The Molecular Basis of Triazine-Herbicide Resistance in Higher-Plant Chloroplasts*

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Triazine herbicides inhibit photosynthesis by blocking electron transport in photosystem II. The target site of the herbicide was identified as a chloroplast thylakoid polypeptide (the Q_B protein) of 32,000 daltons. Studies of triazine-resistant weed biotypes suggested that a subtle change in the Q_B protein caused the resistance. We have cloned the chloroplast gene (*psbA*) that codes this protein from herbicide-resistant and herbicide-susceptible biotypes of *Solanum nigrum*. By DNA sequencing we detected a single base substitution in the *psbA* gene of the resistant plants, resulting in an amino acid change (serine to glycine for the susceptible to resistance conversion). This mutation is exactly the same one which we have described in a herbicide-resistant biotype of *Amaranthus hybridus*.

Introduction

The first report of an atrazine-resistant biotype of *Senecio vulgaris* appeared in 1970 [1]. Since that time, the appearance of triazine-resistance in many other weed species in North America, Europe, and Israel has been documented [2]. In all cases where this phenomenon has been carefully analyzed, the mechanism of resistance has been shown to be a change in the target site for triazine herbicides at the photosystem II (PS II) complex of chloroplast thylakoids. In studies using isolated thylakoids, the herbicide receptor was identified as a 32 kilodalton (kDa) polypeptide through the use of a photoaffinity labeled triazine herbicide [3, 4]. It was hypothesized that this polypeptide underwent a subtle change in resistant chloroplasts, resulting in a selective loss of triazine binding but without a loss of the polypeptide's normal function in the photosynthetic electron transport pathway [5]. Initial attempts to verify this hypothesis *via* the isolation and characterization of the 32 kDa polypeptide were fruitless due to our inability to isolate sufficient quantities of this hydrophobic polypeptide (unpublished data). The availability of recombinant DNA techniques, however, has offered an alternate way to approach this problem. This manuscript reviews the overall approach taken to isolate and clone the gene for the 32 kDa polypeptide and to derive the amino acid sequence of the protein *via* sequencing of the gene from triazine-resistant and susceptible chloroplasts. The material presented is not an exhaustive review of all aspects of this topic, but includes observations from several laboratories as they relate to this central problem of characterizing the 32 kDa protein.

Abbreviations: Atrazine, 2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine; DCMU(diuron), 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PS, photosystem; Q_A, primary quinone electron acceptor of photosystem II; Q_B, secondary quinone electron acceptor; RC, reaction center; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

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Materials and Methods

Seedlings of *Amaranthus hybridus* (seeds kindly provided by Dr. Homer LeBaron, Agricultural Chemicals Division, CIBA-GEIGY corporation, Greensboro, North Carolina, 27409, USA), and *Solanum nigrum* (seeds kindly provided by Dr. J. Gasquez, INRA, Dijon, France) were grown in soil in a glass house for approximately five weeks. For functional characterization and analysis of polypeptides, chloroplasts were isolated as previously described [3]. Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was conducted using a slab gel containing 4 M urea [6]. Azido-atrazine photoaffinity tagging of thylakoids was as previously described [3].

Isolation of Chloroplast DNA, cloning and sequencing

Chloroplast DNA was prepared from five-week-old plants using the procedure described by Palmer [7]. The DNA was digested with the restriction endonuclease *Pst* I and the resulting fragments were ligated to *Pst* I-digested *pBR322* DNA for use in the transformation of *E. coli* (HB101). Tetracycline-resistant colonies were replica-plated on ampicillin plates in order to reveal the bacteria carrying the recombinant plasmids. These clones were screened [8] for homology to the maize *psbA* cloned in *pZmc 427* [9]. Procedures for restriction endonuclease digestion, agarose and acrylamide gel electrophoresis and Southern hybridization, have been described previously [10]. DNA sequencing was performed according to the procedures described by Maxam and Gilbert [11].

Results and Discussion

Chloroplast gene expression

In the 1970's the characterization of protein synthesis by isolated chloroplasts had demonstrated that a prominent product of the cell-free system was a thylakoid-bound polypeptide of about 32 kDa [12, 13]. The fact that there was no stainable polypeptide which accumulated to any significant extent at this molecular weight led to very careful studies by M. Edelman and his colleagues. They concluded that the 32 kDa polypeptide undergoes rapid turnover (reviewed in [14]). In addition, it is apparent that this protein is synthesized as a larger polypeptide precursor which is very rapidly processed to approximately 32 kDa [13–18].

