# Light-induced uptake of inorganic phosphate in cell-free extracts of obligately anaerobic photosynthetic bacteria

ARNON *et al.*[1,2] have demonstrated a light-induced phosphorylation by preparations of whole spinach chloroplasts, and FRENKEL[3] has reported a similar finding with cell-free extracts of *Rhodospirillum rubrum*, a facultatively anaerobic purple bacterium. In both investigations the product of the phosphorylation was identified as adenosine triphosphate (ATP). It seemed desirable to extend this work to other photosynthetic microorganisms, particularly the obligately anaerobic bacteria *Chromatium* and *Chlorobium*.

Suspensions of *Chromatium* strain D and *Chlorobium limicola*, in 0.2 *M* potassium glycylglycine buffer at pH 7.0, were disrupted 10 minutes at 5° C in a sonic oscillator, then centrifuged 10 minutes at 10,000 *g*. The cell-free supernatants were illuminated in a Warburg apparatus at 26° C under nitrogen. The vessels contained 12–16 $\mu M$ potassium phosphate at pH 7.0, 10 $\mu M$ adenosine monophosphate (AMP), 30 $\mu M$ $MgCl_2$, 30 $\mu M$ KF, supernatant to contain about 10 mg protein, volume to 3.0 ml. "Dark" vessels were covered with aluminum foil to exclude light. After illumination, usually for 1 hour, 1 ml of 20% cold trichloracetic acid was added to the vessels and the contents were centrifuged in the cold. The supernatants were analyzed for orthophosphate ($P_i$) and orthophosphate liberated by 7-minute hydrolysis in 1 *N* HCl at 100° C ($P_7$), by a modification of the KING method[4]. In control vessels the trichloracetic acid was added at the same time as the supernatant.

Representative trials are shown in Tables I and II. Fluoride was added to inhibit any adenosine triphosphatase activity. Omission of fluoride decreased $P_i$ uptake of the *Chromatium* extracts by about 75%, but did not markedly affect uptake by the *Chlorobium* extracts. Under an atmosphere of air instead of $N_2$, uptake by extracts of both organisms was decreased 40–60%.

TABLE I

LIGHT-INDUCED PHOSPHORYLATION BY EXTRACTS OF *Chromatium* STRAIN D

10.2 mg protein, 16.0 $\mu M$ $P_i$

| | *Decrease in $P_i$* $\mu M$/*vessel* | *Increase in $P_7$* $\mu M$/*vessel* |
|---|---|---|
| Dark | 0.0 | 0.2 |
| Illuminated | 3.4 | 3.1 |
| Illuminated minus fluoride | 0.9 | 0.7 |
| Illuminated under air | 1.5 | 1.2 |

TABLE II

LIGHT-INDUCED PHOSPHORYLATION BY EXTRACTS OF *Chlorobium limicola*

9.8 mg protein, 16.2 $\mu M$ $P_i$

| | *Decrease in $P_i$* $\mu M$/*vessel* | *Increase in $P_7$* $\mu M$/*vessel* |
|---|---|---|
| Dark | 0.0 | 0.2 |
| Illuminated | 2.3 | 2.6 |
| Illuminated minus fluoride | 2.1 | 2.5 |
| Illuminated under air | 1.3 | 1.2 |

Similar tests of phosphate uptake were made with preparations of *Spirogyra* chloroplast fragments in conjunction with photosynthesis experiments of THOMAS AND HAANS[5], and are described by these authors. An uptake of 1.5 $\mu M$ $P_i$ out of 11.8 $\mu M$ originally present was obtained upon illumination of duplicate vessels containing 1.5 mg chlorophyll in 3.0 ml reaction mixture. Corresponding dark vessels showed no change in $P_i$ concentration.

It will be necessary to identify the product or products of the $P_i$ uptake shown by the bacterial extracts and algal preparations. However, since a concurrent increase in $P_7$ was obtained, and considering the results of FRENKEL and of ARNON, it might be anticipated that ATP formation has taken place. If this proves to be correct, "photosynthetic phosphorylation" is seen to occur even in obligately anaerobic organisms.

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