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Identification of an electron acceptor in reaction centers of *Rhodopseudomonas spheroides* by EPR spectroscopy

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SUMMARY

Photochemically active reaction centers from *Rhodopseudomonas spheroides* R-26 were prepared in which the electron donor is P(865) and the electron acceptor is ubiquinone. The latter was identified by comparing the EPR characteristics of the light-induced signal with those obtained from a ubiquinone radical.

It is now commonly accepted that the primary electron donor in bacterial photosynthesis is the specialized bacteriochlorophyll P(865) [1–5]. The chemical identity of the primary acceptor has, however, so far not been determined with certainty. The presence of iron[6] and the observation of a broad EPR signal[7] has led to the working hypothesis that iron plays a role in the primary process[6]. The recent observation by P.A. Loach of a new EPR signal in subchromatophore preparations from *R. rubrum* (refs 8, 9; and P.A. Loach, personal communication) and our own corroborative observation in specially treated reaction centers from *Rhodopseudomonas spheroides* R-26 [10,11] promised to shed new light on the question of the primary electron acceptor. In this note, we identify the chemical species giving rise to the new EPR signal by comparing its EPR characteristics with those obtained from a ubiquinone radical.

Purified reaction centers containing approximately 1 iron per P(865) were prepared from *Rhodopseudomonas spheroides*, R-26, as described previously[6]. In order to observe the new EPR signal, it was found necessary to treat the reaction centers with a mixture of two detergents. This was accomplished by dialyzing an approx. $3 \cdot 10^{-5}$ M solution of reaction centers in the dark at room temperature for 20 h against 0.05 M Tris chloride buffer, pH 8.0, which contained 0.1% (w/v) sodium dodecyl sulphate, 0.02% lauryl dimethyl amine oxide (supplied by Onyx Chemical Company, Jersey City, N.J.), and 10^{-3} M EDTA. At least 70–80% of the iron, as determined by atomic absorption, was

removed by this treatment. Exposing the reaction centers to sodium dodecyl sulphate alone destroyed their photochemical activity completely. If lauryl dimethyl amine oxide was also present, about 60–70% of the reversible photochemical activity was found at room temperature. At low temperature, the light-induced EPR signal indicated that only 20–30% of the activity was retained. It should be noted that atomic adsorption measures only the average iron content of reaction centers; we do not know, therefore, the iron composition of the fraction that gave rise to the new signal★.

The reversible, light-induced EPR signal from the "double detergent" treated reaction centers is shown in Fig. 1. The EPR spectrum is a composite of signals arising from

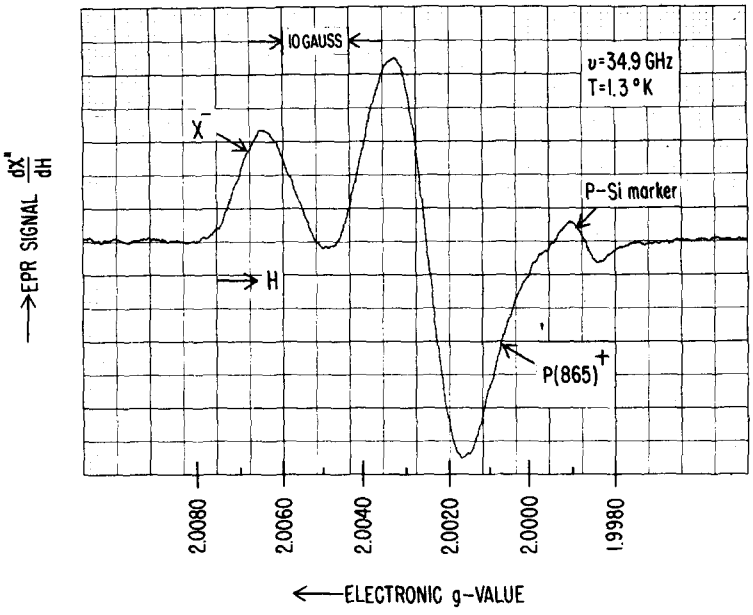

Fig. 1. Light-induced EPR signal from reaction centers from *Rhodopseudomonas spheroides* R-26 treated as described in the text. Note the occurrence of a new signal labeled X^-, part of which is hidden under the $P(865)^+$ signal (see Fig. 2). The phosphorus-doped silicon marker has been introduced as a convenient marker (its electronic g-value is 1.9988 ± 0.0001). The actinic light passed through an interference filter having a pass band of 800–900 nm.

two radical species. The low-field peak arises from the new radical, X^-, while the absorption near $g = 2.0026$ arises from $P(865)^+$, as well as from X^-. In order to unravel the part of the X^- signal which overlaps with the $P(865)^+$ signal, the latter was suppressed by reducing $P(865)^+$ with cytochrome *c*. The EPR signal from X^- alone is shown at two microwave frequencies in the left part of Fig. 2. There is a striking increase in resolved structure and field difference between inflection points at 34.9 GHz, which we attribute to an anisotropic electronic g-value.

