Technical Papers

Phosphoglyceric Acid in Photosynthesis

E. W. Fager and J. L. Rosenberg[1]

Institute of Radiobiology and Biophysics (Fels Fund), University of Chicago

The role of phosphoglyceric acid as an intermediate in the photosynthetic reduction of carbon dioxide in plants has been the subject of conflicting claims. Calvin and Benson (*1*) reported that phosphoglyceric acid was the major radioactive compound formed in short-term photosynthesis with $C^{14}O_2$. Earlier work in our laboratory indicated that this compound was a minor component accounting for only a small percentage of the radioactivity fixed during 30–40 sec of photosynthesis (*2*). As a result of more recent experiments, done with higher CO_2 concentrations, we are now able to confirm the observation of Calvin and Benson. Since our identification was done by direct chemical methods different from those used by the California group, a somewhat detailed description seems in order.

Suspensions of the alga *Scenedesmus obliquus* were illuminated for 10–15 min in the presence of an excess of $C^{12}O_2$. After this period of normal photosynthesis, tracer (HCO_3^-) was added,[2] illumination was continued for 30–40 sec, and then the algae were killed in boiling water. The aqueous extract was combined with natural carrier material provided by the aqueous extract from about 2 pounds of nontagged algae. The solution was dialyzed, decationized on a column of Nalcite HCR, and exchanged onto a column of the weak anion exchange resin Amberlite IR4-B. The eluate obtained from the IR4-B with 1.5 *M* ammonia contained 80% of the total activity. This solution was concentrated, freed of ammonium ions by another cation exchange treatment, exchanged onto a column of 100–250 mesh Amberlite IR4-B, and fractionally eluted with 0.022 *M* ammonia. The center cuts from the activity peak of the eluate were concentrated and refluxed at pH 4.5 to hydrolyze phosphate esters. The material was again decationized, absorbed on Amberlite IR4-B, and fractionally eluted with 0.022 *M* ammonia. Center cuts from this activity peak were further purified by fractional elution from an intermediate-strength anion exchange resin, Ionac A-300,[3] with 1.0 *M* ammonia. The center cuts of the activity peak of the purified material were freed of excess ammonia, concentrated, extracted with ether to remove traces of nitrogenous compounds from the resin, and decationized. In each stage of the purification there was an appreciable increase in specific activity. The final purified material had a specific activity about 60 times that of the original hot-water extract.

From the solution of the purified glyceric acid, the barium salt, the *p*-phenylphenacyl, and the *p*-bromphenacyl esters were prepared. The esters were further purified by twofold chromatographic elutions from a silicic acid column with benzene containing 2.5% of acetone. Analyses of these compounds gave definite proof that the compound we had isolated was D-glyceric acid, formed by hydrolysis from D-phosphoglyceric acid. The compound apparently underwent partial racemization during the isolation. Table 1 gives the physical constants and analyses for the acid and derivatives.

TABLE 1

pK_a:
- Reported for glyceric acid 3.55 (*3*)
- Found for unknown acid 3.65 (by best fit of titration curve, uncorrected)

$[\alpha]_D$:
- Reported for Ba-D-glycerate, molybdate enhanced + 104° (*4*)
- Found for Na salt, molybdate enhanced + 83°

Barium glycerate:[4]
- Calculated Ba, 39.5%; C, 20.7%; H, 2.9%
- Found (after vacuum drying at 110°): Ba, 40.0%; C, 20.6%; H, 3.1%

p-Phenylphenacyl glycerate:
- Calculated C, 68.0%; H, 5.3%
- Found: C, 67.6%, 68.0%; H, 5.2%, 5.3%

p-Bromphenacyl glycerate:
- Calculated C, 43.5%; H, 3.6%; Br, 26.4%
- Found: C, 43.5%; H, 3.5%; Br, 26.4%

Periodic acid oxidation of the *p*-phenylphenacyl ester showed the expected 1 mole of adjacent hydroxyls per mole of ester.

Because of the losses attendant upon selection of only the central portions of the activity peaks during purification, the purified esters accounted for only about 20% of the total activity in the hot-water extract. The determination of the total radioactivity in phosphoglyceric acid was performed with the help of artificial carrier. A known amount of phosphoglyceric acid was added to the algal extract, phosphate was hydrolyzed by incubation at 35° C with intestinal phosphatase (Armour Laboratories), the resultant glyceric acid was oxidized with periodic acid using the amount calculated for oxidation to glyoxylic acid and formaldehyde, and the carbonate-soluble 2,4-dinitrophenylhydrazone of glyoxylic acid was isolated and counted. These analyses showed that 50–60% of the activity fixed in 10 sec of normal photosynthesis was in phosphoglyceric acid.

[1] Atomic Energy Commission postdoctoral fellow in the physical sciences, the National Research Council.

[2] C^{14} was obtained as $BaCO_3$ from the Isotopes Division, U. S. Atomic Energy Commission.

