Effect of Betaine on Enzyme Activity and Subunit Interaction of Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase from *Aphanothece halophytica*

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**ABSTRACT**

The presence of betaine, a quaternary ammonium compound, at a concentration (0.5 molar) reported to accumulate inside *Aphanothece halophytica* in response to increasing external salinity, slightly promoted ribulose-1,5-bisphosphate (RuBP) carboxylase activity. KCl at 0.25 molar inhibited RuBP carboxylase about 55%. Betaine relieved the inhibition by 0.25 m KCl and the original uninhibited activity was restored at 1 M betaine. Other osmoregulatory solutes such as sucrose and glycine also reduced KCl inhibition, though to a lesser extent than betaine. Proline had no effect. The protective effect of betaine against KCl inhibition of RuBP carboxylase activity was also observed in other cyanobacteria, i.e. *Synechococcus* ACMM 323, *Plectonema boryanum*, and *Anabaena variabilis*, in the photosynthetic bacterium *Rhodospirillum rubrum* but not in *Chlamydomonas reinhardtii*. Apart from betaine, other quaternary ammonium compounds, i.e. sarcosine and trimethylamine-N-oxide (TMAO), but not glycine, also protected the enzyme against KCl inhibition and the effectiveness of such compounds appeared to correlate with the extent of N-methylation. Heat and cold inactivation of the enzyme could be protected by either betaine or KCl. However, best protection occurred when both betaine and KCl were present together. The $K_a$ (CO$_2$) was not altered by either betaine or KCl, nor when they were present together. However, the $K_a$ (RuBP) was increased about 5-fold by KCl, but was unaffected by betaine. The presence of betaine together with KCl lowered the KCl-raised $K_a$ (RuBP) by about half. The extent of the dissociation of the enzyme molecule under the condition of low ionic strength was reduced by either betaine or KCl alone and more so when they were present together. Glycine, sarcosine, and TMEO were more effective than betaine or KCl in lowering the extent of the dissociation of the enzyme molecule.

Organisms that thrive in hypersaline environments must possess specific mechanisms to adjust their internal osmotic status according to the salinity of the environment. One such mechanism is the ability to accumulate inorganic ions such as K$^+$, and Na$^+$ to a lesser extent, or some organic solutes like glycero, sucrose, proline, and betaine (11).

Osmoregulation in cyanobacteria grown at high external NaCl concentrations involves the accumulation of organic and inorganic solutes. For several fresh water cyanobacteria the role of low mol wt carbohydrates as osmoregulators has been shown; for example, glucoislylglycerol in *Synechocystis* 6714 (14) and sucrose in *Synechococcus* 6311 (2). A combination of both inorganic and organic solutes (sucrose and glucoislylglycerol) is involved in osmotic adjustment in the euryhaline cyanobacterium, *Synechocystis* PCC 6714, which can grow in both nonsaline and saline media (19).

Betaine (glycinebetaine), a quaternary ammonium compound, was first shown to be the major osmoticum in a halophilic cyanobacterium, *Synechocystis* DUN 52 (16). *Aphanothece halophytica* is a halophilic cyanobacterium, unable to grow at or below 3.5% (w/v) NaCl (sea water salinity) (5). The osmotic adaptation of this organism was first studied by Miller et al. (15) who showed that *A. halophytica* accumulated up to 1 M K$^+$ in response to increasing external salinity. Recently, Reed et al. (18) demonstrated that betaine is the major solute accumulated followed by K$^+$ in four strains of cyanobacteria including *A. halophytica* grown in high salinity. Furthermore, the variation for betaine accumulation in response to changes in extracellular salinity is much greater than that observed with K$^+$. The former group has also recently accepted the idea that betaine is a major osmoticum in *A. halophytica* cells (21). Betaine has also been implicated as a major cytoplasmic osmoticum in shoots of a number of plants (25) as well as in the halophilic photosynthetic bacterium, *Ectothiorhodospira halochloris* (7).

High concentration of salts have been reported to inhibit the activity of many enzymes from both eucaryotic and procaroytic origins (28). Higher plants are able to compartmentalize the accumulated salts, Na$^+$ and Cl$^-$ in particular, in the vacuole (8, 26), and thereby prevent the inhibition of enzyme activities. In contrast, the soluble enzymes in procaroytic organisms have to be directly exposed to any osmoregulatory substances. Based on the findings of Reed et al. (18) it is interesting to investigate how salt and betaine may affect the function of some soluble enzymes in *A. halophytica*.

