Biochimica et Biophysica Acta, 368 (1974) 135—139

BBA Report

BBA 41265

ON THE QUESTION OF THE PRIMARY ACCEPTOR IN BACTERIAL PHOTOSYNTHESIS: MANGANESE SUBSTITUTING FOR IRON IN REACTION CENTERS OF *RHODOPSEUDOMONAS SPHEROIDES* R-26

G. FEHER, R.A. ISAACSON, J.D. McELROY*, L.C. ACKERSON and M.Y. OKAMURA
Department of Physics, University of California, San Diego, La Jolla, Calif. 92037 (U.S.A.)
(Received July 1st, 1974)

Summary

Iron was partially replaced by manganese in a reaction center preparation of *Rhodopseudomonas spheroides* R-26. The reaction centers containing manganese were distinguished spectroscopically (EPR) from those containing iron. The low-temperature photochemical activities were found to be identical for both species. This makes it unlikely that the transition metal by itself is the primary electron acceptor.

The primary acceptor in bacterial photosynthesis has so far not been definitively identified. The observation of a broad EPR signal [1—4] and the presence of iron in reaction centers of *Rhodopseudomonas spheroides* R-26 [2] led to the working hypothesis that iron plays an important role in the primary electron transfer. The discovery by Loach and Hall of a second, narrow EPR signal [5] and its subsequent identification as a ubiquinone [6] raised the possibility that it may be the primary acceptor. A third possibility, that an iron—ubiquinone complex performs the function of the primary acceptor, has also been proposed [6,7]. In this communication we report that iron can be partially replaced by manganese in a preparation of fully active reaction centers of *R. spheroides* R-26. This finding makes it unlikely that iron by itself is the primary acceptor.

Reaction centers from *R. spheroides* were prepared as described previously [2,8]. Manganese was incorporated into reaction centers in vivo by growing the bacteria in Mn-enriched media**. The EPR spectra were obtained by using light modulation and the kinetics of the light-induced EPR signals were measured as described previously [9].

*Present address: Bell Telephone Laboratories, Murray Hill, N.J. 07974, U.S.A.

**The role of manganese on the growth of photosynthetic bacteria has been investigated by R.J. Kassner and M.D. Kamen, Biochim. Biophys. Acta (1968) 153, 270—278.

The Fe and Mn content in the growth medium is reflected in the metal content of the purified reaction centers. This is illustrated for two limiting cases in Fig. 1. It is seen that the total amount of (Fe + Mn) adds up to one mole per mole of P_{865}. This relationship was found to hold also for other ratios of Fe/Mn, indicating that Mn had replaced Fe in some of the reaction centers. (If some reaction centers had both metals and others had none, it would be highly unlikely that the stoichiometry (Fe + Mn)/(P_{865}) = 1 would be maintained for all Fe/Mn ratios).

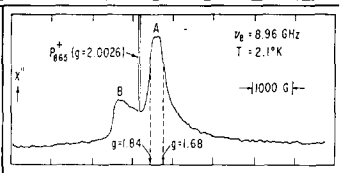
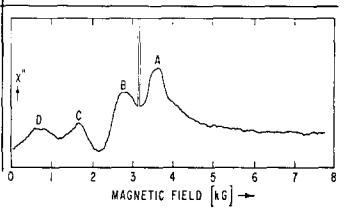
METALS IN MEDIUM (ppm-W/W)		METALS IN REACTION CENTERS (mole fraction)			EPR SPECTRA
Fe	Mn	Fe/P_{865}	Mn/P_{865}	$(Fe+Mn)/P_{865}$	
0.5	<0.02	0.99±0.02	0.02±0.01	1.01±0.03	
0.5	20	0.70±0.02	0.34±0.02	1.04±0.03	

Fig. 1. EPR spectra of reaction centers from *R. spheroides* R-26 for different (Fe/Mn) ratios. Absorbance of sample $A^{1\,cm}_{800} = 5$. The spectra were obtained with 4 Hz actinic light modulation (λ = 855 nm, band width = 50 nm, incident light intensity $\approx$100 mW/cm^2. Microwave power = 60 μW. Peak A is attributed to iron, peaks C, D, and part of B in the lower trace to manganese; peak B in the upper trace is probably due to iron in a different environment (see text). When magnetic field modulation is used, the maxima and minima occur at the *g*-values indicated [4]. The metal content was determined by atomic absorption spectroscopy using the extinction coefficient $\epsilon_{802\,nm} = 288\ mM^{-1} \cdot cm^{-1}$ [10]. The quoted rms errors are associated with the atomic absorption measurements and do not take into account the 5% uncertainty in the value of the extinction coefficient.

