

FIGURE 22.4 Osmotic adjustment occurs when the concentrations of solutes within a plant cell increase to maintain positive turgor pressure within the cell. As the cell actively accumulates solutes, Ψ_s drops, promoting the flow of water into the cell. In cells that fail to adjust osmotically, solutes are concentrated passively, but turgor is lost.

When water uptake by roots closely matches water loss by leaves, the RWC of transpiring leaves typically ranges from 85% to 95%. If the RWC for an organ drops below a critical value, tissue death follows. The value for critical RWC varies among species and tissue types, but it is frequently less than 50%.

22.2.3 Osmotic adjustment is a biochemical mechanism that helps plants acclimate to dry or saline soil

While some plants are highly sensitive to water stress and wilt (dehydrate), others can endure dry or saline conditions without evident loss of turgor. To extract water from the soil, a plant root must establish a water potential gradient so that water flows toward the root surface from the soil (i.e., the water potential must be lower in the root than in the surrounding soil). Many drought-tolerant plants regulate their solute potential (Ψ_s) to compensate for transient or extended periods of water stress. This process, called **osmotic adjustment**, results from a net increase in the number of solute particles in the plant cell. The osmolalities achieved through osmotic adjustment exceed those from passive concentration of solutes by dehydration. By decreasing plant Ψ_s , osmotic adjustment can drive root Ψ_w to values lower than soil Ψ_w , thereby allowing water to move from soil to plant down a potential gradient (Fig. 22.4). Osmotic adjustment is believed to play a critical role in helping plants acclimate to conditions of drought or high salinity.

22.2.4 Compatible solutes and aquaporins play roles in osmotic adjustment

One mechanism for osmotic adjustment is the accumulation of **compatible solutes**, or **compatible osmolytes**, a chemically diverse and highly soluble group of organic compounds that do not interfere with cellular metabolism, even at high concentrations (Fig. 22.5).

Synthesis and accumulation of these compounds are widespread in plants, but their distribution varies among species. For example, whereas one such compatible solute, the amino acid proline, is accumulated by a taxonomically diverse set of plants, the quaternary ammonium compound β -alanine betaine appears to be confined to representatives of a few genera of Plumbaginaceae (Leadwort). Stress can trigger the irreversible synthesis of these compounds (for example, glycine betaine; see Section 22.3.4), alter the balance between their synthesis and catabolism (e.g., proline), or trigger their release from polymeric forms (e.g., monomeric sugars like glucose and fructose can be released from their polymeric forms, starch and fructans). Once the stress is removed, these monomers can be repolymerized to facilitate rapid and reversible osmotic adjustment, or they can be metabolized to produce primary metabolites or energy.

Unlike many inorganic solutes, which may become toxic at high concentrations, the organic character of compatible solutes greatly diminishes their potential toxicity. Many ions found in cells adversely affect metabolic processes at high concentrations, possibly by binding to and altering the

