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"Research in Photosynthesis"

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RESEARCH IN PHOTOSYNTHESIS

The enclosed manuscript entitled, "Observations on the Reducing Side of the O_2 Evolving Photoact," (manuscript presented at the Brookhaven Symposium on Photosynthesis) was completed during the last reporting period. The various subjects touched upon in this manuscript have been studied further in the past three months. Emphasis was on the kinetics of the various reactions of dichlorophenol indophenol in illuminated chloroplasts. Experimental work on fluorescence emission included the design and operation of a multi-flash technique which allowed a direct measurement of the ratio of the pool sizes of the primary and secondary reductants of photosystem II. The number obtained agreed quite well with earlier indirect measurements and indicated a ratio of 1-20. A good deal of time has been spent to organize our earlier fluorescence work and ready it for publication. New lines of research have been initiated; these concerned the conformational changes occurring in chloroplast particles upon illumination, chemiluminescence and the possible role of cytochrome b. Dr.'s P. and A. Joliot (husband and wife) both outstanding workers in the field of photosynthesis have joined us for a period of collaboration. They have installed their sophisticated polarographic equipment which initially will be applied for studies of enhancement and later also for other research concerning the O_2 evolution.

Role of Manganese in Photosynthesis.

As previously reported the restoration by manganese of photosynthesis or the quinone Hill-reaction in manganese deficient *Scenedesmus* is independent of protein synthesis but peculiarly dependent upon light. The requirement for light, using either manganous or permanganate ions, cannot be explained by the light causing an increased flux of these ions into the cell. Concentrations of KCN to completely inhibit photosynthesis do not inhibit the restoration. Thus, complete photosynthesis is not necessary for restoring by manganese full-activity of both photosynthesis and the Hill-reaction.

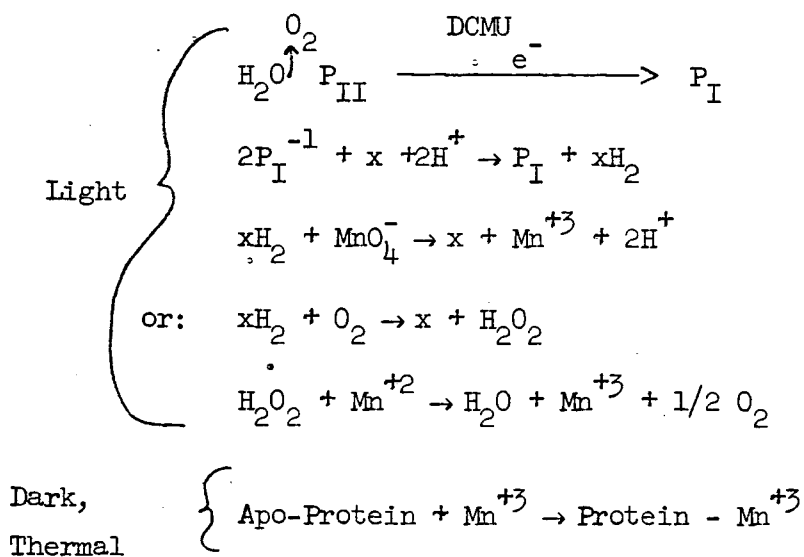
Whereas the restoration of quinone-Hill reaction is completely dependent upon light, a small but significant (10-15%) increase in photosynthesis can be observed with dark preincubation of deficient cells with manganous ions. This small extent of restoration of photosynthesis in the dark appears to be an effect on the CO₂-fixing enzymes. In support of this contention it has been found that the specific activity of the mangano-carboxy-dismutase enzyme in cell-free extracts of these deficient, non-preincubated cells is decreased 10-20% from the control cells. For full restoration of photosynthesis, however, the requirement for light is observed exactly as in the quinone-Hill reaction.

DCMU, an inhibition blocking electron flow between photosystems II and I, prevents restoration in the deficient cells. • Low temperature (4°C) delays the light dependent restoration. From this the suggestion can be made that in the restoration process thermal-dependent collision processes are involved

as well as thermal-independent photoreactions. The experiments with DCMU also eliminate the possibility of non-specific chlorophyll-sensitized photo-oxidative processes being involved.

It is believed, however, that oxidation processes are involved in the restoration by mangannous ions but not in the case of permanganate ions. Thus, illumination of deficient cells under argon with added mangannous ions results in no restoration. Under the same conditions except with permanganate, restoration is observed.

From these type experiments, it is postulated that the reductant of photosystem I is involved in restoration either by Mn^{+2} or MnO_4^{-1} :



Subsequent binding to its enzyme of some oxidation-state of manganese between +5 and +2 leads to full activity of photosystem II and the O_2 -evolving reactions. Experiments are in progress to test this hypothesis.

At the cell-free level studies have been continued on the size of the manganese pool in photosynthetic tissues and the relation of the pool to

Hill-activity. With *Scenedesmus* particles we had reported previously a pool of tightly bound manganese of $1\text{Mn}/45$ Chlorophylls, an unexpectedly high amount. This value was obtained using radio-chemical procedures. Substantiation of this pool size now has been obtained by direct chemical determination of manganese in chloroplasts of spinach as well as particles of *Scenedesmus*. By suitable procedures, however, this pool-size can be decreased 90% without affecting quantum yield or saturation rate of Hill-activity. We are currently investigating the relation of the large pool of manganese to the O_2 -gush ($1 \text{ eq}/40 \text{ Chl.}$) and the small pool of manganese ($1\text{Mn}/500 \text{ Chl.}$) to steady-state O_2 -production.

In support of a very specific function of manganese in photosynthesis electron micrographs of deficient cells ($\sim 90\%$ deficient by growth measurements) reveal no striking dissimilarities from that of normal cells. Minor differences such as decrease in thickness of cell-wall, absence of starch grains and increase in cell-size are observed in the deficient cells but the lamellar-stacking of the chloroplasts is scarcely affected by the deficiency.