We reported previously that KCl strongly inhibits the activity but prevents the dissociation of RuBioS (10) molecules from *A. halophytica* into catalytic core (octamer of large subunit A) and small subunit B (27). In the present study, we examined the effect of betaine and some other solutes in relation to the effect of KCl on the activity of RuBP carboxylase, a key enzyme in...
photosynthesis, from *A. halophytica*. Effects of betaine and KCl on heat and cold inactivation of RuBP carboxylase were also investigated. We demonstrated previously that RuBisCO molecules from *A. halophytica* can be partially dissociated under conditions of low ionic strength and low temperature (12). The protective effect of quaternary ammonium compounds on the dissociation of RuBisCO molecule at low temperature from *A. halophytica* is also reported.

**MATERIALS AND METHODS**

Organisms and Growth Conditions. *Aphanothece halophytica* was grown autotrophically as previously described (27). *Pleoterna boryanum* and *Anabaena variabilis* were grown in a BG 11 medium (23). The culture and growth of *Chromatium visous* have been described elsewhere (13).

Purification of RuBisCO. RuBisCO from *A. halophytica* was purified by sucrose density gradient centrifugation as previously described (12) with slight modification. The 25 to 50% (NH₄)₂SO₄ pellet, after resuspension in 50 mm Hepes-KOH buffer (pH 7.5) containing 1 mM EDTA, 5 mM DTT, and 0.3 M KCl (HEDK buffer) and passing through a Sephadex G-25 column equilibrated with the same buffer (referred to as partially purified enzyme), was layered onto a 38 ml 0.2 to 0.8 M linear sucrose gradient prepared in HEDK buffer containing 10 mM CaCl₂ and 20 mM MgCl₂. The gradient was centrifuged in a Beckman VTi 50 rotor at 242,000g for 2 h at 4°C in a Beckman ultracentrifuge. The enzymically active fractions were pooled and precipitated with 60% (NH₄)₂SO₄. The resuspended pellet was finally passed through a Sephadex G-25 column to remove any low mol wt compounds. Partially purified enzymes from *P. boryanum* and *A. variabilis* were obtained by the method described for *A. halophytica* with the omission of KCl in the purification buffer.

RuBisCO from *C. visous* was partially purified by a modification of the method of Berhow and McFadden (1). About 10 g of cells were suspended in 50 ml of MEMMB buffer (50 mM morpholinopropanesulfonic acid, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, 10 mM MgCl₂, 50 mM NaHCO₃, adjusted to pH 7.2 at 25°C) and sonicated twice, 5 min each at 200 W in a Kubota Insonator model 200 M. The resulting suspension was centrifuged at 94,000g for 1 h. The supernatant was diluted with two volumes of cold MEMMB buffer and was adjusted to 10% (w/v) PEG 4,000 by slow addition of 60% PEG. The solution was stirred in an ice-bath for 30 min before centrifugation at 39,000g for 20 min to remove most of the chromatophores and membranous fractions. Additional protein was precipitated from the supernatant by adding 2 M MgCl₂ to a final concentration of 50 mM. The solution was stirred in an ice-bath for 30 min before centrifugation as before. The final pellet obtained was resuspended in a small volume of 10 mM phosphate buffer (pH 7.6) containing 1 mM EDTA and passed through a Sephadex G-25 column eluted with the same buffer before use.

Assay Method of RuBP Carboxylase and Protein. The standard assay mixture for RuBP carboxylase contained the following components in a total volume of 250 μl: 100 mM Tris-HCl (pH 7.8), 20 mM MgCl₂, 50 mM NaHCO₃ (0.4 mM Ci/mmol), 1 mM RuBP, and appropriate amounts of purified enzyme. After a 10 min activation at 25°C, the catalytic reaction was initiated by the addition of RuBP. The reaction was stopped after 10 min by the addition of 50 μl of acetic acid. The vial contents were evaporated to dryness under an electric lamp, 0.3 ml of water, and 3 ml of scintillation fluid were added, and the acid-stable radioactivity was determined by liquid scintillation counting.

Protein was assayed by using the Bio-Rad protein assay kit and Sigma BSA as a standard.

