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Interaction of methylamine with extrinsic and intrinsic subunits of photosystem II ☆

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The interaction of methylamine with chloroplasts' photosystem II (PSII) was studied in isolated thylakoid membranes. Low concentration of methylamine (mM range) was shown to affect water oxidation and the advancement of the S-states. Modified kinetics of chlorophyll fluorescence rise and thermoluminescence in the presence of methylamine indicated that the electron transfer was affected at both sides of PSII, and in particular the electron transfer between Y_Z and $P680^+$. As the concentration of methylamine was raised above 10 mM, the extrinsic polypeptides associated with the oxygen-evolving complex were lost and energy transfer between PSII antenna complexes and reaction centers was impaired. It was concluded that methylamine is able to affect both extrinsic and intrinsic subunits of PSII even at the lowest concentrations used where the extrinsic polypeptides of the OEC are still associated with the luminal side of the photosystem. As methylamine concentration increases, the extrinsic polypeptides are lost and the interaction with intrinsic domains is amplified resulting in an increased F_0.

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1. Introduction

In bacteria, plants and animals, polyamines are required for cell proliferation and growth. Through their binding to biological molecules, polyamines are considered to take part in the regulation of basic plant physiological processes such as cell division and morphogenesis, as well as responses to environmental and stress conditions [1,2]. Several studies have reported the interaction of polyamines with proteins of photosystem II (PSII[1]) leading to the inhibition of electron transport activity [3–5]. This photosystem is responsible for water oxidation and the consequent oxygen evolution in oxygenic photosynthetic prokaryotes and chloroplasts of higher plants [6]. PSII contains multiple intrinsic and extrinsic subunits. A tetranuclear manganese (Mn) cluster and cofactors such as Ca^{2+} and Cl^- form the oxygen-evolving complex (OEC) together with three extrinsic polypeptides of 17, 23 and 33 kDa [7]. The OEC is associated with the intrinsic proteins D1 and D2, which cross the thylakoid membrane and form the heterodimeric core of PSII that binds the redox-active cofactors involved in electron transfer [8]. Among the pigment–protein complexes in charge of light harvesting, CP47 and CP43 compose the inner light-harvesting complex of PSII. Both subunits have been revealed to interact with the oxygen-evolving site and participate in the stabilization of electron transfer reactions [9].

The photochemical events in PSII are initiated by the capture of incident photons by the antenna complexes. The energy absorbed is quickly transferred to the photochemical reaction centers (RC) where the excited singlet state of the special chlorophyll (Chl) a, P680, reduces a pheophytin (Pheo) molecule. Stabilization of the charge separated state occurs with the electron transfer from $Pheo^-$ to Q_A, the primary plastoquinone of PSII, forming $P680^+Q_A^-$. The $P680^+$ radical oxidizes tyrosine Y_Z (Tyrosine 161 of D1). The latter is re-reduced by electrons originating from the Mn cluster. At this site, water oxidation is performed through the so-called S-state cycle, $S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow (S_4) \rightarrow S_0$, requiring four successive quanta of excitation. Concurrent with the release of dioxygen from water, the S_4-state decays to the S_0-state after the 4th step. At the acceptor side, Q_A^- reduces the secondary plastoquinone, Q_B, in a two step process leading to the formation of plastoquinol (PQH_2).

Polyamines such as spermine and spermidine were shown to strongly interact with the luminal side of PSII causing the release of the three extrinsic polypeptides of 17, 23 and 33 kDa associated with the OEC. The release of the 33 kDa in the presence of polyamines did not affect the content of the Mn cluster but the S-state advancement of the OEC was compromised together with the following electron transfer reactions [5]. Similarly, diamines such as putrescine and cadaverine interacted with PSII proteins (H-bonding) through

Abbreviations: Chl, chlorophyll; FI, chlorophyll fluorescence induction; F_0, basal level of chlorophyll fluorescence; F_m, maximal level of chlorophyll fluorescence; F_v, variable chlorophyll fluorescence; OEC, oxygen-evolving complex; P680, primary electron donor of photosystem II; Pheo, pheophytin; PS, photosystem; PQ, plastoquinone; Q_A and Q_B, primary and secondary quinone acceptors of photosystem II; RC, reaction center; LHC, light-harvesting complex; TL, thermoluminescence; T_m, temperature maximum of thermoluminescence emission; D1 and D2, heterodimeric core of PSII; Y_D, the redox-active tyrosine 160 of D2 protein; Y_Z, the redox-active tyrosine 161 of D1 protein; CP47 and CP43, proximal antenna proteins; MSP, manganese stabilizing protein

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polypeptides C=O groups with no major perturbation of protein secondary structure. This was sufficient to strongly inhibit oxygen evolution and electron transfer was inadequate to reduce the quinone acceptors of PSII thus decreasing the maximum fluorescence yield [4].

