MUTANTS WITH IMPAIRED PHOTOSYNTHESIS IN CHLAMYDOMONAS REINHARDI[1]

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A mutant strain unable to carry out photosynthetic phosphorylation has been described for the unicellular green alga Chlamydomonas reinhardi (Levine, 1960a). Recently, additional mutant strains with impaired photosynthesis have been obtained by induction with ultraviolet light and subsequent screening (Levine, 1960b) for their ability to fix carbon dioxide. The purpose of the present communication is to report some initial findings which relate to seven of these strains. Further work is in progress and a detailed report will be published.

Since each of the mutants with impaired photosynthesis will not grow on minimal medium (Levine and Ebersold, 1958) unless it is supplemented with sodium acetate, they have been referred to as acetate-requiring mutants (abbreviated ac). For the experiments reported here the wild-type strain (137c) and the mutants were grown at 25°C in shake cultures of minimal medium supplemented with sodium acetate and at a light intensity of 2500 lux from daylight fluorescent lamps. Cells were harvested when the cultures were in the logarithmic phase of growth.

Genetic Analysis: The seven mutants strains (ac-16, ac-21, ac-40, ac-46, ac-59, ac-115, and ac-141) segregate in a one to one fashion in all crosses. All of the mutants have been tested with the appropriate marker stocks and, except for ac-46 and ac-59, they have been placed within their respective linkage group. These mutants fall into the following linkage

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groups: ac-16, group X; ac-21, group XI; ac-40, group II; ac-115, group I, and ac-141, group III (see Levine and Ebersold, 1960 for the linkage groups of C. reinhardi). All available tester stocks have been used in an effort to localize ac-46 and ac-59, and though they segregate in the expected manner, they have not been localized to any of the established linkage groups as yet. Their genetic analysis is being continued.

Photosynthetic Reactions: Three photosynthetic reactions have been investigated; the Hill Reaction, carbon dioxide fixation, and the activity of PPNR.* The Hill Reaction using para-benzoquinone as a hydrogen acceptor, as well as carbon dioxide fixation, were measured at 25°C and at a light intensity of 30,000 lux according to the method described in a previous report (Levine, 1960a). In order to determine PPNR activity, a crude PPNR was prepared by breaking cells with the aid of a Mullard ultrasonic disintegrator. The broken cell preparation was centrifuged at 140,000 x G in a Spinco Model L Centrifuge, and the supernatent fluid tested for activity.

The assay for PPNR activity requires the presence of chloroplasts or chloroplast fragments (San Pietro and Lang, 1958). These were prepared from both Chlamydomonas and spinach in the following manner, with all procedures carried aout at 0°C. Cells of Chlamydomonas were suspended in 0.05 M Tris buffer, pH 7.5, and broken in a French pressure cell at a pressure of 224 Kg per sq. cm. The suspension was diluted three-fold with the Tris buffer and centrifuged at 1800 x G for eight minutes to remove whole cells. The resulting supernatent, containing the chloroplast fragments, was then diluted seven-fold with the Tris buffer and centrifuged at 20,000 x G for fifteen minutes. The sediment, which contained the chloroplast fragments was then resuspended in three ml of the Tris buffer. Chloroplast fragments from spinach were prepared from freshly harvested leaves. Slices of leaves were ground in a mortar and pestle in twenty-five

* Abbreviations used: PPNR, photosynthetic pyridine nucleotide reductase; Tris, tris (hydroxymethyl) aminomethane; TPN, triphosphopyridine nucleotide.

ml of 0.02 M Tris buffer, pH 7.5. The slurry was then squeezed through a double layer of cheesecloth and centrifuged three minutes at 2,000 x G. The sediment was resuspended in four ml of the Tris buffer and recentrifuged at 20,000 x G for fifteen minutes. The final suspension, containing chloroplast fragments, was suspended in four to five ml of the Tris buffer. The amount of chlorophyll in both the Chlamydomonas and spinach chloroplast fragments was determined spectrophotometrically by Arnon's (1949) modification of the technique of Mackinney (1941).

The PPNR activity was measured in a Beckman Model DU Spectrophotometer by determining the rate of TPN reduction at 340 mu. The reaction mixture in the test cuvette contained, in μmoles per ml, TPN, 0.1; Tris buffer (pH 7.5), 50; and $MgCl_2$, 0.5. It also contained Chlamydomonas or spinach chloroplast fragments equivalent to 100 μg of chlorophyll and an amount of crude enzyme extract containing twelve to fourteen mg protein. The reaction mixture in the blank cuvette lacked the crude PPNR. The two cuvettes were exposed to light from a reflector-flood lamp at 8000 lux for intervals of thirty seconds. Readings were taken after each exposure to light until the reaction had ceased.