**RESULTS**

Effect of Betaine and Other Solutes on RuBP Carboxylase Activity from *A. halophytica*. A range of organic solutes that have been implicated as cytoplasmic osmoregulators in plants or photosynthetic procaryotes were examined in comparison to KCl for their effects on the activity of RuBP carboxylase from *A. halophytica* (Fig. 1A). Sucrose enhanced enzyme activity even at concentrations up to 1 M. The effect of betaine was similar to that of glycerol, which showed a slight activation of activity. On the other hand, proline and KCl had an inhibitory effect on enzyme activity. Fifty percent of the control activity was observed at 0.25 M KCl, confirming our previous results (27). For betaine and glycerol, our results are in agreement with previous studies that showed slight activation of malate dehydrogenase from *Suaeda maritima* plants by betaine (6) and slight activation by glycerol of glucose 6-P dehydrogenase from the unicellular alga, *Dunaliea* (3). However, striking differences from previous studies were observed with sucrose and proline. Sucrose was slightly (17) or strongly (29) inhibitory to leaf malate dehydrogenase.
whereas proline either had no effect (24) or slightly activated (6) some enzymes from halophytes.

The investigation of the protective effects of betaine and other solutes on inhibition of RuBP carboxylase by KCl revealed that all but proline were effective (Fig. 1B). Betaine was most effective, especially at high concentrations followed by sucrose and glyc erol. Proline did not appear to confer any protection but it is interesting to note that the presence of proline, even at high concentrations, did not further decrease the activity of KCl-inhibited RuBP carboxylase (cf. Fig. 1A).

Effect of Betaine Alone and Together with KCl on RuBP Carboxylase Activity in other Cyanobacteria and Photosynthetic Bacteria. The effect of betaine alone and together with KCl on RuBisCO activity was also studied in five other organisms: three cyanobacteria, *Synechococcus* ACMM323, *Plectonemabor yanum*, *Anabaena variabilis* and two photosynthetic bacteria, *Chromatium vinosum* and *Rhodospirillum rubrum*. Figure 2 compares the responses of RuBP carboxylase activities from the various organisms to increasing betaine concentrations in the presence and absence of KCl. In the absence of KCl, the *Synechococcus* enzyme showed no response to betaine, whereas some activation at intermediate betaine concentrations was observed for the remaining enzymes with the exception of the *Chromatium* enzyme, which was inhibited 50% by 1 mM betaine. Inhibition of enzyme activity by 0.25 mM KCl was greatly relieved by increasing betaine concentrations in all cases but *Chromatium*, which again was inhibited by betaine. The protection afforded by betaine appeared to be most effective with the *Aphanathece* enzyme, especially at high betaine concentrations. It is noted that none of the organisms tested in Figure 2, except *A. halophytica*, have been reported to accumulate betaine. Obviously the stimulation of RuBisCO and the protection against KCl inhibition by betaine is not specific.

Influence of Solute Structure on Protection against KCl Inhibition of RuBP Carboxylase Activity from *A. halophytica*. The effect of varying the N-methylation of glycine on the protection against KCl inhibition is shown in Figure 3. Glycine, with no methyl group, was unable to relieve the inhibition by KCl. TMAO was more effective than betaine and sarcosine, and restored the original uninhibited activity at concentrations below 0.25 M. The relative effectiveness of the solute was correlated with the extent of methylation of the solute's nitrogen atom. These data are in agreement with previous studies on protection against urea inhibition of rabbit phosphofructokinase (10) and against KCl inhibition of barley malate dehydrogenase (6).

Effect of Betaine and KCl on Heat and Cold Inactivation of RuBP Carboxylase Activity from *A. halophytica*. RuBisCO was heated at 50°C or cooled at 0°C in the presence and absence of betaine and/or KCl for various times prior to enzyme activity measurement at 25°C. The enzyme lost about half of the original activity after heating for 1 min in the absence of effectors (Fig. 4A). Betaine conferred slight protection against heat inactivation, whereas better protection was afforded by KCl. The best protection was observed when the enzyme was heated in the presence of betaine plus KCl. Similar responses of the enzyme to the effectors were observed during cold inactivation (Fig. 4B). However, betaine and KCl appeared to protect equally the enzyme against cold inactivation, in contrast to the better protective effect of KCl than betaine against heat inactivation.