The PSII subunits CP47, D1 and D2 contain reactive groups, which covalently bind monoamines such as methylamine and buthylamine [10–12]. Monoamines could represent an interesting simplified model to better understand the action of polyamines. Several monoamines such as Tris, ammonia, methylamine and buthylamine are known to reversibly inhibit water oxidation in PSII and affect the distribution of higher S-states of the OEC [13]. Ammonia induced inhibition involved binding at two sites: the chloride site in a competitive manner and the substrate water binding site at or near the tetranuclear Mn cluster [13,14]. However, it has been reported that amines larger than ammonia do not bind to the Mn cluster directly because of steric reasons [13].

In order to examine how methylamine can interact with extrinsic and intrinsic components of PSII, we have analyzed the interaction of this monoamine with isolated thylakoid membranes. Water oxidation and the advancement of the S-states, the kinetics of Chl fluorescence rise, and the electron transfer at both sides of PSII were affected. We concluded from the experimental data that inhibition of the OEC and the following electron transfer resulted from interaction of methylamine with both extrinsic and intrinsic subunits of the photosystem.

2. Materials and methods

2.1. Materials

Methylamine (40 wt.% solution in water) was purchased from Sigma Chemical Co. (St-Louise, MO) and used as supplied.

2.2. Thylakoid membrane preparation

Thylakoid membranes were isolated from fresh market spinach (*Spinacia oleracea* L.) as described elsewhere [15] and kept in the dark. Chl concentration was calculated following the procedure outlined in Porra et al. [16].

2.3. Isolation of PSII submembrane fractions

PSII submembrane fractions were isolated from thylakoid membranes according to Berthold [17] with minor modifications. After incubation for 90 min in the dark at ice-cold temperature, Triton X-100 was added while gently shaking for 1 min to obtain a final concentration of 2.35% (v/v) and 1 mg Chl ml^{-1}. This solution was incubated 1 min in the dark and centrifuged for 4 min at 600 ×*g*. The supernatants were further centrifuged for 15 min at 35,300 ×*g*. The resulting pellet was suspended in a buffer containing 20 mM Mes–NaOH (pH 6.2), 15 mM NaCl, 10 mM $MgCl_2$, and 400 mM sucrose. The homogenate was centrifuged for 4 min at 4960 ×*g*. The supernatants were centrifuged for 15 min at 35,300 ×*g* and the pellets were suspended in the same buffer. This new homogenate was centrifuged for 15 min at 35,300 ×*g*. Finally the pellet was suspended in the same buffer and the Chl content was determined as described previously [16].

2.4. Polyacrylamide gel electrophoresis

To determine the polypeptides released by methylamine, PSII preparations (100 μg Chl ml^{-1}) were incubated for 1 min at room temperature in the presence of methylamine and harvested immediately by a 5-min centrifugation (12,400 rpm) in an Eppendorf microcentrifuge. The pellets were washed twice in 20 mM Mes–NaOH (pH 6.2) and used for polypeptide analysis. The first supernatants were further centrifuged (12,400 rpm for 5 min) to remove remaining membrane fragments. Tris-alkali extraction of the 17, 23 and 33 kDa polypeptides was carried out following the standard procedure of Nakatani [18]. The supernatants of the methylamine or Tris-alkali treated PSII submembrane fractions were concentrated against sucrose using Spectra/Por Molecularporous membranes (Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA) before analysis by polyacrylamide gel electrophoresis. The latter was performed at room temperature using miniature slab gels (Bio-Rad Laboratories, Hercules, California) containing 13% acrylamide and 6 M urea. The gels were stained with Coomassie brilliant blue and the polypeptide content was analyzed with the Gel-Doc 2000 system (Bio-Rad Laboratories, Hercules, CA, USA).

2.5. Oxygen evolution

Flash-induced oxygen evolution was measured in the thylakoid membranes at 22 ° C using a laboratory built instrument. A complete description of the oxygen electrode system can be found elsewhere [19]. The electrode consists of two compartments separated by a cellophane membrane. The silver anode is filled by an electrolyte buffer containing 400 mM sucrose, 40 mM Hepes–NaOH (pH 7.6), 100 mM KCl, 10 mM NaCl, and 5 mM $MgCl_2$. Thylakoid membranes deposited in the cathode chamber were diluted to 200 μg Chl ml^{-1} in a medium containing 400 mM sucrose, 40 mM Hepes–NaOH (pH 7.6), 10 mM NaCl, 5 mM $MgCl_2$ and the specified concentrations of methylamine in the total volume of 100 μl. After a 3-min incubation, the sample was illuminated by a train of 12 saturating (4J) single turnover flashes (10 μs). The quantitative estimation of photosynthetic oxygen production and the S-state transitions were measured using an analytical solution for the fitting of experimental data as described previously [20].