[3] P. E. Holder, of the American Cyanamid Company, Chicago, kindly provided us with experimental quantities of fine-mesh Ionac A-300.

[4] The microanalyses were performed by William Saschek, Department of Chemistry, University of Chicago.

The total counts in the original extract were determined as $CaCO_3$ formed subsequent to wet combustion. Correspondence between these counts and the hydrazone counts was checked by wet combustion of several samples of the latter. The routine analyses on the total algal extracts were supplemented by an experiment in which phosphoglyceric acid, with carrier, was first eluted with 0.01 *M* hydrochloric acid as a narrow band from a 100–200 mesh column of Amberlite IRA-400 (Cl^-). This band was then subjected to the usual hydrolysis, oxidation, and isolation procedures. It accounted for 80% of the activity found in phosphoglyceric acid by analysis of the total extract. The discrepancy may be due to the presence of some tagged glyceric acid produced by phosphatase activity of the algae.

Our earlier results in which only 5% (15% in some later experiments) of the fixed tracer was found in phosphoglyceric acid may have been due to a deficit of CO_2 during the tagging. If this had been true, tracer would have been fixed first in phosphoglyceric acid and then rapidly transformed photochemically into other substances. In the more recent experiments leading to the isolation, a large excess of CO_2 was provided, so that a steady-state amount of phosphoglyceric acid typical of normal photosynthesis was always present. Further experiments on the kinetics of phosphoglyceric acid in photosynthesis have been reported elsewhere (*5*).

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A Study of Grinding Techniques for Bacterial Cells[1]

W. B. Dockstader and H. O. Halvorson

Departments of Poultry Husbandry* and *Bacteriology and Public Health,
State College of Washington, Pullman,
and *Department of Bacteriology and Immunology,*
University of Illinois, Urbana

It has long been a problem of the cell physiologist to get efficient grinding in the preparation of cell extracts. A recent study of this problem, using some of the common techniques and abrasives, yielded certain data that may prove useful. Three types of grinding equipment were used:

[1] Supported in part by a Hormel Institute Fellowship at the University of Minnesota, Austin, and submitted as a Master's thesis to the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis.

1. An ordinary mortar and pestle of a capacity of 20–25 cc for trituration.

2. A Wood-Werkman mill (*1*), a mechanical arrangement whereby the mixture of organisms and abrasive is forced between 2 concentric conical ground-glass surfaces while the inner cone is rotated and the outer one is held stationary by a vicelike clamp. The rotating cone is hollow and can be filled with ice to provide refrigeration. A tube is fused on the small end of the outer cone in such a way as to form a funnel-like piece.

The pasty-fluid mixture of cells and abrasive is forced through the tube by means of a syringelike plunger, easily made by cutting down and ridging a rubber stopper. The mill is best used by first filling the hollow cone with cracked ice and closing the opening that fits over the drive shaft to avoid spilling the ice. The ice-filled cone is then placed in the funnel-like piece, and the grinding mixture poured into the open end of the tube. The plunger is inserted, and the stopper removed from the ice chamber. The assembled mill is then fitted to the clamping and driving mechanisms. A container is inserted under the lip of the funnel to collect the ground material. This can be cooled by means of an ice-water bath. By a slow, *steady* pressure on the plunger, the material is forced between the ground glass surfaces. At least ¼ hp electric motor should be used as a power source. Variable speed pulleys connect the motor to a gearbox, which drives the shaft that operates the mill. The variable-speed pulleys and gearbox allow adjustment of the speed from about 300 rpm to 600 rpm.

3. A modified Potter's homogenizer (*1*), which is constructed by grinding the inside of a pyrex glass tube and preparing a pestle with prongs on the end from another slightly smaller tube, to which a shaft is fused. The sides of the pestle are ground to a tolerance that permits the tube to slide off slowly when not supported. The modification is simple. The prongs are left off the pestle because the nature of the material to be ground does not require their presence. Two sizes proved suitable, but the longer tube and pestle is more satisfactory because it affords greater ease in handling, and the longer pestle presents a larger grinding surface. For grinding, the shaft of the pestle is best connected to a cone-drive laboratory stirring motor by means of a short piece of pressure tubing. This affords some flexibility, which helps prevent breakage of the homogenizer if the pestle should stick or get out of line. The speed of rotation is set at approximately 2,000 rpm. The speed is not constant because the drag of the pestle in the grinding mixture slows it down considerably, and it varies with the adhesiveness of different abrasives. For refrigeration the homogenizer tube is placed in a suitable container filled with cracked ice and water. The two are then moved simultaneously up and down, grinding the cells by forcing the rotating pestle through the mixture.

Commercial abrasives used were Norton's Abrasive Grain Alundum 600X, Norton's Levigated Alumina, Johns-Manville Hyflo Super-Cel, and silicon carbide 940X, which may be obtained from any optical company. Hyflo Super-Cel can be used as it is received, except that it