Inorganic Fe^{2+} formation upon Fe-S protein thermodestruction in the membranes of thermophilic cyanobacteria: Mössbauer spectroscopy study

Yu.N. Kaurov[1,a], A.A. Novakova[b], L.N. Davletshina[a], A.Yu. Aleksandrov[a], E.A. Khval'kovskaya[b], B.K. Semin[a,*], N.P. Belevich[a], I.I. Ivanov[a], A.B. Rubin[a]

[a] *Department of Biology, Moscow State University, 119899 Moscow, Russia*
[b] *Department of Physics, Moscow State University, 119899 Moscow, Russia*

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Abstract A model description of the Mössbauer spectrum (80 K) of native membranes of the thermophilic cyanobacterium *Synechococcus elongatus* is suggested on the basis of the known values of quadrupole splitting (ΔE_Q) and isomer shift (δ_{Fe}) for the iron-containing components of the photosynthetic apparatus. Using this approach, we found that heating the membranes at 70–80 K results in a decrease of doublet amplitudes belonging to F_X, F_A, F_B and ferredoxin and simultaneous formation of a new doublet with $\Delta E_Q = 3.10$ mm/s and $\delta_{Fe} = 1.28$ mm/s, typical of inorganic hydrated forms of Fe^{2+}. The inhibition of electron transfer via photosystem I to oxygen, catalyzed by ferredoxin, occurs within the same range of temperatures. The data demonstrate that the processes of thermoinduced Fe^{2+} formation and distortions in the photosystem I electron transport in the membranes are interrelated and caused mainly by the degradation of ferredoxin. The possible role of Fe^{2+} formation in the damage of the photosynthetic apparatus resulting from heating and the action of other extreme factors is discussed.

Key words: Mössbauer spectroscopy; Photosystem I; Thermodestruction; Fe-S center; Iron; Thermophilic cyanobacterium

1. Introduction

The iron-containing components of photosynthetic membranes are mainly represented by Fe-S core proteins of the reaction centers and by ferredoxin located at the membrane surface. These proteins account for more than 70% of the total intracellular iron content [1]. The Fe-S proteins are known as highly thermolabile constituents of photosystem I (PS I) in oxygen-evolving plant organisms. For example, the functional activity of ferredoxin in membranes from thermophilic cyanobacteria disappears at early stages of thermally induced impairment of PS I-supported electron transport [2]. At present, the mechanism of inactivation of Fe-S centers is still unclear. While studying the Mössbauer spectra of various photosynthetic organisms, it was frequently noted that heating of plant preparations at 140–360°C led to the formation of reduced iron, with characteristics similar to those of hydrated unbound ions $[Fe(H_2O)_6]^{2+}$. This effect was observed on pods and cotyledons of soy beans, as well as on cells of green algae [3,4] and purple bacteria [5]. Studies on purple bacteria aging under anaerobic conditions indicated that similar forms of iron(II) are also formed at 12–30°C in preparations of bacterial cells and isolated chromatophores [5]. Apparently, the appearance of Fe^{2+} in purple membranes is the result of destruction of the membrane-bound Fe-S proteins. Therefore, it seems reasonable to assume that the loss of functional activity of Fe-S centers upon heating of photosynthetic membranes is related to the formation of reduced iron. To test this assumption, it is first necessary to find out whether the changes in the state of Fe-S centers and functional properties of the electron transport chain induced by thermal treatment are accompanied by accumulation of Fe^{2+} in photosynthetic membranes. The membranes and submembrane complexes of thermophilic cyanobacteria represent convenient material for studying the thermally induced inactivation of the photosynthetic apparatus. In this study we used Mössbauer spectroscopy to investigate the thermally induced changes in the oxidation state of iron in the membrane-bound Fe-S centers of the thermophilic cyanobacterium *Synechococcus elongatus* after thermal treatments causing functional destruction.