2.6. Chl fluorescence induction (FI)

FI measurements were performed at room temperature using the Plant Efficiency Analyser (Hanasatech, King' Lynn, Norfolk, UK). The assay medium contained 250 mM sorbitol, 20 mM Tricine KOH (pH 7.8), 10 mM KCl, 10 mM NaCl, 5 mM $MgCl_2$, 25 μg Chl ml^{-1} and the specified concentrations of methylamine (1 min incubation). Samples were excited with saturating red actinic light (655 nm and an intensity of 3000 μmol m^{-2} s^{-1}) provided by light emitting diodes. As the fluorescence signal during the first 40 μs is ascribed to artifacts due to delay in response time of the instrument, these data were not included in the analyses of FI traces. The signal at 40 μs was taken as F_0, the initial fluorescence. Variable fluorescence, F_v (the difference between F_0 and the maximal fluorescence, F_m, in dark adapted samples), was used to calculate the F_v/F_m and F_v/F_0 ratios.

2.7. Thermoluminescence (TL)

TL measurements were carried out with a laboratory built instrument. The description of the design and functional aspects are presented elsewhere [21,22]. Thylakoid membranes were diluted to 200 μg Chl ml^{-1} in a medium containing 20 mM Tricine–NaOH (pH 7.8), 400 mM sucrose, 10 mM KCl, 10 mM NaCl, 5 mM $MgCl_2$ and the specified concentrations of methylamine. About 200 μl of the suspension was added to the sample compartiment (15 mm diameter) positioned just above the Peltier plate and covered with a Hellma 202-OS disc window. The sample chamber was closed with a holder bearing the light guide connected to the photomultiplier. The sequence of pre-incubation periods and flash illumination of thylakoids is presented below. First, samples were incubated for 120 s at 20 °C. Following this step, the temperature was brought down to 2 ° C within 5–8 s and kept for 60 s. This incubation temperature was selected in order to avoid freezing induced damages to the OEC that may give rise to artefacts in thylakoid membranes without

cryoprotectant [22]. An actinic single turn-over saturating white flash of about 1 µs width (setting 10, XE-STC, Walz, Germany) was applied to initiate charge separation in PSII. During the last step, linear warming of samples in total darkness activated the recombination of PSII charge pairs that can be detected by the appearance of emission bands with characteristic temperature optima [21,22].

2.8. Flash-induced fluorescence

Flash-induced Chl fluorescence was measured by a double-modulation fluorometer (Photon Systems Instruments, Brno, Czech Republic). Thylakoid membranes were diluted to 25 µg Chl ml^{-1} in a medium containing 250 mM sorbitol, 20 mM Tricine KOH (pH 7.8), 10 mM KCl, 10 mM NaCl, 5 mM $MgCl_2$ and the specified concentrations of methylamine. After 1 min incubation, an actinic flash of about 50 µs duration was applied and fluorescence measurements were taken each 1 µs during the flash.

3. Results

The influence of methylamine (added as free methylamine) on the OEC of thylakoid membranes isolated from spinach is shown in Fig. 1A. Samples were illuminated by a group of saturating single turn-over flashes. A periodicity of four in the yield of oxygen evolution is observed in relation to the advancement of the S-states (S_n, where $n=0$, 1, 2, 3, 4) of the OEC [23]. Oxygen evolution after each flash strongly declined in the presence of methylamine and the oscillation pattern was also modified. In the presence of up to 10 mM of methylamine, the largest oxygen evolution yield was observed after the third flash. This maximum was progressively shifted toward the fourth flash as the concentration of methylamine was raised (see Fig. 1A, 16 mM). This shift was accompanied by an increase in the percent of misses (zero-step advance) and a decrease in the percent of hits (one-step advance) (Table 1).

Fig. 1B shows that methylamine inhibits oxygen evolution in the mM range and about 50% of the activity was lost with 10 mM. The inhibitory action is maximal within less than 1 min incubation. Similar observations could be made with PSII submembrane fractions using a Clark-type electrode (result not shown). Inhibition of oxygen evolution by methylamine was previously explained by its interaction with the OEC [11]. In order to establish the site of action of methylamine more precisely, various techniques were used as described below.

Polyacrylamide gel electrophoresis was used to clarify the interaction of methylamine with the extrinsic polypeptides associated with the OEC (Fig. 1C). PSII submembrane fractions were incubated for 1 min with methylamine and then centrifuged to separate the submembrane fractions from free polypeptides. In lane 5 (Fig. 1C) it is shown that incubation for 1 min of the PSII submembrane fractions in the presence of 20 mM methylamine, three polypeptides were depleted at the position that corresponded to the extrinsic polypeptides of 17, 23 and 33 kDa associated with the OEC as compared to the control (lane 2). These polypeptides were recovered in the supernatant of the treated samples (lane 6). This set of polypeptides coincided with the polypeptides released by the incubation of PSII submembrane fractions for 1 min with Tris-alkali (pH 9.2) (lane 7), a treatment known to release the three extrinsic polypeptides of the OEC. At 10 mM, no polypeptide was removed significantly (see lanes 3 and 4). The methylamine concentration that caused polypeptide depletion corresponded to the almost complete inhibition of oxygen evolution observed in thylakoid membranes (Fig. 1B) or in PSII submembrane fractions using a Clark-type electrode (results not shown).

