Proc. Natl. Acad. Sci. USA
Vol. 78, No. 12, pp. 7463–7467, December 1981
Biochemistry

Identification of the triazine receptor protein as a chloroplast gene product

(photosystem II/*Amaranthus*/*Zea mays*/restriction endonuclease)

KATHERINE E. STEINBACK*, LEE MCINTOSH†‡, LAWRENCE BOGORAD†, AND CHARLES J. ARNTZEN*

*MSU–DOE Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824; and †The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

Contributed by Lawrence Bogorad, September 4, 1981

ABSTRACT **The triazine herbicides inhibit photosynthesis by blocking electron transport at the second stable electron acceptor of photosystem II. This electron transport component of chloroplast thylakoid membranes is a protein–plastoquinone complex termed "B." The polypeptide that is believed to be a component of the B complex has recently been identified as a 32- to 34-kilodalton polypeptide by using a photoaffinity labeling probe, azido-[^{14}C]atrazine. A 34-kilodalton polypeptide of pea chloroplasts rapidly incorporates [^{35}S]methionine *in vivo* and is also a rapidly labeled product of chloroplast-directed protein synthesis. Trypsin treatment of membranes tagged with azido-[^{14}C]atrazine, [^{35}S]methionine *in vivo*, or [^{35}S]methionine in isolated intact chloroplasts results in identical, sequential alterations of the 34-kilodalton polypeptide to species of 32, then 18 and 16 kilodaltons. From the identical pattern of susceptibility to trypsin we conclude that the rapidly synthesized 34-kilodalton polypeptide that is a product of chloroplast-directed protein synthesis is identical to the triazine herbicide-binding protein of photosystem II. Chloroplasts of both triazine-susceptible and triazine-resistant biotypes of *Amaranthus hybridus* synthesize the 34-kilodalton polypeptide, but that of the resistant biotype does not bind the herbicide.**

When dark-grown seedlings are transferred to light, a sequence of developmental processes leads to the formation of green, photosynthetically competent chloroplasts (1). Rapid accumulation of a major thylakoid protein of 32 kilodaltons (kDal) parallels the appearance of functional activity (2). In *Zea mays*, this 32-kDal protein is structurally related to, and is presumably derived from, a 34.5-kDal polypeptide that is the major membrane-bound product of protein synthesis by isolated chloroplasts (3). It has been shown to be encoded by a chloroplast gene in *Z. mays* (4). A number of chloroplast-encoded proteins appear to be synthesized in etioplasts of dark-grown maize seedlings (2), but the transcription of the chloroplast gene coding for this polypeptide is light dependent in developing plastids and has thus been described as a "photogene" (5, 6). Until recently, no function had been determined for the *photogene 32* product; it has been identified now as a determinant of the electron transport function of a bound plastoquinone molecule in the photosystem II (PS II) complex (7). Its photoregulated synthesis (2, 8), apparent rapid turnover, and continued synthesis throughout all stages of leaf development (2, 3, 9) suggest a regulatory role for the protein product in PS II function. Purified PS II complexes isolated by detergent fractionation techniques contain a protein component of 32 kDal (10, 11). Certain mutants of *Z. mays* lacking PS II function are deficient in a 32-kDal membrane polypeptide (12). Trypsin treatment of thylakoid membranes (13) or of isolated PS II complexes (10) results in the degradation of the 32-kDal polypeptide with concomitant loss of PS II activity. These lines of evidence also suggest that the 32-kDal polypeptide plays an integral role in PS II electron transport.

A broad range of inhibitors of photosynthetic electron transport, including the triazines, act at the same site on the photosynthetic membrane. Inhibition occurs at the level of a protein plastoquinone complex called B, which functions as the second stable electron acceptor of PS II (14, 15). Triazine herbicides are believed to act by associating with a high-affinity binding site within the PS II complex (16) and interfering directly with the function of the quinone cofactor of B. The identity of this high-affinity site has recently been established through the use of azido-[^{14}C]atrazine, a radiolabeled photoaffinity derivative of the PS II inhibitor atrazine. This photoaffinity probe binds covalently to thylakoid membranes, and binding is specific to membrane polypeptides of 34 to 32 kDal (17, 18).