2. Materials and methods

The methods of cyanobacterial cultivation and isolation of the membranes have been described previously [6,7]. PS I samples were obtained after treatment of the membranes with lauryldimethylamine-*N*-oxide (ratio of detergent to chlorophyll was 2.7:1, w/w) at 4°C for 40 min. Fragments of membranes, enriched in PS I, were pelleted at $144\,000 \times g$ for 50 min. The characteristics of the samples of PS I have been given by Kaurov et al. [7]. HEPES-NaOH buffer (15 mM; pH 7.5) with $MgCl_2$ (10 mM) was used in experiments with photosynthetic membranes and PS I-enriched fragments.

Mössbauer spectra of the membranes were measured at 80 K on the same membrane sample exposed sequentially to 5 min heating at various temperatures. To accomplish this, the sample was incubated for 5 min in a thermostat at a given temperature and then transferred to liquid nitrogen. After recording the spectrum, the sample was slowly thawed, heated at a higher temperature, and prepared for the next Mössbauer measurement. Standard UNIVEM software was used for Mössbauer spectrum fitting. The values of isomer shifts are given with respect to metallic iron.

The temperature dependence of oxygen reduction by PS I was measured using a similar procedure, except that samples were cooled to room temperature after heating and that each measurement was performed with a new sample. The rate of oxygen reduction was measured with a Clark-type electrode in a buffer containing PS I samples at a chlorophyll concentration of 10 μg/ml, dichloropheno-

*Corresponding author. Fax: (7) (095) 939-11-15.
E-mail: semin@mars.biophys.msu.ru

[1] Dr. Yu.N. Kaurov died on 2 October 1998.

Abbreviations: ΔE_Q, quadrupole splitting; δ_{Fe}, isomer shift; PS, photosystem; F_X, F_A and F_B, Fe-S centers of photosystem I; HEPES, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid

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lindophenol (40 μM), methylviologen (0.1 mM) and sodium ascorbate (1 mM).

3. Results and discussion

Fig. 1 shows the Mössbauer spectrum of initial native membranes (A) isolated from the cells of *Synechococcus elongatus*. The spectrum has the shape of an asymmetric broad doublet, which is characteristic of photosynthetic membranes of cyanobacteria [8,9] and purple bacteria [5]. Solid lines represent the computer simulation of the spectrum by the sum of subspectrum doublets. Their parameters closely correspond to those of iron-containing components of PS I and II [9–15] and of non-specifically adsorbed iron Fe^{3+} [16] (Table 1). The major contribution to the Mössbauer spectrum of the membranes is made by four doublets (I–IV) corresponding to the oxidized four iron cations in 4Fe-4S centers F_X, F_A and F_B [10,11]. This is consistent with the high content of P700 reaction centers in the membranes of cyanobacteria (under the conditions of cell growth used in this study, the P700:P680 molar ratio in membranes of *S. elongatus* was equal to 6:1 [6]). Two doublets (V and VI) which correspond to the two iron cations in 2Fe-2S ferredoxin [13] have high intensities too. Their content in the samples should be relatively high since the isolation of the membranes was performed in the buffer systems with a rather low ion strength (see Section 2). Minor doublets VII and VIII are related to the cytochrome b_{559} [9] and non-heme iron of PS II [14] respectivelyly. Their influence on the form of the model spectrum is insignificant.

After heating the membranes of thermophilic cyanobacteria at 70°C, we observed a new doublet with a quadrupole splitting parameter $\Delta E_Q = 3.10$ mm/s and an isomeric shift of $\delta_{Fe} = 1.28$ mm/s (Fig. 1B, Table 1; doublet IX); these parameters correspond to the respective characteristics of the hydrated forms of reduced iron ($\Delta E_Q = 3.35$ mm/s and $\delta_{Fe} = 1.4$ mm/s) [17]. It seems that a sufficiently large width of the new doublet is caused by accumulation of hydrated iron with different surroundings. The ratio of the total spectrum area and the thermally induced doublet area suggests that the process of reduction involves at least 33% of the total amount of iron present in the samples. The major source of this iron is likely associated with Fe-S proteins that account for more than 85% of the total iron in the membranes of cyanobacteria. In this