Effect of Betaine and KCl on Kinetic Properties of RuBP Carboxylase from *A. halophytica*. Apparent Michaelis constants and maximum velocity for both substrates of RuBP carboxylase from *A. halophytica* were determined in the presence and absence of betaine and KCl (Table I). Betaine and KCl increased and decreased $V_{\text{max}}$, respectively, to 1.32 and 0.72 $\mu$mol CO$_2$ fixed $\cdot$ min$^{-1}$·mg$^{-1}$. The reduced $V_{\text{max}}$ caused by KCl was reversed by the further addition of betaine. The $K_m$(CO$_2$) appeared to be unaltered in the presence of betaine or KCl or both. Betaine did not cause a significant change in $K_m$(RuBP). KCl, however, increased the $K_m$(RuBP) about 5-fold and the $K_m$(RuBP) in the presence of KCl was lowered about half by the further addition of betaine.

Protective Effect of Quaternary Ammonium Compounds on the Dissociation of RuBisCO Molecule from *A. halophytica*.

![Fig. 2. Effect of betaine on activity of RuBP carboxylase from six different organisms assayed in the presence and absence of KCl. About 8 to 15 $\mu$g protein of partially purified enzymes (A, C, D, F) and 1 to 2 $\mu$g protein of purified enzymes from *R. rubrum* (E) and *Synechococcus* ACMM 323 (B) were assayed in the presence ( control activity is in the absence of betaine. A, *A. halophytica* (data are derived from Fig. 1); B, *Synechococcus* ACMM 323; C, *P. boryanum*; D, *A. variabilis*; E, *R. rubrum*; F, *C. vinosum.*](www.plantphysiol.org)
Previously we reported that KCl and the substrate RuBP reduced the dissociation of RuBisCO molecule from *A. halophytica* (12). The strong protective effect of betaine against KCl inhibition of RuBP carboxylase (Fig. 1B) prompted us to test the effect of betaine on the dissociation of this enzyme. The partially purified RuBisCO was layered onto sucrose gradients containing various solutes. As shown in Figure 5, A to D, higher enzyme activity was detected around fraction 12 in the gradient containing betaine than that without betaine (*cf.* dark shaded area of Fig. 5, A and B). This higher enzyme activity was attributed to less dissociation of small subunit from the holoenzyme, because when a fixed amount of catalytic core (highly depleted of small subunit) was added to the top fractions (fractions 1–11) the recovered activity was less in the gradient containing betaine than that without betaine (*cf.* light shaded area of Fig. 5, A and B). In this assay, recovered activity was a linear function of the concentration of small subunit (12). The observed shift of enzyme activity peak (fraction 12) from the protein peak (fraction 15) results from the partial dissociation of the small subunit in the sucrose gradient since both catalytic core and small subunit are essential for enzyme activity. It is evident that the protective effect of betaine on the dissociation of the enzyme was not as great as that observed with KCl (Fig. 5C). Interestingly, since the protective effect by KCl was saturated at concentrations higher than 0.3 M (A Incharoensakdi, T Takabe, T Akazawa, unpublished data), the protective effect appeared to be additive when betaine was added together with KCl in the gradient (Fig. 5D). The results may suggest that KCl and betaine reduce the extent of the dissociation of the enzyme by different mechanisms. To examine whether the hydrophobicity of the solute molecule has any effect on the dissociation of the enzyme subunits, solutes having different methyl groups on the nitrogen atom were tested for their effects on the dissociation of the enzyme. Glycine, sarcosine and TMAO all showed similar effectiveness in reducing the extent of the dissociation of RuBisCO molecule (Fig. 5, E–G), judging by the same peak area under the curve around the catalytic core (fraction 12) together with the same amount of dissociated small subunit (light shaded area of Fig. 5, E–G). The effectiveness of the methylamine solutes in reducing the extent of the dissociation of the enzyme did not appear to correlate with the number of methyl groups on the molecule, since glycine was even more effective than betaine (*cf.* Fig. 5, B and E), suggesting the involvement of complicated mechanisms due to the structure of each quaternary ammonium compound.

