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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 for isolation and storage 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).

$$\underset{\text{choline}}{(CH_3)_3N^+CH_2CH_2OH} \rightarrow \underset{\text{gylcine betaine aldehyde hydrate}}{(CH_3)_3N^+CH_2CH(OH)_2} \rightarrow \underset{\text{Glycine betaine}}{(CH_3)_3N^+CH_2COO^-}$$

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 monooxygenase 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; Brouquise 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 monooxygenase (Brouquise 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).

This observation suggests that glycine betaine might migrate through chloroplast envelope membranes and through cell membranes. However, it is very likely that the transport of glycine betaine across the cell membranes is regulated, since glycine betaine serves as a cytoplasmic regulator when the cells are stressed under salt conditions. It seems quite probable that glycine betaine can pass through thylakoid membranes and can, thus, act on the inner side of such membranes.

The oxygen-evolving Photosystem II complex

The exciting discoveries of the last two decades have led to proposals of detailed models of the oxygen-evolving Photosystem II complex (for recent selected reviews, see Klein et al. 1993; Renger 1993; van Gorkom and Schelvis 1993; and Vermaas et al. 1993). According to the prevailing model, water is oxidized at the so-called oxygen-evolving Photosystem II complex, a functionally defined entity that is organized on two thylakoid membrane-spanning proteins (known as D1 and D2). These proteins also bind the photochemical reaction center II, the electron carrier (Tyr_Z) on the oxidizing side of the photochemical reaction center, and the plastoquinone electron carriers (Q_A and Q_B) on the reducing side. The oxygen-evolving complex of higher plants consists of six intrinsic proteins, namely, D1, D2, CP47, CP43, and the α and β subunits of cytochrome b_{559}, and three extrinsic proteins of 18, 23 and 33 kD, respectively. The catalytic center in which decomposition of water occurs, is a cluster of four Mn ions, with valences of III and IV. The clustered ions are coordinated by ligands provided by the D1, D2 and, possibly, the CP47 proteins. The three extrinsic proteins play an auxiliary but essential role in the photosynthetic evolution of oxygen. Their role is 'auxiliary' in the sense that oxygen is photo-inducibly evolved even in the absence of these three proteins and 'essential' because only when they are properly attached does the evolution of oxygen occur with optimal efficiency and in a stable manner.

Dissociation of extrinsic proteins

In recent years, researchers studying photosynthesis have focused their attention on the roles of the extrinsic proteins. These proteins are released from isolated Photosystem II membranes (Kuwabara and Murata 1983; Miyao and Murata 1983; Ono and Inoue 1984) and from inverted thylakoid vesicles (Åkerlund and Jansson 1981) in the presence of chlorides and other salts of high concentrations. Dissociation of the 18 and 23 kD proteins, but not of the 33 kD protein, occurs in the presence of 1 M NaCl (Kuwabara and Murata 1983), while 1 M $MgCl_2$ and 1 M $CaCl_2$ dissociate all three extrinsic proteins (Ono and Inoue 1984). The latter result can also be achieved by treating Photosystem II particles with 2 to 3 M urea (Miyao and Murata 1984a). These treatments release the extrinsic proteins without damaging the structure or impairing the function of the Mn cluster. Thus, although the depleted Photosystem II complex is inactive in the evolution of oxygen, the depleted complex can be reactivated by the addition of millimolar levels of Ca^{2+} ions together with Cl^- ions in the 100 mM range (Miyao and Murata 1984b,c).

Photosystem II complexes that have been depleted of all three extrinsic proteins tend to release two of the four manganese ions in the Mn cluster as Mn^{2+} ions. The loss of manganese is irreversible and causes the irreversible cessation of oxygen evolution. However, retention of the 33 kD protein alone is sufficient to stabilize both the Mn cluster and the photosynthetic evolution of oxygen (Miyao and Murata 1984a). These observations led to the suggestion that the 33 kD protein stabilizes the clustered manganese ions. This suggestion was widely accepted and the 33 kD protein was designated the manganese-stabilizing protein (Burnap and Sherman 1991).

