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Structural Relationship in Chloroplast Membranes

Final Report

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ABSTRACT

Methodology has been developed for the isolation and characterization of pigment-proteins from chloroplast membranes. Characterization of these pigment-proteins has increased our understanding of mechanisms regulating the efficiency of photosynthetic light harvesting during photosynthesis. Incorporation of isolated pigment-protein complexes into model membranes has allowed simulation of grana stacks; these structural features of chloroplasts play a key role in maintaining appropriate interactions among light-harvesting assemblies to regulate photosynthetic solar energy conversion.

FINAL REPORT

The first step in all biological solar energy conversion is the capture of radiant energy by chlorophyll and conversion of this energy into a stable chemical form via the photosynthetic light reactions. The enzymes which catalyze these reactions, as well as the pigments which absorb the radiant energy, are localized within the lamellae of higher plant chloroplasts. It is now generally accepted that the photosynthetic process, when working under conditions of optimal efficiency, requires 8-10 quanta to drive the photosynthetic fixation of one CO₂ molecule. Extensive studies in many laboratories over the last 25 years have laid out a general framework for the biochemical/biophysical steps which influence the quantum efficiency of the initial stages of solar energy conversion. A general conclusion that has come out of all these studies is that a large number of components (both pigments and enzymes catalyzing electron transport) must interact via specific mechanisms which are imposed by the structural organization of the membrane system in which they are embedded. A purpose of our research has to define the diversity of proteins which are involved in regulating membrane organization features, to understand the mechanisms by which lipids and proteins associate and interact, and to understand possible control mechanisms that might influence the assembly, turnover, and dynamic changes in interactions among and within the protein complexes that form the basic structure of the chloroplast membrane.

Chloroplast membranes consist of a lipid bilayer in which are embedded protein complexes. The proteins comprise approximately 60% of the total mass of the membrane; they function both as specific enzymes catalyzing electron transfer steps and as structural components which bind and orient the pigments which are active in solar energy capture. Any procedure, be it biochemical, genetic, or damage induced by environmental stress, which significantly alters the organization of proteins in chloroplast membranes results in a block and/or a decrease in efficiency of the related photosynthetic processes. From a practical standpoint, it has now been shown in several systems that one of the earliest effects of environmental stress upon plant physiological function is a decrease in the quantum efficiency of the photosynthetic reactions. It has been our goal to more precisely define specific protein interactions within chloroplast membranes, and to then evaluate how changes in these interactions might influence the photosynthetic process.

In 1932, Emerson and Arnold investigated the involvement of chlorophyll molecules in the absorption of quanta utilized during the photosynthetic process. Their data provided evidence for the functional interaction of approximately 2,400 chlorophyll molecules in absorbing quanta necessary to fix one molecule of CO_2 or in the evolution of one molecule of O_2 . By the early 1960's, these data had been expanded to lead to the concept that approximately 600 chlorophyll molecules are structurally and functionally organized to interact with the components of one functional electron transport chain.

Furthermore, these 600 chlorophylls are subdivided among two photosystems which act in series to drive electron flow from water to the terminal electron acceptor NADP. Each photosystem, with its associated electron transport carriers, exists as a separate structural entity.

Light reactions of photosynthesis are known to be able to function at high quantum efficiency under optimal environmental conditions. Based on our fundamental knowledge of the photosynthetic process, we can pose certain questions regarding how this efficiency is achieved. First, since protein components of the photosynthetic membrane are derived from both cytoplasmic and chloroplast synthesis steps, there must be a mechanism which regulates the rate of synthesis of these various components to give rise to a proper stoichiometry of pigment and enzyme complexes (for both photosystem I and photosystem II). Secondly, since photosystems I and II operate in series, and since each is driven by a separate pigment bed, optimal efficiency of noncyclic electron flow could only be achieved if a mechanism is available to insure equal partitioning of absorbed excitation energy between the two photosystems. This last supposition is based, in part, upon experimental evidence for chromatic adaptations in algae, whereas the first supposition is based, at present, on intuitive logic. Experimental evidence is needed to simply catalog the degree of variability that exists in rates of synthesis of specific membrane components, as well as their turnover, and maintenance of specific stoichiometric ratios of membrane components. It has been the long range goal of this task to identify specific control mechanisms which regulate the basic processes of photosynthetic quantum efficiency.

Grana stacks are the predominant and characteristic structural features of higher plant chloroplast membranes. In these stacks, 2-20 or more disc-shaped thylakoids lie appressed in a structure resembling a stack of coins. It is common that these grana stacks are larger in shade-adapted plants. It has therefore been suggested, by implication, that the stacking phenomenon is a process which is involved in increasing light harvesting efficiency. Our most recent studies have presented a major step forward in understanding the grana stacking process. An isolated, light-harvesting pigment-protein complex (LHC) has been isolated and purified from detergent-solubilized chloroplast membranes. The complexes were dialyzed to remove detergents and then examined by freeze-fracture electron microscopy. The material was found to consist of planar sheets of 80 Å subunits which interacted via edge-to-edge contact. To further understand the mechanism by which this pigment-protein mediates grana stacking, the isolated complexes were incorporated into phospholipid vesicles. The LHC was found to span the lipid bilayer. The complexes were arranged in either random patterns or in hexagonal crystalline lattices. Removal of surface-exposed segments of the pigment-protein by protease treatment resulted in a reorientation of a portion of the population of particles within the lipid bilayer.

Addition of monovalent or divalent cations to phospholipid vesicles containing the LHC caused vesicle aggregation. The cation concentration requirement for this process mimicked exactly the levels of cations needed to elicit grana stacking in intact chloroplast membranes. We have, therefore, simulated the in vivo grana stacking process with a model membrane system containing only one chloroplast-derived protein complex. This now allows a detailed molecular evaluation of the mechanism of membrane adhesion.

A number of research laboratories have previously correlated salt effects on membrane appression with cation-induced changes in excitation energy distribution between photosystems I and II. This regulation of energy distribution is thought to involve the LHC. Our studies using LHC in a model membrane system have provided new evidence explaining how altered pigment-protein interactions could influence the regulatory steps controlling light harvesting efficiency.

The research conducted under the auspices of this contract is not fully complete. One of the investigators (C. J. Arntzen) has assumed a new position at Michigan State University where he is the director of the DOE Plant Research Laboratory. In this capacity he will continue studies of factors regulating photosynthetic efficiency and will extend the findings reported above.

Publications Issued During the Contract Period:

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Staehelin, L. A. and C. J. Arntzen: Effects of Ions and Gravity Forces on the Supramolecular Organization and Excitation Energy Distribution in Chloroplast Membranes. *In: Chlorophyll Organization and Energy Transfer in Photosynthesis*, Ciba Foundation Symposium 61, ISBN Elsevier/North-Holland. 147-175. 1979.

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