The characterization of chloroplast development in the laboratory of L. Bogorad during the 1970's included the analysis of light-induced changes in chloroplast gene expression. Transfer of dark-grown maize plants to light was found to induce transcription of several chloroplast-encoded genes (termed "photogenes"; [19]). One of these genes was identified as the gene that codes for the rapidly-turned over 32 kDa-thylakoid polypeptide [9, 19]. This gene is now designated *psbA*. The maize *psbA* was cloned in the plasmid *pBR322* and the recombinant plasmid was designated *pZmc427* [9]. The corresponding genes from spinach, tobacco and soybean have been cloned and sequenced [20, 21]. This data revealed a very highly conserved amino acid sequence for the polypeptide.

Functional role of 32 kDa polypeptide(s) in chloroplast thylakoids

A large number of commercial herbicides have been known to inhibit PS II-mediated reactions [5, 22]. The development of techniques for measuring binding of radioactive herbicides to chloroplast membranes led to the concept of defined herbicide binding sites with characteristic binding constants [23]. It was demonstrated that there is one binding site per electron transport chain for the herbicides diuron (DCMU) and atrazine [24] which are commonly utilized in membrane analyses. Treatment of isolated thylakoids with proteolytic enzymes such as trypsin resulted in a selective loss of herbicide inhibition of electron transport [25] and loss of herbicide binding [26]. These observations led to the idea that the herbicide receptor is a protein which is present in stoichiometric amounts with respect to photosynthetic electron transport chains.

Several approaches have been taken to identify the thylakoid herbicide receptor protein(s) for diuron and atrazine. Initial studies utilized a combination of isolated PS II sub-membrane particles and protein extraction *via* detergents or proteolysis. In all cases the loss of a 32 kDa polypeptide was directly correlated with loss of herbicide (diuron or atrazine) receptor sites [27–28]. Analyses of a maize mutant blocked in PS II-mediated electron transport indicated that a loss of the 32 kDa polypeptide was correlated with the loss of atrazine binding sites [29]. Lastly, the photoaffinity herbicide azido-atrazine definitively identified a 32-kDa polypeptide as the triazine receptor [3, 4, 28, 30].

We should note that the identification of the triazine-receptor protein as a 32 kDa polypeptide does not fully identify the binding protein in intact chloroplast membranes. Thylakoids contain more than one polypeptide of this general size class. In a series of recent studies [30–33] we have attempted to resolve these proteins and clarify their function. Figure 1 compares the one-dimensional separation of thylakoid polypeptides (lane 1) by SDS-PAGE with a purified, oxygen-evolving PS II preparation (lane 2). The latter is depleted of photosystem I (PS I) polypeptides (note the loss of CPI) as well as the coupling factor α and β subunits. When the

