Pigment Protein Complex from Gonyaulax

Abstract. *A water-soluble peridinin-chlorophyll-protein complex from the dinoflagellate* Gonyaulax polyedra *was found to have a molecular weight of about 38,000. The complex could be disrupted by digestion with proteolytic enzymes. No electron transfer was observed when the complex was irradiated.*

The pigments of the chloroplasts of algae and higher plants are probably complexed with lipoprotein. Numerous attempts have been made to extract such complexes intact, with varying degrees of success (*1*). Most of the extracts containing chlorophyll and protein were suspensions rather than true solutions, and the chlorophyll could be sedimented from them by centrifugation unless the pigments were made soluble by the addition of detergents (*2*).

During the preparation of luciferin and luciferase from *Gonyaulax polyedra,* Bode noted that extracts of these cells in buffer solutions were deep orange in color (*3*). He identified the source of this color as the xanthophyll peridinin, the principal carotenoid of dinoflagellates, and suggested that it might be bound to a protein. We have prepared a similar orange solution from *Gonyaulax* and have found that the pigment could not be sedimented even by centrifugation at 150,000*g* for 8 hours. The ease with which this soluble carotenoid could be prepared prompted us to study its properties further.

Cultures of *Gonyaulax polyedra* (*4*) were harvested at the end of the logarithmic phase of growth by centrifugation for several minutes at 1000*g*. The cells were suspended in 0.01*M* phosphate buffer at a *p*H of either 6.8 or 8, and were broken by treatment with high frequency sound for 6 minutes in an ice-water bath in a Branson Sonifier. The resultant extract was centrifuged at 20,000*g* for 20 to 30 minutes in the cold, and the sediment was discarded. The supernatant was treated with streptomycin sulfate (0.3 volumes of a 5-percent solution) for 30 minutes, the precipitated nucleic acids were removed by centrifugation, and the supernatant solution was dialyzed overnight against 0.01*M* phosphate buffer. The absorption spectrum of such an extract is shown in Fig. 1*A*. The presence of chlorophyll *a* and a xanthophyll are manifest by absorption maxima at 440, 480, and 680 mμ.

When two volumes of acetone were added to the crude pigment solution, there was an immediate color change from brick red to yellow. A similar color change occurred when the extract was heated. When the pigments were extracted into ether and the ether was evaporated to dryness, the pigments were no longer water-soluble. The absorption maxima of the carotenoid were those characteristic of peridinin when the pigment was taken up in benzene, carbon disulfide, or petroleum ether.

Chlorophyll was always present in the complex. This complexed chlorophyll represented about one-fifth of all the chlorophyll present in the cell extract. The remainder was associated with small particles.

Precipitation of the orange complex in 90 percent saturated ammonium sulfate or in 10 percent trichloroacetic acid suggested that a pigment-protein complex was present. Freezing and thawing tended to cause precipitation of a part of the complex.

The molecular weight of the pigment-protein complex was estimated by chromatography on Sephadex (*5*) with cytochrome *c* (mol. wt. 12,400), ovalbumin (mol. wt. 45,000), and gamma globulin (mol. wt. 160,000) as standards, and was found to be about 38,000. About the same molecular weight has been reported by Wolken (*6*) for "chloroplastin," a chlorophyll-protein complex isolated in digitonin from *Euglena.*

Digestion of the pigment complex with a mixture of trypsin and chymotrypsin for 4 hours brought about considerable change in the spectrum (Fig. 1*B*). A difference spectrum before and after digestion showed that most of the peridinin and some of the chlorophyll was lost during digestion.