Triazine-resistant biotypes of various weed species have been found in the United States, Canada, and Europe (19–21). In cases studied to date, triazines fail to inhibit photosynthetic electron transport in plastid membranes isolated from resistant weeds. In plants that have been tested in reciprocal crossing experiments, this resistance is maternally inherited (22), suggesting it is carried in the chloroplast genome.

The data presented here indicate that the 34-kDal polypeptide to which azido-atrazine binds is identical to a rapidly synthesized 34-kDal membrane polypeptide product of both chloroplast-directed protein synthesis *in vitro* and protein synthesis *in vivo* in peas and *Amaranthus hybridus*.

METHODS

Plants. Seedlings of dwarf pea (*Pisum sativum* Linnaeus va. Progress 9) were grown as described (23). Leaves were harvested from 12- to 20-day-old seedlings. Plants of susceptible and resistant biotypes of *A. hybridus* (seed collected in the state of Washington) were grown in growth chambers as described (17).

Chloroplast Isolation. Intact chloroplasts were obtained from pea leaves by following the method of Blair and Ellis (24). Leaves (15 g) were homogenized with 100 ml of 0.34 M sorbitol/50 mM *N*-[tris(hydroxymethyl)methyl]glycine (Tricine)–NaOH, pH 7.8/2 mM sodium ascorbate/2 mM Na_2EDTA/5 mM

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: atrazine, 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine; azido-atrazine, 2-azido-4-ethylamino-6-isopropylamino-*s*-triazine; B, a protein-bound quinone serving as the second stable acceptor for photosystem II; Chl, chlorophyll; diuron, 3-(3,4-dichlorophenyl)-1, 1-dimethyurea; kDal, kilodalton(s); LHC, light-harvesting complex of photosystem II; PS II, photosystem II; Tricine, *N*-[tris-(hydroxymethyl)methyl]glycine.

‡ Present address: MSU-DOE Plant Research Laboratory, Michigan State Univ., E. Lansing, MI 48824.

$MgCl_2$. Stroma-free thylakoid membranes were isolated as described (23).

Trypsin Treatment. Trypsin incubations were carried out at room temperature in dim light. Thylakoids were brought to a final concentration of 100 μg of chlorophyll (Chl) per ml with the Tricine resuspension buffer (20 mM Tricine–NaOH, pH 7.8/100 mM sorbitol/5 mM $MgCl_2$). Trypsin (treated with L-1-tosylamido-2-phenylethyl chloromethyl ketone, 223 enzyme units/mg of protein) was added to a final concentration of 2, 10, or 40 μg/ml and, after 15 min, a 20-fold excess of soybean trypsin inhibitor (type 1-S, Sigma) was added. Membranes were pelleted by centrifugation at 4000 × *g* for 5 min and were washed twice with the Tricine resuspension buffer to remove trypsin and trypsin inhibitor.

Photoaffinity Labeling. The photoaffinity labeling of pea thylakoid membranes with azido-[^{14}C]atrazine was carried out as described (17). The compound was generously provided by Gary Gardner (Shell Development, Modesto, CA).

Radioactive Amino Acid Incorporation. For labeling of proteins synthesized *in vivo*, leaves of pea seedlings and of *A. hybridus* were painted with 100–150 μCi of [^{35}S]methionine (New England Nuclear, 1200–1400 mCi/mmol; 1 Ci = 3.7 × 10^{10} becquerels) in a 100-μl solution containing 0.1% Tween-80. Uptake and incorporation of the radiolabel into protein was allowed to proceed for 4 hr prior to isolation of the thylakoid membranes.

For synthesis of radiolabeled proteins *in vitro* by isolated intact chloroplasts (chloroplast-directed synthesis), plastids (0.5 mg of Chl per ml) were incubated with 100 μCi of [^{35}S]methionine for 40 min in an illuminated constant-temperature (20°C) water bath. After incubation, chloroplasts were broken in a low osmotic strength buffer containing 20 mM Tricine–NaOH (pH 7.8) and centrifuged at 4000 × *g* for 5 min. The resulting pellet containing stroma-free thylakoids was washed once in the same buffer prior to trypsin treatment.

Polyacrylamide Gel Electrophoresis. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis was carried out with a 10–17.5% linear polyacrylamide gradient slab gel with a 1-cm 5% stacking gel incorporating the buffer system of Laemmli (25). Samples were prepared for electrophoresis and gels were run, fixed, and stained for protein as described (26).

