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THE RIESKE IRON-SULFUR CENTER IN MITOCHONDRIAL AND PHOTOSYNTHETIC SYSTEMS: E_m/pH RELATIONSHIPS

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1. Introduction

One approach to the elucidation of the mechanisms of energy coupling and electron flow has been the thermodynamic characterisation of the electron and hydrogen carriers involved. A considerable literature has accumulated concerning the half reduction potentials (E_m) of components which can be monitored either with optical or electron spin resonance spectroscopy (see [1,2] for reviews). However, while the pH dependancy of the half reduction potentials of the cytochromes has been extensively studied in photosynthetic, mitochondrial and bacterial [1] systems, the pH/E_m relationships of the iron-sulfur proteins have not yet received much attention. An important candidate for such an examination is the iron-sulfur protein which, in the reduced state, is responsible for the EPR-detectable g 1.90 signal. This component was first described by Rieske et al. [4] in preparations from beef heart mitochondria, and has since become colloquially known as the 'Rieske iron-sulfur center'. Recent exploratory work indicates that the protein may be ubiquitous in energy conserving organelles; it has been found in animal [3], yeast [2] and avian [4] mitochondria, chloroplasts (R. Malkin, personal communication), the purple sulfur photosynthetic bacterium *Chromatium* D [5,6], and in the purple non-sulfur photosynthetic bacteria *Rhodopseudomonas spheroides* [7] and *Rps. capsulata* [8]. In all cases, the 'Rieske centers' have a half-reduction potential in the 250–310 mV redox potential range at pH values near 7.0. Rieske et al. [4] demonstrated that in functional terms, the iron-sulfur protein was physically associated with the succinate-cytochrome *c* reductase, was actively involved in succinate oxidation [3,7], and was located on the electropositive side of the site of inhibition by Antimycin A. It has also been implicated in electron flow in *Chromatium* D [6].

Recently it has been suggested [9,10] that the Rieske iron-sulfur protein might act as a hydrogen carrier in the mitochondrial site II. Such a role would demand that the half reduction potential be sensitive to pH to the extent of –59 mV per pH unit. In this report we examine the pH dependancy of the half reduction potential of the center, both in mitochondria and the photosynthetic bacteria *Rps. spheroides* and *Rps. capsulata*. In addition, we present some data on iron-sulfur centers with signals at g 1.94 in *Rps. spheroides*, and show that the 'Rieske center' of *Rps. spheroides* appears to be actively involved in photosynthetic electron transport.

2. Materials and methods

Pigeon heart mitochondria [11] and chromatophores from *Rps. spheroides* Ga and *Rps. capsulata* St. Louis [12] were prepared as previously described.

The oxidation–reduction potential measurements were performed in the vessel described earlier [13], samples being removed at poised potentials and transferred to 3 mm i.d. quartz tubes before being frozen in iso-octane cooled to near liquid nitrogen temperatures [4]. The EPR spectra were run at near liquid

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helium temperatures in a Varian E4 spectrometer equipped as previously described [5]; the instrument settings are given in the figure legends. The redox titrations were performed over the pH 6–8 range, the equilibration time with each buffer at the experimental pH being at least 30 min. This time allows the internal pH of the vesicles to equilibrate with the external buffer solution, and has been used to give reliable pH dependant titrations [14]. The redox titrations took between 45 and 60 min, during which time samples were taken at different E_h values during the oxidative and reductive phases of the titration to check that redox equilibrium had been achieved. The chromatophore titrations were performed in the dark.

3. Results

3.1. *The pH dependancy of the 'Rieske center' in pigeon heart mitochondria*

Wilson and Leigh [4] previously measured the half-reduction potential of the Rieske iron-sulfur center in both mitochondria and sub-mitochondrial particles from pigeon heart. They showed that in both preparations, at pH 7.0, the center titrated as a one-electron (n=1) redox component with a half reduction potential ($E_{m7.0}$) of +280 mV. We have repeated their redox titrations, but at pH 6.3 and pH 8.3. The results are shown in fig.1a, and the E_m/pH relationship is plotted in fig.1b. It is clear that no appreciable change in the measured half reduction potential of the Rieske center occurs over the 100-fold range of hydrogen ion activity examined. Thus the oxidation and reduction of the Rieske iron-sulfur protein does not appear to involve the hydrogen ion in the physiological range of pH. Furthermore, at the EPR spectrometer settings used (the signal was not microwave power saturated), the fully reduced signals were the same height at pH 6.3, 7.2 and 8.3, showing that the line shape of the reduced iron-sulfur protein is insensitive to pH.

