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Applications of biotechnology to agricultural chemistry

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Abstract - Near term applications of biotechnology to agricultural chemistry will include modification of the responses of plants to chemicals, identification of new target sites for chemical action, and the introduction of mechanisms for removing chemicals from the environment. The production of plants resistant to sulfonylurea herbicides is presented as an example of the first two of these applications. Mutants of tobacco resistant to sulfonylurea herbicides were isolated by selection in cell culture. Biochemical studies of these plants identified acetolactate synthase (ALS), the first enzyme of the biosynthetic pathway for isoleucine, leucine, and valine, as the site of action of the sulfonylurea herbicides. Molecular cloning of the mutant form of the tobacco ALS gene will permit resistance to sulfonylurea herbicides to be transferred into a wide range of crop species.

INTRODUCTION

In the initial excess of enthusiasm that invariably accompanies the birth of a new field, biotechnology was hailed as a panacea that would ultimately displace a traditional agricultural chemical industry beleaguered by stricter regulation, concern about environmental pollution, and a declining efficiency of new product discovery. But now that the dust raised by those initial predictions is settling, a more realistic view of the role of biotechnology is emerging. That role is one of support rather than supplantation, of synergy rather than rivalry, in which the techniques, developments, and products of biotechnology will enable the agricultural chemical industry to produce more effective and environmentally safe products at reduced costs. Occasionally, as in the case of microbial pesticides, it may appear that a substitute for a traditional chemical product has been produced through biotechnology. But, even in such cases, it is more likely that the greatest effectiveness will be achieved through use of the biological agent in combination with, rather than instead of, a chemical agent.

In the near term, important applications of biotechnology to agricultural chemistry can be expected in at least the following three areas:

1. Modification of plant responses to chemicals
2. Identification of (new) target sites for chemical action
3. Degradation of chemicals introduced into the environment

The last of these three applications has been discussed extensively elsewhere by others more qualified than myself and, therefore, will not be considered here. Examples of the first two applications are found in the genetic modification of plants to introduce herbicide tolerance in general and in the production of plants resistant to sulfonylurea herbicides in particular.

GENETIC MODIFICATION OF PLANT RESPONSE

The traditional method of herbicide discovery is based on the synthesis and screening of large numbers of compounds. Further directed synthesis then strives to increase the activity of promising leads. This process, which relies exclusively on introducing selective phytotoxicity as a chemical property of the herbicide, is both expensive and time-consuming. As many as 20,000 compounds may have to be synthesized and examined at a

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cost of tens of millions of dollars before a herbicide worthy of commercial development is identified. Such numbers provide impetus to seek other methods by which to achieve the desired selectivity of herbicidal activity. One attractive alternative to chemical modification of the herbicide is genetic modification of the response of the crop. By widening the spectrum of applicability of existing compounds to include crops for which they were not originally developed, or by permitting development of new herbicides for use on several, rather than single, crops, genetic introduction of tolerance offers the potential of reducing herbicide development costs. Genetic enhancement of tolerance would also enlarge the safety margin for herbicide application and increase the flexibility of herbicide use in crop rotational systems.

The feasibility of genetically modifying herbicide sensitivity has been demonstrated in studies with the two Du Pont herbicides, Glean® and Oust®. The active ingredients of these compounds, chlorsulfuron (Glean) and sulfometuron methyl (Oust), are sulfonylurea compounds that are characterized by high herbicidal activities and low mammalian toxicities.

Mutants of tobacco (Nicotiana tabacum) resistant to these two herbicides were selected directly in vitro by transferring cell cultures to medium supplemented with either compound at 6 nM (ref. 1). Extensive crosses with plants regenerated from twelve isolates demonstrated that in all cases resistance resulted from a single dominant or semidominant nuclear mutation. Genetic linkage studies of six mutants identified two loci, which were designated SuRA and SuRB. Although mutations at both loci confer resistance at the whole plant level, one mutation (S4) residing at the SuRB locus was studied in the greatest detail. Plants homozygous for the S4 mutation were at least 100-fold more resistant to damage by chlorsulfuron than were nonmutant plants of the parental variety. This level of resistance completely protected mutant plants in the field from injury by a post emergence application of Glean® sufficient to provide excellent weed control (Fig. 1).

Passage of homozygous S4/S4 mutant callus tissue through a second cycle of selection in the presence of 600 nM sulfometuron methyl yielded an even more highly resistant cell line. Genetic studies with regenerated plants revealed that this enhanced level of resistance resulted from the occurrence of a second mutation (Hra), which was genetically linked to the S4 mutation and which, therefore, resided at or near the SuRB locus (ref. 2). Plants homozygous for both mutations (S4 Hra/S4 Hra) were at least 500 times more resistant to chlorsulfuron than were nonmutant plants.

Fig. 1. Normal (left) and homozygous S4/S4 mutant (right) tobacco plants in the field without herbicide application (background) and following treatment with sulfometuron methyl at 30 gm/acre (courtesy of B. Smeeton and K. Bridle; R. J. Reynolds Tobacco Co.).

Certain sulfonylurea herbicides alone or in combination with other herbicides would provide excellent and cost effective control of weeds endemic to tobacco fields. However, the sensitivity of tobacco to sulfonylurea herbicides precluded their use in tobacco cultivation. Introgression of the S4 and Hra mutations into commercial tobacco cultivars by conventional backcross breeding methods will produce new resistant cultivars with which sulfonylurea herbicides can be used safely for effective weed control. Thus, a potential new use for sulfonylurea herbicides was created by altering the sensitivity of a crop to these compounds through genetic intervention.