Fluorography and Autoradiography. Analysis of polyacrylamide gels for azido-[^{14}C]atrazine-protein complexes or [^{35}S]methionine-labeled proteins was accomplished by x-ray fluorography as described (27), using Kodak SB-5 x-ray film. Kodak XR-5 film was used for detecting ^{32}P in hybridization experiments.

Chloroplast DNA Isolation and Restriction Endonuclease Analysis. Chloroplasts were isolated from 100- to 200-g lots of leaves from 6- to 8-week-old *A. hybridus* seedlings. Chloroplast DNA was isolated from DNase I-treated intact chloroplasts by phenol extraction and further purified by sucrose density gradient centrifugation as described (28). Purified chloroplast DNA was digested with *Bam*HI restriction endonuclease and separated by agarose gel electrophoresis (28).

DNA Hybridization. DNA nitrocellulose blots of *Bam*HI digests of chloroplast DNA were prepared according to Southern (29), but filters were preincubated for 3 hr in Denhardt's PM solution (30) containing 50% (vol/vol) deionized formamide at 37°C. Hybridization was performed in the same solution in the presence of 0.1% sodium dodecyl sulfate, denatured calf thymus DNA at 20 μg/ml, and a radioactive labeled cRNA probe. The probe was made by synthesizing ^{32}P-labeled copy RNA from DNA template as described (28). Hybridization was at 37°C for 16 hr, after which the filters were washed at 37°C for 20 min in three changes of 0.45 M NaCl/0.045 M sodium citrate and then exposed for autoradiography (see above).

RESULTS

Chloroplast Membrane Polypeptides. The proteolytic enzyme trypsin was used as a selective membrane modifier to alter surface-exposed polypeptides of pea chloroplast membranes. Analysis of the trypsin-induced changes in membrane polypeptides was carried out in three parallel experiments. In the first, isolated pea thylakoid membranes were irradiated with ultraviolet light in the presence of azido-[^{14}C]atrazine to covalently label the triazine receptor site (17). Membranes were then treated with 2, 10, or 40 μg of trypsin per ml for 15 min. In a second experiment, leaves of pea seedlings were allowed to take up and incorporate [^{35}S]methionine for 4 hr; this resulted in labeling of rapidly synthesized thylakoid polypeptides. Chloroplasts were then isolated and the thylakoid membranes were treated with trypsin as indicated above. In the third experiment, intact chloroplasts were isolated from pea seedlings, incubated with [^{35}S]methionine in the light for 40 min to permit chloroplast-directed protein synthesis, and then shocked osmotically. Thylakoids that were recovered were treated with trypsin. The results of these experiments are shown in Figs. 1 and 2. Control and trypsin-treated samples for each condition were subjected to sodium dodecyl sulfate/polyacrylamide gel

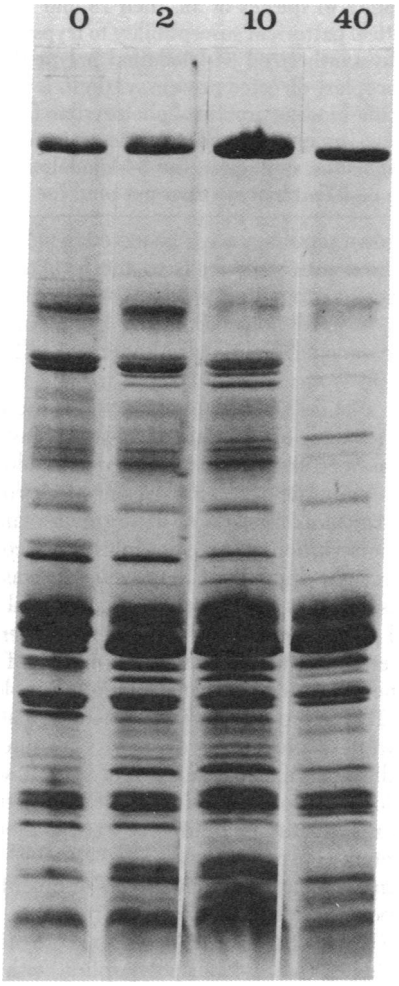

FIG. 1. Polyacrylamide gel, stained for protein, showing the effects of trypsin treatment on chloroplast thylakoid membrane polypeptides. Incubation was with 0, 2, 10, or 40 μg of trypsin per ml.