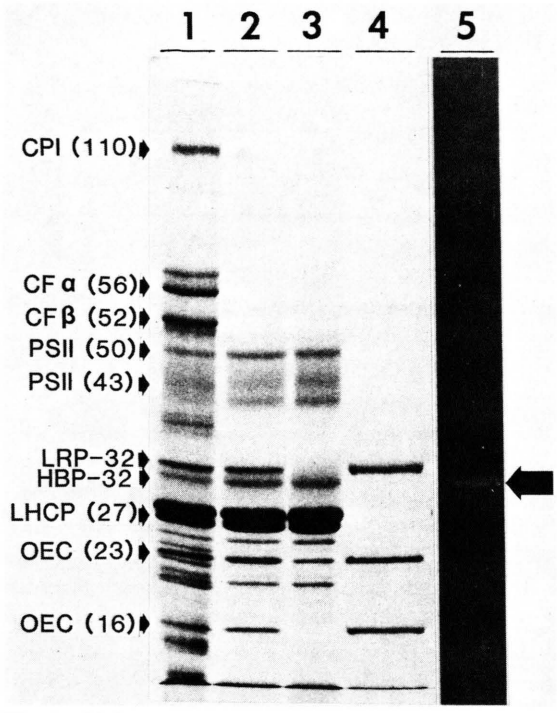

Fig. 1. SDS-PAGE analysis of chloroplast thylakoids (lane 1); oxygen-evolving PS II particles (lane 2); alkaline salt-washed PS II particles (lane 3), and 32, 23, and 16 kDa polypeptides released from PS II by the alkaline salt-wash (lane 4). Autoradiograms were prepared of each of the samples in lanes 1–3, all of which had been tagged with azido-^{14}C-atrazine. All were virtually identical; the autoradiogram for the sample of lane 3 is shown in lane 5. The only polypeptide showing evidence of radioactivity (arrow) is labeled HBP-32 (Herbicide Binding Protein of 32 kDa). Other thylakoid polypeptides include CP-I (the Chlorophyll Protein of PS I), the α and β subunits of the coupling factor, the PS II reaction center polypeptides of 50 and 43 kDa, and the light-harvesting chlorophyll-protein (LHCP) of 27 kDa. Approximate molecular weights are indicated by numbers in parenthesis.

PS II particles were washed with an alkaline salt solution (0.5 M NaCl, pH 9.0), the resulting PS II preparation (lane 3) had lost oxygen evolution activity and extracted polypeptides of approximately 32, 23, and 16 kDa (lane 4) were recovered in the supernatant. The larger of these three has been further purified and found to contain 10 mol% lysine [32]; it is therefore designated as the lysine-rich protein (LRP-32) in Fig. 1. It appears to be identical to the "33 kDa protein" thought to participate in oxygen evolution reactions [34–36].

In one dimensional SDS-PAGE, a polypeptide which is tagged by azido-atrazine changes its relative mobility when increasing concentrations of urea are added to the gel system [30–33]. In zero molar urea gels, the polypeptide runs as a diffuse, poorly staining (with Coomassie Brilliant Blue) region of 32–34 kDa. In the presence of 4 M urea, as in Fig. 1, the polypeptide migrates on SDS-PAGE as a more distinct band of lower apparent molecular weight. We have labeled this as the herbicide binding protein (HBP-32) on Fig. 1. The autoradiogram of the azido-^{14}C-atrazine tagged PS II particles is shown in lane 5 (arrow indicates that the position of the radioactivity corresponds to the HBP-32 of lane 3).

To summarize, the thylakoid polypeptide which binds azido-atrazine displays an anomalous electrophoretic migration pattern in SDS-PAGE, dependent upon the presence of urea in the gels. We cannot, therefore, accurately assign a molecular weight to the mature protein even though we continue to follow convention and identify it as a 32 kDa polypeptide.

Mechanism of herbicide action

The mechanism by which herbicides such as diuron or atrazine exert their inhibitory action upon photosynthetic electron flow has been considered by several investigators. Velthuys [37] and Wraight [38] independently hypothesized that the herbicides cause the displacement of a bound plastoquinone molecule which functions as the secondary electron acceptor on the reducing side of the PS II reaction center; this redox component is now designated as Q_B (formerly identified as B or R). This concept has been supported by several lines of experimentation [39, 40]. One approach, involving the reaction of thylakoids with a photo-affinity azido-quinone, resulted in blockage of Q_B function as well as a dramatic reduction in atrazine binding affinity [40]. In these latter studies it was concluded that the photo-affinity quinone was covalently affixed in the Q_B binding site, thereby limiting atrazine binding. The fact that the number of atrazine binding sites remained unchanged in the treated thylakoids indicated that the binding determinants for quinones and triazines are not completely identical. However, the fact that proteolytic digestion of thylakoids resulted in a parallel pattern of loss of herbicide binding and loss of Q_B function provided evidence that binding determinants for both quinones and triazines were on the same polypeptide [26].

In summary, the analyses described above have led to an agreement (reached at the 1983 Wageningen Workshop on Modes of Action of Herbicides in Photosynthesis) that the 32 kDa polypeptide, which is tagged with azido-atrazine, shall be designated as the Q_B protein. We will utilize this terminology in the remainder of this report.