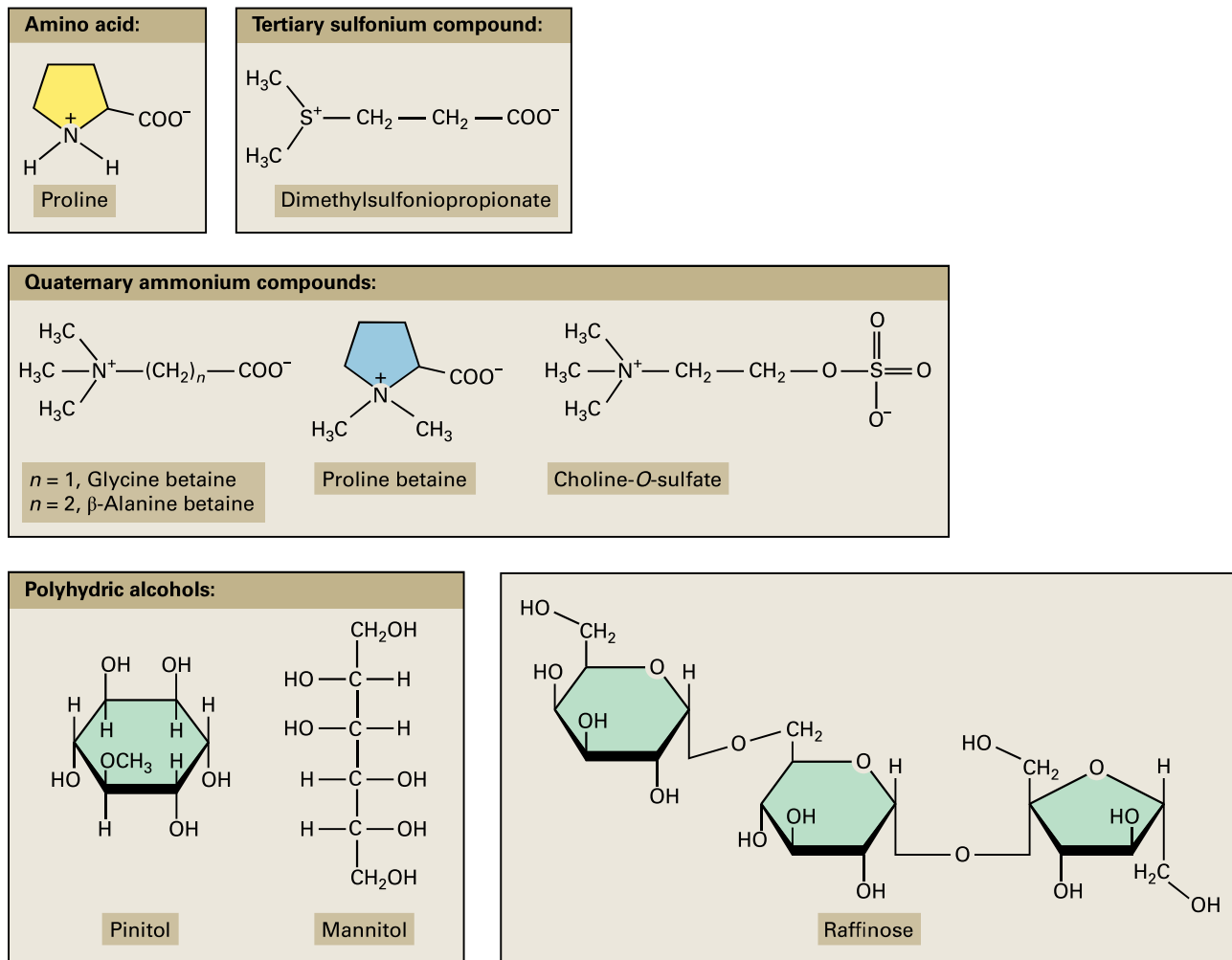


FIGURE 22.5 Chemical structures of several important compatible solutes (osmolytes) that accumulate in plant cells in response to water deficit. The organic character and nonionic or zwitterionic nature of compatible solutes greatly diminishes their potential toxicity.

properties of cofactors, substrates, membranes, and enzymes. Furthermore, many ions can enter the hydration shells of a protein and promote its denaturation. In contrast, compatible solutes tend to be neutrally charged at physiological pH, either nonionic or **zwitterionic** (dipolar, with spatially separated positive and negative charges), and are excluded from hydration shells of macromolecules (Fig. 22.6).

In addition to stereotypical charge characteristics, compounds active in osmotic adjustment show distribution patterns that support water potential equilibria among the various membrane-bound compartments of the cell. Vacuoles tend to accumulate charged ions and solutes that would perturb metabolism if present in the cytoplasm. Compatible solutes in the cytoplasm, however, allow the cytosol to achieve osmotic balance with the vacuole.

