPN-dependent GPD was found only in green tissues, while the DPN-dependent enzyme occurred in both photosynthetic and non-photosynthetic plant tissues. It was suggested that the TPN-dependent GPD functioned in photosynthesis, while the DPN-dependent GPD participated only in carbohydrate metabolism during respiration. This concept was supported by studies of the relative activities of the two enzymes during the growth of pea plants (Hageman and Arnon, 1955) and by the finding that photosynthetic phosphorylation was TPN specific (Jagendorf, 1956).

Recently, Smillie and Fuller (1960) have followed the cellular levels of a number of enzymes in growing pea leaves. They found that both the DPN-dependent and TPN-dependent GPD showed developmental patterns characteristic of photosynthetic enzymes. This finding constituted further

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[1]Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

[2]Abbreviations used: DPN for diphosphopyridine nucleotide; TPN for triphosphopyridine nucleotide; GPD for glyceraldehyde 3-phosphate dehydrogenase; GTR for glyceraldehyde 3-phosphate TPN reductase; and 3-PGA for 3-phosphoglyceric acid.

evidence for the involvement of TPN-dependent GPD in photosynthesis, but also indicated that the DPN-dependent enzyme participated in photosynthesis.

The third plant enzyme (GTR) acting on glyceraldehyde-3-phosphate was also TPN dependent. The reaction proceeded irreversibly to 3-PGA and was independent of the coupled phosphorylation of adenosine diphosphate (Rosenberg and Arnon, 1955).

This communication presents additional data on the distribution of the three enzymes in plants and photosynthetic bacteria and their possible roles in photosynthesis.

Results and Discussion

DPN-dependent GPD.--As was indicated above, the results of Smillie and Fuller (1960) suggested that this enzyme may play a role in photosynthesis. Evidence supporting this suggestion has been elicited from intracellular distribution studies, which disclosed that most, but not all of the cellular DPN-dependent GPD in green pea leaves was associated with the chloroplasts.

Quantitative measurements of the DPN-dependent GPD in a number of different organisms provided further pertinent information on the possible roles of this enzyme. In extracts prepared from nearly fully expanded pea leaves, the activity of the DPN-dependent GPD was comparable with a number of photosynthetic enzymes including the TPN-dependent GPD and was from 40-50 times as active as glycolytic enzymes such as phosphohexokinase, enolase, and pyruvate phosphokinase. In autotrophically-grown Euglena the activity of the DPN enzyme was considerably higher than the TPN enzyme (Fuller and Gibbs, 1959). This finding has been confirmed and ratios as high as 8 to 1 (Table 1) have been obtained with extracts from rapidly growing cells. From these results it would appear unlikely that in Euglena the TPN-dependent GPD is solely responsible for the conversion of 3-PGA to glyceraldehyde 3-phosphate following CO_2 fixation in photosynthesis.

Table 1.--GPD Activities of Some Photosynthetic Organisms

Organism	GPD Activity (μM/min/gm protein)	
	DPN	TPN
Euglena gracilis, strain Z	1780	230
Rhodopseudomonas spheroides, strain L	860	Trace
Rhodomicrobium vannielii	540	Trace
Chromatium, strain D	1830	0
Chlorobium thiosulfatophilum	114	Trace

The reaction mixture contained the extract, Tris, pH 8.0 (33 μM/ml), sodium arsenate (17 μM/ml), potassium fluoride (20 μM/ml), cysteine, pH 8.0 (4 μM/ml), pyridine nucleotide (0.1 μM/ml)(test curvette only). The reaction mixtures were incubated at 23° C for 8 minutes and then D-glyceraldehyde-3-phosphate (2 μM/ml) was added to both control and test curvettes. Trace activity was less than 3 μM/min/gm protein and was probably not significant.

Table 1 also shows activities found in cell-free extracts of four photosynthetic bacteria. In each case a high GPD activity was obtained with DPN, but little or no activity was evident if TPN was substituted for DPN. These results pointed to the DPN-dependent enzyme being the dominant, or perhaps the only GPD active in the photosynthesis of these organisms. That the DPN-dependent enzyme may function generally in carbohydrate synthesis was indicated by the studies of Trudinger (1956), who showed that the chemoautotroph Thiobacillus denitrificans contained the DPN-linked, but not the TPN-linked GPD. This bacterium has a CO_2 fixation cycle similar to that operating in photosynthetic cells.

TPN-dependent GPD.--This enzyme appears to be present in actively photosynthesizing cells which contain definite chlorophyll-containing

lamellae. When the function of chloroplasts was suppressed, such as when certain autotrophic algae were converted to heterotrophic growth, the activity of the enzyme disappeared (Fuller and Gibbs, 1959). Intracellular distribution experiments of the type described for carboxydismutase (Smillie and Fuller, 1959) and alkaline C-1 fructose 1,6-diphosphatase (Smillie, 1960) indicated that the TPN-dependent GPD of pea leaves was localized exclusively in the plastids. The apparent lack of significant amounts of this enzyme in anaerobic chemosynthetic and photosynthetic bacteria may indicate that the enzyme is only functional in aerobic cells.

GPD activity with TPN is not restricted to chlorophyll containing cells, since this activity has been demonstrated in extracts of albino barley leaves (Fuller and Gibbs, 1959) and Alcaligenes faecalis (Brenneman and Volk, 1959).

GTR.--Although GTR has been studied less than the above two enzymes, there are a number of reasons for thinking that this enzyme plays either a minor or no role in photosynthesis. At the stage in the development of pea leaves when the rate of photosynthesis was most rapid, the activity of the enzyme was quite low (0.57 μM/min/gm fresh weight; this was 1.1% the activity of the TPN-dependent GPD). Intracellular distribution studies of leaf fractions isolated in both aqueous and non-aqueous media indicated that the enzyme was not localized in chloroplasts. Using the assay described by Rosenberg and Arnon (1955), GTR activity was noted in extracts from pea root, etiolated pea stem, and streptomycin-bleached Euglena. Negative results were obtained when the GTR assay was applied to extracts of Anacystis nidulans and Chromatium.

Conclusions

1. In contrast to previous notions, the DPN-linked GPD may participate actively in photosynthesis. In anaerobic photosynthetic bacteria it appears to be the only GPD operating during photosynthesis. The DPN-dependent GPD of pea leaves is associated with both the chloroplasts

and cytoplasm.

2. TPN-dependent GPD probably functions in the photosynthesis of plants and algae. The enzyme is localized in the vicinity of the lamellae in these organisms. However, its distribution in nature is not confined exclusively to photosynthetic cells, since it has been found in at least two non-chlorophyll containing organisms.

3. GTR is not localized in the chloroplasts of pea leaves. The enzyme appears to be present in some non-photosynthetic tissues and absent from certain photosynthetic cells. It is deemed improbable that this enzyme has a direct role in photosynthesis.

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