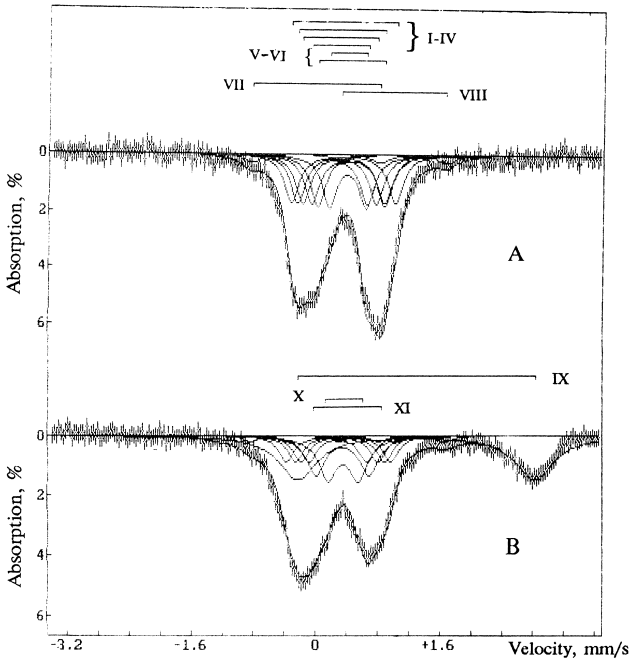

Fig. 1. Mössbauer spectrum (80 K) of *S. elongatus* membranes after a 5 min incubation at room temperature (A) and 75°C (B).

case, the formation of Fe^{2+} may occur at the expense of 4Fe-4S centers in the core of PS I (F_X, F_A and F_B; doublets I–IV) and/or at the expense of ferredoxin (doublets V and VI).

In a special series of experiments performed on isolated PS I complexes containing 4Fe-4S centers, F_X, F_A and F_B, we observed that heating of these complexes under similar conditions was not accompanied by a rise of the reduced iron doublet in the Mössbauer spectrum (data not shown). We have seen only the appearance of doublets in the central part of the spectrum which correspond to the clusters of oxidized iron non-specifically adsorbed to the membranes or clusters of iron-accumulating proteins such as ferritin. The presence of these clusters might be caused by the binding of ^{57}Fe to membranes, which can appear during destruction of labile 4Fe-4S centers, F_X, F_A and F_B. The same is seen in the spectrum of heated membranes (Fig. 1B, doublets X and XI). The above data suggest that ferredoxins are the main source

Table 1
Characteristic parameters of the components used for the simulation of Mössbauer spectra of membranes from *S. elongatus*

Doublet	Isomer shift[a], δ_{Fe} (mm/s)	Quadrupole splitting, ΔE_Q (mm/s)	Component	Reference
I	0.31	1.35	F_X, F_A and F_B oxidized forms [4Fe-FS]	[10]
II	0.31	1.15		
III	0.3	0.99		
IV	0.3	0.76		
V	0.4	0.48	ferredoxin [2Fe-2S]	[12]
VI	0.44	0.87		
VII	−0.01	1.065	cytochrome b_{559}	[9]
VIII	0.99	1.035	non-heme iron	[14]
IX	1.28	3.1	hydrated Fe^{2+}	[17]
X	0.36	0.4	iron-accumulating substances	[16]
XI	0.35	0.71		

[a]The values of isomer shifts are given relative to α-Fe.