★This point was not fully appreciated in a preliminary report of this work[11] in which we loosely referred to the reaction centers as "iron-free".

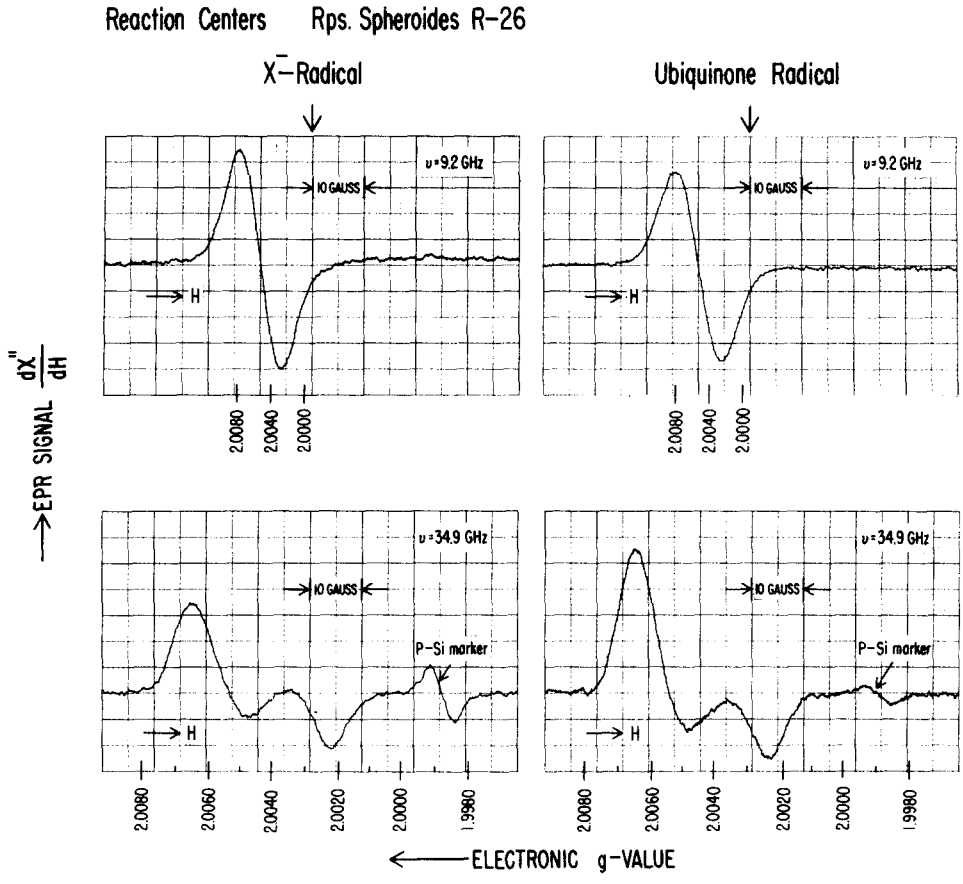

Fig. 2. Comparison of the light-induced EPR signal from the electron acceptor in reaction centers of *Rhodopseudomonas spheroides* R-26 (left) and the ubiquinone radical (right) at 9.2 GHz and 34.9 GHz (T = 1.3 °K). Note the increased resolution at 34.9 GHz. The $P(865)^+$ radical has been reduced with cytochrome *c* in order to reveal the entire spectrum of X^-. The experimental conditions are described in Table I.

The three peaks of the EPR spectrum of Fig. 1 decay with identical kinetics when the actinic light is turned off★. The relative amounts of $P(865)^+$ and X^- formed during illumination were obtained by double integration of the EPR spectra. [$(P(865)^+$ was obtained from Fig. 1 by subtracting X^- from it.] The ratio $P(865)^+/X^-$ was found to be 1.0 ± 0.1. These two pieces of evidence lead to the conclusion that $P(865)^+ - X^-$ form a donor–acceptor pair.