Identification of the gene encoding the Q_B protein

As described in the Introduction to this report, it was of practical interest to determine the molecular change in chloroplasts that caused triazine-herbicide resistance. Since we could not develop protocols to isolate satisfactory quantities of the Q_B protein to characterize it, we instead set out to isolate the gene encoding the polypeptide. One critical phase of research in this direction had already been completed; it was known that the triazine resistance trait was inherited in a non-Mendelian pattern [41]. In studies of chloroplasts isolated from weed seedlings resulting from reciprocal crosses between susceptible and resistant plants, it was found that a slower rate of electron transfer from Q_A to Q_B was inherited in parallel to a loss of triazine binding properties of isolated thylakoids; both traits were inherited maternally [42]. This led to the obvious suggestion that the Q_B protein was chloroplast-encoded, and could, in fact, be the gene product of *psbA*.

It is possible to label, rather specifically, the *psbA* gene product *in vivo* by feeding radioactive amino acids to intact, fully developed leaves. Since the mRNA for this protein is very abundant in chloroplasts, and the protein turns over more rapidly than other thylakoid polypeptides, newly synthesized 32 kDa protein accumulates the predominance of the label incorporated in thylakoids following a short pulse of label [14, 43]. This procedure was carried out to isolate [^{35}S]methionine-labeled membranes from both triazine susceptible and resistant membranes. These were then treated with a graded series of trypsin concentrations. In parallel, a second thylakoid membrane sample was tagged with azido-^{14}C-atrazine and treated with an identical trypsin series. Analysis of the samples by SDS-PAGE showed that the *psbA* gene product (the rapidly turned over 32 kDa protein) behaved identically to the azido-atrazine tagged protein [43–46]. Both samples were cleaved by trypsin at thylakoid surface-exposed sites resulting in the initial removal of a peptide fragment of about 1–2 kDa, followed by reduction in size of the membrane protein to 18–19 kDa and then to 16–17 kDa [43, 44]. This trypsin "finger-print" identity revealed that the *psbA* gene product was the herbicide-binding Q_B protein (discussed in more detail in [43]).

Isolation, cloning, and mapping the psbA gene from triazine-resistant and susceptible weeds

As outlined above, it is concluded that: a) triazines act by binding to the Q_B protein and displacing the bound quinone cofactor, b) the chloroplast gene *psbA* codes for the Q_B protein, and c) triazine resistance results from a subtle change in the triazine receptor protein of the PS II complex. From these data we predicted that triazine resistance was due to a mutation in the *psbA* gene. Our goal was to isolate this gene from susceptible and resistant biotypes of the weed *Solanum nigrum* and to com-

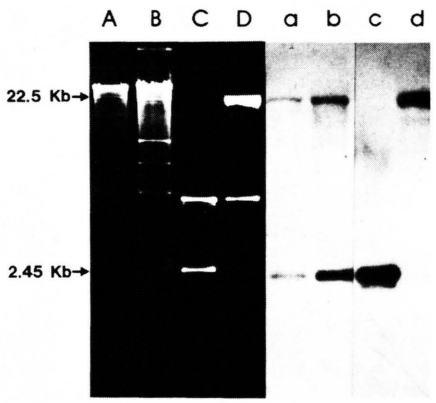

Fig. 2. Agarose gel eletrophoresis and Southern-hybridization analysis of chloroplast DNA (cpDNA) from *Solanum nigrum* and the plasmids *pSnsI* and *pSnr68*. cpDNA was digested by the restriction endonuclease *Pst*I and the resulting fragments were separated by agarose (1.0%) gel electrophoresis and stained with ethidium bromide. Lane A: cpDNA from an atrazine-resistant biotype; lane B: cpDNA from an atrazine susceptible biotype; lane C: *Pst*I-digested *pSnsI* and; lane D: *Pst*I-digested *pSnr68*. The gel was blotted to nitrocellulose and then hybridized to a ^{32}P-labeled *Bgl*II insert from the plasmid *pZmc 427* [9]. Two fragments (of 22.5 Kb and 2.45 Kb) in both the resistant (lane a) and susceptible (lane b), hybridized to the *psbA* gene from maize. The amount of DNA from the susceptible plant which was loaded on the gel (lanes B, b) was approximately five times higher than the resistant DNA (lanes A, a). The sizes of the *Pst*I-inserts of the plasmids (lanes C and D) and their hybridization to the *psbA* probe (lanes c and d) indicate that plasmid *pSnsI* contains the 2.45 kb *Pst*I fragment (from the susceptible biotype) and plasmid *pSnr68* contains the 22.5 kb *Pst*I fragment (from the resistant biotype).

pare DNA sequences. A similar approach was taken with resistant and susceptible biotypes of *Amaranthus hybridus* and some aspects of that study have been described in a recent report [47].

