The effects of spermine and spermidine on the structure of photosystem II proteins in relation to inhibition of electron transport

A. Bograh, Y. Gingras, H.A. Tajmir-Riahi, R. Carpentier*

Groupe de Recherche en Énergie et Information Biomoléculaires, Université du Québec at Trois-Rivières, C.P. 500, Trois-Rivières, Qué. G9A 5H7, Canada

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Abstract Polyamines (PAs) are ubiquitous in cells of higher plants and play an important role in many biological functions. Because PAs affect photosynthetic oxygen evolution, this study is designed to investigate the interaction of spermine (Spm) and spermidine (Spd) cations with proteins of photosystem II (PSII) using PSII-enriched submembranes fraction with polyamine concentrations of 0.01–10 mM. Fourier transform infrared (FTIR) difference spectroscopy with its self-deconvolution and second derivative resolution enhancement as well as curve-fitting procedures was applied, in order to determine the cation binding mode, the protein conformational changes and the structural properties of cation-protein complexes. It is shown that at low polyamine concentration, cation-protein interaction (H-bonding) is through the polypeptide C = O groups with no major perturbation of the protein secondary structure. As cation concentration increases, the polyamine complexation causes significant alterations of the protein secondary structure with a decrease of the α-helical domains from 47% (uncomplexed PSII) up to 37% (cation complexes) and an increase in the β-sheet structure from 18% (uncomplexed PSII) up to 29% (cation complexes). Correlations between the effects of polyamines on protein secondary structure and on the rate of oxygen evolution in PSII are also established.

Key words: Polyamine; Photosystem II; Protein; Oxygen evolution; Secondary structure; FTIR spectroscopy

1. Introduction

The polyamines (PAs) are organic cations, products of the decarboxylation of basic amino acids. Putrescine, spermine (Spm), and spermidine (Spd) are widely distributed in bacteria, plants, and animals [1–3]. They occur in three different forms: (a) in the free form as cations, (b) conjugated with small molecules such as phenolic acids, and (c) bound to various macromolecules such as proteins and nucleic acids. Their mechanism of action is based on their chemical specificity as polycations and is possibly connected to their chemical and physical interactions with the nucleic acids, proteins, and phospholipids [1].

The binding of these polyamines to biological molecules is considered to constitute a mechanism of regulation of basic processes in plants such as cell division, morphogenesis, stability and responses to environmental and stress conditions [4]. Although some of their effects are similar to those of the inorganic cations Mg^{2+} and Ca^{2+}, they cannot be replaced by them [5]. At very low concentration, they show a positive effect on photosynthetic activity and oxygen evolution, while at higher concentration, they reduce the rate of oxygen production [6].

In recent years, several studies have been concentrated on the effects of polyamines on the functional activity of thylakoid membranes including cell cycle, chlorophyll degradation, and the rate of photosynthetic oxygen evolution [6–10]. While these investigations were mainly concerned with the effects of polyamines on the functional activity of thylakoid membranes in general, there has been no report on the exact cation binding mode and on the effects of polyamine interaction on the structural point of view. We have previously used FTIR spectroscopy with its self-deconvolution and derivative methods to determine the cation binding mode and the protein secondary structure of photosystem II (PSII) and extrinsic polypeptides [11–14]. In the present study the above approach is used to study the interaction of polyamines with PSII submembrane fractions and to determine the polyamine binding mode (with membrane proteins and lipids) and the effects of polycation complexation on the protein secondary structure. The correlation between the effects of polyamine interaction on the protein structural changes and on the photosynthetic activity of PSII is examined.

2. Materials and methods

The isolation of PSII-enriched submembrane fractions from fresh spinach was carried out by the standard method with minor modifications [15]. Fresh leaves are homogenized in a medium containing 50 mM Tricine-NaOH (pH 7.6), 10 mM NaCl, 5 mM $MgCl_2$, 0.4 M sorbitol, 6 mM ascorbate, and 1 mM PMSF (phenylmethylsulfonyl fluoride). The homogenate is filtered through 12 layers of cheesecloth and the filtrate is centrifuged for 5 min at $2000 \times g$. The pellet is suspended in the same buffer but without sorbitol and PMSF and then recentrifuged under the same conditions. The resulting pellet is resuspended in a buffer containing 20 mM MES-NaOH (pH 6.5), 1.5 mM NaCl, 10 mM $MgCl_2$, and 4% Triton X-100 with a chlorophyll (Chl) concentration of 1 mg/ml. After incubation for 20 min in the dark at ice-cold temperature with constant stirring, the mixture is centrifuged for 10 min at $3600 \times g$. The PSII submembrane fractions are collected from the supernatant by centrifugation for 30 min at $3600 \times g$ and resuspended in the same buffer (without Triton X-100) at a Chl concentration of 2 mg/ml. The Chl content is determined as reported [16].

