TECHNICAL PAPERS

Photosynthetic Studies With Mutant Strains of *Chlorella*[1]

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In 1946 experiments were started in this laboratory with the aim of developing a new method for the study of photosynthesis. It was hoped that the method developed by Beadle and Tatum (*1*) with *Neurospora* for the study of physiological genetics could be adapted to green organisms for the study of photosynthesis. If individual reactions in the photosynthetic mechanism of an organism could be blocked through gene mutations, it is possible that intermediate stages in the process could be traced and a series of reactions built up for the process such as Tatum, Bonner, and Beadle (*5*), Bonner (*2*), and Srb and Horowitz (*4*) have done in the study of other biochemical syntheses.

A green alga, *Chlorella* sp., originally isolated and obtained in pure culture from a single cell by Dr. Hempstead Castle, was used in this study. The cells were grown in Moore's solution (*3*) at one-fifth dilution with the addition of trace elements, glucose, and tryptone. Mutant strains were obtained in the following manner: Cells were suspended in quartz tubes in a complete medium containing one-fifth Moore's solution, trace elements, glucose, tryptone, yeast extract, and a vitamin mixture. The suspensions were irradiated with ultraviolet light at a wave length of 2,537 A. The irradiation time was varied, and dilution plates of the irradiated suspensions were made on a solid complete medium containing the same components as the medium in which the cells were irradiated. The plates were placed under continuous light. Only colonies obtained on plates which had 0.1% the number of colonies contained in nonirradiated control plates were used for subsequent studies. Transfers were made from widely spaced colonies to agar slants of the complete medium. Inoculations from the complete slants were then made to minimal liquid medium tubes containing one-fifth Moore's solution and trace elements, in which wild type *Chlorella* grows normally. The presence or absence of growth in the minimal medium was used as the basis for the detection of biochemical mutants. The absence of growth on the minimal medium indicates that the cells are unable to synthesize some component contained in the complete medium. Growth on both the complete and minimal media was used to indicate that a deficiency had not been induced.

Three strains were isolated which have absolute deficiencies, *i.e.* are completely unable to grow on the minimal medium. (These strains shall hereafter be referred to as nongrowers.) Seven others were obtained which exhibit a negligible amount of growth compared with the wild type on the minimal medium. Two of these are also morphological variants. All of these strains are green when grown on the complete medium. The following will deal only with the nongrowers.

An analysis was made of each mutant's ability to grow on media containing the minimal medium plus individual components of the complete medium, *i.e.* glucose, tryptone, yeast extract, and a vitamin mixture. In addition to these, casein hydrolysate and a combination of casein hydrolysate and vitamins were tested. None of these media except that containing glucose supported growth of the three nongrower strains. During the past two years the mutant strains have not regained the ability to exist autotrophically and have continued to remain completely heterotrophic.

Comparative growth studies were made with the wild type and two of the nongrowers. These were grown both in the light and in the dark in minimal solutions containing glucose which was varied from 0 to 2%. The growth of the mutants in the light and dark and of the wild type in the dark was about the same at the lower glucose concentrations. However, the growth of the mutants in the light was superior to that of the mutants and of the wild type in the dark at the higher glucose concentrations. As the glucose supply is depleted, the mutant cultures gradually lose their green color until they become colorless. The green color of such cultures can be restored by the addition of glucose to the medium.

There is evidence to indicate that failure to grow in the minimal medium is not caused by blocks in the production of chlorophyll. The total pigment absorption spectra of the mutants and wild type reveal peaks at identical wave lengths. Further evidence comes from the fact that the cells are green when grown in the presence of glucose. It thus appears that all of the reactions following the synthesis of glucose to the formation of chlorophyll are present and operative.

The mutants were tested for their abilities to liberate oxygen when suspended in a 0.1 molar carbonate-bicarbonate mixture and exposed to light. Oxygen uptake was noted rather than oxygen liberation, which occurs with wild type cells under similar circumstances. The fact that oxygen liberation due to photosynthesis has not been demonstrated for two of the mutant strains suggests that there are blocked reactions in the photosynthetic mechanisms of these mutants.

[1] This work was supported by a fellowship from the F. A. Bartlett Tree Expert Company, Stamford, Connecticut.

[2] The author wishes to express his gratitude to Drs. A. W. Naylor, E. L. Tatum, David Bonner, and P. R. Burkholder for their helpful suggestions and discussions.