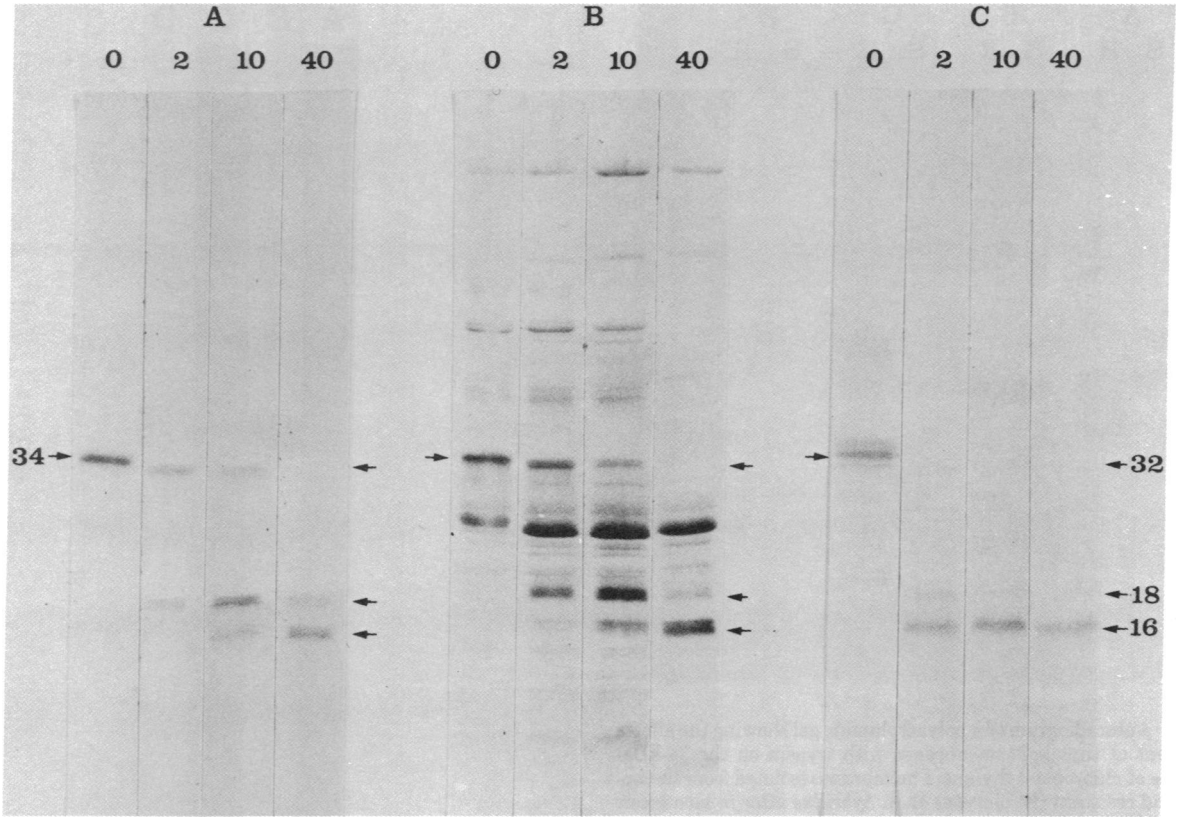

FIG. 2. Autoradiograms of polyacrylamide gels showing effects of treating pea thylakoids with trypsin on the 34-kDal polypeptide radiolabeled by covalent addition of azido-[^{14}C]atrazine (*A*), *in vivo* incorporation of [^{35}S]methionine in whole leaves (*B*), and *in vitro* light-dependent incorporation of [^{35}S]methionine in isolated chloroplasts (*C*). After radiolabeling, isolated thylakoid membranes (100 μg of Chl per ml) were treated with trypsin at 0, 2, 10, or 40 μg/ml, solubilized, and analyzed by gel electrophoresis. Sizes of proteins are indicated in kDal.

electrophoresis. After staining for protein, each set of samples was analyzed for radiolabel incorporation by fluorography.