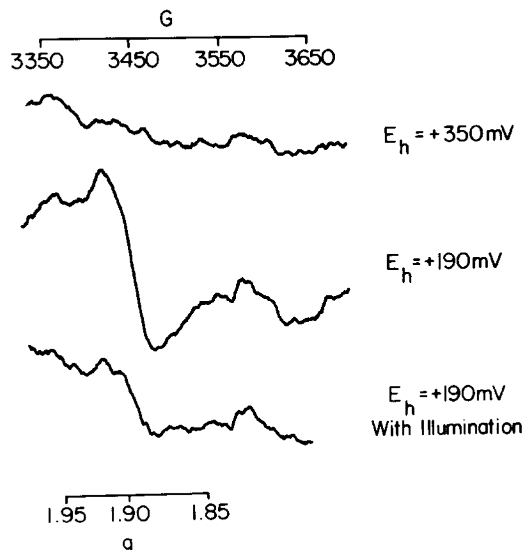

Fig.2. The g 1.90 signal of *Rps. spheroides: Rps. spheroides* Ga chromatophores (763 μM BChl) were suspended in 100 mM KCl, 20 mM morpholinopropanesulfonic acid (MOPS) 1 mM $MgCl_2$ pH 6.9, 20 μM PMS, 30 μM DAD, and samples taken and rapidly frozen in the dark at the potentials indicated. A second sample was taken at 190 mV and illuminated for 30 sec prior to its rapid freezing. Spectrometer settings, Sample temperature = 12° K, microwave power = 20 mW.

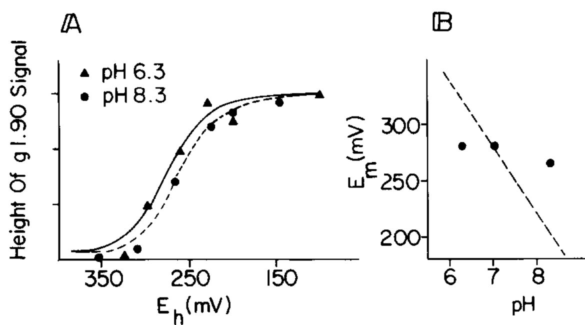

Fig.1. Redoc titration of the g 1.90 signal in pigeon heart mitochondria: A) Pigeon heart mitochondria were suspended to 8 mg/protein per ml in 220 mM mannitol, 70 mM sucrose, 100 mM buffer (Tris at pH 8.3, morpholino-ethanesulfonic acid (MES) at pH 6.3) in the presence of the following mediators: phenazine methosulfate (PMS), phenazine ethosulfate (PES) and diaminodurene (DAD), all at 100 μM. Spectrometer settings; sample temperature = 11° K, microwave power = 20 mW. The lines drawn through the points are theoretical curves for a one-electron redox couple at the extremes permitted by our data. B) Plot of E_m versus pH. The dotted line represents the theoretical line expected if the redox reaction involved a proton. The value for pH 7 is taken from [4].

3.2. *The pH dependancy of the 'Rieske center' in the photosynthetic bacteria Rps. spheroides and Rps. capsulata*

We have previously reported [8] that chromatophores from *Rps. capsulata* have a g 1.90 signal of similar line shape and half reduction potential to the mitochondrial 'Rieske center'; fig.2 shows the signal found in *Rps. spheroides.* We have titrated this signal in both bacterial species at three different values of ambient pH (fig.3). The absence, in both species, of an appreciable variation of the half reduction potential with pH, indicates that the redox reactions of these 'Rieske centers' do not involve coupled protonation/deprotonation in the physiological pH range. The properties of the Rieske iron-sulfur protein in photosynthetic and mitochondrial sources thus appear to be similar.

In addition, fig.2 shows that the 'Rieske center' in *Rps. spheroides* can be photo-oxidised if the chromatophores are illuminated briefly, immediately prior to freezing. We may thus assign a functional role to the Rieske center in photosynthetic electron flow.

3.3. *The g 1.94 signals of Rps. spheroides*

In addition to the signal at g 1.90, other components with signals at g 1.94 appear as the ambient redox potential of the chromatophores is lowered below +100 mV, both in *Rps. spheroides* [7,15] and *Rps. capsulata* [8]. Redox titrations of these signals

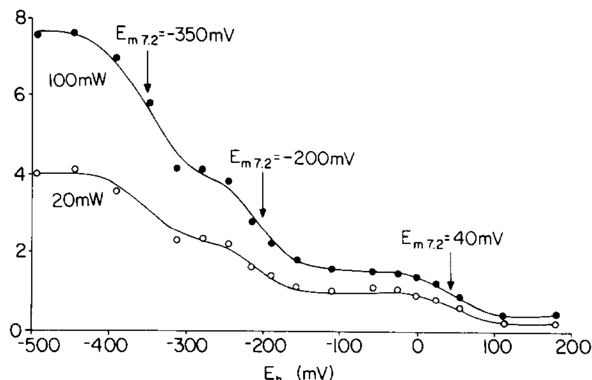

Fig.4. Redox titration of the g 1.94 signals of *Rps. spheroides.* Conditions as for fig.3A at pH 7.20 with the addition of 100 μM 2-hydroxy 14 naphthoquinone, duroquinone, pyocyanine, benzyl viologen and methyl viologen as redox mediators. Spectrometer settings: sample temperature 15°K, microwave power 20 or 100 mW as indicated.