In order to determine the chemical identity of X^-, we used the "model compound" approach which has been successfully applied in the past in the identification of $P(865)^+$ (refs 4, 5). The model compound that we chose was the ubiquinone radical

★The decay of the amplitude $A(t)$ of each of the three peaks could be fitted within experimental error (approx. 10%) by the same expression: $A(t) = A_0\ (0.6\ e^{-t/0.26} + 0.4\ e^{-t/1.8})$, where t is in seconds.

prepared in two different ways: by ultraviolet irradiation of an ethanolic solution of ubiquinone (Q-50) at 77 °C (G. Tollin, personal communication) and by reduction of Q-50 in ethanol by potassium borohydride at room temperature with subsequent freezing[12]. The EPR spectra of the radicals prepared in the two ways were the same and are shown in the right hand side of Fig. 2. The EPR characteristics of X^- and the ubiquinone radical were found to be identical within experimental error (see Table I). This leaves little doubt that X^- has a ubiquinone-like structure and is most probably the ubiquinone radical. Preliminary assays (solvent extraction and thin-layer chromatography) show that reaction centers prepared in the standard way contain of the order of one ubiquinone molecule per P(865) (M.Y. Okamura, unpublished results).

TABLE I

COMPARISON OF THE EPR CHARACTERISTICS OF X^- FROM REACTION CENTERS FROM *RHODOPSEUDOMONAS SPHEROIDES* R-26 AND THE UBIQUINONE (Q-50) FREE RADICAL

Reaction centers, after being exposed to a mixture of two detergents (see text), were illuminated in the presence of 10^{-3} M reduced cytochrome *c* (horse heart) and 10^{-2} M dithiothreitol and frozen during illumination. Ubiquinone (Q-50) 10^{-3} M in ethanol was reduced by the slow dissolution of a chip of potassium borohydride placed at the bottom of the quartz EPR tube. Sample temperature 1.3 °K.

Paramagnetic species	*Microwave frequency (GHz)*	*Effective g-value*★ *(± 0.0002)*	*Linewidth ΔH*★★
X^-	9.2	2.0046	8.1 ± 0.5
	34.9		27 ± 1
Ubiquinone radical	9.2	2.0047	8.5 ± 0.5
	34.9		26 ± 1

★The effective *g*-value was obtained from the value of the magnetic field at which $d\chi''/dH$ is zero. The effect of the *g*-anisotropy in our case is negligibly small at 9 GHz, and the effective *g*-value of the ubiquinone radical equals within experimental error ro the average *g*-value obtained at room temperature[13]

★★Total width between inflection points of $d\chi''/dH$. At 34.9 GHz, this width is a measure of the *g*-anisotropy.

A question that remains to be answered concerns the role of iron and ubiquinone in the primary photochemical act. The experimental findings to date are consistent with several models: In the simplest one, *iron serves as the primary acceptor*, as postulated earlier[6]. Treatment of the reaction centers with the sodium dodecyl sulphate–lauryl dimethyl amine oxide mixture may allow an exogenous ubiquinone to move into the acceptor position, taking over the role from iron as the primary acceptor. Alternately, the ubiquinone may move in the presence of detergents close to the iron and serve as a secondary electron acceptor – a role that several authors have assigned to it previously[14–16] In the second model, we postulate that reaction centers prepared in the standard way contain an *iron–ubiquinone complex*. The observation of a broad EPR signal is compatible with the electron being localized predominantly on the iron. In the presence of detergent, the iron may be removed and the electron becomes localized on the ubiquinone and gives

rise to the signal X^-, described in this note. Which, if any of these schemes is operative in the intact photosynthetic system, remains to be determined.

In summary: We have found that under certain conditions, photochemically active reaction centers can be prepared in which the electron donor is P(865) and the electron acceptor is ubiquinone. Additional experiments remain to be performed in order to establish the relevance of this donor–acceptor pair in the photochemical act *in vivo* and to elucidate the role of iron in this process.

We are indebted to Dr P. Loach for many stimulating discussions on the presence and origin of the X^- signal, and to Dr D. Mauzerall for his active interest in this work. We would like to thank Dr G. Tollin and Mr M. Weissman for helpful advice on the preparation of the ubiquinone radical. This work was supported by Public Health Service Grant GM13191, National Institutes of Health. M.Y.O. would like to acknowledge the support of a National Science Foundation Postdoctoral Fellowship, and J.D.M., the support of a National Institute of Health Special Fellowship (GM44394).

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