The action of methylamine on Chl fluorescence properties of thylakoid membranes was studied. The results are shown in Fig. 2. The initial fluorescence level F_0 observed with the reaction center (RC) in an open state was strongly increased with methylamine (Fig. 2A). The

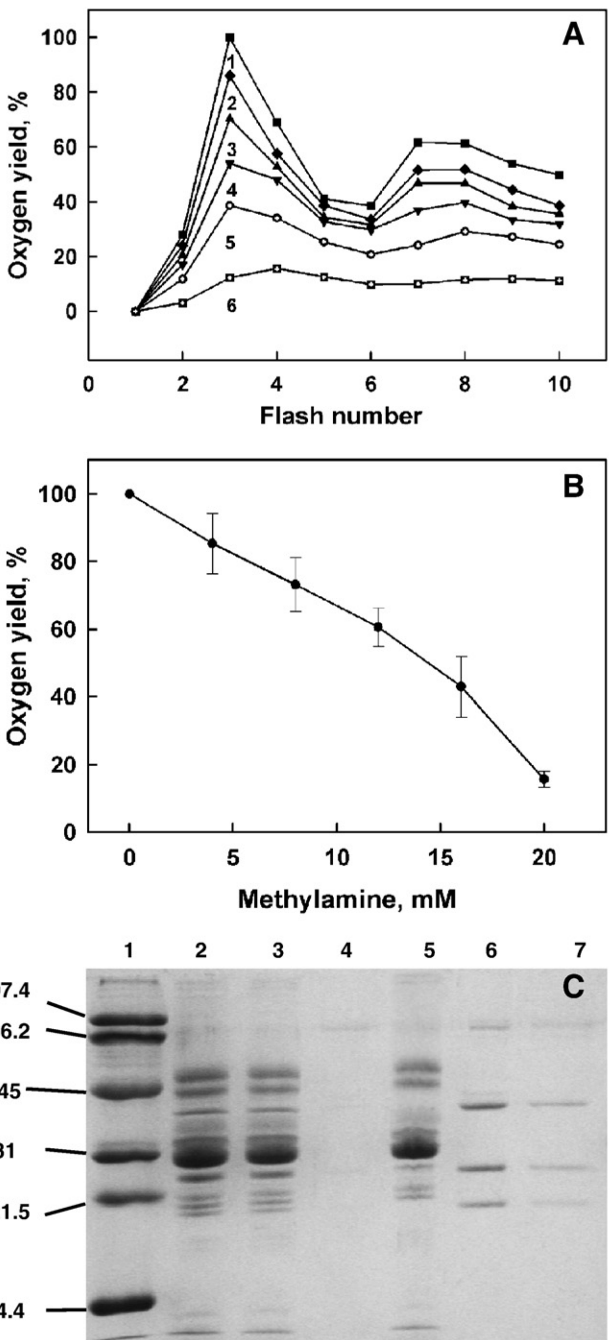

Fig. 1. (A) Period-four oscillation of the yield of oxygen evolution in thylakoid membranes using a train of 12 saturating (4J) single turn-over flashes (10 µs) after a 3-min incubation with various concentrations of methylamine: (1) control; (2) 4 mM; (3) 8 mM; (4) 12 mM; (5) 16 mM; (6) 20 mM. All traces were normalized at the third flash of the control. Assays were carried out in media containing 400 mM sucrose, 40 mM Hepes–NaOH (pH 7.6), 10 mM NaCl, and 5 mM $MgCl_2$. Details are given in Materials and methods. (B) Inhibition of oxygen evolution in thylakoid membranes after a 3-min incubation with various concentrations of methylamine. Each point represents the total yield of the first four flashes from the experiment of panel A given as the percent of oxygen evolution of the untreated sample (100%). (C) Depletion of extrinsic polypeptides in PSII submembrane fractions after a 1-min incubation in the presence of methylamine. Lane 1, molecular weight standards; lane 2, control PSII; lane 3, 10 mM methylamine-treated PSII; lane 4, supernatant of 10 mM methylamine-treated PSII; lane 5, 20 mM methylamine-treated PSII; lane 6, supernatant of 20 mM methylamine-treated PSII; lane 7, supernatant of the Tris-alkali-treated PSII. Numbers on the left indicate apparent molecular masses (kDa) of the markers.

Table 1
Values of parameters determined from the oxygen yields induced by a train of single turn-over flashes in the presence of various concentrations of methylamine.

Parameters	Methylamine, mM					
	0	4	8	12	16	20
Misses, %	18	14	12	20	25	40
Hits, %	74	77	77	71	69	59
Double-hits, %	1.6	2.1	3	2.8	3.6	1

Remaining fraction is due to inactivations and backward-transitions.

increase in F_0 was mainly observed above 15 mM of methylamine, which coincided with a decrease in the maximal PSII photochemical yield, F_v/F_m, where $F_v = F_m - F_0$, (Fig. 2B). The maximal fluorescence F_m observed with the RC in closed state, was also greatly decreased (data not shown). The decline in F_m corresponded with a decrease in F_v/F_0 observed above 5 mM (Fig. 2C), a parameter that accounts for