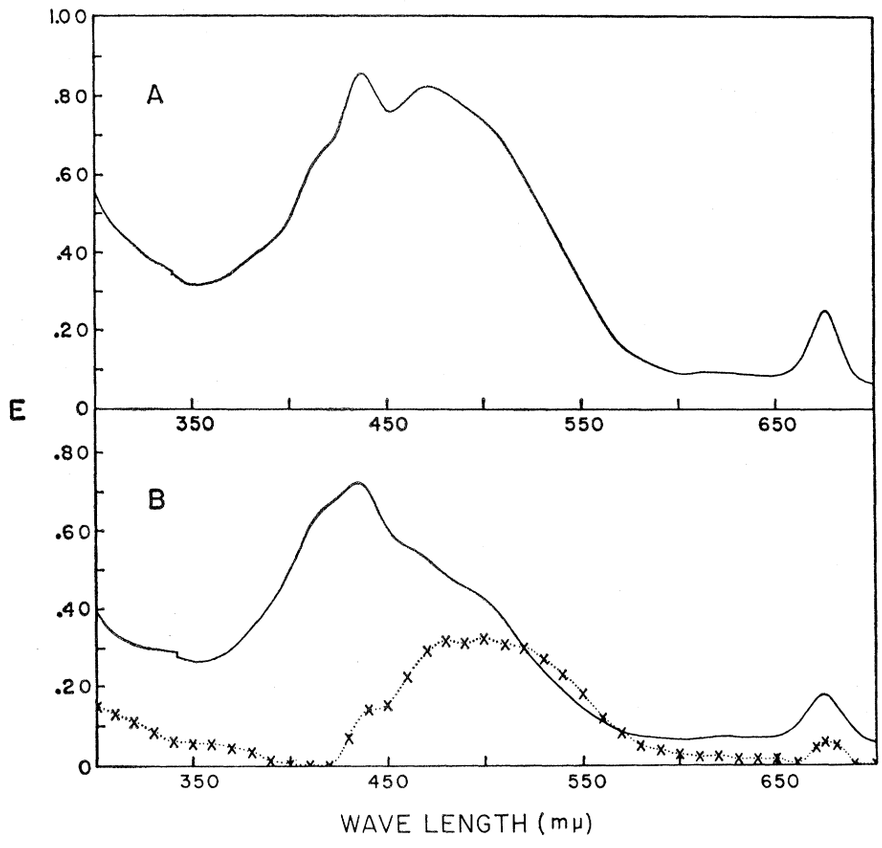

Fig. 1. Spectrum of the peridinin-chlorophyll-protein complex from *Gonyaulax polyedra* before and after digestion with trypsin and chymotrypsin. (*A*) Spectrum of the extract treated with streptomycin sulfate and dialyzed against 0.01*M* ammonium carbonate buffer, *p*H 8.3, containing 2*M* urea. This spectrum is indistinguishable from that of the undialyzed extract. (*B*) Spectrum of the same extract after digestion for 4 hours with 5 μg of trypsin and 2.5 μg of chymotrypsin (solid line), and the spectrum for the digested material calculated from the difference between spectra before and after digestion (dashed line).

Chromatography of the digested complex on Sephadex indicated a decrease in the amount of high-molecular-weight, colored material.

The pigment-protein complex from *Gonyaulax* did not catalyze the photoreduction of ferricyanide or 2,6-dichlorophenol indophenol. No enhancement of photoreduction by spinach chloroplasts was observed in the presence of the complex.

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Ethylene Formation in Rat Liver Microsomes

Abstract. *Reduced triphosphopyridine nucleotide and pyrophosphate-dependent peroxidation of lipids in rat liver microsomes were coupled to the generation of ethylene in the presence of cuprous ions. This system suggests a model for the biogenesis of ethylene in cells.*

The almost ubiquitous presence of endogenous ethylene in minute amounts in plant cells and the profound physiological effects of exogenous ethylene on plant tissue have raised the question of the origin of ethylene in plant metabolism (*1*). There is no known metabolic pathway to which the formation of ethylene can be assigned. Lieberman and Mapson (*2*) described a model system in which ethylene was produced during the cuprous-catalyzed degradation of peroxidized linolenic acid. This model suggests that the origin of ethylene may be associated with peroxidation of lipids in the plant cell. It also raises the possibility that ethylene may be formed by a similar process in animal cells.