Growth studies and gas exchange measurements have revealed differences in the capabilities of the mutant strains. It is probable that different reactions are blocked in each. By determining which reactions are blocked in these mutants, together with similar studies on others, it is possible that a number of intermediate steps in the photosynthetic mechanism can be positively determined.

A more complete report including supporting data will be published at a later date.

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Fractionation of Amino Acids From Hydrolysates in Nonaqueous Systems

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All of the amino acids which are present in an acid hydrolysate of a protein can be brought into solution in acetone by the action of certain organic acids which form with them salts or complexes which are soluble in this solvent. Thus far we have found no other solvent as good as acetone for this purpose. Alcohols react with the reagents, and dioxane, although of possible usefulness in special cases, is less satisfactory than acetone. Methyl-ethyl ketone is greatly inferior to acetone as a solvent for the amino acids with the organic acids which we have tested as reagents. We have explored the possibilities of fractionation of amino acid mixtures in several nonaqueous systems, making use of differences in solubility of individual amino acids in acetone solutions of certain organic acids.

A large number of acids have been examined for their power to cause solution of amino acids in acetone. The following possess this property to a useful degree: trichloroacetic acid, dichloroacetic acid, *p*-toluenesulfonic acid, 4-nitrochlorbenzenesulfonic acid, *d*-camphorsulfonic acid, *dl*-camphorsulfonic acid, benzenesulfonic acid, and *n*-butylsulfonic acid.

The ammonium salts of these acids are soluble in acetone. On addition of dry ammonia gas, the amino acids are precipitated from such solutions when trichloroacetic acid, benzenesulfonic acid, or *dl*-camphorsulfonic acid is employed, the basic amino acids being exceptions. Arginine, histidine, and lysine separate in combination with the reagent acid. When *p*-toluenesulfonic acid, 4-nitrochlorbenzenesulfonic acid, or *d*-camphorsulfonic acid is employed as reagent, the amino acids separate in great measure in the form of complexes when dry ammonia gas is introduced in excess.

When an acid hydrolysate of casein is dissolved in acetone with the aid of trichloroacetic acid, benzenesulfonic acid, or *dl*-camphorsulfonic acid, and an excess of ammonia is introduced, about 88% by weight of the sample is recovered in the resulting precipitate. In the case of individual amino acids brought into solution in this way, recovery on precipitation is not far from 100%.

Table 1 shows the molecular ratios between reagent acid and amino acid necessary to bring the latter into solution in acetone when the concentration of acid in acetone is as indicated.

TABLE 1

Amino acid	Moles of acid reagent / Mole of amino acid		
	Trichloroacetic acid*	*dl*-Camphorsulfonic acid†	Benzenesulfonic acid‡
Proline	4.5	1.1	1.0
Threonine	5.5	1.4	1.6
Tyrosine	68.0	1.4	1.4
Isoleucine	5.1	1.0	1.6
Alanine	5.0	1.6	1.8
Valine	5.1	1.4	1.8
Aspartic acid	53.0	1.6	2.0
Phenylalanine	5.2	0.9	3.2
Serine	20.0	0.8	3.4
Hydroxyproline	25.0	2.1	3.6
Leucine	5.4	1.6	4.2
Norleucine	5.4	1.3	7.4
Tryptophan	3.0	28.6	10.6
Cystine	Insol.	10.0	13.4
Glycine	4.2	> 22.5	16.2
Glutamic acid	55.0	1.7	44.0
Methionine	6.2	0.9	64.0
Histidine	6.8	3.7	128.0

* The trichloroacetic acid in acetone was a 0.24 N solution.

† The *dl*-camphorsulfonic acid in acetone was a 0.2 N solution.

‡ The benzenesulfonic acid in acetone was a 0.4 N solution.

Fractionation of hydrolysates or other mixtures of amino acids has been accomplished by the following procedures:

(1) Fractional solution: Increments of reagent (acid in acetone) are brought successively into contact with dry hydrolysate or other mixture of amino acids, in the finely ground state, contained in a filter funnel or crucible. After brief contact the reagent, containing some dissolved amino acids, is removed by applying suction. The receiver is changed, and another increment of reagent is applied to the undissolved portion of the hydrolysate, and this is then removed by suction. In this way a hydrolysate can be separated within 2 or 3 hrs into as many as 50 or 60 fractions.

(2) The hydrolysate can be completely dissolved in the minimum amount of the acid-acetone reagent, and fractions of amino acids can then be dropped out by the stepwise introduction of dry ammonia to precipitate successive fractions of the dissolved amino acids. Ammonia can be introduced in the form of a strong solution