Chloroplast DNA was isolated from both triazine susceptible and triazine-resistant biotypes of *S. nigrum*, and digested with the restriction endonucleases *PstI* and *Eco*RI. The resulting fragments were separated by agarose gel electrophoresis. No difference in the *PstI* (Fig. 2A, B) or *Eco*RI (data not shown) restriction pattern could be detected between the herbicide-resistant and herbicide-susceptible biotypes. This indicates that there are no major rearrangements in the chloroplast genome of the resistant biotype, which is in agreement with the earlier observations with *A. hybridus* [43, 47].

The *Pst* I-generated chloroplast DNA fragments from susceptible and resistant plastids were analyzed by Southern hybridization using, as a probe, a ^{32}P-labeled *Bgl*II DNA fragment from the plasmid *pZmc427* (containing the *psbA* gene from *Zea mays*; [9]). Two fragments of 2.45 kilobases (kb) and 22.5 kb were identified as showing homology to the probe (Fig. 2A, B, a, b). These fragments, from both the susceptible and resistant biotypes, were then cloned in the plasmid *pBR322*. *Pst* I cleaved DNAs of the recombinant plasmids are shown in Fig. 2C, D. Hybridization analysis of these plasmids (Fig. 2c, d) indicates the presence of the *S. nigrum psbA* gene in these cloned chloroplast DNA fragments. The presence of two hybridizing fragments indicates the conservation in *S. nigrum* of the single *Pst* I restriction site, internal to the coding region of the gene, which was also found in other species [20, 21, 47]. This is confirmed by the restriction map shown in Fig. 3. The restriction map was found to be similar to both *Nicotiana debneyi* [20] and *Amaranthus hybridus* (shown in Fig. 3), and indicated that the 2.45 kb *Pst* I fragment contained the 3′ end of the *psbA* gene of *S. nigrum*.

Comparative sequence analysis of the entire *psbA* gene of susceptible and resistant biotypes of *A. hybridus* revealed only one base substitution resulting in an amino acid change in the resistant biotype [47]. The location of this base shift (from adenine in the susceptible to guanine in the resistant biotypes of *A. hybridus*) is indicated by an arrow in the upper portion of Fig. 3. The relative location of the base shift in the gene can be determined from the codons indicated for the NH_2 and