A number of alterations in electrophoretic mobility of stainable polypeptides were observed in all trypsin-treated samples (Fig. 1); these alterations have been discussed in detail elsewhere (3, 13). In general it is difficult to establish a relationship between the original polypeptides observed in control membrane samples and the multiple bands that appear or disappear as a result of trypsin treatments. An exception is the major apoprotein of the light-harvesting complex (LHC; 25 kDal in the untreated membranes); its increase in electrophoretic mobility corresponds to an apparent decrease in mass of 2 kDal (26).

Irradiation of thylakoids with ultraviolet light in the presence of azido-[^{14}C]atrazine resulted in covalent binding to a single polypeptide of 34 kDal (Fig. 2A). A polypeptide of this molecular mass has been identified as the high-affinity triazine-binding site in *A. hybridus* (17) and peas (18). At 2 μg of trypsin per ml, the lowest concentration utilized, the ^{14}C label shifted to comigrate with the stainable polypeptide of 32 kDal. Treatment of membranes labeled with the azido-[^{14}C]atrazine with trypsin at 10 μg/ml resulted in loss of 34-kDal polypeptide and the appearance of both a polypeptide of 32 kDal and a doublet band of approximately 18-kDal polypeptides. With the highest trypsin concentration used (40 μg/ml) the ^{14}C label was associated with 18-and 16-kDal polypeptide species. A doublet near 16 kDal predominated; the 32-kDal polypeptide was entirely lacking.

In vivo protein synthesis, monitored by [^{35}S]methionine incorporation into thylakoid proteins (Fig. 2*B*), resulted in incorporation of label into two major polypeptide species—the apoprotein of the LHC at 25 kDal and a polypeptide at 34 kDal. After trypsin treatment, the radiolabel associated with the LHC polypeptide is altered in electrophoretic mobility by 2 kDal, following the pattern observed for the stainable polypeptides (Fig. 1). The rapidly synthesized 34-kDal polypeptide is also susceptible to trypsin; its disappearance is paralleled by the appearance of new labeled bands of 32 and 18 kDal. At the highest trypsin level, the labeled 32-kDal polypeptide was absent and a double band near 16 kDal was observed.

The major membrane polypeptide produced by pea chloroplasts *in vitro* is a 34-kDal species [also identified in early studies as "peak D" in peas (31)]. In the fluorogram of control pea membranes (Fig. 2*C*) label was also observed in a polypeptide of 34.5 kDal. This may correspond to a precursor of the 34-kDal species that is rapidly processed in pea chloroplast (3). Trypsin degraded this major synthesized species to lower molecular weights. No 32-kDal intermediate proteolytic product was detected with the trypsin concentrations used. However, the accumulated 18- and 16-kDal digestion products migrated identically to those generated by trypsin treatment of thylakoids labeled *in vivo* (Fig. 2 *B* and *C*).

Chloroplasts isolated from triazine-resistant weed biotypes have been shown to have drastically reduced affinities for triazine herbicides (13). It was of interest to determine if the polypeptide responsible for herbicide sensitivity in chloroplasts of normal, triazine-susceptible plants was also synthesized in the resistant biotypes. The data shown in Fig. 3 for *A. hybridus* demonstrate that the 34-kDal polypeptide is synthesized in both herbicide-susceptible and resistant plants. Furthermore, the