Another possible mechanism for osmotic adjustment is increasing movement of water into the cell. The hydrophobic nature of the lipid bilayer presents a considerable barrier to the free movement of water into the cell and between intracellular compartments. However, plasma membranes and tonoplasts can be rendered more permeable to water by

proteinaceous transmembrane water channels called **aquaporins** (see Chapter 3). Water movement through aquaporins can be modulated rapidly, and evidence suggests these channels may facilitate water movement in drought-stressed plant tissues and promote rapid recovery of turgor upon watering. The abundance of aquaporin mRNA is correlated with turgor changes in leaves subjected to osmotic stress. Higher transcript levels, enhanced translation, and activation of existing proteins may constitute multiple mechanisms for regulating aquaporin abundance and activity, which may be advantageous for plants coping with water deficit.

22.2.5 Some compatible solutes may have protective functions in addition to osmotic adjustment

The role that compatible solutes play in osmotically stressed plants is often defined as “**osmoprotection**”—solute accumulation is believed to have a protective function against water

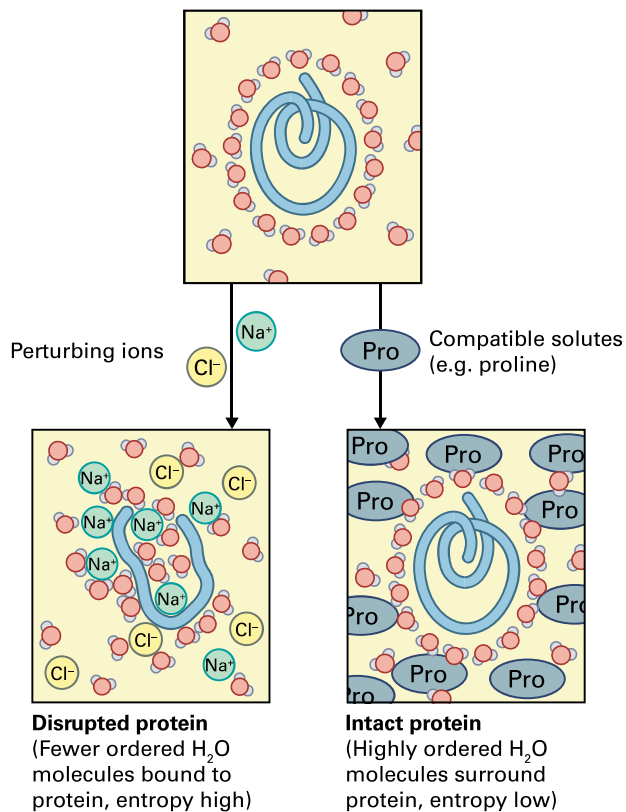


FIGURE 22.6 Hydration shells of macromolecules are not disrupted by compatible solutes. Depicted is a protein with a hydration shell (i.e., surrounded by ordered H_2O molecules). Ions such as Na^+ and Cl^- can penetrate these shells and interfere with the noncovalent interactions that maintain the structure of the protein. Unlike ions, compatible solutes, such as proline and glycine betaine, do not penetrate the protein's hydration shell, so protein and solute do not come into direct contact. The hydration shells of the ions and compatible solutes are not shown.

deficit. However, this term should be applied with discretion. Historically, direct physiological evidence for osmoprotection has been obtained for bacteria, but not for plants. Salt-sensitive bacteria, such as *Escherichia coli*, can be induced to grow in high-salt media supplemented with specific compounds, including the osmolytes shown in Figure 22.5. Therefore, the presence of concentrated compatible solutes in drought-tolerant and halophilic (“salt-loving”) plants has been taken as compelling, albeit indirect, evidence for the role of osmolyte in plant osmoprotection.

Compatible solutes that accumulate in plants may also act as antioxidants to minimize the impact of abiotic stress in plants. In vitro, many of these compounds directly offset the deleterious, perturbing effects of ions. For example, glycine betaine prevents salt-induced inactivation of Rubisco and destabilization of Photosystem II (PSII). Sorbitol, mannitol, *myo*-inositol, proline, and raffinose can also scavenge hydroxyl radicals in vitro, though glycine betaine cannot. This antioxidant activity suggests a protective role for these compounds in osmotic stress tolerance distinct from osmotic adjustment.

