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BARD

FINAL REPORT

PROJECT No. US-80-79

... A Study of the Mechanism of
... Photosynthetic Herbicide Action with the
Aim of Improving Weed Control and
Thereby Increasing the Efficiency of
Water and Soil Utilization

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A Study of the Mechanism of Photosynthetic Herbicide Action with the Aim
of Improving Weed Control and Thereby Increasing the Efficiency of Water
and Soil Utilization

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III. ABSTRACT:

BARD Research Project No. US 80-79 was set up to characterize the molecular mechanism for herbicide resistance. This goal has been accomplished. In studies of higher plants a 32 kilo Dalton has been identified as the triazine receptor protein. This polypeptide has been found to be maternally inherited. In studies with the algae Chlamydomonas the presence of herbicide polypeptide has been correlated with high affinity herbicide binding sites. Developmental studies with Chlamydomonas have further demonstrated that while the herbicide binding protein is required for binding, other polypeptides of the system II complex confer important attributes to the herbicide binding site, especially those effecting the binding constant for different classes of herbicides. In both higher plants and algae it has been demonstrated that protein phosphorylation alters herbicide binding affinity. The studies completed under the auspices of this project have provided insights into the biochemistry of the herbicide binding site in chloroplast membranes that should allow rapid progress to be made in the transfer of herbicide resistance to crop plants.

IV. BODY OF THE REPORT:

Introduction

A large number of chemical compounds are in commercial use today as herbicides; several major classes have their primary site of action on the photosynthetic process. Of the compounds which inhibit photosynthesis, a large number have a direct affect on the membrane-bound enzymatic processes which are involved in the light reactions of photosynthesis -- these include ureas, uracils, triazines, and pyridazones.

Studies in many laboratories over the last five years have greatly increased our understanding of the reactions of photosystem II (the target site of many of the above-mentioned herbicides) as well as the mode of action of these chemicals. The following material will focus specifically on details of the research that relate to the objectives of our original proposal, and will describe the progress we have made in reaching these objectives. The literature citations used refer to our published reports and manuscripts (listed in section IX of this report).

Results

A. Identification of Herbicide Target Sites.

The first objective of our research proposal was to identify the components of the chloroplast which determine herbicide binding. These studies are now completed. There are four lines of evidence which have indicated a 32 Kilo-dalton (Kd) polypeptide is the primary determinant of triazine (atrazine) or phenylurea (diuron) binding:

- 1) Selective modification of proteins in isolated membranes by proteases removes a 32 Kd polypeptide and, in parallel, causes loss of herbicide binding sites (1).
- 2) Photosystem II particles can be prepared which contain a 32 Kd protein and are herbicide sensitive; removal of the protein causes loss of herbicide activity (2).
- 3) A photoaffinity triazine has been found to react specifically with a polypeptide of approximately 32 Kd (3, 4, 5).
- 4) In developing chloroplast of Chlamydomonas depleted of 32-35 Kd polypeptides there is a loss of high affinity herbicide binding sites (5, 6).

In addition, several observations provide evidence that components of the thylakoid other than the 32 Kd protein are involved in the herbicide receptor site; more specifically, the core (or reaction center) polypeptides of PS II -- approximately 43 to 50 Kilodaltons -- are required for high affinity binding. We have shown that in Chlamydomonas with incompletely developed thylakoids (containing the 32 Kd protein but lacking the reaction center proteins), only low affinity herbicide binding was detected (5, 6).

B. Regulation of Binding Specificity at the Herbicide Receptor Site of Chloroplast Thylakoids.

The second objective of our BARD proposal was to study the binding mechanism and specificity for binding among different classes of herbicides. This objective is now nearly complete, although it has led to our recognition that the site is not "constant" -- there is biological variability over time due to covalent modification of the binding protein by protein phosphorylation.

Our concept of the herbicide binding process has changed considerably over the last three years. The original idea indicated that herbicides bind to PS II and allosterically change the environment around a bound quinone (B) which serves as a PS II electron carrier (discussed in our original proposal). We have now provided evidence for an alternate concept (5); this is based upon the hypothesis that herbicides displace the bound quinone (originally formulated by Velthuys at a "quinone conference" in 1981). The major idea of our recent experiments is that a photoaffinity quinone can be covalently linked to the quinone binding site in "B". When irreversibly held in this site, the affinity of the site of herbicides is dramatically reduced. Since all classes of PS II-directed herbicides compete with one another for binding, we conclude that all act by quinone displacement at B. The mutually competitive interaction at a single site explains the competition among the many classes of

chemicals for binding. It is important to note, however, that we believe that different amino acid determinants around the binding site selectively influence the binding affinities of different chemicals.