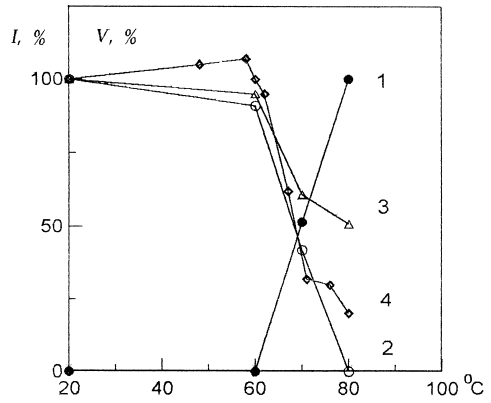

Fig. 2. Temperature dependences of the intensities of Mössbauer spectrum components (*I*) associated with reduced iron (1), ferredoxin (2), and centers F_X, F_A and F_B (3), and of the oxygen reduction rate (*V*) in the membranes from *S. elongatus* cells (4). The maximum values of *I* and *V* taken as 100% correspond to the highest intensity (doublet area) of the respective components and to the rate of oxygen reduction at 25°C, respectively.

of Fe^{2+} in the membranes. However, it should be noted that PS I samples contain residual amounts of detergent (see Section 2), which might affect the process of iron transformation.

The main role of ferredoxins in the process of Fe^{2+} formation is confirmed by the comparative study of temperature dependences of the Mössbauer spectra, in particular the intensities of their main components (doublets I–IV, V–VI and IX) and of the electron transport rate in PS I (Fig. 2). As shown in Fig. 2, the accumulation of reduced iron in the cyanobacterial membranes upon heating (curve 1) is paralleled by the practically complete disappearance of the component related to the ferredoxin (curve 2). Conversely, the component corresponding to F_X, F_A, and F_B centers of PS I (curve 3) decreased by 50% only. The increase in the amount of reduced iron and the respective decrease in the amount of ferredoxins were accompanied by 80% inhibition of PS I-dependent electron transport to oxygen (curve 4). It is known that ferredoxins are the main terminal donors of electrons to hydrophilic electron acceptors. The temperature corresponding to the 50% change in the amplitude characteristics of curves 1, 2 and 4 was about 68°C. In all the experiments, this temperature was close to 75°C, the temperature at which oxidation-reduction reactions of ferredoxins were inhibited by 50% in the membranes from *Synechococcus* sp. [2]. Our data suggest that the thermally induced accumulation of reduced iron and inhibition of PS I-dependent electron transport in membranes of thermophilic bacteria are interrelated processes caused primarily by degradation of Fe-S centers of ferredoxins. However, the iron atoms in the Fe-S components of photosynthesizing organisms are mostly present in the oxidized state [1,3,4,8–14]; therefore, the inactivation of membrane ferredoxins should be associated with the reduction of trivalent iron by some donors. The chemical nature of these donors remains unclear. It can be assumed that ferredoxins themselves can serve as such donors. For instance, the results of the research on succinate dehydrogenase have demonstrated that thermodenaturation of this protein is accompanied by the formation of label sulfur-containing fragments, which are capable of donating electrons to the endogenous oxidized iron and exogenous acceptors [18]. It is possible that a similar process occurs during the thermodegradation of ferredoxins or some other proteins of the cyanobacterial membranes.

Thus, the thermoinactivation of PS I in membranes of thermophilic cyanobacteria results in the destruction of Fe-S centers in ferredoxins and is accompanied by accumulation of reduced iron. It is known that Fe^{2+} ions are effective catalysts promoting the transformation of reactive oxygen species as well as lipid and protein radicals in the reactions of oxidative destruction of membrane components (see, e.g. reviews [19,20]). The initiation of Fe^{2+} of various reactions such as the Fenton reaction, the Haber-Weiss reaction, free radical disintegration of lipid hydroperoxides, etc., may substantially accelerate the thermally induced impairment of the electron transport chain and the suppression of functional activity of the membranes. It seems reasonable to assume that the destruction of Fe-S centers and the accumulation of reduced iron forms contribute to the inactivation of photosynthetic membranes under a variety of stressful conditions. It was shown that the photodestruction of PS I in plants during cold shock mainly affects the Fe-S centers which are likely to be the main generators of active forms of oxygen [21,22]. It was also found that reduced non-heme iron of PS II initiates D1 protein destruction as a result of the treatment of thylakoid membranes with H_2O_2 in the dark and possibly under photodestruction [23].

These effects suggest an important role of destruction of Fe-S centers in the impairment and inactivation of the photosynthetic apparatus.

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