Stabilization of the extrinsic proteins and the Mn cluster by glycine betaine

Glycine betaine decreases the ability of metal chlorides and urea to dissociate the extrinsic proteins from the Photosystem II complex. Glycine betaine obstructs the NaCl-induced dissociation of the 18 and 23 kD proteins (Papageorgiou et al. 1991; Murata et al. 1992), as well as the $CaCl_2$-, $MgCl_2$- and urea-induced dissociation of the 33 kD protein (Murata et al. 1992) (Fig. 1). At the same time, glycine betaine protects the oxygen-evolving machinery from salt-induced inactivation (Papageorgiou et al. 1991). However, the osmolyte is less effective in protecting the association of extrinsic proteins with the Photosystem II complex in the presence of salts that have a tendency to randomize protein structure (chaotropic salts). In fact, the closer the position of a salt to the chaotropic end of the Hofmeister series (Collins and Washabaugh 1985) the less likely is glycine betaine to prevent the dissociation caused by this salt. For example, the ability of 1.2 M glycine betaine to protect the 23 kD protein

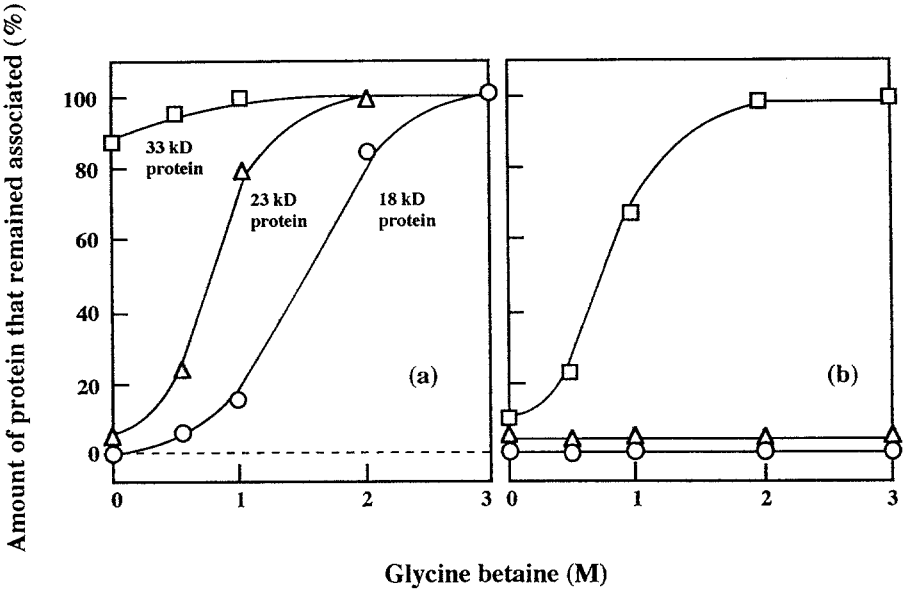

Fig. 1. Protection by glycine betaine of association of 18-kD, 23-kD and 33-kD extrinsic proteins to spinach PS2 particles spinach under salt conditions. Incubation was performed at 0 °C for 10 min. (a) In the presence of 1.2 M NaCl; (b) in the presence of 1.0 M $MgCl_2$. (*Source:* Murata et al. 1992)

from dissociation from the oxygen-evolving complex in the presence of sodium salts at 0.4 M was found to diminish in the following order (Papageorgiou et al. 1991):

$$\text{No salt} = Na_2SO_4 = NaCl > NaBr > NaNO_3$$

(strong protection) (weak protection)

In an analogous experiment, we compared the chlorides of different metals in terms of their ability to dissociate the extrinsic proteins from the oxygen-evolving complex in Photosystem II membranes suspended in a solution that contained 1 M glycine betaine. The protection by the osmolyte of the binding of these proteins to the complex decreased in the following order (Murata et al. 1992):

$$\text{No salt} > NaCl > MgCl_2 > CaCl_2$$

(strong protection) (weak protection)

As mentioned in the previous section, the Photosystem II complex tends to release manganese ions from the cluster and to become inactive when depleted of all three extrinsic proteins of the oxygen-evolving complex. However, when Photosystem II complexes depleted of extrinsic proteins are suspended in solutions that contain glycine betaine (1 M or higher), both