The activity of crude PPNR from wild-type Chlamydomonas was assayed with chloroplast fragments from spinach, wild-type Chlamydomonas, and from each of the mutants. That of each mutant was measured using chloroplast fragments from either spinach, wild-type Chlamydomonas, or from mutant itself.

Results and Discussion: The results of the experiments are summarized in Tables I and II. Each figure represents the average value for at least three replicate experiments. The data given in column three of Table II show that carbon dioxide fixation in the mutants is significantly below the level found in wild-type. From the results given in Table I it can be concluded that the mutants fall into one of two classes.

Table I

PPNR activity of wild-type Chlamydomonas and of mutants with impaired photosynthesis (expressed as μmoles TPN reduced/min/g protein).

Source of crude PPNR	Source of Chloroplast Fragments								
	spinach	wild-type	ac-16	ac-21	ac-40	ac-46	ac-59	ac-115	ac-141
wild-type	9.58	4.01	0	0	8.36	9.04	7.68	0	0
ac-16	8.41	5.41	0	-	-	-	-	-	-
ac-21	12.10	5.85	-	0	-	-	-	-	-
ac-40	9.35	3.58	-	-	8.61	-	-	-	-
ac-46	14.20	4.84	-	-	-	5.56	-	-	-
ac-59	10.14	4.61	-	-	-	-	4.02	-	-
ac-115	19.07	5.08	-	-	-	-	-	0	-
ac-141	11.45	9.20	-	-	-	-	-	-	0

The first class, ac-40, ac-46, and ac-59, consists of mutants that possess PPNR activity with chloroplast fragments derived from spinach, wild-type, or from their own chloroplast. The second class of mutants, ac-16, ac-21, ac-115, and ac-141, consists of those mutants that possess PPNR activity, equivalent to that of wild-type, but lack activity with their own chloroplast fragments. In addition, PPNR derived from wild-type is inactive with chloroplast fragments derived from these mutants. In cases where the enzyme was found to be active, its specific activity was relatively low. In recent experiments, to be published shortly, partially purified enzyme has been obtained by acetone precipitation of the crude extract after the procedure of San Pietro and Lang (1958). Partial purification has increased the specific activity of the enzyme around twenty-fold.

It is suggested that the PPNR activity of ac-16, ac-21, ac-115, and ac-141 is ineffective in the reduction of TPN because of a genetic block in some step of electron transfer within their chloroplasts which preceeds that of TPN reduction.

It is important to note that this class of mutants can be further subdivided (Table II).

Table II

A comparison of the rates of the Hill Reaction and carbon dioxide fixation in wild-type Chlamydomonas and in mutants with impaired photosynthesis.

strain	Hill Reaction μliters O_2/Hr./mgChl.	CO_2 fixation cpm x 10^5/Hr./mgChl.
wild-type	1715	8.20
ac-16	7.4	0.43
ac-21	548	0.13
ac-40	1712	0.65
ac-46	66	1.00
ac-59	1260	1.60
ac-115	0	0.08
ac-141	0	0.19

The Hill Reaction is missing or negligible in three of the mutants (ac-16, ac-115, and ac-141), while it is present, though somewhat reduced, in the fourth mutant ac-21. Recent experiments, however, have shown that the Hill Reaction of this mutant is equivalent to that of wild-type when measured spectrophotometrically with 2,6-dichlorophenol indophenol or potassium ferricyanide. That of ac-16, ac-115, and ac-141 is zero with these reagents. The distinction between ac-21, on the one hand, and ac-16, ac-115, and ac-141, on the other hand, suggests that the inability of the former to reduce TPN is due to a genetic block which lies at a point of electron transfer subsequent to the Hill Reaction. The latter three mutants are unable to reduce TPN because the genetic block, reflected in their inability to carry out the Hill Reaction, lies at a step in electron transfer preceeding the Hill Reaction.

The remaining three mutants, ac-40, ac-46, and ac-59, require additional study since their PPNR activity is indistinguishable from

wild-type. These mutants may be blocked in their ability to carry out photosynthetic phosphorylation, or in one of the numerous dark reactions of the reductive pentose phosphate cycle. Both of these possibilities are presently under investigation.

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