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Glycinebetaine stabilizes the association of extrinsic proteins with the photosynthetic oxygen-evolving complex

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The photosynthetic oxygen-evolving activity of the photosystem 2 complex, prepared from spinach, was labile when the complex was exposed to high-salt conditions under which the extrinsic proteins were dissociated from the complex. Glycinebetaine prevented the dissociation of the 18-kDa and the 23-kDa extrinsic proteins from the photosystem 2 complex in the presence of 1 M NaCl. It also prevented the dissociation of the 33-kDa extrinsic protein from the complex in the presence of 1 M $MgCl_2$ or 1 M $CaCl_2$. The oxygen-evolving activity of the photosystem 2 complex was stabilized by glycinebetaine when the complex was subjected to treatment with NaCl and $MgCl_2$.

Glycinebetaine; Oxygen evolution; Photosynthesis; Photosystem 2 complex; Salt effect

1. INTRODUCTION

Thylakoid membranes of chloroplasts contain the oxygen-evolving photosystem 2 (PS2) complex which is composed of a number of intrinsic proteins and 3 extrinsic proteins with relative molecular weights of 18, 23 and 33 kDa [1,2]. In PS2 particles the extrinsic proteins can be dissociated sequentially from the complex in the presence of high concentrations of salts [3–5] or urea [6]. The dissociation of these proteins results in an increase in the requirements for chloride [7] and calcium ions [5,8,9] for the evolution of oxygen, and eventually the release of 2 Mn atoms from the 4-atom Mn cluster of the oxygen-evolving complex occurs [10].

Halophilic higher plants accumulate glycinebetaine (referred to hereafter as betaine) in their chloroplasts [11,12]. We have shown that betaine at 1 M protects the PS2 complex from disruption of its oxygen-evolving activity by NaCl [13]. In the present study, we examined the effects of a wide range of concentrations of betaine in stabilizing the association of all 3 extrinsic proteins with the PS2 complex in the presence of NaCl, $MgCl_2$, $CaCl_2$ and urea.

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2. MATERIALS AND METHODS

PS2 particles, i.e. fragments of thylakoid membrane containing PS2 complexes but practically no other protein components, were prepared from spinach by the method of Kuwabara and Murata [14]. They were highly active in terms of evolution of oxygen (400–500 μmol O_2·mg chlorophyll^{-1}·h^{-1}) and contained, per P680, 220 chlorophyll (Chl) molecules, 4 Mn atoms, and 1 molecule each of the 18-, 23- and 33-kDa extrinsic proteins [15].

The effects of salts, urea and betaine on the dissociation of the extrinsic proteins were examined by incubating PS2 particles for 10 min at 0°C in darkness in the designated medium at a particle concentration that corresponded to 10 μg Chl·ml^{-1}. To determine whether levels of extrinsic proteins remained associated with PS2 particles, the particles were collected by centrifugation at 33 000 × *g* for 10 min. The levels of extrinsic proteins were measured by densitometry of polyacrylamide gel electrophoresis of the proteins in the pelleted PS2 particles, as described previously [13].

Oxygen-evolving activity of the treated PS2 particles was assayed with a Clark-type oxygen electrode after the incubated suspension had been warmed to 25°C. The reaction mixture contained 0.3 mM phenyl-1,4-benzoquinone, 0.3 M sucrose, 0.01 M NaCl and 0.025 M 2-(*N*-morpholino)-ethanesulphonic acid (MES)/NaOH (pH 6.5), and PS2 particles that corresponded to 6–8 μg Chl·ml^{-1}.

3. RESULTS AND DISCUSSION

The incubation of PS2 particles with 1.0 M NaCl resulted in dissociation of almost all of the 18- and 23-kDa proteins, but practically none of the 33-kDa protein, and decreased the oxygen-evolving activity. When 1.0 M betaine was present in the incubation medium, it inhibited the dissociation of the extrinsic proteins from the complex and the impairment of oxygen-evolving ability (Table I). The incubation with 1.0 M $MgCl_2$ completely or almost completely dissociated the 3 extrinsic proteins from the complex, and it totally halted the evolution of oxygen. In the presence of 1.0

Table I

Effects of betaine on the dissociation of the extrinsic proteins and the oxygen-evolving activity of PS2 particles under high-salt conditions

Salt (1.0 M)	Betaine (1.0 M)	Relative amounts of extrinsic proteins that remained associated (%)			Evolution of oxygen (μmol O_2·mg Chl^{-1}·h^{-1})
		18 kDa	23 kDa	33 kDa	
–	–	100	100	100	480 (100)
–	+	100	100	100	520 (110)
NaCl	–	0	5	90	100 (20)
NaCl	+	20	75	97	310 (65)
$MgCl_2$	–	0	0	10	0 (0)
$MgCl_2$	+	0	5	75	210 (45)
$CaCl_2$	–	0	0	5	0 (0)
$CaCl_2$	+	0	5	50	0 (0)

Before the measurement of relative amounts of associated proteins and oxygen-evolving activities, PS2 particles were incubated in media which contained 0.3 M sucrose, 0.01 M NaCl, 0.025 M MES/NaOH (pH 6.5) plus 1.0 M NaCl, 1.0 M $MgCl_2$ or 1.0 M $CaCl_2$ in the presence or absence of 1.0 M betaine. The numbers in parentheses indicate relative oxygen-evolving activities.

M betaine, the dissociation of the 33-kDa protein and the impairment of the evolution of oxygen by $MgCl_2$ were only partial (Table I). These observations suggest that betaine prevents the dissociation from the complex of the 3 extrinsic proteins in the presence of NaCl and $MgCl_2$. As a result, it stabilizes the oxygen-evolving activity.

