Investigation of the iron components in photosystem II by Mössbauer spectroscopy

Vasili Petrouleas and Bruce A. Diner*

Physics Division, NRC Democritos, Athens, Greece and **Institute de Biologie Physico Chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France*

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1. INTRODUCTION

In photosystem II of green plant photosynthesis (review [1]) the absorption of a quantum of light results in the transfer of an electron from the primary donor P680 (a chlorophyll-containing species) to the stable primary acceptor Q, a plastoquinone [2,3]. It has been shown [4–6] that a pheophytin, I, acts as an intermediate acceptor. The situation in this respect is analogous to the better understood bacterial photosystem [7–10]. This similarity is further exemplified in [5,11,12] which suggest that Q is an Fe^{2+}–plastoquinone complex. An EPR doublet, similar to that observed in bacterial reaction centers, has been detected in samples containing photosystem II in which both Q and I are reduced; the intensity of the doublet has in turn been related to the Fe^{2+} concentration in Fe extraction/reconstitution experiments [5,11]. Also, in photosystem II preparations from a mutant of *Chlamydomonas* which lacks photosystem I [13] a broad EPR signal at $g = 1.84$ analogous to the bacterial case has been chemically or photochemically induced and attributed to Q^- [12].

To investigate the role of iron in the acceptor side of electron transfer and also to detect other possible iron components in photosystem II, e.g., cytochrome *b*-599, we applied Mössbauer spectroscopy to the study of photosystem II particles isolated from the double mutant F54-14 of *Chlamydomonas reinhardtii* [13]. Preliminary studies of both intact and DEAE-purified particles indicate the presence of at least 2 iron components:

(i) An Fe^{2+} component with parameters similar but not identical to those of the bacterial acceptor; this component, which is light sensitive, is attributed to the Fe^{2+}–Q complex;

(ii) A low-spin Fe^{3+} component which probably arises from cytochrome *b*-559.

2. MATERIALS AND METHODS

Photosystem II particles were prepared as in [13], except that iron in the growth medium was ~90% enriched in ^{57}Fe. Intact particles, isolated from the sucrose density gradient after isopycnic centrifugation, and DEAE particles were diluted 2.5–3-fold with 20 mM MES/KOH (pH 5.9) containing 1 mM EDTA and 200 μM phenylmethane–sulphonyl–fluoride and centrifuged at 200 000 × *g* for 5 h. The pellets were resuspended in 20 mM MES/KOH (pH 5.9). Samples for Mössbauer measurements contained ~0.5–0.8 μmol RC/ml.

Mössbauer measurements were obtained with a conventional constant acceleration spectrometer. Illumination at 4.2 K was accomplished by the use of 9 light-emitting diodes of the GaAsP type placed in the periphery of the sample; the quantum efficiency for light emission (in the red) of these devices is high at low temperatures. For the illumination of the sample at room temperature, two 50 W white-light projection lamps were used.

3. RESULTS AND DISCUSSION

Fig.1a shows representative spectra of photosystem II particles at ~130 K and 4.2 K, respec-

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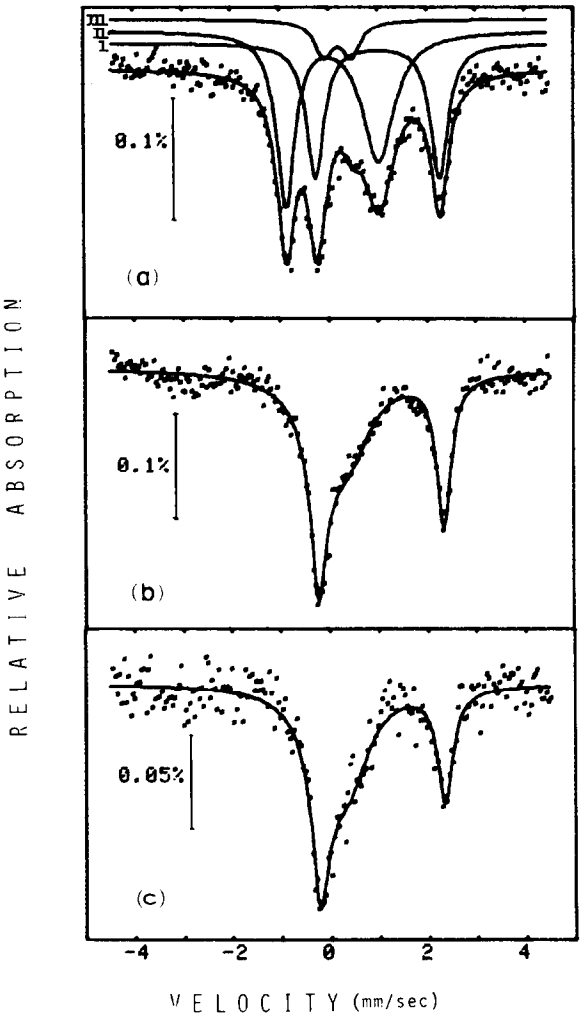

Fig.1. Mössbauer spectra of intact photosystem II particles: (a) spectrum at ~ 130 K; (—) computer fit consisting of the subspectra I,II,III; (b) spectrum at 4.2 K; (c) spectrum at 4.2 K following addition of benzidine and illumination while cooling.

tively. Two main components are apparent in the spectra, while the computer analysis has indicated the presence of an additional small component in the central part of the spectrum. The solid line over the data points represents the computer fit while the component subspectra are illustrated separately in fig.1a. The parameters of the analysis are summarized in table 1.