The EPR signal of the two samples is also shown in Fig. 1. The peak labeled A has been associated earlier with iron [2]. Since light modulation is used in observing the spectra, the output (ordinate) is proportional to the radio frequency susceptibility, χ'', instead of the commonly observed derivative $d\chi''/dH$. The maximum and minimum slopes of the signal occur at magnetic fields corresponding to electronic *g*-values of 1.84 ± 0.01 and 1.68 ± 0.02, respectively (measured both at 9 GHz and 35 GHz). The same *g*-values have been determined by Dutton et al., from the positions of the maxima and minima of the derivative curve [4]. Peak B was absent in manganese-free chromatophores, but appeared in reaction centers prepared from them (see top trace in Fig. 1). Its amplitude increased during the course of purification. We attribute, therefore, this peak in manganese-free reaction centers to an altered environment at the iron site. In chromatophores containing Mn, a small peak in the B position was also observed.

The amplitudes of peaks C and D (see bottom traces in Fig. 1) were found to be proportional to the amount of manganese present in the reaction centers. Furthermore, when the Mn was preferentially extracted in 1 M $LiClO_4$, 1 mM EDTA (T = 21 °C, 2 hours), peaks C and D were eliminated. They reappeared when Mn^{2+} was added to the reaction centers. We attribute, therefore, peaks C and D to manganese. Thus, the EPR technique provides

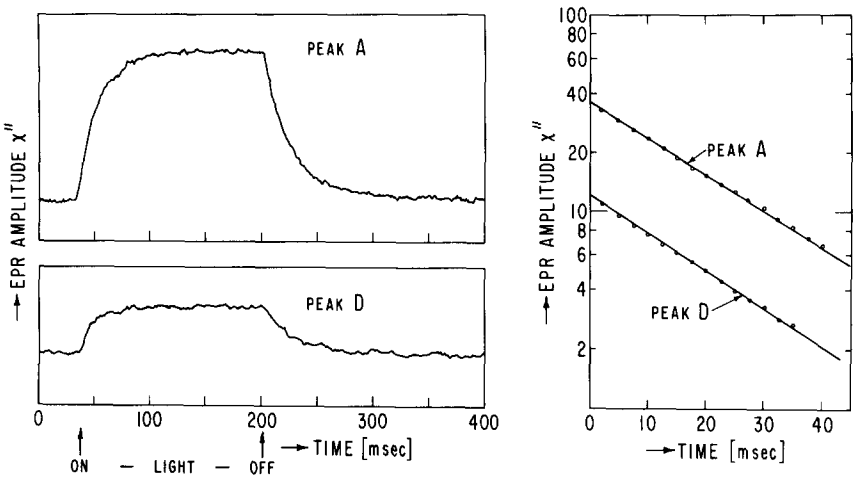


Fig. 2. Light-induced kinetics of the different peaks of the EPR spectrum obtained from reaction centers of *R. spheroides* R-26 in which iron had been partially replaced by manganese (see lower trace of Fig. 1). The left side shows the experimental traces; after expanding the time scale they were replotted on a logarithmic scale, as shown for the decay on the right side. The rise and decay kinetics of Peaks A (iron) and D (Mn) were found to be identical.

us with a method of spectroscopically separating reaction centers containing manganese from those containing iron.