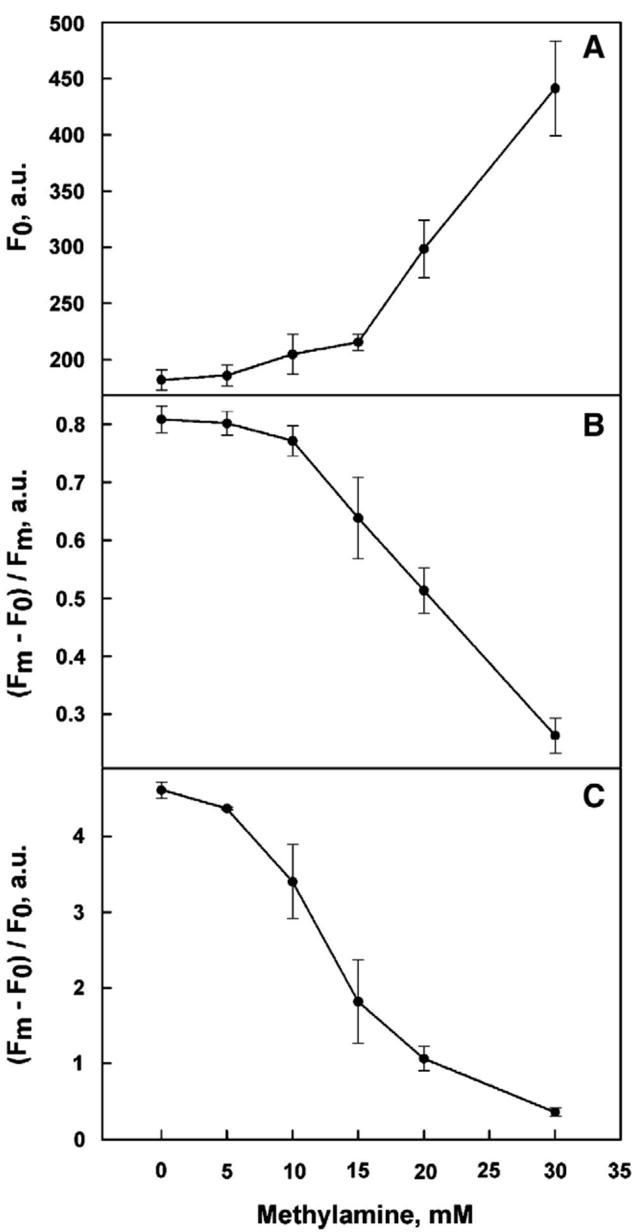

Fig. 2. Effect of increasing methylamine concentration in thylakoid membranes on the Chl fluorescence parameters (A) F_0, (B) F_v/F_m, (C) F_v/F_0. Each point is the average of 3 independent experiments (6–9 assays per point).

