References

1. SCHÜRCH, A. F., LLOYD, L. E., and CROMPTON, E. W. *J. Nutrition*, **41**, 629 (1950).
2. CARROLL, R. W., HENSLEY, G. W., and GRAHAM, W. R., JR. *Science*, **115**, 36 (1952).
3. BOLIN, D. W., and STAMBERG, O. A. *Ind. Eng. Chem.*, Anal. Ed., **16**, 345 (1944).
4. BOLIN, D. W. *Proc. N. Dakota Acad. Sci.*, **1**, 25 (1948).

Manuscript received June 2, 1952.

Glyceraldehyde Phosphate Dehydrogenase of Green Plants [1,2]

Daniel I. Arnon

Division of Plant Nutrition,
University of California, Berkeley

The operation of the glycolytic cycle in the metabolism of green plants has received strong experimental support from the findings of Benson and Calvin (*1*) that known intermediates of glycolysis are the principal products of short-time photosynthesis. In very short periods of photosynthesis, of the order of several seconds, most of the newly fixed $C^{14}O_2$ was found in the carboxyl of phosphoglyceric acid (*1, 2*). With increasing exposure to light, the proportion of total radioactivity fixed in the phosphoglycerate decreased, and newly fixed carbon dioxide was detected in a relatively large array of compounds, including triosephosphate, hexose diphosphate, and sucrose (*1*).

$$\text{Glyceraldehyde-3-phosphate} + H_2PO_4^- + DPN^+ \rightleftharpoons 1,3\text{-phosphoglycerate} + DPNH + H^+. \quad (1)$$

The identification of phosphoglycerate as the main early product of photosynthesis pointed to the special importance in green plants of glyceraldehyde phosphate dehydrogenase, the enzyme catalyzing the reversible reduction of phosphoglycerate to triosephosphate according to Eq. (1).[3]

Without this enzyme, the path from phosphoglycerate to carbohydrate synthesis would be blocked. However, the occurrence of glyceraldehyde phosphate dehydrogenase in green plants was recently called in question. Tewfik and Stumpf (*3*), studying the degradation of fructose diphosphate, found that this enzyme, although present in the seed, disappeared from the leaves of pea plants, and Stepka (*4*) concluded from inhibition studies of *Chlorella* with iodoacetamide that reaction 1 is not involved in sugar formation in photosynthesis.

The distribution of glyceraldehyde phosphate dehydrogenase in mature green leaves of sugar beets, spinach, sunflower, and tobacco was studied in our laboratory. Plants were grown in synthetic nutrient solutions in a greenhouse until mature leaves were formed. Immediately following the harvest, the leaves were washed in glass-distilled water and stored in a turgid condition in the cold (5° C). The midribs were removed and 60 g of leaf blades prefrozen at −35° C were blended for 30 sec with 150 ml 0.1 *M* K_2HPO_4. To the blend was added slowly, with stirring, 450 ml cold acetone (−35° C), and the slurry was filtered with suction. The filtrate was discarded, and the residue (acetone powder) was rinsed several times with cold acetone, sucked dry until the odor of acetone could no longer be detected, and dried *in vacuo* over P_2O_5. The dry acetone powder was stored at −35° C; 0.5 g acetone powder was extracted with 10 ml cold water for 10 min, centrifuged, and the residue discarded. The supernatant fluid was filtered, and the clear filtrate, designated acetone powder extract, was used as a source of the glycolytic enzymes. Similar acetone powder extracts were prepared, using either 0.05 *M* phosphate or 0.2 *M* tris (hydroxymethyl) amino methane buffer at pH 7.3. The activity of glyceraldehyde phosphate dehydrogenase was measured by a slight modification of the optical test of Warburg and Christian (*5*) in a Beckman Model DU spectrophotometer. This test is based on the increase in optical density at 340 mμ of the reaction mixture, as a result of the reduction of the pyridine nucleotide, when the reaction proceeds from left to right in accordance with Eq. (1).