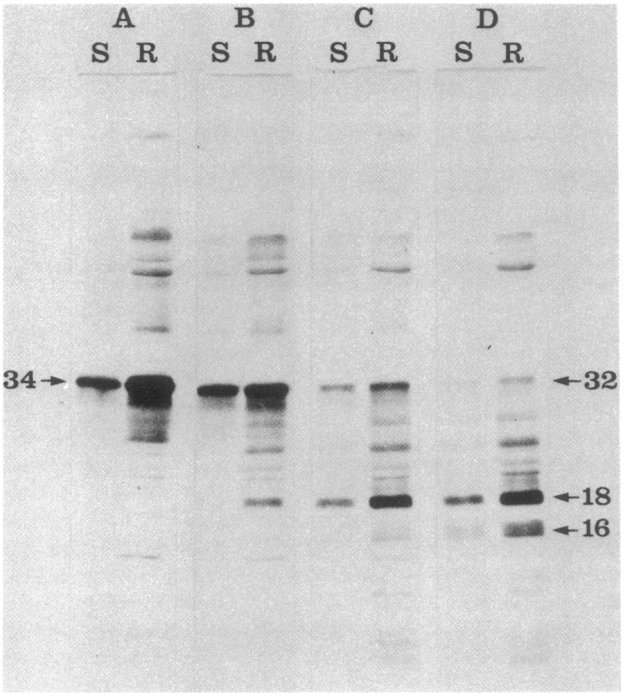

FIG. 3. Autoradiogram of a polyacrylamide gel showing the effects of treatment of thylakoid membranes with trypsin on the 34-kDal polypeptide of chloroplast thylakoid membranes isolated from susceptible (S) and resistant (R) biotypes of *A. hybridus* after *in vivo* incorporation of [^{35}S]methionine in whole leaves. After radiolabeling, isolated thylakoid membranes (100 μg of Chl per ml) were treated with trypsin at 0 (*A*), 2 (*B*), 10 (*C*), or 40 (*D*) μg/ml before sodium dodecyl sulfate/polyacrylamide gel electrophoretic analysis.

polypeptide in resistant membranes and the 34-kDal polypeptide in the susceptible membranes respond to trypsin treatment identically. It is evident that the polypeptide is synthesized and present in both susceptible and resistant membranes, but there is no apparent size difference or change in membrane orientation that can account for variability in triazine affinity.

Chloroplast DNA of Triazine Resistant and Susceptible *A. hybridus*. Chloroplast DNA was isolated from both herbicide-resistant and susceptible biotypes of *A. hybridus* and analyzed by *Bam*HI restriction endonuclease digestion and agarose gel electrophoresis. No major structural changes were detected by using this restriction endonuclease when DNA from the resistant biotype was compared to DNA from the susceptible chloroplast (data not shown).

For further study of the physical basis of herbicide resistance, [^{32}P]cRNA of cloned *Z. mays* chloroplast DNA (pZmc 427) containing photogene 32 (5) was used as a hybridization probe to locate the structural gene for the triazine-binding polypeptide. Hybridization was to a 6-kilobase-pair fragment of chloroplast DNA from the atrazine-resistant biotype of *A. hybridus* (Fig. 4).

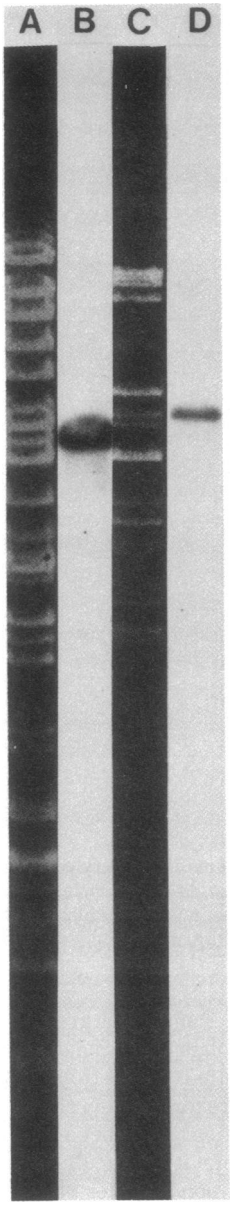

FIG. 4. Chloroplast DNA from *Z. mays* (lane A) and chloroplast DNA from the resistant biotype of *A. hybridus* (lane C) were digested with *Bam*HI and separated by agarose gel electrophoresis. A Southern (29) blot of this gel was then hybridized with a radioactive probe from a recombinant plasmid, pZmc 427, that contains the region of the maize chloroplast genome encoding photogene *32*. Lane B, the control, shows hybridization of the probe, which contains part of the *Bam*HI fragment 8, to *Z. mays* fragment 8. Lane D shows the hybridization of the maize photogene probe to a single 6.0-kilobase-pair *Bam*HI fragment from *A. hybridus*.