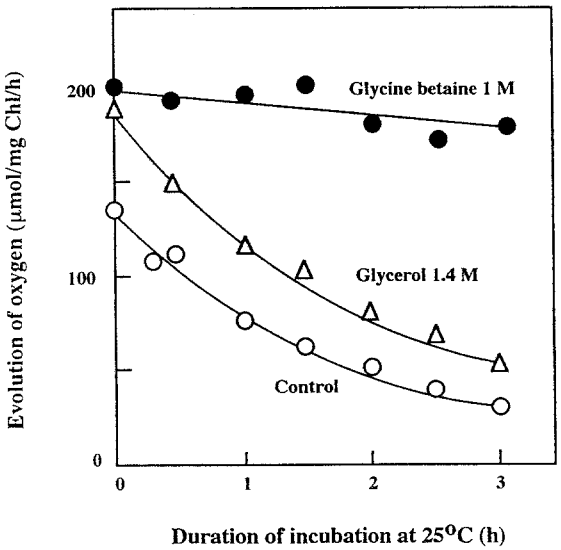

Fig. 2. Effects of glycine betaine on the inactivation of oxygen evolution in spinach PS2 particles depleted of the 18-kD, 23-kD and 33-kD proteins. Oxygen-evolving activity was measured after addition of 100 μM phenylbenzoquinone. (*Source*: Mohanty et al. 1993.)

the Mn cluster and the oxygen-evolving activity are stabilized (Mohanty et al. 1993; Fig. 2).

Glycine betaine also prevents the inactivation of the photosynthetic evolution of oxygen by alkaline

pH (Mohanty et al. 1993), by submillimolar concentrations of Cl^- ions (Kalosaka and Papageorgiou 1992) and by prolonged storage in darkness at 25 °C (Williams and Gounaris 1992).

Cyanobacteria

Glycine betaine in cyanobacteria

Glycine betaine behaves both as a compatible and as a counteracting solute in solutions of soluble cyanobacterial enzymes in vitro (Warr et al. 1984; Incharoendsakdi et al. 1986; Azaria-Gabbay et al. 1988). According to Mackay et al. (1984), cyanobacteria found in hypersaline habitats (2 M NaCl) accumulate either glycine betaine or both glycine betaine and disaccharides as osmoregulatory solutes. In less saline habitats (marine and freshwater) cyanobacteria accumulate disaccharides and glucosides. Glycine betaine is either synthesized de novo or is imported in response to changes in salinity.

The cyanobacterial Photosystem II complex

According to the fossil record, cyanobacteria are the oldest oxygenic photosynthetic organisms (Schopf and Walter 1982). However, their photosynthetic apparatus is essentially the same as that of green plants. The cyanobacteria have three extrinsic proteins of 9–12 kD, 17 kD and 33 kD in the oxygen-evolving complex, (Stewart et al. 1985a; Shen et al. 1992) as green plants do (18 kD, 23 kD and 33 kD proteins).

Isolated Photosystem II membrane particles from cyanobacteria are less stable than thylakoid fragments obtained from the same source and the latter are less stable than Photosystem II membrane particles and thylakoid fragments obtained from chloroplasts of higher plants. The reason for these differences appears to be the unstable binding of the 9 kD extrinsic protein to the cyanobacterial oxygen-evolving complex. This protein dissociates more readily when the complex is exposed to a dilute aqueous suspension medium (as in the case of suspensions of membrane particles) than when it is exposed to the lumen space of thylakoids (as in the case of vesicular thylakoid fragments). By contrast, the extrinsic proteins of the oxygen-evolving complex of higher plants are not as prone to dissociate spontaneously from the complex. These proteins dissociate from the complex only in the presence of metal chlorides at elevated concentrations (see section on 'Dissociation of extrinsic proteins').

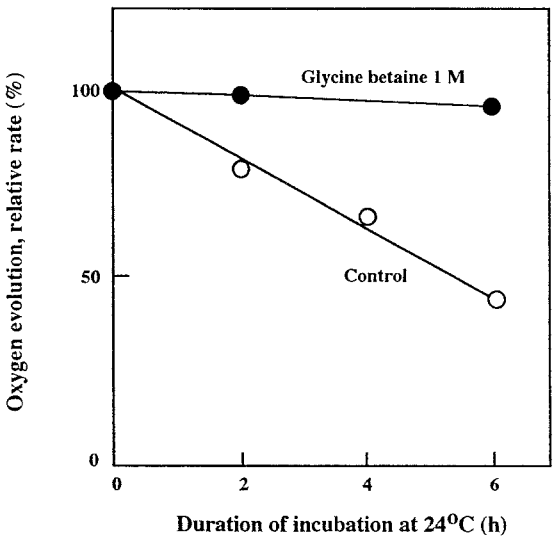

Fig. 3. Effects of glycine betaine on the inactivation of oxygen evolution of thylakoid membranes from *Synechocystis* sp. PCC 6803. Oxygen-evolving activity was measured after addition of 100 μM phenylbenzoquinone. (*Source:* Mamedov et al. 1991)