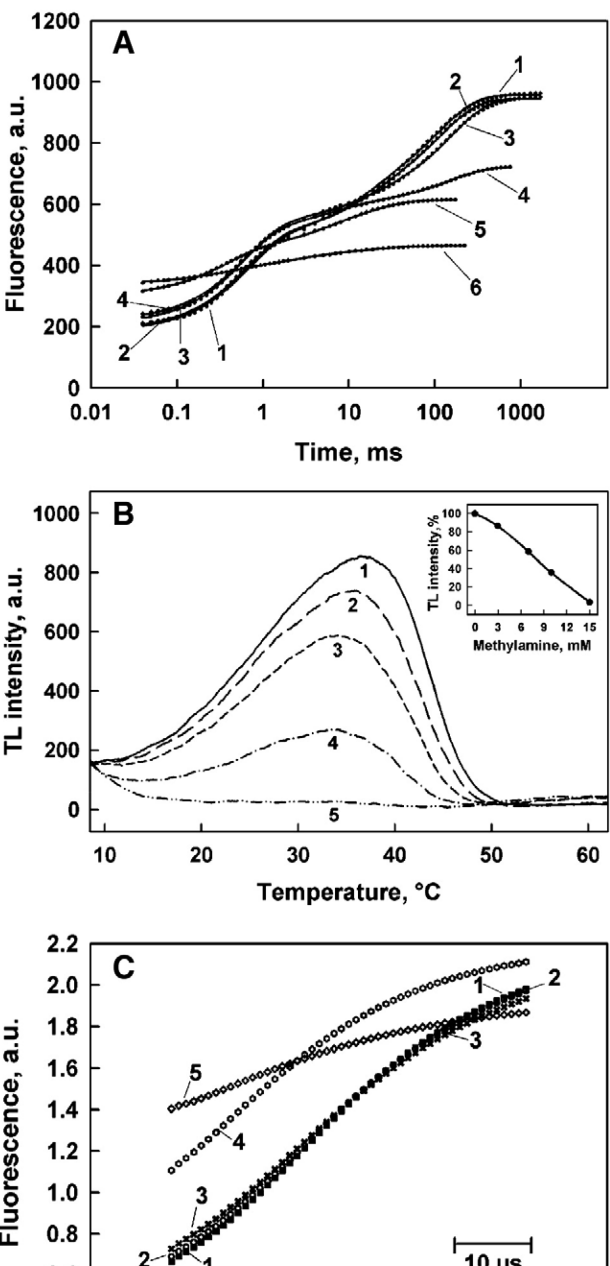

Fig. 3. (A) Chl fluorescence induction traces of thylakoid membranes treated with various concentrations of methylamine: (1) control; (2) 5 mM; (3) 10 mM; (4) 15 mM; (5) 20 mM; (6) 30 mM. (B) Thermoluminescence glow curves (B-band) from thylakoid membranes after treatment with different concentrations of methylamine: (1) control; (2) 3 mM; (3) 7 mM; (4) 10 mM; (5) 15 mM. The samples were heated from 2 °C to 62 °C at a rate of 0.5 °C/s. Inset: Relative intensity of the B-band at various methylamine concentrations. (C) Chl fluorescence induction provided by a single saturating flash in the presence of methylamine: (1) control; (2) 5 mM; (3) 10 mM; (4) 20 mM; (5) 30 mM. The arrow indicates the onset of the flash. Fluorescence measurements were taken each 1 µs during the flash (50 µs). The points are the average of 3 independent experiments (9 assays per point).

the simultaneous variations in F_m and F_0 in determinations of the maximum quantum yield of PSII [24,25]. Thus, the drop of F_v/F_0 observed above 5 mM methylamine and of F_v/F_m above 15 mM was correlated, respectively, with the pattern of inhibition of oxygen evolution and the depletion of the three extrinsic polypeptides of the OEC (Fig. 1B and C).

In order to have more information on FI properties of methylamine-treated samples, we analyzed the O–J–I–P induction traces. These traces display the progressive reduction of the plastoquinones located at the acceptor side of PSII with three main phases corresponding to OJ, JI and IP [26–28] (Fig. 3A). Fig. 3A shows the progressive damping of FI upon treatment with methylamine suggesting that the OEC failed to provide electrons for PSII to reduce the quinone acceptors. Addition of calcium chloride ($CaCl_2$, up to 10 mM) or the artificial electron donor diphenylcarbazide (2–5 mM) could not restore the kinetics of Chl fluorescence in methylamine-treated samples (data not shown), despite the loss of the extrinsic polypeptides associated with the OEC and the dysfunctional Mn cluster.

Thermoluminescence was used to determine the effect of methylamine on charge recombination processes in PSII. Fig. 3B shows the TL glow curves supplied with a linear increase in temperature from 2 °C to 62 °C at the rate of 0.5 °C/s following a 1-µs single turn-over white flash. The amplitude of TL signal attains its maximum (T_m) at 37 °C in control thylakoid samples (Fig. 3B trace 1). This major TL emission band corresponds to the temperature optimum of the B-band (30 °C–40 °C) attributable to charge recombination between the oxidized Mn cluster predominantly in the S_2 state and Q_B^- [29]. This band was progressively decreased as the concentration of methylamine was raised (Fig. 3B traces 2–4). About 50% of the TL intensity was abolished with 7–10 mM methylamine (see inset of Fig. 3B). Above 15 mM methylamine the B-band was totally suppressed (Fig. 3B trace 5). The effect of methylamine on the intensity of the B-band was also accompanied by a small shift of the T_m toward lower temperatures that reached 2.5 °C at 10 mM methylamine. Probably, the strong inhibitory effect of methylamine on charge recombination was due to the loss of the donor side partners for the radiative recombination pathways.

Fig. 3C shows the flash-induced Chl fluorescence measured at a 1-µs time resolution simultaneously with the duration of an actinic saturating flash of about 50 µs. In this experiment, the electron transfer between Q_A^- and Q_B^- is not observed because the time needed for this transfer is greater than 50 µs [30]. The shape of this fluorescence rise contains information concerning the fluorescence when RCs are opened (F_0) and the variable fluorescence during the flash (F_v 50 µs) pertaining to electron transport between P680 and Q_A. These traces display the progressive reduction of Q_A, which was slowed as the concentration of methylamine increased (Fig. 3C, traces 1–5). Indeed, with 20 mM methylamine F_v 50 µs was diminished by approximately 25%. In parallel with this loss, F_0 was increased by nearly 70% compared to control (Fig. 3C traces 4). At 30 mM methylamine, F_0 was augmented by about 100% compared to control while F_v 50 µs was completely abolished (Fig. 3C traces 5).

4. Discussion

In the present study, we have shown that methylamine is able to affect PSII activity in the mM range of concentration and within only a minute of incubation. Indeed, below 10 mM, up to 50% of the oxygen yield was lost. At these methylamine concentrations, no significant perturbation in the advancement of the S-states of the Mn cluster was observed in the OECs that were still unaffected by the inhibitor (Fig. 1A and B). Similar results were obtained with several anions or Lewis bases [31,32]. This inhibitory effect is explained by the interaction between methylamine and the OEC at or near to the Mn cluster. The loss of oxygen yield was accompanied by the inhibition of electron transfer to the acceptor side that was reflected in a decreased F_v/F_0 ratio. Hence, the incapacity of PSII to reduce adequately the plastoquinone pool resulted in seriously damped fluorescence induction kinetics (Fig. 3A).

