Photoreduction of 2,4-dinitrophenol by chloroplasts

Recently we have shown that DNP is able to catalyse the generation of ATP by illuminated spinach chloroplasts[1]. We explained this effect provisionally by suggesting that DNP, just as the other compounds effective as cofactors in photosynthetic phosphorylation, acts as an intermediate electron carrier across some gap in the electron-transport chain of isolated chloroplasts. This would imply that DNP can be transformed by chloroplasts into a reversible oxidation-reduction system. We have now found that illuminated chloroplasts are able to reduce DNP to 2-amino-4-nitrophenol, and that the latter compound can serve as a cofactor of photosynthetic phosphorylation.

The reaction was carried out at 15° in an illuminated Warburg respirometer. Anaerobic conditions were maintained by flushing the Warburg manometer vessels with pure N_2 and introducing $CrCl_2$ in a side-arm. The reaction mixture consisted of 1 ml of a suspension of chloroplasts in 0.1 *M* Tris buffer, pH 7.5, containing 1 mg chlorophyll and prepared as described previously[2], 0.6 μmole DNP, and deionized water to give a final volume of 3.0 ml. The reaction was terminated after 60–90 min by turning off the light. Chloroplasts were removed by centrifugation, and the absorption spectrum of the orange supernatant was determined in the Unicam spectrophotometer. A rather broad absorption band was observed with a maximum at 430–440 mμ. The absence of DNP in the supernatant was indicated by the complete disappearance of the characteristic absorption maximum of this compound at 360 mμ.

The transformation of DNP proved to be dependent on light and on anaerobic conditions. When the mixture was kept in the dark or when the chloroplasts were illuminated in air, DNP could be recovered nearly quantitatively even after 2 h. This was also the case when boiled chloroplasts were illuminated in the presence of DNP. The transformation of DNP proceeded approximately twice as fast if phosphorylating reagents (phosphate, ADP, $MgCl_2$, glucose and hexokinase) were present. The reaction was strongly inhibited by 10^{-4} *M* *o*-phenanthroline, 4.10^{-6} *M* 3-(4-chlorophenyl)-1,1-dimethylurea and 10^{-3} *M* hydroxylamine, which are known to inhibit the Hill reaction, and was accompanied by oxygen evolution (1.35 μmole of O_2 per μmole of DNP). This suggests that DNP serves as a Hill oxidant and can be reduced by illuminated chloroplasts. As 3.10^{-4} *M* *p*-chloromercuribenzoate showed no inhibitory effect, we think that photoreduction of TPN, which is very sensitive to this poison, is not involved in this process.

In order to identify the product of the photoreaction, we collected the contents of a great number of Warburg vessels in which the reaction had been performed, and removed the chloroplasts by centrifugation. The supernatant was acidified to pH 5 by addition of 1 *N* H_2SO_4, and extracted with ether. The ethereal extract was thoroughly extracted with NaOH solution of pH 9. The aqueous solution was then again brought to pH 5 by addition of 1 *N* H_2SO_4, extracted with ether, and the ether was removed by vacuum distillation. The residue was recrystallized twice from water, and the crystalline solid (needles) dried in a vacuum desiccator over conc. H_2SO_4. The orange-yellow crystals melted at 143°. The melting point was not depressed by mixing the substance intimately with synthetically prepared 2-amino-4-nitro-

Abbreviations: DNP, 2,4-dinitrophenol; FMN, flavin mononucleotide; ATP, adenosine triphosphate; ADP, adenosine diphosphate; TPN, triphosphopyridine nucleotide; Tris, tris-(hydroxymethyl)aminomethane.

phenol[3]. The compounds were found to be identical also with respect to ultraviolet and infrared spectra, and to paper chromatography in four different solvents.

2-Amino-4-nitrophenol was shown to be capable of catalyzing photosynthetic phosphorylation. We therefore may conclude that the ability of DNP to catalyze ATP synthesis by illuminated chloroplasts is due to photoreduction of this compound.

We have found that photoreduction of DNP did not occur in the presence of phosphorylating agents and vitamin K_3 or FMN. This indicates that the transformation of DNP into 2-amino-4-nitrophenol cannot account for the insensitivity of photosynthetic phosphorylation to DNP. It seems evident, therefore, that DNP itself is unable to effect uncoupling of photosynthetic phosphorylation.

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Announcement

Vth International Congress of Biochemistry

The Vth International Congress of Biochemistry, organized by the U.S.S.R. Biochemical Society under the auspices of the International Union of Biochemistry, is to be held from August 10th–16th, 1961, in Moscow. Details concerning this Congress may be obtained from the Secretary-General of the Organizing Committee, Professor N. M. Sissakian, Leninsky prospekt 33, Moscow B-71, U.S.S.R