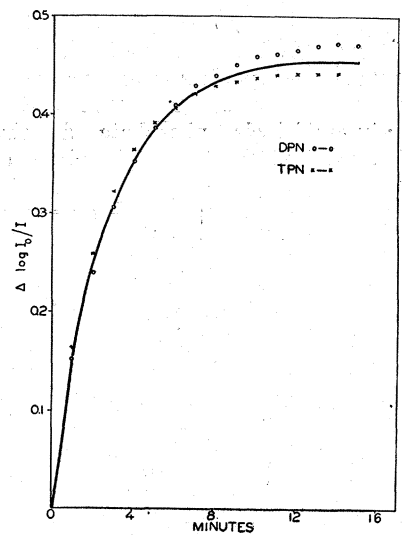

FIG. 1. Oxidation of glyceraldehyde-3-phosphate by crude leaf glyceraldehyde phosphate dehydrogenase. The reaction mixtures contained each 0.3 mg of a dioxane compound of DL-glyceraldehyde-1-bromide-3 phosphoric acid, 0.3 ml of the enzyme preparation and the following in μm: DPN, 0.214 *or* TPN, 0.218; sodium arsenate, 15; sodium fluoride, 10; tris (hydroxymethyl) amino methane buffer pH 8.2, 100; and water to 3.0 ml final volume. The reaction was started by adding the glyceraldehyde phosphate. Quartz cuvettes, $d = 10$ mm.

The acetone powder extract was found to contain aldolase, isomerase, and glyceraldehyde phosphate dehyrogenase (*6*). Glyceraldehyde phosphate dehydro-

[1] Aided by a grant from the U. S. Public Health Service.

[2] After this paper was submitted for publication, M. Gibbs reported independent observations on glyceraldehyde phosphate dehydrogenase in green plants (*Nature*, **170**, 164, [1952]).

[3] The following abbreviations will be used: DPN+—oxidized diphosphopyridine nucleotide (coenzyme I); DPNH—reduced diphosphopyridine nucleotide; TPN+—oxidized triphosphopyridine nucleotide (coenzyme II); TPNH—reduced triphosphopyridine nucleotide.

genase from yeast (*5*) and from rabbit muscle (*7*) is DPN-specific. It was found, however, that the acetone powder extract oxidized glyceraldehyde-3-phosphate with either DPN or TPN. The identity of the reduced DPN and TPN at the completion of the reaction was established by their reoxidation with acetaldehyde and oxidized glutathione, respectively (*6*), thus ruling out a possible interconversion of the two pyridine nucleotides. The acetone powder extract contained alcohol dehydrogenase, which is specific for DPN, and glutathione reductase (*8, 9*), which is specific for TPN. It appears, therefore, that unlike the reaction in yeast and muscle, glycolysis in the leaf can proceed with either TPN or DPN. The reversible oxidation of 3-phosphoglyceraldehyde in leaves can thus be also represented by Eq. (2).

$$\text{Glyceraldehyde-3-phosphate} + H_2PO_4^- + TPN^+ \rightleftharpoons \text{3-phosphoglycerate} + TPNH + H^+. \quad (2)$$

The oxidation of glyceraldehyde-3-phosphate[4] with either DPN or TPN is shown in Fig. 1. In these experiments the acetone powder extract was treated (*5*) to remove aldolase and isomerase. The resulting partly purified glyceraldehyde phosphate dehydrogenase preparation oxidized fructose diphosphate (Fig. 2)

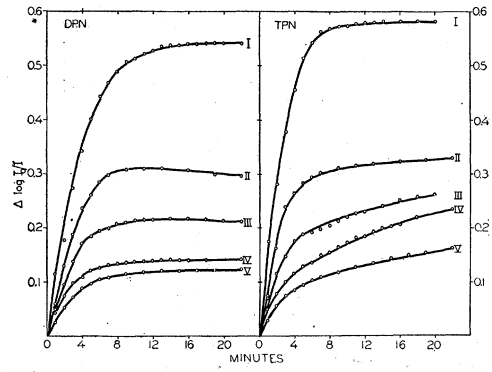

FIG. 2. Effect of phosphate concentration and pH on leaf glyceraldehyde phosphate dehydrogenase. Each reaction mixture contained 0.24 ml of the enzyme preparation, 0.1 ml of a crystalline aldolase solution, and the following in μm: DPN, 0.26 *or* TPN, 0.28; sodium fluoride, 10; fructose diphosphate, 3; and tris (hydroxymethyl) amino methane buffer, 100; water to a final volume of 3 ml. I—pH 8.5, 15 μm sodium arsenate, II—pH 8.5, 90 μm potassium phosphate, III—pH 8.5, 30 μm phosphate, IV—pH 8.5, 3 μm phosphate, V—pH 7.3, 90 μm phosphate. Reaction was started by adding the fructose diphosphate. Quartz cuvettes, $d = 1.0$ cm.

only upon addition of aldolase. Crystalline aldolase was prepared (*10*) by R. H. Hageman and recrystallized once.