In the early work of Sandusky and Yocum [13,14], it was concluded that methylamine competed with chloride for the binding site but was not directly ligated to the Mn cluster. It was proposed that this site was accessible only to ammonia. In addition, a previous study had shown that bulkier amines such as Tris, AEPD, and methylamine did not affect the S_2 state multiline EPR signal [33]. In contrast, another report [34] suggested that methylamine competed with substrate water and did not bind to the chloride binding site due to steric effects. Our results indicate that the action of methylamine is more deleterious than simple binding to the chloride site because the addition of calcium chloride had no effect on the degree of inhibition by methylamine.

A recent FTIR study showed that small amines (including ammonia and methylamine) can modify the structural and electronic properties of the Mn cluster [35]. Accordingly, TL measurements revealed a progressive loss of the B-band emission after incubation with raising concentrations of methylamine (Fig. 3B). This decrease is explained by the strong disruption of the back-flow of electrons from Q_B^- to the S_2/S_3 states of the Mn cluster. In parallel, a shift of T_m by about 2.5 °C towards lower temperatures was observed. This shift is opposite to the shift observed in the presence of polyamines [5]. The upshift observed with polyamines was proposed to be due to partial disorganization of the Mn_4Ca complex and stabilization of the S_2 state during an intermediate step of this disorganization [5]. In the present case, such an intermediate step is probably not observed as methylamine may interact further with the donor side of PSII (see below). A decline in T_m in the presence of inhibitors, such as observed here, was previously interpreted by a faster inhibition of the PSII centers having a greater activation energy requirement for charge recombination and thus a higher T_m [22]. This is likely to reflect the heterogeneity in the populations of PSII centers (e.g. spatially segregated in stromal and granal membranes or Q_B non-reducing centers) and their differential sensitivity toward inhibitory effects [36].

During our experiments we have observed that the addition of 5 mM diphenylcarbazide did not restore the slower electron transfer from Y_Z to the PQ pool inhibited by methylamine. Diphenylcarbazide is an efficient electron donor to Y_Z when the OEC is perturbed [37] and 1 mM diphenylcarbazide could indeed restore significantly the photoreduction of the plastoquinone pool in PSII affected by polyamines such as spermine, spermidine [5] or by diamines [4]. The inefficient electron donation by diphenylcarbazide observed in the present study suggests that methylamine exerts a deeper effect in the OEC compared to polyamines. As the inhibition by methylamine can be overcome by neither calcium chloride nor diphenylcarbazide, methylamine is likely to interact either directly with Y_Z (Tyr 161 of D1) or with amino acids located nearby thus causing a dysfunctional electron transfer from Y_Z to $P680^+$. Specific conformational characteristics are required for the active electron transfer between Y_Z to $P680^+$ as the photooxidation of Y_Z requires the simultaneous proton exchange with His^{190} of D1 [38,39]. Perturbation of the OEC, even milder than that observed with methylamine, was shown to disturb this electron transfer reaction [32]. Also, the oxidation of Y_Z is pH-dependent and much slower in Mn-depleted PSII [40,41]. Thus, the binding of methylamine with amino acids at or near the OEC not only affects oxygen evolution but also the direct reduction of $P680^+$.

At 20 mM, methylamine almost totally suppressed the oxygen yield (Fig. 1A and B). This inhibition was correlated with the release of the three extrinsic polypeptides of 17, 23 and 33 kDa associated with the OEC (Fig. 1C). The polyamines spermine and spermidine, and several cations such as Ni^{2+}, Hg^{2+}, Cu^{2+}, Zn^{2+} and Pb^{2+} were also shown to release the extrinsic polypeptides of PSII to various extents [5,32,42,43]. Removal of the two extrinsic polypeptides of 17, 23 kDa decreases the binding affinity of Ca^{2+} and Cl^- for the OEC [44]. These cofactors are essential to maintain the active conformation of the OEC preserving the proper advancement of the S-states [13,45,46]. The other extrinsic polypeptide of 33 kDa is known as the manganese stabilizing protein (MSP). In its absence, two or four Mn ions are released and the oxygen-evolving activity is abolished [47]. At this concentration of methylamine, we have observed an almost complete loss of the electron transfer from donor side to acceptor side of PSII.