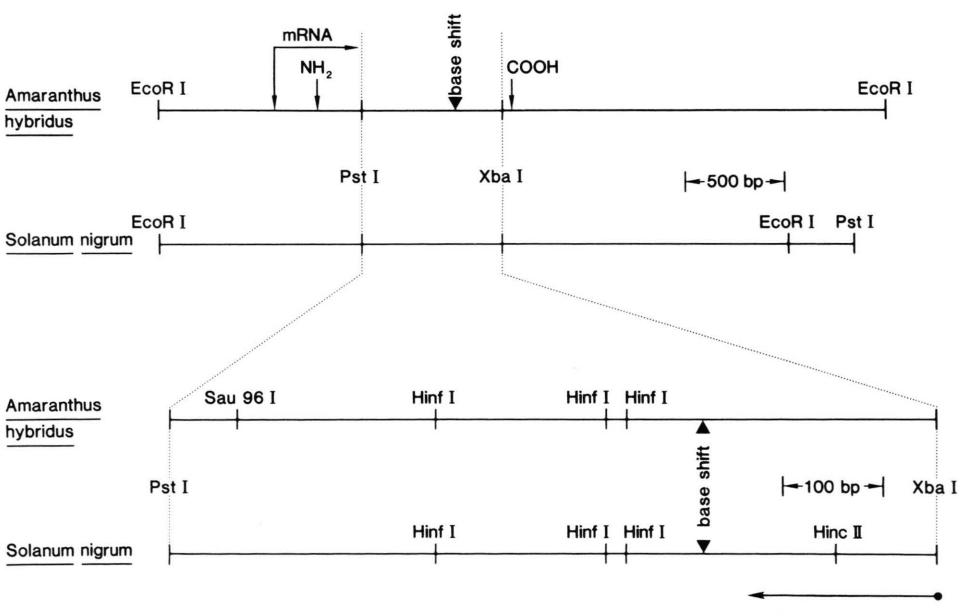

Fig. 3. Restriction mapping of the *psbA* gene from *Amaranthus hybridus* and *Solanum nigrum*. The upper map shows the location of the protein-coding region and the position of the base shift that was found in the atrazine-resistant biotype of *A. hybridus*, and the parallel region of the *S. nigrum* cpDNA. High conservation of restriction sites enabled us to identify in the *S. nigrum* cloned gene, a 309 *bp Hinf* I–*Xba* I fragment (shown in the lower map) which contains the region with the mutation in *A. hybridus*. Arrows below the map indicate the strategy of sequencing of this region [11].

COOH termini of the protein. We investigated whether or not a similar point mutation had occurred in *S. nigrum* by sequencing the DNA in this region of both resistant and susceptible genes. The strategy for this procedure is diagramed in the lower half of Fig. 3. A *309 bp* DNA fragment that was generated by digesting with endonucleases *Hinf* I and *Xba* I was identified as homologous to the region of the *A. hybridus* gene containing the mutation. This internal region of the *S. nigrum psbA* gene was then sequenced. The data obtained, as well as the derived amino acid sequence of this region of the Q_B protein, are shown in Fig. 4.

The amino acid sequence for the Q_B protein from *S. nigrum* indicated a primary sequence change from serine in the susceptible biotype to glycine in the herbicide-resistant biotype (shown within the box in Fig. 4). This was the only change found within the region sequenced for the two genes. This change was caused by a single base substitution in the DNA – from adenine to guanine. This is *identical* to the base substitution and amino acid alteration previously found for *A. hybridus* susceptible and resistant biotypes [47].

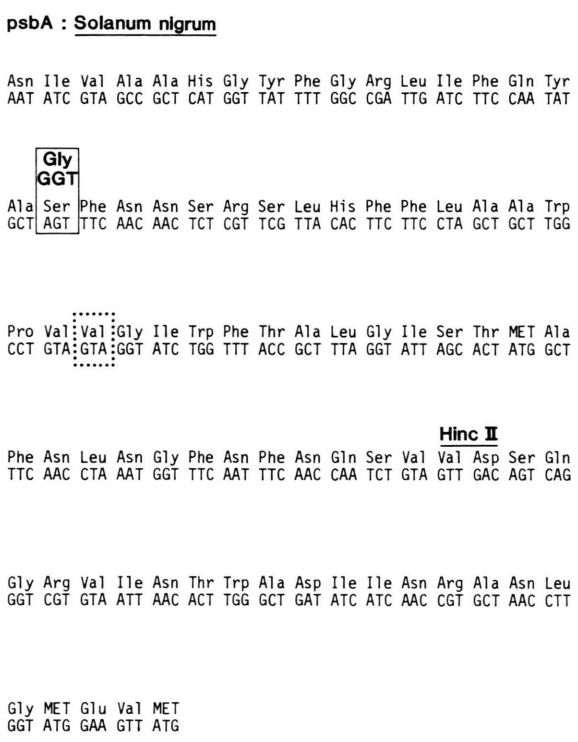

Fig. 4. Nucleotide sequence of the *Hinf*I-*Xba* I fragment (Fig. 3) of the *psbA* gene from a herbicide-susceptible biotype of *S. nigrum* and the deduced amino acid sequence of that region in the Q_B protein. The boxed codon marks the only difference found in the atrazine-resistant biotype. A change from adenine in the susceptible to guanine in the resistant leads to a substitution of serine for glycine. The dotted box marks the amino acid difference between *A. hybridus* [47] and *S. nigrum*. As in tobacco, spinach [20], maize (McIntosh and Bogorad, unpublished), and soybean [21], *S. nigrum* Q_B has valine while in *A. hybridus* there is isoleucine in this position.