The rate of oxygen evolution in the PSII submembrane fractions is measured at 22°C, using a Clark-type electrode [17]. Continuous saturating white light is employed to illuminate the samples. The medium contains 20 mM MES-NaOH (pH 6.5), 0.35 mM DCBQ (2,5-dichlorobenzoquinone) as PSII electron acceptor, 15 μg Chl/ml and the mentioned additives. In the absence of additive, the oxygen evolution rate (100%) is between 350 and 400 μmol O_2/mg Chl·h.

Spermine and spermidine were obtained from Sigma Chem. Co. and used as supplied. Polyamine-PSII complexes are prepared by the addition of an appropriate amount of spermine or spermidine

*Corresponding author. Fax: (1) (819) 376-5057.

Abbreviations: PSII, photosystem II; PAs, polyamines; Spm, spermine; Spd, spermidine; Chl, chlorophyll; PMSF, phenylmethylsulfonyl fluoride; DCBQ, 2,5-dichlorobenzoquinone; FTIR, Fourier transform infrared

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chloride salt solution to the PSII preparation to obtain the desired concentrations of 0.01, 0.1, 1, 5, and 10 mM polycations. 100 μl of each cation-PSII complex solution is then transferred onto an AgBr window and air dried at room temperature under green light to form a uniform (hydrated) film.

The infrared spectra are recorded on a BOMEM DA3-0.02 Fourier transform infrared spectrometer equipped with a liquid nitrogen cooled HgCdTe detector and a KBr beam splitter. The spectra are taken with a resolution of 2 cm^{-1}. The difference spectra [(PSII+polyamine)−(PSII)] are generated, using protein C-H stretching bands centered at 2900–2800 cm^{-1} as internal and the Triton band at 1285 cm^{-1} as external references. These vibrations exhibited no spectral changes (shifting or intensity variations) on cation complex formation and they are cancelled upon spectral subtraction. The accuracy of this method of subtraction was tested using several control samples of the same PSII and cation concentrations, which resulted in a similar flat baseline formation. However, a similar absorption scale is used for the spectra of free PSII and their cation complexes, as well as for the difference spectra produced here. This allows us to obtain a meaningful spectral subtraction. Such generated spectral differences are used in order to analyze the nature of cation-protein interaction, with regard to different binding modes (such as protein C=O and C-N) as well as cation-lipid and cation-tyrosine complexation. The detailed spectral manipulations and data treatments were given in our recent publication [12]. The spectra presented here are not smoothed.

The determination of the protein secondary structure was carried out as reported [18,19,21,22]. The secondary structure of PSII proteins was determined from the shape of the amide I band at 1657 cm^{-1}. Fourier self-deconvolution and second derivative resolution enhancement and curve-fitting procedures were applied to determine quantitatively the protein conformation in both the uncomplexed PSII and their polyamine complexes. The detailed spectral treatments and calculations of protein secondary structure were reported in our recent publication [11] and will not be described here. The spectral manipulations were performed with Spectra Calc program (Galactic Industries Co., Salem, NH).

3. Results and discussion

The infrared spectrum of the uncomplexed (untreated) PSII submembrane fractions shows a strong and broad band at 3298 cm^{-1}, which is assigned to the polypeptide amide A (N-H stretching vibrations) [20]. The symmetric and antisymmetric C-H stretchings [20] of the peptide groups are observed as three sharp absorption bands at 2955, 2926 and 2871 cm^{-1}, in the spectrum of PSII submembrane fractions (spectrum not shown). The protein amide I band (polypeptide C=O stretchings [20]) is observed as a strong band at 1657 cm^{-1}, while a band with medium intensity at 1545 cm^{-1} of the PSII preparations is attributed to the amide II (C-N stretching and N-H bending modes) vibrations (Fig. 1). A weak absorption frequency at 1515 cm^{-1} of the untreated PSII is assigned to the tyrosine amino acid side-chain vibrations [23–29]. The lipid ester carbonyl stretching vibration [30] is also observed as a band with medium intensity at 1736 cm^{-1} (Fig. 1). Polyamine-PSII interaction resulted in no major shiftings of the amide I, amide II, tyrosine and lipid vibrational modes (1740–1500 cm^{-1}). Hence, the difference spectra [(PSII+polyamine)−(PSII)] are produced, in order to measure the intensity variations of these vibrational frequencies in the presence of polyamine cations.

3.1. Polyamine-PSII interaction

At low concentration of spermine and spermidine (0.01 mM), a weak positive feature was observed at 1655 cm^{-1}, in the difference spectra of polyamine-PSII complexes, which is attributed to an increase in the intensity of the amide I band at 1657 cm^{-1} as a result of cation interaction (H-bonding) with the protein C=O groups (Fig. 1, 0.01 mM). As polyamine concentration increased to 0.1 mM, the positive feature at 1656 cm^{-1} gained intensity in the difference spectrum of the spermidine-PSII complex, while two weak negative peaks were emerged at 1659 and 1548 cm^{-1} in the difference spectra of the spermine complexes (Fig. 1, 0.1 mM). The increase in the intensity of the amide I band in the spermidine spectrum is due to a major polyamine interaction with the polypeptide C=O groups, while the loss of the intensity of the amide I and amide II bands in the spectrum of the spermine complex are due to protein secondary structural changes upon spermine complexation.

The perturbation of the protein conformation by spermine complexation at low cation concentration is consistent with a major inhibition of oxygen evolution (74%) for spermine in comparison with the minor inhibition (6%) induced by spermidine with the same cation concentration (Table 1, 0.1 mM). This observation is also consistent with the larger induction kinetics of delayed fluorescence of thylakoid membranes observed for spermine with respect to spermidine with a similar

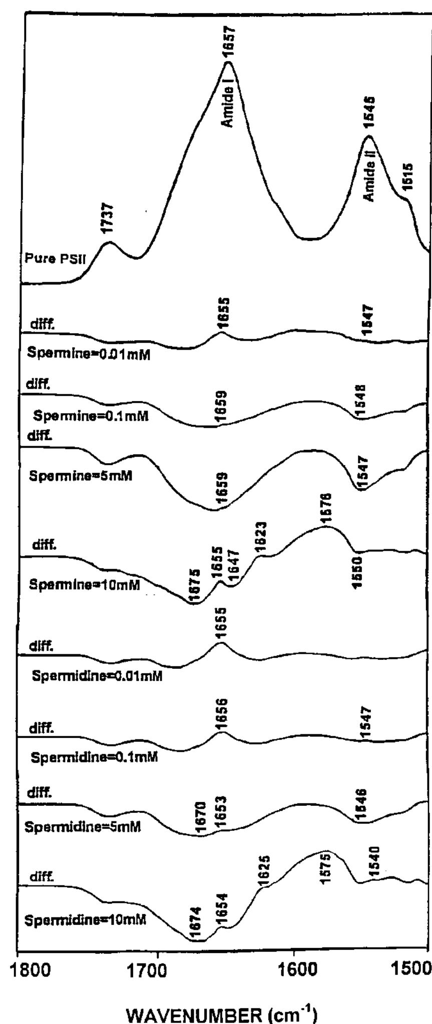

Fig. 1. FTIR spectra (top curve) and difference spectra [(PSII+polyamine)−(PSII)] (bottom eight curves) of uncomplexed PSII and its spermine and spermidine complexes in the region of 1800–1500 cm^{-1} with different polyamine concentrations.

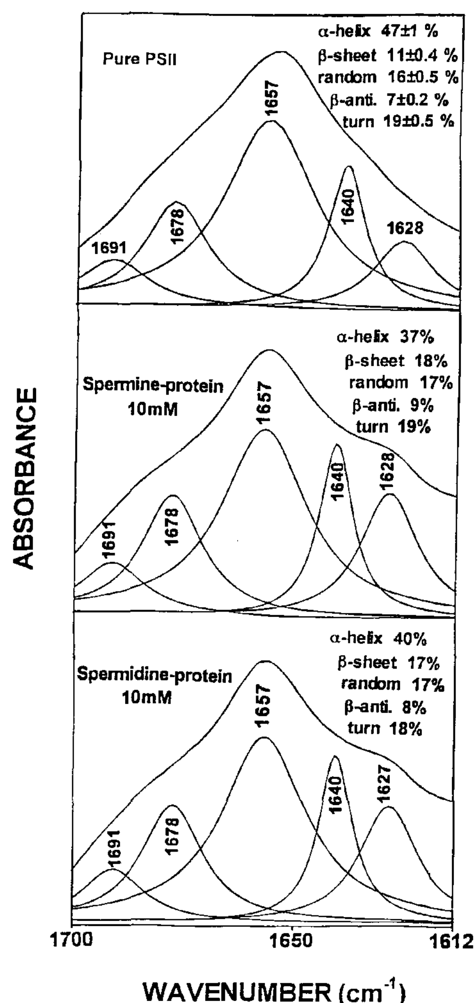

Fig. 2. Curve-fitted amide I band region (1700–1600 cm^{-1}) and secondary structure determination of the band in uncomplexed PSII and in its spermine and spermidine complexes with the cation concentration of 10 mM.

cation concentration [7]. This can be attributed to the greater positive charge distributed on the spermine cation (4+) in comparison with spermidine (3+). The greater positive charge induces stronger electrostatic interaction with protein donor groups that results in local perturbations of protein structure.

However, at higher cation concentrations (5 and 10 mM), major alterations of the protein conformation occur in the presence of both spermine and spermidine cations. Evidence for this comes from the presence of broad and strong negative derivative features at 1675–1647 cm^{-1}, for the amide I band, in the difference spectra of the spermine- and spermidine-PSII complexes (Fig. 1, 5 and 10 mM). The negative features are due to the reduction of the intensity of the amide I band at 1657 cm^{-1} as a result of major changes of the protein secondary structure in the presence of polyamines at high concentrations. It should be noted that the negative peaks at 1555–1540 cm^{-1} observed in the difference spectra of polyamine-PSII complexes also arise from the loss of the intensity of the amide II band at 1545 cm^{-1} as a result of modification in the protein secondary structure (Fig. 1, 5 and 10, mM).

A quantitative secondary structure analysis of the uncomplexed proteins of PSII and its polyamine complexes with different cation concentrations is presented in Table 1. The untreated PSII proteins showed α-helix 47% (1657 cm^{-1}), β-sheet 18% (1628 and 1691 cm^{-1}), turn 19% (1678 cm^{-1}) and random 16% (1640 cm^{-1}) (Table 1 and Fig. 2). Upon spermine complexation, the α-helix component was reduced to up to 35% and the β-sheet increased up to 29%, while no major alterations were observed for the β-anti, turn and random structures (Table 1 and Fig. 2). Similarly, in the presence of spermidine, α-helix was reduced to 37% and β-sheet increased to 28%, whereas no major changes occurred for the other minor conformational components (Table 1 and Fig. 2). The observed structural changes at the various polyamines concentrations studied are indicative of a larger perturbation of the protein conformation by spermine than spermidine. This is consistent with the effects of spermine and spermidine complexation on the rate of the photosynthetic oxygen evolution. At low cation concentrations (0.01 and 0.1 mM), the inhibition is negligible for spermidine, while spermine induces a large reduction on the rate of oxygen evolution (18% and 74%, respectively) with similar cation contents (Table 1). At higher polyamine concentration (1 mM), the inhibition rate was increased to 100% for spermine and 50% for spermidine cations (Table 1). However, a complete inhibition (100%) was also observed for the spermidine cation at concentrations higher than 1 mM (Table 1).

The above thus demonstrates a clear relationship between the inhibition of photosynthetic electron transport in PSII by polyamines and the modification of the protein secondary structure, α-helical domains being modified to β-sheet structures. It is likely that the proteins affected by these polycations are either extrinsic polypeptides or portions of integral polypeptides protruding at the surface of the PSII membranes. Toxic cations such as Pb^{2+}, Hg^{2+} and Zn^{2+} were shown to produce the depletion of extrinsic PSII polypeptides that are involved in the regulation of a Ca^{2+} and Cl^{-} cofactor requirement and in maintaining an active Mn cluster for oxygen

Table 1
Secondary structure determination of PSII proteins in the presence of spermine and spermidine, and inhibition of oxygen evolution in PSII submembrane fractions at various concentrations

Amide I component (cm^{-1})	Conformation	% of conformation Pure PSII	% of conformation Spermine (mM)						% of conformation Spermidine (mM)					
			0.01	0.1	1	5	10	20	0.01	0.1	1	5	10	20
1657	α-helix	47 ± 1	43	42	41	40	37	35	45	43	42	41	40	37
1626–1640	β-sheet	11 ± 0.4	14	15	16	18	18	20	12	14	14	16	17	21
1641–1648	random	16 ± 0.5	15	15	15	15	17	17	15	15	16	17	17	17
1670–1678	turn	19 ± 0.5	20	20	20	19	19	19	20	20	20	18	18	18
1685–1691	β-antiparallel	7 ± 0.2	8	8	8	8	9	9	8	8	8	8	8	7
% of inhibition			18	74	100	100	100	100	0	6	54	100	100	100

evolution [31–33]. The action of polyamines on these extrinsic polypeptides and their possible interference with the roles of Ca, Cl and Mn is currently being studied in our laboratory.

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