Minireview

The unusually strong stabilizing effects of glycine betaine on the structure and function of the oxygen-evolving Photosystem II complex

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Abstract

Natural osmoregulatory substances (osmolytes) allow a wide variety of organisms to adjust to environments with high salt and/or low water content. In addition to their role in osmoregulation, some osmolytes protect proteins from denaturation and deactivation by, for example, elevated temperature and chaotropic compounds. A ubiquitous protein-stabilizing osmolyte is glycine betaine (N-trimethyl glycine). Its presence has been reported in bacteria, in particular cyanobacteria, in animals and in plants from higher plants to algae. In the present review we describe the experimental evidence related to the ability of glycine betaine to enhance and stabilize the oxygen-evolving activity of the Photosystem II protein complexes of higher plants and cyanobacteria. The osmolyte protects the Photosystem II complex against dissociation of the regulatory extrinsic proteins (the 18 kD, 23 kD and 33 kD proteins of higher plants and the 9 kD protein of cyanobacteria) from the intrinsic components of the Photosystem II complex, and it also stabilizes the coordination of the Mn cluster to the protein cleft. By contrast, glycine betaine has no stabilizing effect on partial photosynthetic processes that do not involve the oxygen-evolving site of the Photosystem II complex. It is suggested that glycine betaine might act, in part, as a solute that is excluded from charged surface domains of proteins and also as a contact solute at hydrophobic surface domains.

Introduction

Osmolytes

Living organisms employ various defensive strategies to ensure homeostasis in water-deficient or electrolyte-abundant environments. Examples of such strategies are provided by water-impermeable tissues, low passive permeability of cells to ions, sequestration of toxic solutes into vacuoles and the active pumping of ions out of cells. Evidence converging from a variety of sources indicates that, when these defenses fail to prevent loss of water from the cell or the invasion of undesirable solutes, organisms resort to the accumulation of certain low-molecular-weight organic compounds in the cytoplasm (for reviews, see Yancey et al. 1982; Csonka 1989). Since these solutes enable cells to adjust to variations in external osmotic pressure, they are known as osmoregulatory substances or osmolytes. Depending on the organism, osmolytes accumulate as a result of synthesis de novo, of importation, or of both processes. They are essential components of a major short-term defensive mechanism that enables certain organisms and tissues to adapt to environments with high and fluctuating salt content and/or with low and fluctuating water content.

Three important properties enable cytoplasmic osmolytes to fulfill their mission. (a) Being small and highly soluble molecules, they are significant contributors to the colligative properties of the cytoplasm, neutralizing differences in osmotic pressure. (b) Although present at unusually high concentrations, they do not perturb cell functions. (c) They protect delicate macromolecular structures from both chemical and physical structure-randomizing factors. To describe these properties, Brown and Simpson (1972) introduced the term...
'compatible osmolytes'. The compatibility attribute later came to be applied exclusively to the nonperturbing property of such compounds and the term 'counteracting osmolytes' was introduced to emphasize the ability of the osmolytes to protect macromolecules from denaturants, such as urea (Yancey et al. 1982).

Glycine betaine

In terms of chemical structure, cytoplasmic osmolytes can be classified as alkylamines (certain amino acids and derivatives of amino acids) and as polyhydroxylic compounds (polyols, saccharides and glucosides). Usually members of one group do not occur alone in cells but are accompanied by members of the other group. Glycine betaine (N-trimethyl glycine) is the most widespread osmolyte. It occurs in all three taxonomic kingdoms, the animal, plant and bacterial kingdoms, in particular in organisms that are exposed to conditions of extreme salinity.

When cells are broken in the laboratory, the cytoplasmic enzymes are suddenly exposed to environments that are as stressful as or even more stressful than the most extreme natural habitats. In their unnatural new surroundings, enzymes become inactive either immediately or gradually. To overcome this tendency towards inactivation and to optimize the recovery and the longevity of active enzymes in vitro, special media have been designed. These media invariably include a pH-buffering solute (e.g., phosphate, aminoalkyl carboxylate or aminoalkyl sulfonate), a stabilizing solute (e.g., mannitol, sorbitol or sucrose) and, often, one or more 'special-mission' solutes (e.g., antioxidants, reductants and peroxide scavengers). A recent addition to the ranks of the stabilizers of the structure and function of biological preparations in vitro is glycine betaine (Papageorgiou et al. 1991). We have studied the properties of this compound using thylakoid and subthylakoid preparations isolated from higher plants and from cyanobacteria.

According to Bowlus and Somero (1979) a compatible biological osmolyte must not interfere in any way with enzymes, their substrates and their reaction products. This condition is fulfilled by glycine betaine in the case of the oxygen-evolving enzyme complex of photosynthesis. At concentrations as high as 4 M, glycine betaine interferes neither with the photosynthetic evolution of oxygen (Papageorgiou et al. 1991) nor with the solubility of oxygen in aqueous media (Stamatakis and Papageorgiou 1993). Glycine betaine is unique in this sense even though this zwitterionic compound carries positive and negative electric charges. Moreover, glycine betaine does not react with water, the substrate of the oxygen-evolving complex although it is known to be strongly hydrated (the ratio of water to glycine betaine is 8.6:1, mol/mol) in aqueous media (Nakaya et al. 1991).

Higher plants

The accumulation and localization of glycine betaine

Higher plants accumulate glycine betaine in response to both water stress and salinity stress (Wyn Jones and Storey 1981). Several taxonomically distant plants have been identified as accumulators of glycine betaine but the most extensively studied plants are spinach (family Chenopodiaceae) and barley (family Gramineae). These plants synthesize glycine betaine by oxidizing choline first to glycine betaine aldehyde and subsequently to glycine betaine (Hanson et al. 1985).

\[(\text{CH}_3)_3N^+\text{CH}_2\text{CH}_2\text{OH} \rightarrow (\text{CH}_3)_3N^+\text{CH}_2\text{CH(OH)}_2 \rightarrow \text{choline} \]

\[(\text{CH}_3)_3N^+\text{CH}_2\text{COO}^- \quad \text{glycine betaine aldehyde hydrate} \]

\[(\text{CH}_3)_3N^+\text{CH}_2\text{COOH} \quad \text{Glycine betaine} \]

The first oxidation is catalyzed by a ferredoxin-dependent monooxygenase (Brouquisse et al. 1989) and the second by an NAD\(^+\)-dependent glycine betaine aldehyde dehydrogenase (Weigel et al. 1986). Choline monoxygenase has not been fully characterized but glycine betaine aldehyde dehydrogenase has been shown to be a homodimer (monomer MW 54, 267) that is encoded by a nuclear gene of known nucleotide sequence (Weretilnyk and Hanson 1990). Upon exposure of plants to salt stress, the activities of both enzymes and the level of betaine rise (Ladyman et al. 1980; Hanson et al. 1985; Brouquisse et al. 1989). With the exception of a minor cytosolic isozyme of glycine betaine aldehyde dehydrogenase (Weigel et al. 1986), the activities of both choline monoxygenase (Brouquisse et al. 1989) and glycine betaine aldehyde dehydrogenase (Weigel et al. 1986) are found in the chloroplast stroma.

Water-stressed plants accumulate glycine betaine primarily in mature leaves, but after the stress has been relieved glycine betaine can also be found in young leaves and in other plant organs (Ladyman et al. 1980).