IDENTIFICATION OF A HERBICIDE SITE OF ACTION

As mentioned earlier, the traditional method of herbicide discovery rarely either relies on or utilizes information about the mode of action of the compounds being screened. Although this approach affords a certain advantage by preventing the limitations of our knowledge from constraining the scope of discovery, it is not very efficient. Knowledge of the site of action of a particular class of chemical compounds permits rational design of herbicides through studies of structure-activity relationships. Three dimensional studies of the interaction between a protein and an inhibitor would ultimately identify more effective inhibitors. Moreover, not only the inhibitor, but the protein itself could be restructured through such studies. That is, in contrast to an exclusively chemical approach, which can only hope to improve the function of the inhibitor, the techniques of biotechnology permit modification of the target protein itself to realize the desired effect. This ability to modify the second, and previously inaccessible, component of the protein-inhibitor complex will make possible even further enhancement of the potency and specificity of inactivation.

Acetolactate synthase (ALS), the first enzyme of the biosynthetic pathway for isoleucine, leucine, and valine (Fig. 2), was identified as the site of action of the sulfonylurea herbicides by physiological and biochemical studies of Salmonella typhimurium (ref. 3), and of mutants of Saccharomyces cerevisiae (ref. 4) and Nicotiana tabacum (ref. 5). Resistant mutants contained a form of ALS that was less sensitive than the normal enzyme to inhibition by sulfonylurea herbicides and that cosegregated with the resistance phenotype in genetic crosses.

DNA sequence analysis of the cloned yeast ALS gene revealed extensive homology between the deduced amino acid sequences of the yeast enzyme and the ALS II and ALS III isozymes of Escherichia coli in three distinctly conserved domains (ref. 6). The persistence of this sequence conservation through to the plant kingdom permitted use of the yeast gene as a heterologous hybridization probe to isolate the tobacco gene (ref. 7). At least in the case of one yeast mutant and one E. coli mutant, but presumably in the case of plants as well, herbicide resistance resulted from single amino acids in the enzyme (Fig. 3), (ref. 8).

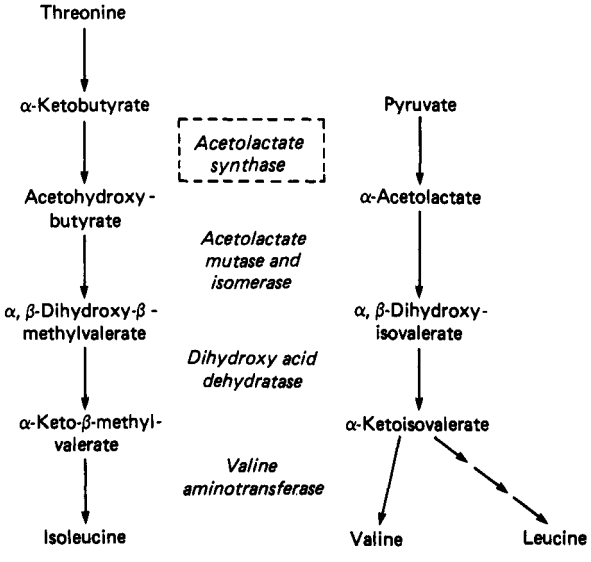

Fig. 2. Pathway for the biosynthesis of isoleucine, leucine, and valine.

	Enzyme	Sequence
A.		
	E. coli ALS II	V F G Y P G G A I m P V Y D A L
	Mutant ALS II	V F G Y P G G v I m P V Y D A L
	E. coli ALS I	V t G i P G G s I L P V Y D A L
	E. coli ALS III	V F G Y P G G A v L d i Y D A L
	Yeast ALS	V F G Y P G G A I L P V Y D A i
B.		
	Yeast ALS	T G Q V p t S a I G T D A F Q E
	Mutant yeast ALS	T G Q V s t S a I G T D A F Q E
	E. coli ALS I	T G Q V p a S m I G T D A F Q E
	E. coli ALS II	T G Q V s a p f I G T e A F Q E
	E. coli ALS III	s G Q V a t S l I G y D A F Q E

Fig. 3. Deduced amino acid sequences in the vicinities of mutations to sulfometuron methyl resistance in E. coli ALS isozyme II (A) and yeast ALS (B). Lower case letters indicate nonconserved amino acids, and underlined letters denote amino acid substitutions in mutant enzymes (ref. 8).

Now let us briefly reconsider the modification of plant response to agricultural chemicals in light of these accomplishments in identifying and cloning the gene encoding the target protein of sulfonylurea herbicides. We have until now only discussed the modification of plant response by means of mutant selection. Such mutant selection programs require the generation and screening of large numbers of alleles repetitively in individual crops to identify a mutant form of each crop that responds in the desired way. In contrast, the techniques of molecular cloning, in vitro mutagenesis, and genetic transformation afford a potentially more efficient and powerful means of introducing the desired phenotype. In this molecular approach to the modification of plant response, a gene encoding a target protein would be cloned and a mutant allele conferring the desired degree and specificity of response created in vitro. This mutant allele could than be introduced into crops by any of several transformation methods. Another important advantage of this molecular approach is that it makes possible the construction of alleles that could not be isolated by random mutation.

This article has attempted to provide a brief description of the methods, rationale, and advantages of identifying the molecular basis of plant responses to agricultural chemicals and of modifying those responses in a directed way. This powerful application of biotechnology in the service of the agricultural chemical industry should be welcomed enthusiastically.

But we should keep in mind that it does not represent a new conceptual discovery. Variation in the responses of plants to herbicides and pesticides has for decades provided an awareness of the genetic dependency of those responses. Biotechnology is simply granting experimental access to those plant responses. Now we must discard our view of plants as an immutable component of an interactive system that could be made more effective only through changes in the chemical component. Instead, we must fully utilize our newly developed capability to modify plants as a means to improve the effectiveness of that interaction.

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