Effectiveness of glycine betaine

Glycine betaine protects the photosynthetic oxygen-evolving machinery of thylakoid and subthylakoid preparations from cyanobacteria against thermally induced (Mamedov et al. 1991, 1993; Stamatakis and Papageorgiou 1993; Fig. 3) and salt-induced (Stamatakis and Papageorgiou 1993) inactivation. When cells of the unicellular cyanobacterium *Synechocystis* PCC6803 are disrupted in the presence of 1 M glycine betaine, the oxygen-evolving activity is quantitatively retained in the resulting fraction of thylakoid membranes (Mamedov et al. 1991).

The presence of glycine betaine is beneficial only to those reactions of cyanobacterial photosynthesis that involve participation of the oxygen-evolving complex (Mamedov et al. 1993). For example, glycine betaine shifts the temperature for 50% inactivation of the oxygen-evolving machinery of *Synechocystis* sp. PCC6803 thylakoids from 36 °C to 42 °C. Partial photosynthetic processes that do not involve the evolution of oxygen are unaffected by glycine betaine. Examples of such processes are the photo-induced transport of electrons from diphenylcarbazide to dichlorophenolindophenol (across Photosystem II) or from duroquinol

to methyl viologen (across Photosystem I). Moreover, glycine betaine protects phenazine methosulfate-mediated cyclic photophosphorylation from the harmful effects of prolonged incubation at 34 °C (Mamedov et al. 1991). However, the temperature for 50% inactivation of such cyclic photophosphorylation was found to be 48 °C both in the absence and in the presence of glycine betaine (Mamedov et al. 1993).

Photosystem II membrane particles obtained from the thermophilic filamentous cyanobacterium *Phormidium laminosum* retain the 9 kD protein, as well as their oxygen-evolving activity, when 1 M glycine betaine (Stamatakis and Papageorgiou 1993) or 25% (v/v) glycerol is present in the incubation medium (Stewart et al. 1985b; Stamatakis and Papageorgiou 1993). Nevertheless, as an additive, glycine betaine has two additional remarkable effects that glycerol does not: first, it protects the 9 kD protein against NaCl-induced dissociation from the complex; and, second, it reactivates the evolution of oxygen in the Photosystem II complex that has been depleted of the 9 kD protein. In contrast, the 12-kD protein of *Synechococcus vulcanus* (corresponding to the 9-kD protein of *P. laminosum*) is more tightly bound to the oxygen-evolving complex (Shen et al. 1992). Its dissociation, together with two other extrinsic proteins (the 33-kD protein and cytochrome *c*-550 with low redox potential), is accomplished with 1 M $CaCl_2$. Full restoration of oxygen-evolving activity requires the reconstitution of all the three extrinsic proteins to the depleted Photosystem II core of *S. vulcanus* (Shen and Inoue 1993).

Comparison between the evolution of oxygen by preparations derived from higher plants and those derived from cyanobacteria

The results described below may shed some light on certain as yet unexplained effects of glycine betaine on the oxygen-evolving activities of preparations of thylakoid membranes. It has been observed frequently that thylakoid and subthylakoid preparations from higher plants are more active when assayed in media that contain glycine betaine than in media without it (Papageorgiou et al. 1991; Kalosaka and Papageorgiou 1992; Mohanty et al. 1993). The enhancement of the evolution of oxygen by glycine betaine is even more conspicuous in thylakoid membrane particles isolated from cyanobacteria (Mamedov et al. 1991, 1993; Nishiyama et al. 1993; Stamatakis and Papageorgiou 1993).