**DISCUSSION**

The results obtained in this study suggest that betaine acts as a useful osmoticum inside *A. halophytica* cells. The fact that betaine is not inhibitory to RuBisCO activity of *A. halophytica* also points to the characteristic compatibility of this solute. According to Reed *et al.* (18), K⁺ concentrations inside *A. halophytica* are in the range of 180 to 280 mM, depending on the external salinity, whereas the concentration of betaine is between 300 and 1000 mM. Our results, employing 0.25 M KCl in the assay, clearly demonstrate that the inhibitory effect of KCl on RuBP carboxylase can be overcome by betaine and that the higher the concentration of betaine, the greater is the effect observed. The value of protection by betaine against salt inhibition, mostly NaCl, of some enzymes as reported in higher plants (17) has not been critically evaluated when compared with *A. halophytica* because of the evidence that Na⁺ and Cl⁻ ions are compartmentalized in the vacuole (8, 26) and the fact that salt levels in the cytoplasm and chloroplast have not yet been clearly understood.
Table 1. Apparent $K_m$ and $V_{max}$ Values for RuBP Carboxylase from A. halophytica Determined under Various Conditions

The purified enzyme was used and the basic experimental procedures
were as described in the text except that the reaction was stopped after 1
min. For the determination of $K_m$ (CO₂), a fixed 2.5 mM RuBP and
varying NaHCO₃ concentrations (5–50 mM) were employed, whereas for
$K_m$ (RuBP), 50 mM NaHCO₃ and 10 to 400 μM RuBP were employed.
Since activity of the enzyme was sensitive to changes in ionic strength,
the difference caused by varying NaHCO₃ was corrected for by an
equivalent amount of NaCl. This was not necessary for the case of RuBP
due to the low concentrations of RuBP employed. $K_m$ and $V_{max}$ (determined
when NaHCO₃ was the variable substrate) together with SE were
determined by the statistical method of Wilkinson (30). CO₂ concentra-
tions were calculated using a pK value of 6.3 for the CO₂-HCO₃⁻
interconversion.

<table>
<thead>
<tr>
<th>Addition</th>
<th>$K_m$ (CO₂)</th>
<th>$K_m$ (RuBP)</th>
<th>$V_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mm</td>
<td>μM</td>
<td>μmol/min-mg</td>
</tr>
<tr>
<td>None</td>
<td>1.17 ± 0.18</td>
<td>92 ± 13</td>
<td>0.99 ± 0.15</td>
</tr>
<tr>
<td>0.25 M KCl</td>
<td>1.29 ± 0.22</td>
<td>435 ± 55</td>
<td>0.72 ± 0.20</td>
</tr>
<tr>
<td>0.5 M betaine</td>
<td>1.12 ± 0.16</td>
<td>70 ± 12</td>
<td>1.32 ± 0.31</td>
</tr>
<tr>
<td>0.25 M KCl plus 0.5 M betaine</td>
<td>1.32 ± 0.22</td>
<td>195 ± 32</td>
<td>0.96 ± 0.26</td>
</tr>
</tbody>
</table>

determined. Other possible compatible solutes, e.g. proline, glycerol,
and sucrose, which accumulate in other organisms (11) have,
with the exception of proline, somewhat similar effects to
betaine in regard to the protective effect against salt inhibition
of RuBisCO from A. halophytica. However, glycerol and sucrose
were less effective than betaine and this may make them unlikely
osmotica in this halophilic cyanobacterium. In fact, it has been
reported that the carbohydrate content of A. halophytica in
response to salinity changes accounts for only about 1% of the
betaine content (18).

The presence of both betaine and KCl proved to be efficient
in protecting RuBisCO from A. halophytica against heat and
cold inactivation (Fig. 4). This phenomenon may be of some
importance in terms of the adaptive mechanism employed in vivo
by A. halophytica so that conformational changes of the
enzyme caused by extreme heat or cold are reduced, thus
minimizing the activity loss.

The study of the effect of betaine on the kinetic properties and
on the dissociation behavior of RuBisCO was intended to delineate
the molecular interaction of betaine with the enzyme. The results
showed no alteration of the $K_m$ for either substrate (CO₂
and RuBP) which suggests that betaine does not directly bind to
active sites on the enzyme. The observation that KCl increased
the $K_m$ (RuBP) (Table I) is in general agreement with other
enzymes from marine invertebrates as well as from mammal (4, 9).
The reduced $V_{max}$ in the presence of KCl appears to be caused
by an anion (Cl⁻) rather than a cation (K⁺) since the substitution
of CH₃COO⁻ for Cl⁻ caused little or no inhibition of enzyme
activity and that potassium salts with different monovalent anions
(Cl⁻, Br⁻, NO₃⁻) at the same concentration showed different
degrees of inhibition of enzyme activity (data not shown).
Therefore, one of the factors contributing to the inhibition of RuBP
carboxylase by salts is the competition of anions with RuBP.
Since high contents of K⁺ and Na⁺ are present inside A. halo-
phytica (18), comparable anion contents should also be present
to balance the charge difference. High concentration of anions,
especially Cl⁻ (about 100 mM), has been reported to accumulate
in the chloroplasts of the halophyte Suaeda australis (20) and
spinach (Table 1 of Ref. 20).