$CaCl_2$, like $MgCl_2$, dissociated all 3 extrinsic proteins from the complex and halted the evolution of oxygen. Betaine in the incubation medium prevented the dissociation of the 33-kDa protein from the complex, but not that of the other 2 extrinsic proteins. However, the oxygen-evolving activity was not sustained by the presence of betaine. There is no explanation as to why this activity was not sustained by betaine in spite of the stabilization of the association of the complex with the 33-kDa protein.

Fig. 1 shows the effects of various concentrations of betaine on the dissociation of the 3 extrinsic proteins from PS2 particles during incubation with 1.2 M NaCl. The proportion of the 18- and 23-kDa proteins that remained associated with the PS2 particles became larger as the concentration of betaine increased. Over the range of concentrations of betaine tested, the extent of association of the 18-kDa protein was lower than that of the 23-kDa protein. At concentrations of betaine above 2 M, however, the association of all the 3 extrinsic proteins with the PS2 particles was complete even in the presence of 1.2 M NaCl.

Fig. 2 shows the effects of betaine at various concentrations on the dissociation of the 3 extrinsic proteins from the complex by $MgCl_2$. Without betaine, all the 3 extrinsic proteins were dissociated from the PS2 particles. With increase in the concentration of betaine, the association of the 33-kDa protein with PS2 particles became stronger, and above 1.5 M betaine the binding of this protein was fully stabilized. However, the dissociation of the 18- and 23-kDa proteins from the complex by $MgCl_2$ was not affected by betaine. A similar type of

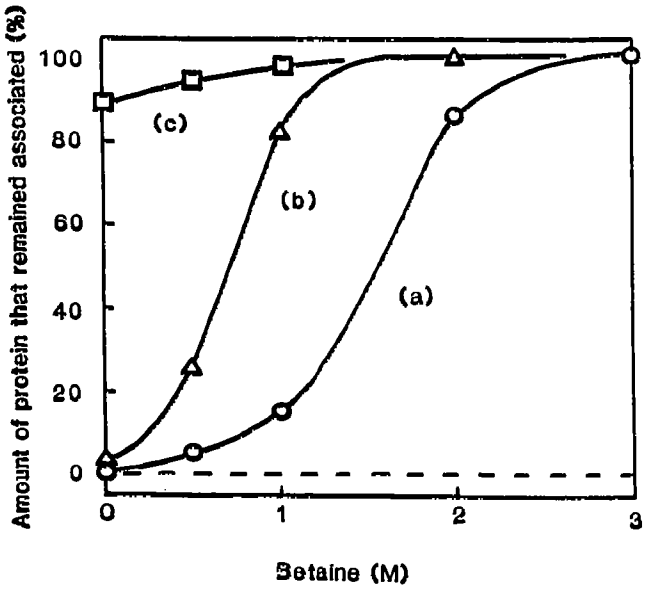

Fig. 1. Effects of betaine on the dissociation of 18- (a), 23- (b) and 33- (c) kDa extrinsic proteins from PS2 particles by NaCl. PS2 particles were incubated in media that contained 1.2 M NaCl, 0.3 M sucrose, 0.025 M MES/NaOH (pH 6.5) plus indicated concentrations of betaine.

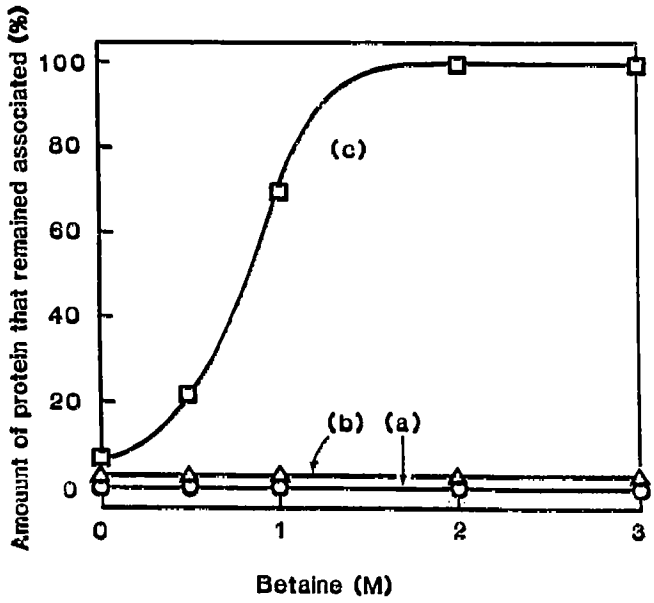

Fig. 2. Effects of betaine on the dissociation of 18- (a), 23- (b) and 33- (c) kDa extrinsic proteins from PS2 particles by $MgCl_2$. PS2 particles were incubated in media that contained 1.0 M $MgCl_2$, 0.3 M sucrose, 0.025 M MES/NaOH (pH 6.5) plus indicated concentrations of betaine.

experiment was conducted with 1.0 M $CaCl_2$ or 3 M urea instead of 1.0 M $MgCl_2$ in the incubation medium; essentially the same results as shown in Fig. 2 were obtained.

Betaine is synthesized and accumulated in chloroplasts of halophilic plants, such as Chenopods, when they are exposed to drought or to high salinity [11,12]. Although it has been clearly demonstrated that NaCl is a stimulus for the biosynthesis of betaine [16], we do not know whether the chlorides of divalent metals, such as $MgCl_2$ and $CaCl_2$, act in a similar manner. Since $MgCl_2$ and $CaCl_2$ are much more chaotropic than NaCl, with a greater ability to intrude into the highly ordered structures of proteins [16], it can be anticipated that $MgCl_2$ and $CaCl_2$ induce the biosynthesis of betaine more efficiently than does NaCl. A further study is being conducted to examine this possibility.

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