It is reasonable to assume that the enhancement observed in cyanobacterial preparations simply reflects the two roles of glycine betaine that were mentioned in the previous section. First, glycine betaine stabilizes the association of the 9 kD protein with the intrinsic part of the Photosystem II complex. The proper attachment of this protein is critical for the evolution of oxygen by the cyanobacterial thylakoid membranes. Second, glycine betaine reactivates oxygen-evolving complexes that have been rendered inactive as a result of the spontaneous dissociation of the 9 kD extrinsic protein from the complexes. Compared to cyanobacterial preparations, Photosystem II membranes from higher plants are less prone to the spontaneous thermal dissociation of the extrinsic proteins from the oxygen-evolving complex and they are, therefore, inherently more stable. The enhancement of the evolution of oxygen observed in the latter preparations when glycine betaine is present in the suspension medium might reflect a further stabilization of the higher-order structure in the oxygen-evolving complex, in particular of the binding of the 33 kD extrinsic protein and the Mn cluster.

The physical basis of the effects of osmolytes

Hypotheses that explain the effects of osmolytes

A great deal of attention has been focused on the way in which glycine betaine and other compatible osmolytes act to protect macromolecular structures and enzymic activities from structure-randomizing and inactivating factors. However, no rigorous physicochemical theory that is capable of explaining all relevant observations has yet been developed. Interpretations, whenever attempted, have all been qualitative, having no predictive value. There are, basically, two types of hypothesis. The most widely considered is the 'solute exclusion hypothesis' of Timasheff and coworkers, which is based on studies of protein hydration (Arakawa and Timasheff 1985 and references cited therein). Surface hydration increases the hydrodynamic volume of a protein (Tinoco et al. 1978). In principle, the degree of hydration of a protein can be estimated from its density. By employing high-precision densimetry of defined three-component solutions that consisted of water, a globular protein and an additional organic solute, Timasheff and coworkers found evidence that suggested that several commonly used additive organic solutes, such as sugars (Arakawa and Timasheff

1982), amino acids (Arakawa and Timasheff 1983) and amino acid derivatives (Arakawa and Timasheff 1983, 1985), induced the preferential hydration of the surface of proteins, while, at the same time, the additive organic solutes were preferentially excluded from such surfaces. The same solutes are known to increase the surface tension of water (Arakawa and Timasheff 1983), forcing water-protein interfaces into contact. To satisfy this condition, protein molecules assume a more folded (more 'native') configuration in the presence of the additive organic solute. In other words, the equilibrium represented by

$$\text{Native protein} \rightleftharpoons \text{Denatured protein}$$

is displaced to the left. Indeed, it has been shown that additive solutes shift the range of temperatures over which globular proteins unfold upwards by 4–8 °C (Arakawa and Timasheff 1983, 1985; Santoro et al. 1992). An exception to this phenomenon is provided by glycine betaine. Although it lowers the surface tension of water, it still induces preferential hydration of proteins (Arakawa and Timasheff 1983).

The second hypothesis relies on ideas elaborated by Schobert (1977) who argued that the most critical function of osmolytes is to preserve the native structures of proteins rather than to balance internal and external osmotic pressures. In the cytoplasm, water exists as free water (bulk water) that serves as a solvent and it also exists as bound water that is held tenaciously by proteins and other biopolymers. The presence of bound water is critical to the structural stability of proteins.

The surface of a protein is composed of hydrophilic and hydrophobic domains. The latter domains are the vulnerable sites in the protein because the water molecules that are weakly held by these domains are the first to depart when cells encounter a water-deficient environment. By contrast, water is more strongly bound to hydrophilic surface domains and, therefore, such domains are less likely to be affected by a water deficiency. Accordingly, a hyperosmotic environment will primarily destabilize the hydrophobic surface domains of proteins.

Structural protection by amphiphilic osmolytes, such as proline and glycine betaine, and hydrophilic osmolytes, such as polyols, can be visualized in terms of two mechanisms, as follows. Amphiphilic osmolytes bind to hydrophobic surfaces via their hydrophobic moieties and convert them to hydrophilic surfaces. Since much more water is involved in the stabilization of a hydrophobic surface domain than in the stabilization of a hydrophilic surface domain, this conversion means that the cell can now preserve the structural integrity of cytoplasmic proteins under conditions of water deficiency. Alternatively, polyols replace some of the water molecules in the vicinity of the hydrophobic surface. During water deficiency these vicinal polyols replace water by contributing to the 'hydrophobically enforced structure' of the protein surface.