Conclusions and Speculations

A series of experiments, described above, have made it possible to identify a single amino acid change in the Q_B protein that corresponds to a triazine susceptible to resistance conversion of two weed biotypes. It has recently been discovered that a diuron and atrazine-resistant strain of *Chlamydomonas* had an alteration of the same amino-acid in the Q_B protein (from a serine to alanine in the susceptible to resistance conversion) (J.-D. Rochaix, pers. communication). We conclude that the repeated loss of a specific serine residue within the Q_B protein, in all three instances where the triazine resistance has been investigated, is highly significant. It is likely that this serine plays a functional role in triazine binding. One probable explanation is that the hydroxyl group of serine can form a hydrogen bond with an electron-rich region of the triazine ring to stabilize the latter in its binding site. We would note that the Q_A to Q_B electron transport properties (in the absence of herbicides) are also known to be influenced by the Q_B-protein modification [5, 48]. It seems possible that the serine which is lost in resistant chloroplasts may normally function *via* hydrogen bonding to stabilize the Q_B semiquinone anion. Absence of this capacity can then be related to the change in equilibrium constant for the Q_A- to Q_B-electron transfer (*i.e.* $Q_A^- Q_B \leftrightarrows Q_A Q_B^-$) in the triazine resistant thylakoids [49].

An ultimate, complete understanding of both herbicide binding and quinone binding to the Q_B protein will require determination of the secondary, tertiary, and perhaps quaternary structure of the polypeptide in the thylakoids membrane. This is not directly possible, at present, although computer modeling of the polypeptide has been attempted [50]. We have utilized the latter information to draw the protein model, shown in Fig. 5. The intent of this figure is not to imply an exact structure of the

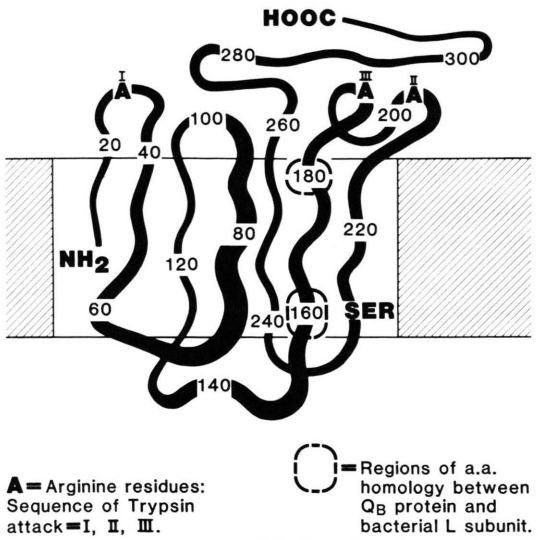

Fig. 5. A schematic model for the three dimensional organization of the Q_B protein in thylakoid membranes. This model uses the second methionine in the open reading frame of the *psbA* gene as the N-terminus of the protein. Numbers within the protein chain incidate the amino acid positions. The hatched area designates the thickness of the hydrophobic domain of the membrane. The serine (SER) which undergoes conversion to glycine in the triazine resistant biotypes of *A. hybridus* and *S. nigrum*, is indicated. Three of the arginines in this protein are designated (A) since they probably correspond to sites of trypsin cleavage as previously described [43, 44]. The segments of the protein which carry amino acid homology to the L-subunit of the *Rhodopseudomonas capsulata* reaction center [56] are indicated by dotted circles near amino acid residues 160 and 180 (see text for further details).

protein, but only to provide a visual framework for the follwoing discussion of our degree of present understanding of this protein.

The DNA sequence of the *psbA* gene has now been obtained for spinach, tobacco [20], soybean [21], maize (McIntosh and Bogorad, unpublished), *A. hybridus* [47], and partially for *S. nigrum* (this manuscript). There is an open reading frame capable of coding for a protein of approximately 38 950 daltons [20, 21, 47]. Since studies of the Q_B protein precursor have indicated its size to be approximately 34–35 kDa [14–18], we have selected the second methionine in the open reading frame as the most appropriate *N*-terminus for the protein [47]. This possibility was also recognized in the earlier studies of spinach, tobacco, and soybeans [20, 21] (choosing the second methionine results in a primary translation product of the *psbA* gene containing 317 amino acids, with a molecular mass of 34.6 kD). It has recently been postulated that the Q_B protein is post-translationally processed at the carboxy-terminus of the polypeptide (M. Edelman, personal communications, and [51]), giving a mature Q_B protein of approximately 32 kDa (see Results and Discussion for details on size uncertainty of the mature polypeptide).