In order to compare the photochemical activity of the Fe- and Mn-containing reaction centers, we measured the low-temperature, light-induced kinetics of the different peaks of the EPR spectrum. The results of these measurements on peaks D (manganese) and A (iron) are shown in Fig. 2. The decay (in the dark) is expected to be very sensitive to the structure of the donor—acceptor pair [9]; it was found to obey first-order kinetics (i.e., $A = A_0 \exp(-t/\tau_D)$). The decay constants of all the peaks were identical, with τ_D = 26 ± 3 ms (T = 2.1 °K), in agreement with previous measurements on the narrow signal P^+_{865} [9]*. The rise times at high light intensities are indicative of the quantum yield. They were also found to be the same for all the peaks. In addition, low-temperature optical light saturation and kinetic measurements were performed; the results were consistent with the EPR measurements.

The identity of the kinetic behavior of the Mn and Fe is striking. It leaves little doubt that Mn can replace iron without impairing the activity of

*The longer spin-lattice relaxation rate of P^+_{865} makes the narrow signal exhibit a longer decay time[9]. This effect can be avoided by working under conditions of microwave saturation. In this case the spin system is driven to its steady state by the microwave power rather than the spin-lattice relaxation process.

the reaction centers. Furthermore, it gives some insight into the possible role of iron. If the transition metal ions alone were to serve as primary electron acceptors, one would expect that their different electronic structures and potentials would result in different kinetics. The identity of τ_D implies that the metal ion does not determine the electron transfer rate. One possible explanation is the formation of a metal—ubiquinone complex [6,7]. In this case the electron may be predominantly located on the ubiquinone which determines the decay kinetics. It should be noted that the EPR results do not exclude at present the possibility that upon illumination the spin state of the transition metal complex changes without affecting the valence state of the metal. Mossbauer and ENDOR experiments are in progress to elucidate the electronic structure of the iron. A second possibility is that ubiquinone is the primary electron acceptor and that the metal ion either facilitates the electron transfer or itself serves as a secondary acceptor. We have obtained evidence supporting the second alternative from measurements on an Mn-free sample in which all but 5% of the iron had been removed with $LiClO_4$ and *o*-phenanthroline. The low-temperature kinetics of the narrow EPR signal* in this sample were found to be biphasic and to obey the relation $A = A_0$ $(0.7 \exp(-t/28 + 0.3 \exp(-t/100))$, where t is in ms ($T = 2.1\ ^\circ K$). Thus, the predominant decay rate was the same as observed in reaction centers that contain a stoichiometric amount of iron. These findings are consistent with the results of Loach et al. [11] on photoreceptor units that have a low iron content.

We wish to thank D. Mauzerall for his active interest and collaboration in the early phases of this work, and E. Moskowitz for measuring the optical kinetics. This work was supported by grants from the National Science Foundation (GB-33340X) and the National Institutes of Health (GM-13191).

Addendum

(Received September 4th, 1974)

Reaction centers of *R. spheroides* R-26 were enriched in Fe^{57} ($1.0\ Fe/P_{865}$) and their Mossbauer parameters were determined at $4\ ^\circ K$ by P. Debrunner (private communication). A reaction center sample under aerobic conditions with the primary reactants in the neutral state gave no EPR signal in the dark and a quadrupole doublet Mossbauer spectrum with a splitting of 2.22 ± 0.02 mm/s and an isomer shift (relative to metallic iron) of 1.15 ± 0.05 mm/s. A sample in which at least 90% of the acceptors were reduced by the addition of dithionite gave a broad EPR signal (peak A, Fig. 1) and a similar quadrupole doublet with a splitting of 2.19 ± 0.05 mm/s and an isomer shift of 1.11 ± 0.05 mm/s. The identity of the Mossbauer parameters in the two samples shows that the valence of the iron does not change after

*The *g*-value of the narrow signal was shifted from 2.0026 to 2.0037 ± 0.0002, indicating the presence of a ubiquinone radical [5,6].

the primary photochemical act; the value of the isomer shift indicates the valence of the iron to be 2. We conclude, therefore, that the primary acceptor is ubiquinone, whose unpaired electron is magnetically coupled to Fe^{2+}. The structure of the reaction center ubiquinone was determined by mass spectroscopy to be that of Coenzyme Q_{10} (i.e., Ubiquinone-50). We are indebted to P. Debrunner for his permission to quote the Mossbauer data prior to publication and to J. Wright for his help with the mass spectroscopic analysis.

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