The two hypotheses complement each other in providing a qualitative physicochemical explanation of the stabilization of biopolymers by osmolytes. However, the solute exclusion hypothesis incorporates the less specific of the two approaches because it makes no distinction between hydrophilic and amphiphilic solutes and because it disregards the hydrophobic surface domains on a protein surface. This hypothesis precludes, furthermore, any direct interaction of additive solute molecules with the surface of proteins. However, Schobert's hypothesis relies on such direct interactions in order to explain the stabilizing effects of hydrophilic and amphiphilic solutes. Both hypotheses totally ignore surface electrostatic interactions between ionic groups on proteins (mostly negative) and electrically charged additive solutes. Accordingly, both are inherently unable to distinguish the protein-stabilizing effects of an electrically charged quaternary alkylammonium (such as glycine betaine) from those of, for example, an uncharged polyol.

If we are to gain even the simplest qualitative insight into the stabilizing effects of osmolytes, it is necessary first to categorize the existing data related to the dissociation of proteins into heat-induced and chaotropic effects. Chaotropic compounds can be either electroneutral (e.g., urea) or electrically charged (e.g., anions).

With regard to the heat-induced destabilization of soluble enzymes, glycine betaine seems to be more effective, albeit not in every case, than polyols and amino acids (Pollard and Wyn Jones 1979; Laurie and Stewart 1990). It is of particular interest that at concentrations below 100 mM, glycine betaine was superior to sucrose in protecting thylakoid membranes from freezing damage, while above 100 mM the converse was true (Coughlan and Heber 1982). In the urea-induced destabilization of proteins, glycine betaine was more or less as effective as other alkylamines (Yancey et al. 1982). However, in cases of destabilization by electrolytes (e.g., NaCl) glycine betaine was nearly always the most effective stabilizing agent (Pollard and Wyn Jones 1979; Warr et al. 1984; Mane-

tas 1990). Moreover, glycine betaine was proved to be a singularly effective additive solute in the protection of the photosynthetic oxygen-evolving complex from heat-induced and salt-induced inactivation (Papageorgiou et al. 1991; Murata et al. 1992; Mohanty et al. 1993).

Specific effects of glycine betaine on the oxygen-evolving complex

What makes glycine betaine such an effective protector of the structure and the activity of enzymes, in general, and of the photosynthetic oxygen-evolving complex in particular? The answer to this question is not clear. Clues can be found, however, in the unique properties of this osmolyte. Glycine betaine is the smallest amino-acid zwitterion. Hence, cells can achieve osmoregulation via biosynthesis of this osmolyte with the comparatively low expenditure of metabolic energy. Being a zwitterion throughout the physiological range of pH values, glycine betaine requires no counterions for electric neutrality in the cytoplasm. An electrically charged but not zwitterionic osmolyte would, by contrast, require disastrously high levels of neutralizing counterions in the cytoplasm. Glycine betaine is also an amphiphilic compound with a hydrophobic positive end (i.e., the tetralkylammonium group) and a hydrophilic negative end (i.e., the carboxylate group). This property enables it to neutralize anionic groups situated within hydrophobic protein domains. Moreover, since its tetravalent nitrogen atom lacks the characteristic lone electron pair that is present in trivalent nitrogen, glycine betaine cannot interact with electrophilic centers (e.g., the Mn cluster) and, therefore, it does not alter their electric properties.

Synergistic effects of glycine betaine and polyols

It is worth noting before we leave this subject that glycine betaine is usually present together with polyols in cells of water-stressed or salt-stressed plants and microalgae. The simultaneous presence of alkylamines and polyols suggests the possibility of a synergistic effect whereby a polyol enhances the effectiveness of the alkylamine osmolyte. Such a possibility remains, however, an open question, although some supporting evidence has been obtained with isolated thylakoid membranes from spinach (Papageorgiou and Murata, unpublished experiments).

Conclusion

Glycine betaine has two apparently different effects on the oxygen-evolving Photosystem II complex: (1) it enhances the oxygen-evolving activity; and (2) it stabilizes the oxygen-evolving activity by protecting the oxygen-evolving complex from dissociation of extrinsic proteins and the Mn cluster. Therefore, we strongly suggest that glycine betaine should be added at a concentration of 1 M to buffers used for the preparation, storage and assays of thylakoid membranes from leaves of higher plants and cyanobacterial cells. The presence of glycine betaine results in highly active preparations of thylakoid membranes and enhances the stability of these preparations during their storage at and below room temperature.

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