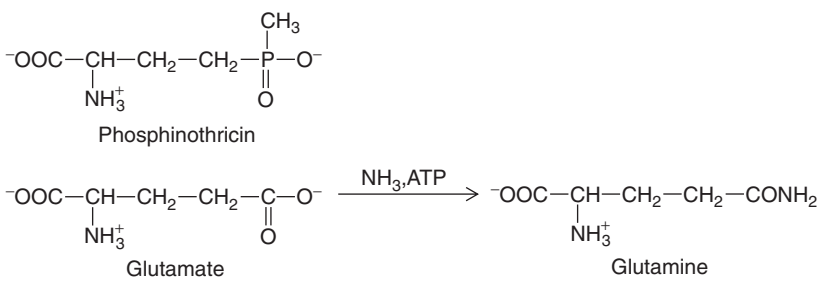
strains of bacteria were not encouraging because the enzyme produced lacked the N-terminal sequence that guided its transport into chloroplasts ("chloroplast transit peptide"). The synthesis of aromatic amino acids occurs mainly in chloroplasts, and the plant enzyme used in the earlier experiments contained this sequence and was therefore effectively moved into chloroplasts. The final commercial products were thus made by introducing a DNA containing a strengthened CaMV 35S promoter, a sequence coding for a chloroplast transit peptide, a sequence coding for a bacterial glyphosate-resistant enzyme, and a nopaline terminator. The gene coding for the enzyme came from a wild-type strain of *Agrobacterium*, and it binds the substrate with an affinity similar to the plant enzyme, whereas its affinity to glyphosate is 5000-fold lower. It is interesting that none of the mutants of normally glyphosate-susceptible enzymes from bacteria or plants displayed resistance even remotely comparable to this naturally occurring enzyme. Herbicide-resistant soybean was planted on nearly 48 million hectares in 2004, accounting for nearly 60% of all the cropland worldwide planted with transgenic plants. Among these, glyphosate-resistant plants presumably represent the overwhelming majority.

Altering the levels or nature of the enzymes targeted by herbicides is thus an effective approach to producing herbicide-resistant plants. However, this approach has several limitations. First, theoretically it cannot produce an absolute level of resistance because greater amounts of herbicide will still inhibit an overproduced or less sensitive target enzyme. Second, the overproduction of an endogenous enzyme may produce unexpected and undesirable results. For example, glyphosate-resistant soybean is known to suffer, in hot weather, from splitting of the stems, which is thought to be caused by the higher lignin content of these plants. The overproduced enzyme catalyzes the synthesis of aromatic compounds, which are predominant building blocks in lignin (see Chapter 12). Third, the overproduction of one enzyme in a complex pathway may disturb the balance in such a pathway and may lead to the slowing of growth. Considerations such as these have prompted the exploration of alternative methods for developing herbicide resistance in plants.

One technique for producing resistant transgenic plants is the introduction of genes coding for herbicide-detoxifying enzymes. The development of glyphosate-resistant plants through this approach is described on p. 410. Another important example involves phosphinothricin, an analog of glutamic acid that inhibits glutamine synthetase (Figure 6.15).

FIGURE 6.15

Phosphinothricin and its mode of action. Phosphinothricin is an analog of glutamate. It binds to glutamine synthetase to inhibit the synthesis of glutamine. Bialaphos, an antibiotic produced by a *Streptomyces* species, is a tripeptide (phosphinothricinyl-alanyl-alanine) that is converted into phosphinothricin by plant peptidases.