22.2.6 Genetic engineering provides an opportunity to test the adaptive significance of compatible solutes

Drought- or salt-sensitive plants can be transformed with genes encoding enzymes that are critical for the synthesis of a putative osmoprotectant. Transformed plants can then be assayed for enhanced accumulation of compatible solutes and ability to adjust osmotically to conditions of drought and high salinity. In fact, biotechnological approaches for enhancing drought tolerance typically involve dissection of the biosynthesis pathways of specific osmolytes and subsequent manipulation of enzymes operating in those pathways. Among the compounds studied to date are proline, glycine betaine, oligosaccharides such as raffinose, and polyhydric alcohols, including mannitol and pinitol. The results from these studies indicate a role for these compounds not only in responses to water and salinity stress, but to other types of abiotic stress as well.

Upon exposure to abiotic stress, many plants accumulate carbohydrates such as mannitol, trehalose, *myo*-inositol, fructan, galactinol, and raffinose. These metabolites not only serve as energy storage but are thought to play roles in balancing osmotic strength, stabilizing macromolecules, and preserving the membrane. Genetic manipulations of key enzymes involved in the biosynthetic pathways of these carbohydrates can improve abiotic stress tolerance in transgenic plants. Metabolite profiling using different types of mass spectrometry has revealed that various types of metabolite, including proline and branched chain amino acids such as valine, leucine, and isoleucine, accumulate in response to abiotic stress and serve a protective function in cells (see Fig. 22.6). The mechanisms that facilitate proline accumulation in response to environmental conditions are discussed in Chapter 7.

Glycine betaine (*N,N,N*-trimethylglycine, GB) is synthesized under various types of environmental stress and functions as a major osmoprotectant. It stabilizes the quaternary structures of the PSII protein-pigment under high salinity and maintains the ordered state of membranes at extreme temperatures. GB is synthesized mainly from choline via betaine aldehyde (BA), with the first and second steps in the pathway catalyzed by choline monoxygenase (CMO) and betaine aldehyde dehydrogenase (BADH), respectively (Fig. 22.7). A wide variety of plant species accumulate GB, but *Arabidopsis*, rice (*Oryza sativa*), and tobacco (*Nicotiana*) are considered “nonaccumulators.” Genetic engineering to introduce GB-biosynthetic pathways into nonaccumulator species is a promising approach to increase abiotic stress tolerance. Transgenic rice plants overexpressing the barley *BADH* gene convert applied BA into GB more efficiently than wild-type plants and exhibit significant tolerance to salt, cold, and heat stress.

Raffinose family oligosaccharides (RFOs), such as raffinose and stachyose and their precursor galactinol, play important roles in drought stress tolerance in plants and seeds. RFOs are important for reducing **reactive oxygen species** (ROS) that accumulate and cause oxidative stress under conditions of