The two main features of the betaine molecule are its dipole
character at physiological pH values, and the crowding of three
methyl groups at the positively charged end of the molecule.
Betaine may help prevent the dissociation of the subunits (Fig.
5B), by virtue of its zwitterion characteristic, by binding to
charged groups in the large and small subunits of the enzyme.

![Fig. 5. Effect of various solutes on the dissociation of RuBisCO molecule from A. halophytica during sucrose gradient centrifugation. Partially purified RuBisCO derived from about 1 g cell was layered on top of a linear sucrose gradient (0.2–0.8 M, 38 ml) and centrifuged in a Beckman VTi 50 rotor for 2 h at 242,000g and 4°C. The gradient solution was prepared in (A), 50 mM Hepes-NaOH (pH 8.0), 1 mM EDTA, 5 mM DTT; (B), same as (A) plus 0.5 M betaine; (C), same as (A) plus 0.3 M KCl; (D), same as (C) plus 0.5 M betaine; (E), same as (A) plus 0.5 M glycine; (F), same as (A) plus 0.5 M sarcosine; (G), same as (A) plus 0.5 M TMAO. All the gradient solutions were adjusted to pH 7.8 at 25°C. After centrifugation, 1.5 ml fractions were collected and 25 μl aliquots were used for the assay of activity as described in the text (C). Separately, 25 μl aliquots of fraction 14 in (A) were added to aliquots (10 μl) of the upper fractions (fractions 1–11) in experiments (A) to (G) and enzyme activities were assayed to estimate the magnitude of the dissociation of small subunit (G). The values in the figure represent activities after subtracting the residual activity of that without added fraction 14 and that of fraction 14 itself. The refractive index (A) patterns were identical in all seven gradients. The dark shaded peak represents the remaining activity of the RuBisCO molecule which reflects the extent of the dissociation of the enzyme. The lightly shaded peak represents the magnitude of the dissociated small subunit since the activity recovered after supplementation of the catalytic core is proportional to the concen-
tration of small subunit (12).]

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Although the nature of the subunit binding domain has been suggested to be hydrophobic, as exemplified by the effectiveness of salt and high temperature in reducing the extent of the dissociation of the subunits (12), the ease of dissociation of the enzyme molecule bears no relation to the degree of methylation of the compound (Fig. 5). For the protective effects afforded by betaine against KCl inhibition of RuBP carboxylase, the correlation exists between the extent of protection and the degree of methylation (Fig. 3). This might be explained in terms of the energy and volume changes occurring during catalytic conformational changes as suggested by Somero et al. (22). KCl, a salting-in salt, promotes protein-water interaction with a concomitant increase in activation volume accompanied by a reduced maximum velocity. The more hydrophobic group, e.g., the three methyl groups on trimethylamino-N-oxide and betaine, can act at the protein-water surface resulting in a decrease of bound water at the surface, and hence, a decrease in activation energy during catalysis. However, the influence of concentrated salts on proteins and other macromolecules may involve extremely complex physical chemistry among salts, macromolecules, and water.

In conclusion, the overall results indicate that the accumulation of high concentrations of both betaine and KCl in A. halophytica has obvious advantages with respect to RubisCO. KCl is more effective than betaine in keeping the two-subunit structure of the enzyme but the drawback is its inhibition on the enzyme activity. This, however, is offset by betaine, which serves as a compatible solute, thus maintaining the internal osmotic balance of the organism. The combined effect of both betaine and KCl in protecting the enzyme against heat and cold inactivation is another example of the advantage of the simultaneous accumulation of these two solutes.

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LITERATURE CITED

9. Greany GS, GN Somero 1980 Contributions of binding and catalytic rate constants to evolutionary modifications in K_m of NADPH for muscle-type (M_m) lactate dehydrogenase. J Comp Physiol 137: 115–121