DISCUSSION

The Atrazine-Binding Site Is on a Chloroplast-Synthesized Polypeptide. Triazine herbicides, including atrazine, inhibit photosynthetic electron transport through the plastiquinone–protein complex B of PS II (14, 15). Azido-[^{14}C]atrazine binds specifically to polypeptides in the 32- to 34-kDal size range (17, 18). There are a number of polypeptides in this size range in thylakoid membranes (3); among them is a chloroplast photogene product that is absent from plastids of dark-grown maize but that accumulates upon illumination of the plants and is labeled rapidly even in fully green plants provided with [^{35}S]methionine *in vivo*. When thylakoid membranes are treated with trypsin over a range of concentrations the rapidly labeled photogene product (34 kDal in pea chloroplast membranes) yields characteristic proteolytic products of 32, 16, and 18 kDal. The present experiments demonstrate that the azido-atrazine-binding protein is identical to the photogene *32* product, because the two respond identically to mild trypsin treatment.

In the present studies azido-[^{14}C]atrazine was used at 2.5 μM to optimize binding to the highest affinity site in the PS II complex. This concentration inhibits PS II electron transport in pea

chloroplasts by 85%. Under these conditions, only a 34-kDal species was labeled by azido-[^{14}C]atrazine. Mild trypsin treatment of membranes labeled in our experiments either by administering [^{35}S]methionine to leaves or by exposing chloroplasts to azido-[^{14}C]atrazine *in vitro* resulted in the production of a 32-kDal labeled species. Alteration of the 34-kDal polypeptide to a 32-kDal form by trypsin results in a decrease in the affinity of the herbicide-binding site within the PS II complex (13).

Treatment of thylakoids with higher concentrations of trypsin results in more drastic alterations of the 34-kDal polypeptide. In membrane samples that had been previously labeled covalently with azido-[^{14}C]atrazine, sequential tryptic digestion products that remained associated with the thylakoid membranes were of 32, 18, and 16 kDal (Fig. 2*A*). We conclude from this that the site for covalent attachment of azido-atrazine is in a hydrophobic region of the membrane, and we suggest that hydrophobic sites determine, at least in part, the binding domain for atrazine. Previous (13) functional studies with trypsin-treated membranes suggested that proteolytic digestion of a 32-kDal polypeptide, stainable after electrophoretic separation, was correlated with the loss of the herbicide-binding site for atrazine and diuron. We suggest that the rapid decrease in affinity for triazine herbicides during trypsin treatment corresponds with the proteolytic cleavage of the 34-kDal polypeptide to 32 kDal, while the loss of atrazine-binding sites correlates with the tryptic alteration of the larger polypeptides (34 or 32 kDal) to the 18- and 16-kDal forms. Perhaps a surface exposed portion of the 32- or 34-kDal polypeptides participates in the formation of a high-affinity binding site and is required for electron transport on the reducing side of PS II.

As shown in Fig. 2, azido-atrazine binding does not influence the proteolytic action of trypsin at the membrane surface—i.e., the herbicide does not protect the binding protein against trypsin. These results are in contrast to those of Matoo *et al.* (32), who reported recently that high concentrations of diuron (10 μM) protect thylakoid proteins against modification by trypsin.

The Chloroplast Gene Coding for the Triazine-Binding Protein Is Expressed in Both Triazine-Susceptible and Triazine-Resistant Plants. Azido-[^{14}C]atrazine does not bind to thylakoid membranes of chloroplasts isolated from triazine-resistant weeds even though these membranes contain a stainable polypeptide of 32 kDal (17). In the present experiments we have found that both susceptible and resistant biotypes of *A. hybridus* produce a rapidly labeled 34-kDal thylakoid polypeptide (Fig. 3). Partial tryptic digestion products of the 34-kDal polypeptides from susceptible and resistant plants are indistinguishable from one another and from the azido-atrazine-labeled polypeptide (Fig. 2*A*). It seems most likely that triazine resistance in *A. hybridus* results from a primary or secondary alteration in the photogene product rather than from its absence. That the gene is present in the resistant strains is also shown by the hybridization of cRNA of the cloned photogene *32* of maize to a 6-kilobase-pair endonuclease *Bam*HI-generated fragment of *A. hybridus* chloroplast DNA from resistant and susceptible biotypes. The identification of this fragment should open the way to gaining an understanding of the mechanism of resistance to triazine herbicides by alterations in their binding site.

We thank Susan A. Benard and Jan Watson for technical assistance. This research was supported, in part, by U.S. Department of Agriculture Binational Agricultural Research and Development Grant US 80-79 to C.J.A. and by grants to L.B. from the National Science Foundation and the U.S. Department of Agriculture Competitive Research Grants Office. This work was also aided in part by the Maria Moors Cabot Foundation of Harvard University.