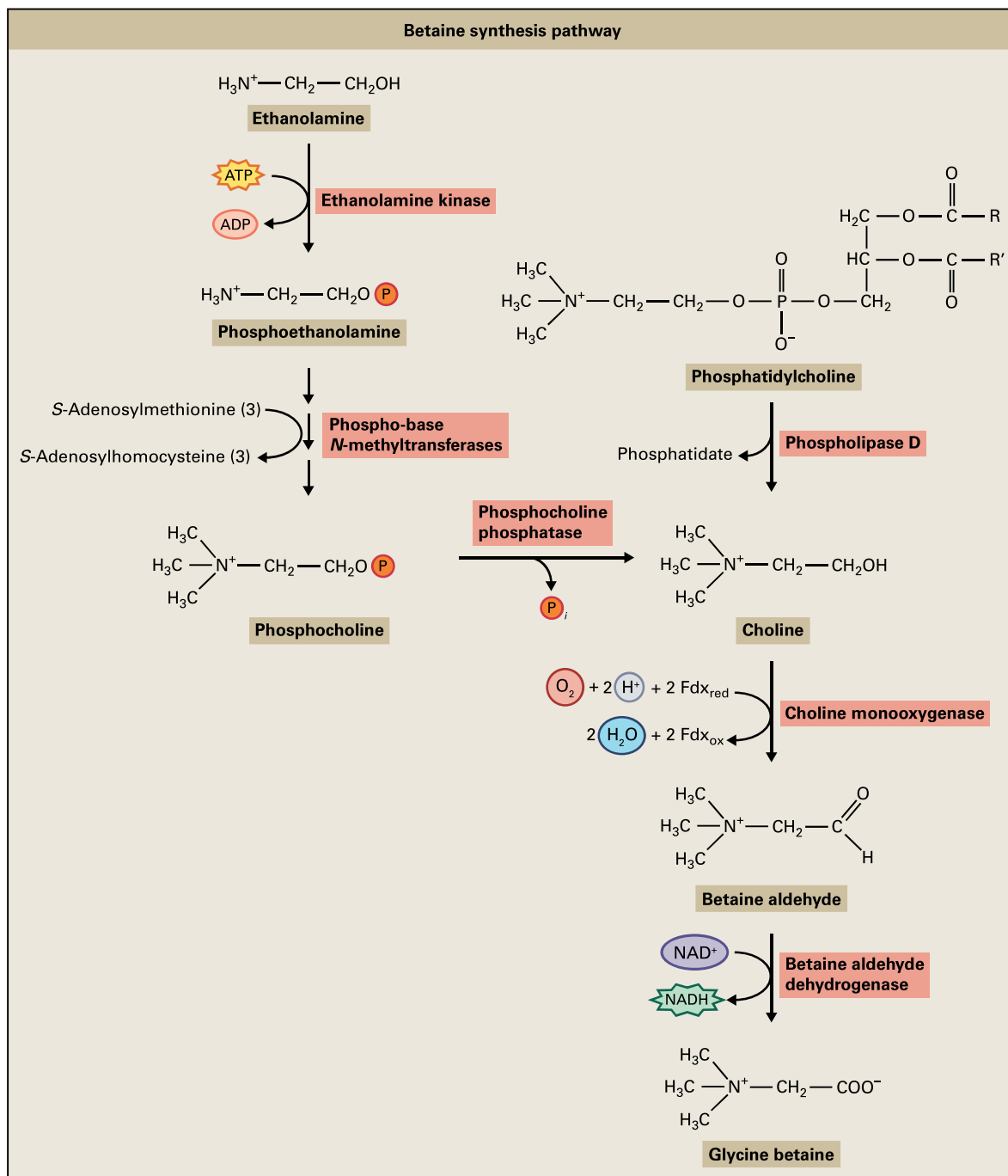


FIGURE 22.7 Proposed biosynthetic pathways of glycine betaine from choline. Introduction of such pathways into nonaccumulator species is a promising approach for increasing abiotic stress tolerance.

abiotic stress. They are synthesized from UDP-galactose (Fig. 22.8), which is converted into galactinol by galactinol synthase (GolS). Raffinose synthase then produces raffinose from the galactinol. Both raffinose and galactinol accumulate in *Arabidopsis* in response to drought, high salinity, and cold stress. These stressors induce expression of the genes for GolS and raffinose synthase, resulting in the production of RFOs. Overexpression of the stress-inducible GolS gene improves drought tolerance by activating the accumulation of galactinol and raffinose in transgenic *Arabidopsis* plants.

22.2.7 Some proteins protect macromolecules and membranes against damage

Large numbers of proteins, such as late embryogenesis abundant (LEA) proteins and heat shock proteins (HSPs), protect plant cells directly against water or osmotic stress damage. They exert their protective effects either as highly hydrophilic proteins that retain water or as molecular