The model of Fig. 5 is consistent with the known actions of trypsin upon isolated thylakoids. This protease cleaves polypeptides at lysine or arginine residues. The Q_B proteins of all higher plants thus far examined contain no lysine [14, 20, 21, 47]. The three-step reduction in size of the Q_B protein by trypsin has been well characterized [43, 44], and requires the surface-localized presence of at least three arginines *in situ*. Sequential trypsin action on the arginines at residues 28, 202, and 189 (shown by the "A" I, II, III of Fig. 5) satisfy this requirement. Unfortunately, at present, there are few other tests of the validity of the lipid bilayer-spanning features of the model of Fig. 5. These details will be revealed as site-specific probes and protein sequence-specific antibodies are used to characterize surface-exposed segments of the protein in right-side-out and inverted thylakoids; we can anticipate the availability of these data in the future.

A powerful way of analyzing enzyme active sites is to compare the enzyme's protein structure from different species. When the Q_B protein primary sequences are compared among the higher plants analyzed to date, there is a remarkable conservation of structure. The amino acid sequence of spinach, tobacco, maize and the triazine-susceptible *S. nigrum* sequence now available (Fig. 4) shows total conservation of primary structure. In both biotypes of *A. hybridus* a "neutral" substitution of isoleucine for valine at residue 245 was observed [47]; this position is indicated by the dotted box in Fig. 4. In the soybean Q_B protein, only two amino acids, at three and seven residues internal to the carboxy-terminus of the protein (which is believed to be processed), were different [21]. In short, there is extreme conservation of protein structure in higher plant Q_B proteins, with the exception of the single amino acid substitution (serine to glycine) which occurs in triazine resistant biotypes.

Recently, two genes of the cyanobacterium *Anabaena* 620 and *Anabaena* 625 have been sequenced [52]. These were largely homologous to the higher plant *psbA* chloroplast gene with only 15% differ-

ence in the primary protein structure shown in Fig. 5. Many of these changes occur in the *N*-terminal half of the protein. However, there may be significance in the fact that two long segments of the protein (residues 133–175 and 272–309) are totally conserved between the Q_B proteins of the cyanobacterium and higher plant chloroplasts.

Another recent approach to the analysis of quinone-binding proteins has centered around the analysis of the reaction center (RC) polypeptides of the purple photosynthetic bacteria. These organisms undergo charge separation reactions which have a great deal of similarity to photosystem II of higher plants [53, 54], including the fact that these reactions are inhibited by triazines *via* a quinone-displacement mechanism apparently identical to that occurring in PS II of chloroplasts [55]. We have used azido-^{14}C-atrazine to identify the L-subunit of the RC of *Rhodopseudomonas sphaeroides* as the triazine-receptor protein (Brown, Gilbert, and Arntzen, manuscript submitted). In separate studies Hearst and coworkers [56, 57] have isolated and sequenced the gene encoding the analogous polypeptide from *R. capsulata.* The derived amino acid sequence shows homology in two segments: a Pro-Phe-His-Met-Lys at residues 160–165 of Fig. 5, and Ala-Met-His-Gly at 177–180. The former sequence falls within the highly conserved segment of the *Anabaena* derived protein sequence. Hearst and Sauer [56] have proposed a model by which the His-Met protein sequence is involved in charge stabilization of the Q_B anion. We note, using the model of Fig. 5, that the serine which is lost in triazine-resistant plants can lie immediately adjacent to the conserved residues 160–165. This may be consistent with the serine participating in hydrogen bonding to the quinone anion as was hypothesized above.

The M subunit of the reaction centers of the purple sulfur bacteria has previously been suggested to contain binding sites for both Q_A and Q_B [58, 59]. The gene for the M subunit of *R. sphaeroides* was recently sequenced and the derived amino acid sequence has considerable homology to the Q_B protein of higher plant chloroplasts [60]. The M subunit of *R. capsulata* has also been analyzed [56]. The homology of M subunit to the Q_B protein was found to be less than that of the L subunit to the Q_B protein. As more information is obtained on these polypeptides, we can anticipate being able to derive a better understanding of the quinone binding sites in the reaction center polypeptides.

In summary, we note that the Q_B protein is fast becoming the best characterized protein of higher plant chloroplasts. Continued advances in these studies will not only contribute to our understanding of electron transport processes involving quinone-proteins, but also should have practical importance for the more knowledgeable design and use of herbicides which inhibit quinone-protein function.

Acknowledgements

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