The highly specific involvement of proteins in controlling herbicide binding to the PS II site has been dramatically indicated in recent studies of chloroplast thylakoid phosphorylation. In both algae (7, 8) and higher plant chloroplasts there is a light-activated protein kinase which phosphorylates several thylakoid proteins, including the 32 Kd herbicide receptor protein. Protein phosphorylation increases herbicide binding affinity (5, 9). We believe the protein phosphorylation may also affect thylakoid protein turnover.

C. Genetic Basis for Herbicide Resistance.

The third objective of our USDA/BARD US 80-79 grant was to understand the genetic basis for triazine-resistance found in many naturally occurring weeds. This section of the research is also nearly completed based upon our own studies (5, 11, 12) as well as those at other locations, particularly at the University of Guelph. It is clear that the triazine-resistance trait, due to a chloroplast membrane alteration, is maternally inherited (10). Furthermore, based upon identical properties of a chloroplast-encoded gene product and the protein labeled by azido-atrazine, the triazine receptor protein has actually been located upon the chloroplast genome (11, 12). In breeding studies, Beversdorf and colleagues at Guelph have utilized the knowledge that the resistance is maternally inherited and have succeeded in transferring the triazine resistance trait into commercial varieties of Brassica. Either Brassica napus or Brassica campestris (L.) (oil-seed rape) were utilized as the paternal parent and B. campestris (atrazine-resistance wild mustard) as the female parent in these experiments. After repeated backcrossing of the progeny using the commercial variety as the male parent,

seed has been released for commercial use carrying the resistance trait (i.e., the chloroplast of the weed but the nuclear genotype of the commercial crop). This is truly a portend of the future for genetic engineering of herbicide resistance.

V. EVALUATION OF RESEARCH ACHIEVEMENTS WITH RESPECT TO THE ORIGINAL RESEARCH PROPOSAL

The evaluation of the research achievements are very simple. The proposal asked three general questions: What are the chloroplast components which bind herbicides?, How do they bind the herbicides?, and Where are these components encoded? We have answered these questions. In addition, we have demonstrated the complexity of the herbicide binding phenomena, but have formulated the general concept by which herbicide susceptibility and resistance can be generated. This information will be useful in future studies to genetically engineer herbicide resistance in crop plants.

VI. DESCRIPTION AND RESULTS OF COOPERATION

This project has allowed extensive and fruitful collaboration between the two principal investigators in the United States and Israel. This collaboration has been culminated by a six months stay by Dr. Itzhak Ohad in the United States in the laboratory of C.J. Arntzen. This period of collaborative research has allowed us to complete all aspects of the project as originally proposed. We hope that a mechanism will be available in the future to continue this fruitful collaboration.

VII. THESIS

A detailed understanding of the molecular mode of action of herbicides affecting the photosystem II complex is now available. Utilizing this information, and the understanding of the genetic basis for herbicide resistance, we hypothesize that it is possible to genetically engineer herbicide resistance in crop plant.

VIII. INVENTIONS

None

IX. PUBLISHED REPRINTS AND MANUSCRIPTS

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10. Uniparental inheritance of a chloroplast photosystem II polypeptide controlling herbicide binding. Sylvia Darr, V. Souza Machado and Charles J. Arntzen. Biochim. Biophys. Acta 634, 219-228 (1981).

11. Identification of the triazine receptor protein as a chloroplast gene product. Katherine E. Steinback, Lee McIntosh, Lawrence Bogorad and Charles J. Arntzen. Proc. Natl. Acad. Sci. USA 78, 7463-7467 (1981).
12. Identification of the receptor site for triazine herbicides in chloroplast thylakoid membranes. Katherine E. Steinback, Klaus Pfister and Charles J. Arntzen. In "Biochemical Responses Induced by Herbicides" ACS Symposium Series No. 181 (D.E. Moreland, et al., eds.) pp. 37-55 (1982).