At 4.2 K (fig.1b) component II splits into a multiline pattern, apparently due to slow electronic relaxation compared to the Larmor precession frequency of the nucleus, and its absorption intensity is lost in the background; measurements on an extended velocity scale showed no detectable absorption intensity outside the range of fig.1b. Only component I is, therefore, reliably analyzed at this temperature. Included in table 1 are also the parameters of this component in DEAE-treated photosystem II particles.

The parameters of component I (table 1) are typical of high-spin Fe^{2+} [17]. The spectrum of component II at ~ 130 K (fig.1a) shows an asymmetry in the two absorption lines. This can be due either to intermediate electronic relaxation or to two closely spaced doublets. These alternatives should be distinguished by studies of the temperature dependence of component II or by the application of small magnetic fields at 4.2 K. In either case the slow relaxation at 4.2 K in conjunction with the low value of the isomer shift imply an Fe^{3+} low spin state. Little can be said about component III which is probably a contribution by impurities. A similar component has been observed in photosystem II preparations from higher plants [18] and its origin has been attributed, at least in part, to superparamagnetic iron cluster, perhaps, iron storage material. Here, care was taken to decrease the iron concentration in the culture medium to the minimum needed for undiminished growth. The parameters of component III are consistent with Fe^{3+}. It should be noted that optical determinations indicate that in the photosystem II particles cyt. b_6 and cyt. f account together for ~ 10–20% of the total iron. Therefore, part of the absorption intensity of components II and III may be due to these cytochromes.

Considering [5,11,12] and these data it is likely that the Fe^{2+} component (I) is associated with the primary acceptor Q. To provide further support for such an assignment we looked for light-induced spectral changes. The inherent difficulty in these experiments is that drastic changes are not expected in the spectrum. Furthermore, the considerable density of the Mössbauer sample does not permit homogeneous illumination. With these reservations in mind we illuminated while cooling intact PS II particles in the presence of benzidine at concentrations ~ 7-times that of the reaction center (fig.1c). Although the signal:noise is not as good as in fig.1a,b, the asymmetry between the

Table 1

Mössbauer parameters of the iron components in photosystem II preparations compared with those of the bacterial reaction centers

Sample	Temp. (K)	Component	Relative abundance (%)	*QS* (mm/s)	*IS* (mm/s)	Linewidth (mm/s)	Oxidation state	Spin state (*S*)
Photosystem II particles	130	I	35	2.54(1)	1.12(1)	0.42	Fe^{2+}	2
		II	55	1.89(1)	0.20(1)	0.44, 0.78	Fe^{3+}	1/2
		III	10	0.56(5)	0.30(5)	0.46	Fe^{3+}	
	4.2	I		2.58(1)	1.17(1)	0.36	Fe^{2+}	2
DEAE-particles	4.2	I		2.55(1)	1.18(1)	0.45	Fe^{2+}	2
Bacterial reaction centers	4.2			2.16–2.22	1.16–1.18	0.31–0.35	Fe^{2+}	2

Isomer shifts are given with respect to metallic iron at room temperature. Numbers in parentheses represent errors in the last significant digit

2 prominent lines is enhanced relative to that in fig.1b. This result is expected if we assume that a fraction of the sample has been reduced and reduction results in a larger shift of the right peak. The same change is expected if reduction results in a somewhat larger broadening of the peak at right. A similar change but of smaller extent was observed in an experiment in which the sample was illuminated at 4.2 K but without addition of artificial electron donors.

The Mössbauer parameters of component I are compared in table 1 with those of the iron–quinone complex in bacterial reaction centers [14]. The isomer shifts at 4.2 K are practically identical; that, probably, indicates that Fe^{2+} has similar ligands in the two cases; possibly oxygens and/or nitrogens in a hexacoordinate arrangement [14,17,19,20]. The difference in the quadrupole splitting can be explained if we assume that the extent of radial expansion of the 3*d* orbitals and/or the distortion from octahedral symmetry is different in the two cases. However, a study of the temperature dependence of the quadrupole splitting parameter has to be made before the latter conclusion can be reliably established.

Reduced-minus-oxidized difference optical spectra have shown that in these preparations cyt. *b*-559 is in the low potential redox form and is the only cytochrome present in concentrations comparable to P680 ($\sim$1 cyt. *b*-559/PS II center, [21]). EPR investigations have also shown that in the oxidized form the iron of cyt. *b*-559 is in the low-spin state [15,16]. It would, therefore, be reasonable to assign the other major component of the spectrum, component II, to this cytochrome. Whether this assignment accounts for the whole absorption area of component II or for only part of it will be the subject of future experiments.

The present Mössbauer studies of photosystem II particles from a mutant of *Chlamydomonas reinhardtii* offer positive evidence for the presence of an Fe^{2+}–Q acceptor; the results are consistent with a ligand environment at the iron site similar to that of the bacterial acceptor. An Fe^{3+} low-spin component has also been identified and is attributed to cyt. *b*-559.

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