The enzymatic tests described so far were carried out under nonphysiologic conditions in the presence of arsenate. The question arises as to which of the two coenzymes, DPN or TPN, is responsible for the reduction of phosphoglyceric acid and the oxidation of phosphoglyceraldehyde in the green cell. Are there two enzymes in the leaf, one DPN- and one TPN-dependent, or is there one enzyme which is capable of using either DPN or TPN? A full answer to these questions must await further purification of the enzyme. This work is now in progress. The following observations which can be cited at this time appear relevant, however.

The reversibility of reactions 1 and 2 was studied by a method analogous to that used by Warburg and Christian (*5*) for the study of the reversibility of the crystalline yeast glyceraldehyde phosphate dehydrogenase. The oxidation of glyceraldehyde-3-phosphate was measured at different pH values and at different concentrations of phosphate. The results are presented in Fig. 2. Curve I for DPN and TPN represents the arsenate systems in which the oxidation of glyceraldehyde-3-phosphate proceeded irreversibly until all the pyridine nucleotide present was reduced. Curves II, III, IV, and V in the DPN series show characteristic equilibrium reactions, which, in accordance with Eq. (1), are dependent on the concentration of inorganic phosphate and hydrogen ion. In the TPN series, however, only Curve II, with the highest phosphate concentration at pH 8.5, shows an approach to equilibrium. At pH 7.3 with this phosphate concentration equilibrium has not been attained in the same period of time (Curve V). This was also the case at pH 8.5 with the lower phosphate concentrations (Curves III and IV).

Whatley (*11*) and Anderson (*12*) found that TPN, rather than DPN, was the dominant pyridine nucleotide in green leaves. It is possible, but by no means certain until the enzyme is purified, that the glyceraldehyde phosphate dehydrogenase of green leaves is normally a TPN-dependent enzyme which can also act through DPN. If there is only one glyceraldehyde phosphate dehydrogenase in leaves, its properties are different from those of the yeast (*5*) and muscle (*7*) enzymes. Warburg and Christian (*5*) concluded that the yeast enzyme does not combine with TPN; the crude leaf enzyme can act with either DPN or TPN, and under physiologic conditions its activity through TPN is greater. Cystein, which was found essential for the DPN reduction by the crystalline enzyme from either muscle or yeast (*7*), was not required for the reduction of either DPN or TPN by the crude leaf enzyme. In certain preparations, however, a marked activating effect of cystein was observed, especially on the DPN reduction. The leaf glyceraldehyde phosphate dehydrogenase is strongly inhibited by iodoacetamide, but the TPN reduction is more resistant to the action of the inhibitor (*6*). The last observation may also perhaps account in part for the previously cited results of Stepka (*4*).

The leaf glyceraldehyde phosphate dehydrogenase was found to be localized in the cytoplasmic fluid, rather than in the chloroplast. This finding provides additional support for the view (*13*) that photosynthesis involves an interaction between the particles of chloroplasts, in which the initial photochemical events take place, and the soluble enzymes of the surrounding cytoplasmic fluid.

[4] Kindly given us by E. Baer, through the courtesy of H. O. L. Fischer.

References

1. BENSON, A. A., and CALVIN, M. *Ann. Rev. Plant Physiol.*, **1**, 25 (1950).
2. GAFFRON, H., FAGER, E. W., and ROSENBERG, J. L. *Symposia Soc. Exptl. Biol.*, **5**, 262 (1951).
3. TEWFIK, S., and STUMPF, P. K. *J. Biol. Chem.*, **192**, 519, 527 (1951).
4. STEPKA, W. Ph.D. diss., Univ. California, Berkeley (1951).
5. WARBURG, O., and CHRISTIAN, W. *Biochem. Z.*, **303**, 40 (1939).
6. ARNON, D. I. In W. D. McElroy and B. Glass, Eds., *Phosphorus Metabolism*, II. Baltimore, Md.: Johns Hopkins Press (in press).
7. CORI, G. T., SLEIN, M. W., and CORI, C. F. *J. Biol. Chem.*, **173**, 605 (1948).
8. MAPSON, L. W., and GODDARD, D. R. *Nature*, **167**, 975 (1951).
9. CONN, E. E., and VENNESLAND, B. *Ibid.*, 976.
10. TAYLOR, J. F., GREEN, A. A., and CORI, G. T. *J. Biol. Chem.*, **173**, 591 (1948).
11. WHATLEY, F. R. *New Phytologist*, **50**, 244 (1951).
12. ANDERSON, D. G. Paper presented before Am. Soc. Plant Physiologists, Minneapolis (1951).
13. ARNON, D. I. *Nature*, **167**, 1008 (1951).