In this report we describe experiments which demonstrate that ethylene formation can be coupled to the reduced triphosphopyridine nucleotide ($NADPH_2$) and pyrophosphate-dependent peroxidation of lipids in rat liver microsomes (*3*, *4*) with a cuprous-generating system.

Suspensions of rat liver microsomes incubated with $NADPH_2$ and adenosine triphosphate (ATP) produced lipid peroxides but no ethylene. However, addition of a cuprous-generating system (5 μmole of cupric ions and 50 μmole of ascorbate) resulted in formation of physiologically significant amounts of ethylene (0.1 to 0.5 nmole/hr per milligram of protein). The amount of ethylene formed was approximately the same in systems containing the cuprous-generating system from the start of the reaction and in those to which the cuprous-generating system was added after peroxidation was virtually complete. Rate of ethylene production in these systems and its relation to lipid peroxidation are shown in Fig. 1. The course of ethylene production is similar to malonaldehyde formation, which is a measure of lipid peroxidation. However, malonaldehyde is not the precursor of ethylene, since there is a much greater increase in the production of ethylene (about 45 percent) than of malonaldehyde (about 25 percent) following a further addition of ascorbate 2 hours after starting the reaction. We have also found that malonaldehyde is a very inefficient source of ethylene in the cuprous-catalyzed system when compared directly with peroxidated linolenate. An intermediate that appears earlier in the lipid peroxidation sequence must be the precursor of ethylene.

Suppression of lipid peroxidation by incorporating vitamin E into the reaction system sharply reduced ethylene production and malonaldehyde formation (Table 1). When vitamin E and the cuprous-generating system were added to the lipid peroxidation system after a 1-hour incubation, during which malonaldehyde accumulated, there was relatively little ethylene formed on further incubation for 1 hour. This indicates that vitamin E can inhibit the formation of ethylene not only by preventing peroxidation but also during the cuprous-catalyzed reaction. Since vitamin E acts as a free-radical terminator (*5*), the data suggest that a free-radical mechanism may be involved in the formation of ethylene in these systems.

Table 1. Effect of vitamin E on lipid peroxidation and ethylene formation in $NADPH_2$-induced lipid peroxidation of rat liver microsomes coupled to a cuprous-generating system. The lipid peroxidation and cuprous-generating systems were as described in the legend to Fig. 1. C_2H_4, ethylene; MA, malonaldehyde.

Addition	Product (nmole/hr per milligram of protein)	
	C_2H_4	MA
None	0.210	630
Vitamin E, 1 mg	.045	220
Vitamin E, 1 mg, and cuprous system (after lipid peroxidation)*	.066	356

* Lipid peroxidation system minus cuprous system incubated for 1 hour, followed by addition of vitamin E and the cuprous-generating system, as indicated, and again incubated for 1 hour.

When the concentration of microsomes was increased above 6 mg of protein per flask in these reactions a decrease in ethylene formation resulted. No ethylene production occurred with about 50 mg of protein in the incubation mixture. The explanation of this phenomenon may lie in the copper-sequestering capability of the microsomes. The proteins of these microsomes contain many sulfhydryl groups which tie up the copper ions by forming –S–Cu–S– moieties. Consequently, no cuprous ions remain to catalyze the formation of ethylene from peroxidated fatty acids. It is also possible that redox reactions, with higher concentrations of microsomes, oxidize ascorbate and thus leave little or no reducing agent for the formation of cuprous ions.

Production of ethylene by homogenates of rat livers was recently reported by Ram Chandra and Spencer (*6*). Whether or not ethylene can be formed by intact animal cells is speculative. However, the system reported here presents a model which provides a mechanism for ethylene formation in microsomes by way of lipid peroxidation, followed by reaction with a hypothetical copper enzyme.

The physiological significance of ethylene in animal cells is not known, but the fact that it has for many years served as an anesthetic in surgery (*7*) attests to its physiological