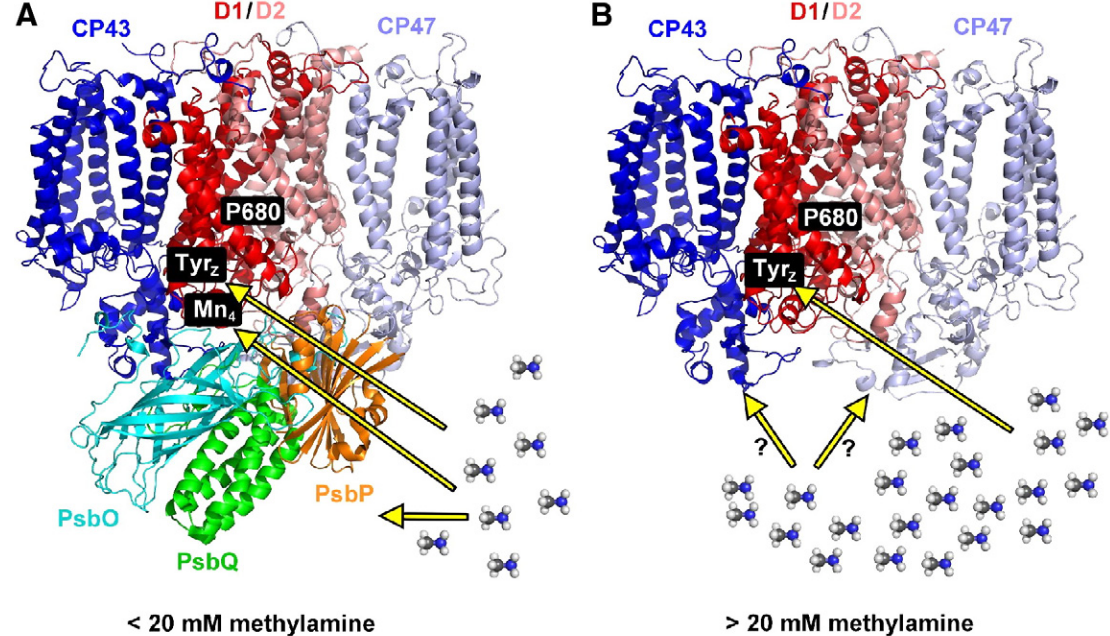

Fig. 4. Proposed model of interaction of methylamine with PSII (A) at a low concentration (<20 mM) and (B) at a high concentration of methylamine (>20 mM). Arrows indicate the sites of interaction of methylamine. At low concentration, methylamine interacts with extrinsic proteins of the OEC, the Mn_4 cluster, and Tyr_Z. At high concentration, extrinsic proteins of the OEC are removed. Methylamine interacts with Tyr_Z and possibly with the hydrophilic loop of CP43 and/or CP47 on the luminal side of PSII. D1 (red), D2 (pink), CP43 (blue), CP47 (light blue), and PsbO (cyan) subunit structures from *Thermosynechococcus elongatus* are drawn from coordinates obtained from Guskov et al. [52] (PDB entry: 3BZ1). PsbP (orange) and PsbQ (green) subunit structures from *Spinacea oleracea* and *Nicotiana tabacum* obtained from Ifuku et al. [53] (PDB entry: 1V2B) and Balsera et al. [54] (PDB entry: 1VYK), respectively, were placed to their approximate position using UCSF Chimera software [55].

This was shown by the drastic decrease in F_v/F_m ratio, damped FI and the totally suppressed TL emission (Figs. 2B, 3A and B). The total dysfunction of the OEC above 20 mM methylamine corresponds with the loss of Q_A reduction by the primary electrons observed during the 50 µs flash-induced fluorescence induction (Fig. 3C). This loss is attributable to the formation of $P680^+$, which is known to occur with the inhibition of electron transfer from Y_Z to $P680^+$ [48] and provides for the fast charge recombination. PSII centers with $P680^+$ are thus characterized by a fluorescence yield close to F_0 [49].

Interestingly, after incubation with 20 mM methylamine, a strong raise of F_0 (nearly 70%) was observed compared to the control (Fig. 3A). The increase of F_0 was further confirmed by experiments using flash-induced Chl fluorescence at a 1-µs resolution (Fig. 3C), which was previously used to provide information regarding the heterogeneity of light harvesting in PSII, with α-centers having large and interconnected antenna systems and β-centers having smaller isolated antenna [50,51]. In our study, the raise of F_0 is suggested to originate from a decreased energy transfer from the antenna complexes to the reaction centers. This idea is supported by an increase in the percent of misses and a decrease in the percent of hits calculated from the yield of flash-induced oxygen evolution (see Table 1, Fig. 1A) that were observed in parallel with the rise of F_0. We propose that, owing to the depletion of extrinsic polypeptides, the interaction between methylamine and the amino acids of the large hydrophilic loops of the proximal antenna protein of CP47 and/or CP43 produces a conformational change perturbing the transfer of excitation energy from these complexes to the reaction centers (see Fig. 4).

In conclusion, methylamine at low concentration (mM range) was shown to affect oxygen evolution and inhibit electron transfer between Y_Z and $P680^+$ (Fig. 4). As the concentration of methylamine was raised, the extrinsic polypeptides of the OEC were lost and energy transfer between PSII antenna complexes and RCs was impaired. These actions indicate that methylamine is able to affect both extrinsic and intrinsic subunits of PSII even at the lowest concentrations used where the extrinsic polypeptides of the OEC are still associated with the luminal side of the photosystem (Fig. 4A). The extrinsic polypeptides are depleted at higher methylamine concentration. This facilitates the interaction with the proximal antenna resulting in an increased F_0 (Fig. 4B).

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