1. Arntzen, C. J. & Briantais, J. M. (1975) in *Bioenergetics of Photosynthesis*, ed. Govindjee (Academic, New York), pp. 51–113.
2. Grebanier, A. E., Steinback, K. E., & Bogorad, L. (1979) *Plant Physiol.* **63**, 436–439.
3. Grebanier, A. E., Coen, D. M., Rich, A. & Bogorad, L. (1978) *J. Cell Biol.* **78**, 734–746.
4. Bedbrook, J. R., Link, G. & Bogorad, L. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3060–3064.
5. Bogorad, L., Jolly, S. O., Link, G., McIntosh, L., Poulsen, C., Schwarz, Z. & Steinmetz, A. (1980) in *Biological Chemistry of Organelle Formation*, eds. Bucher, T., Sebald, W. & Weiss, H. (Springer, Berlin), pp. 87–96.
6. Bogorad, L., Jolly, S. O., Kidd, G., Link, B. & McIntosh, L. (1980) in *Genome Organization and Expression in Plants*, ed. Leaver, C. (Plenum, London), pp. 291–304.
7. Arntzen, C. J., Darr, S. C., Mullet, J. E., Steinback, K. E. & Pfister, K. in *Function of Quinones in Energy Conserving Systems*, ed. Trumpower, B. (Academic, New York), in press.
8. Weinbaum, S. A., Gressel, J., Reisfeld, A. & Edelman, M. (1979) *Plant Physiol.* **64**, 828–832.
9. Edelman, M. & Reisfeld, A. (1978) in *Chloroplast Development*, eds. Akoyunoglou, G. and Argyroudi-Akoyunoglou, J. H. (Elsevier, Amsterdam), pp. 641–652.
10. Croze, E., Kelly, M. & Horton, P. (1979) *FEBS Lett.* **103**, 22–26.
11. Mullet, J. E. & Arntzen, C. J. (1981) *Biochim. Biophys. Acta* **635**, 236–248.
12. Leto, K. J. & Miles, D. (1980) *Plant Physiol.* **66**, 18–24.
13. Steinback, K. E., Pfister, K. & Arntzen, C. J. (1981) *Z. Naturforsch.* **36**, 98–108.
14. Bouges-Boquet, B. (1973) *Biochim. Biophys. Acta* **314**, 250–256.
15. Velthuys, B. R. & Amesz, J. (1974) *Biochim. Biophys. Acta* **333**, 85–94.
16. Tisher, W. & Strotman, H. (1979) *Biochim. Biophys. Acta* **460**, 113–125.
17. Pfister, K., Steinback, K., Gardner, G. & Arntzen, C. J. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 981–985.
18. Gardner, G. (1981) *Science* **211**, 937–940.
19. Ryan, G. F. (1970) *Weed Sci.* **18**, 614–616.
20. Radosevich, S. R. & DeVilliers, O. T. (1976) *Weed Sci.* **24**, 229–232.
21. Machado, V. S., Arntzen, C. J., Bandeen, J. D. & Stephenson, G. R. (1978) *Weed Sci.* **26**, 381–322.
22. Machado, V. S., Bandeen, J. D., Stephenson, G. R. & Lavigne, P. (1978) *Can. J. Plant Sci.* **58**, 977–981.
23. Burke, J. J., Ditto, C. L. & Arntzen, C. J. (1978) *Arch. Biochem. Biophys.* **187**, 252–263.
24. Blair, G. E. & Ellis, R. J. (1973) *Biochim. Biophys. Acta* **319**, 223–234.
25. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
26. Steinback, K. E., Burke, J. J. & Arntzen, C. J. (1979) *Arch. Biochem. Biophys.* **195**, 546–557.
27. Lasky, R. A. & Mills, A. D. (1975) *Eur. J. Biochem.* **56**, 335–341.
28. Bedbrook, J. R. & Bogorad, L. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 4309–4313.
29. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
30. Denhardt, D. T. (1966) *Biochem. Biophys. Res. Commun.* **23**, 641–646.
31. Ellis, R. J. (1977) *Biochim. Biophys. Acta* **463**, 185–215.
32. Matoo, A. K., Pick, U., Hoffman-Falk, H. & Edelman, M. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1572–1576.