Manuscript received April 25, 1952.

Formation of Large Bodies in a Member of the Genus *Bacillus*

Moselio Schaechter[1, 2]

Department of Bacteriology, University of Kansas, Lawrence

Cultures of a spore-forming *Bacillus* of the brevis-polymyxa group were examined on thin blocks of serum agar. A Bausch & Lomb phase contrast microscope attachment was used on a Leitz "Panphot" microscope with a 97× objective and a 15× ocular. Time-sequence pictures were taken with Kodak Microfile film. "Large bodies" (of the Dienes and Klieneberger-Nobel type) were often found in great numbers in cultures that had completed logarithmic growth. Such large body formation continued for one or two days afterward. Fig. 1 shows pictures of the same

FIG. 1. Formation of two large bodies from a single rod.

cell taken at the indicated time intervals. It demonstrates the direct transformation of a rod into two separate large bodies, one of them rodlike in shape and the other ovoid. Both elements show clearing of the cellular material and abundant granules. These granules were highly motile, and their movement appeared to follow a definite pattern, like a chain that is pulled from one end. This movement was observed for periods of time up to 48 hr, after which it gradually ceased. The granules were also found outside the cells, swimming freely on the agar, singly or in chains. Their fate could not be determined by direct observations. In Fig. 2, the cell labeled *1* shows a central swelling that is followed by swelling of the rest of the cell, with the formation of a round, ovoid-shaped large body. Cell 2 also has undergone a change into an element that is similar to, but smaller than, the preceding. Cell 3 developed into a rod-shaped element of similar structure. In Fig. 3, the culture was transferred from a 24-hr culture that showed an abundance of large bodies. Over a period of 30 min the cell undergoes a clearing and shows granules; granules are also seen outside and adjacent to the cell.

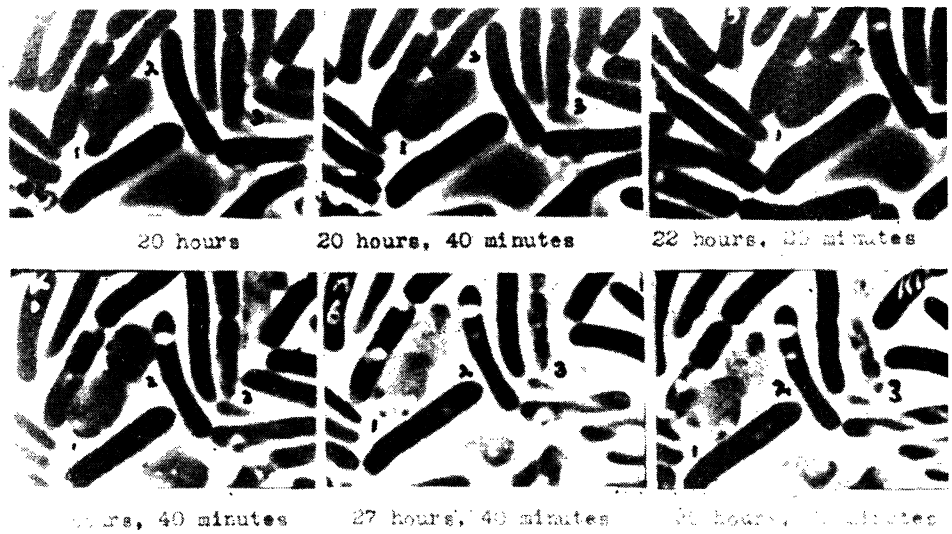

FIG. 2. Formation of balloonlike large bodies from rods (*1* and *2*) and of rodlike elements (*3*).

The formation of large bodies was observed to occur

[1] The author wishes to thank David Paretsky for helpful advice and encouragement.

[2] Present address: Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia.