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Effect of formate on Mössbauer parameters of the non-heme iron of PS II particles of cyanobacteria

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Mössbauer spectra were measured for PSII particles having an active water-splitting system. The particles were isolated from the thermophilic cyanobacterium *Synechococcus elongatus* enriched in ^{57}Fe. The Mössbauer resonance absorption spectrum is a superposition of 3 doublets with the following quadrupole splitting and chemical shift: 1, $\delta = 0.40$, $\Delta = 0.85$; II, $\delta = 1.35$, $\Delta = 2.35$; III, $\delta = 0.25$, $\Delta = 1.65$. The δ and Δ values of doublets I, II, III are characteristic of proteins with iron-sulphur center, non-heme iron of the reaction center of higher plants and of the oxidized cytochrome *b*-559. Treatment with sodium formate to remove bicarbonate affects only the doublet of non-heme iron, causing its quadrupole splitting to reduce to 1.75 and the chemical shift to reduce to 0.90. After washing out the formate, the Mössbauer spectrum of non-heme iron is restored. The data suggest that bicarbonate is a ligand for the non-heme iron of the reaction center of cyanobacteria.

Photosystem II; Non-heme iron; Bicarbonate; Mössbauer spectroscopy

1. INTRODUCTION

Bicarbonate is a necessary component for the operation of electron transport on the acceptor side of photosystem II (PS II) both in higher plants [1] and cyanobacteria [2]. The removal of this formate anion causes a slowing of electron transfer from Q_A^- to Q_B [3] and blocks the $Q_B \longrightarrow PQ$ pathway [4]. In the absence of HCO_3^-, Q_B^{2-} protonation is a rate-limiting reaction the consequence of which is inhibition of exchange between $Q_B^{2-}(2H^+)$ and the PQ pool at the site of Q_B binding to apoprotein [5].

The location of the HCO_3^- binding site in PS II is unknown yet. It is supposed that HCO_3^- induces conformational changes in D1, a herbicide-binding protein, making Q_B reduction and exchange between $Q_B^{2-}(2H^+)$ and the quinones of the plastoquinone pool easier [6]. Arginine-257 has been proposed as a probable candidate for the binding component [7]: a conformation favorable for binding herbicides (known for their influence on the Q_A–Q_B electron transfer) was suggested to be formed by way of compensating the positive charge of the arginine by bicarbonate. An ESR study of the properties of the acceptors of PS II under the bicarbonate effect has suggested that the site of HCO_3^- binding is somewhere about the non-heme iron or is the iron itself [2].

In an attempt to identify the HCO_3^- binding site in PS II, we investigated the influence of formate on the Mössbauer spectra of PS II particles of cyanobacteria enriched in ^{57}Fe.

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2. MATERIALS AND METHODS

The thermophilic cyanobacterium *Synechococcus elongatus* was grown in Kratz-Mayer's medium [8] abundant in ^{57}Fe. Photosynthetic membranes and PS II particles were isolated by a modification of the method described in [9]. The isolation medium was buffer A containing 15 mM Hepes-NaOH (pH 7.5) and 10 mM $MgCl_2$. PS II particles were extracted by LDAO treatment with a detergent: chlorophyll ratio of 2.5 in buffer A containing 25% glycerol by volume and sedimented by centrifugation at 150000 × *g* for 90 min.

A Clark electrode was used to measure O_2 evolution. As an electron acceptor, use was made of a mixture of potassium ferricyanide (2 mM) and *p*-benzoquinone (1 mM). The rate of O_2 evolution was measured to be 1 mM/mg Chl per hour. For the isolated preparation the P680:P700:Chl ratio was 6:1:500.

Formate treatment of PS II particles was done as described elsewhere [10]. The PS II particles were incubated in a buffer containing 50 mM Mes (pH 6.0), 7 mM $MgCl_2$, 25 mM Na-formate, 25% glycerol (v/v) for 1 h under nitrogen at room temperature. The mixture was centrifuged at 150 000 × *g* for 60 min and suspended in the same buffer, C_{Chl} = 24 mg per cuvette. To remove the formate, the PS II particles were washed out with buffer A containing 25% glycerol and blown out with CO_2.

Mössbauer spectra were recorded on a spectrometer with a uniform acceleration of the Co^{57} source in Cr at a rate of ~7 mm/s and ~30 mm/s. Isomeric shifts δ were given relative to sodium nitroprusside. Measurements were made over the temperature range 80–265 K. The spectra (more than 50) were computer processed and decomposed to get constituent doublets.

3. RESULTS

The Mössbauer spectra of native photosynthetic membranes [11,12] are an unresolved doublet with δ = 0.6 mm/s and Δ = 0.7 mm/s. This spectrum consists of two components, which is characteristic for iron-sulphur centers: A with δ = 0.45 mm/s, Δ = 0.85 mm/s, and B with δ = 0.5 mm/s, Δ = 0.6 mm/s.

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Table I

Mössbauer parameters of iron-containing components of PS II particles isolated from thermophilic cyanobacteria

Sample	Component	Fe relative content (%)	Chemical shift (mm/s)	Quadrupole splitting (mm/s)	Valence	Spin
PS II particles	I	62	0.40	0.85	Fe^{3+}	5/2
	II	17	1.35	2.35	Fe^{2+}	2
	III	21	0.25	1.65	Fe^{3+}	1/2
PS II particles treated with formate	I	60.5	0.40	0.85	Fe^{3+}	5/2
	II	18.6	0.90	1.75	Fe^{2+}	2
	III	20.9	0.20	1.65	Fe^{3+}	1/2
PS II particles after formate removal	I	66.5	0.40	0.85	Fe^{3+}	5/2
	II	15.5	1.35	2.20	Fe^{2+}	2
	III	18	0.20	1.65	Fe^{3+}	1/2

Mössbauer spectra of PS II particles isolated from membranes were taken over the temperature range 80–265 K. They are more complex in shape. Presented in Fig. 1a is the Mössbauer spectrum taken at T = 90 K. A computer simulation gave a reliable decomposition yielding 3 doublets with parameters presented in Table I. It follows from the tabulated data and Fig. 1a that doublet I corresponds to component A of the spectrum for the membrane preparation. The δ and Δ values of this component suggest that it arises from iron-sulfur centers.

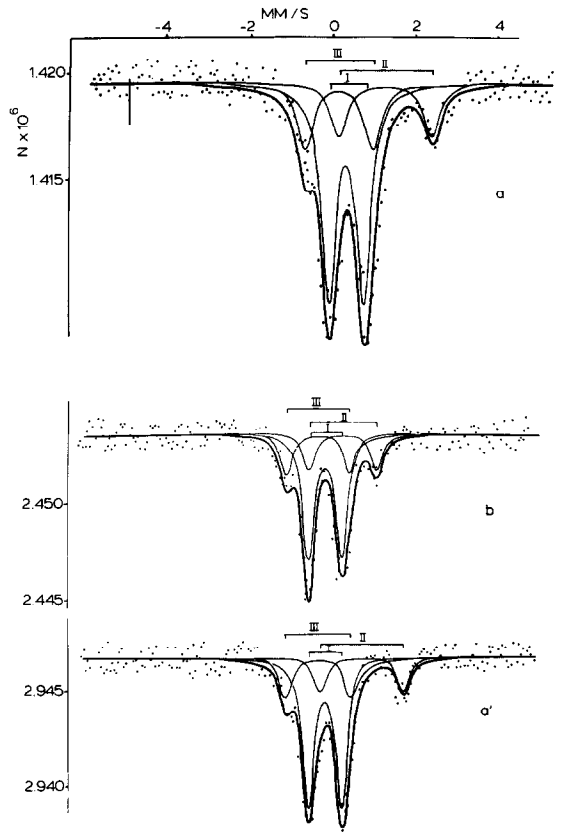

Fig. 1. Mössbauer spectra of PS II particles from the *Synechococcus elongatus* cyanobacterium at T = 90 K. (a) Control; (b) after Na formate treatment; (a') after Na formate removal.

The iron of component III is a trivalent low-spin state. Bearing in mind that: (1) the PS II preparation has the greatest amount of cytochrome *b*-559 per reaction center [13], (2) in oxidized cytochrome *b*-559 the iron is in a low-spin state [14]; and (3) the Mössbauer parameters of oxidized cytochrome *b*-559 are very similar to those of component III [15], there are good reasons to believe that cytochrome *b*-559 is the main component that absorbs in this region. Values of the chemical shift and quadrupole splitting of component II are typical of high-spin Fe^{2+} [16]. They correspond to the parameters of the non-heme iron of PS II of *Chlamydomonas reinhardtii* [15,17].

To investigate the effect of bicarbonate on non-heme, the PS II particles were treated with formate and their oxygen evolution and Mössbauer spectra were measured. After the removal of bicarbonate, the rate of O_2 evolution was seen to slow markedly (by 65%) and the Mössbauer resonance absorption spectra changed significantly (Fig. 1b). The chemical shift changed by 0.45 mm/s (from 1.35 to 0.90 mm/s), and the quadrupole splitting, by 0.6 mm/s (from 2.35 to 1.75 mm/s). The δ and Δ values of the two other components remained unchanged. The effect of formate is reversible: after its removal, the spectra are restored (Fig. 1a').

4. DISCUSSION

The results show that the removal of bicarbonate by formate from PS II particles from cyanobacteria largely affects the Mössbauer spectra of non-heme iron. The changes in the quadrupole splitting and chemical shift suggest that bicarbonate interacts with the iron atom. Changes similar to those observed by us were seen when varying ligands site VI of the iron atom in hemoglobin [18]. In bacterial reaction centers, 4 histidine residues of the L and M subunits and glutamyl residue of the M

subunit [19] are involved in the iron atom binding. A comparison of the amino acid sequences of these subunits with those of proteins D1 and D2 – that constitute the reaction center in higher plants – reveals homologous histidines, suggesting a similar structure of the binding site [20]. However, no amino acid was found in D1 and D2 that can serve as ligand V. In [20], bicarbonate has been suggested to act as ligand V on the ground of the known role of HCO_3^- in PS II-driven electron transport in higher plants.

Recent investigations have shown that in cyanobacteria the reaction centers have a structure analogous to the reaction centers of higher plants, and are, hence, composed of protein D1 and D2 [21]. The genetic sequence of these proteins suggests that they cannot differ much from the corresponding proteins of eukaryotes [22]. A herbicide-binding protein has 96% homology with protein D1 of higher plants [23]. The data above suggest that the iron and HCO_3^- binding sites are identical for cyanobacteria and higher plants and that bicarbonate interacts directly with the reaction center iron in cyanobacteria, according to the supposed reaction center structure, as deduced from the amino acid sequence data.

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