in *Rps. spheroides* are shown in fig.4. The resolved components have half reduction potentials ($E_{m7.2}$) of −350 (±20) mV, −200 (±30) mV and +40 (±20) mV. Similar components have been titrated in *Rps. capsulata* [8] with $E_{m7.0}$ of −335 (±20) mV, −235 (±20) mV and +30 (±20) mV. These are within the redox span of the substrate couples NADH/NAD$^+$ and succinate/fumarate, so may be tentatively assigned to the bacterial NADH and succinate dehydrogenases (c.f. [2]), and perhaps indicate that these organisms possess a complement of iron-sulfur

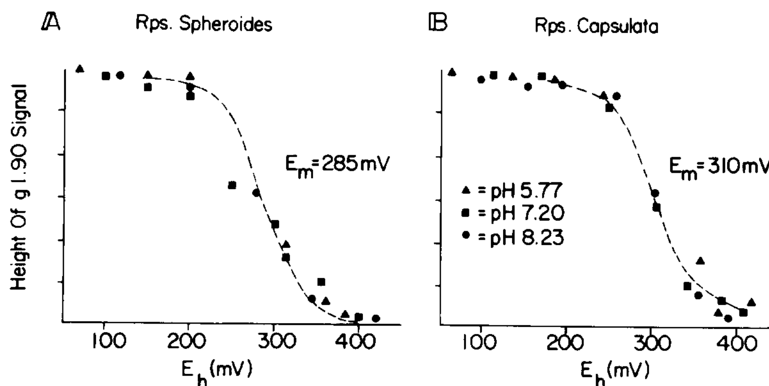

Fig.3. Redox titration of the g 1.90 signal in chromatophores from *Rps. spheroides* and *Rps. capsulate:* A) Chromatophores from *Rps. spheroides* Ga (1.32 mM BChl) were suspended in 75 mM KCl, 75 mM buffer (MES at pH 5.77, MOPS at pH 7.20 and Tris at pH 8.23) with 200 μM DAD, 150 μM PMS and PES. B) As A) buth with chromatophores from *Rps. capsulata* St. Louis (1.25 mM BChl). In both cases the spectrometer settings were those for fig.2.

proteins analogous to those found in the site I region of mitochondria [2]. However, it is obvious from the elegant and thorough work of Ohnishi [2], that in making the analogy with the mitochondrial system, many more iron-sulfur centers may be expected to be associated with the bacterial dehydrogenases.

4. Discussion

4.1. *The pH dependancy of the midpoint potential of the 'Rieske iron-sulfur center'*

It is now generally accepted that the 'Rieske center' is involved in electron flow in the cytochrome b-c_1 region of the mitochondrial electron transport chain, being reduced by succinate in an Antimycin A sensitive reaction, and oxidised by cytochrome *c* [3]. It also appears to play a similar role in *Rps. spheroides* [8], and our results implicate it in photosynthetic electron flow in this organism as well. The recent reports of the g 1.90 signal from a wide variety of biological sources [5–8] suggest that the 'Rieske center' is a ubiquitous member of electron transport chains in biological systems. On a functional level, it has recently been suggested [9,10] that the 'Rieske center' might act as the chemiosmotic hydrogen carrier in the site II region. Simple theoretical considerations (footnote* and [1]) predict that if this were the case, the half reduction potential would vary by −59 mV per unit increase of pH. The absence of a significant pH dependancy in either mitochondria or the purple non-sulfur bacteria do not support the suggestion, and in addition, the independent titrations of the purple sulfur bacterium *Chromatium* D by Dutton and Leigh [5] ($E_{m7.2}$ = 280 mV) and Evans et al. [6] ($E_{m8.0}$ = 285 mV) suggest that the protein has a pH independant half reduction potential in this organism as well. This must rule out the 'Rieske center' as a hydrogen carrier in any of these systems.

* In the reaction: $Ox + e^- + H^+ = Red\ H$

$$E_h = E_o + \frac{0.59}{n} \log \frac{[ox]\ [H^+]}{[Red\ H]}$$

where E_h is the redox potential related to the standard hydrogen electrode, E_o is the half reduction potential at pH 0, and n is the number of electrons involved in the redox reaction (in the case of the Rieske iron-sulfur center, n = 1). We can define the E_m at a pH other than zero $[E_{m(pH)}]$ as follows:

$$E_{m(pH)} = E_o + \frac{0.59}{n} \log [H^+]$$

$$= E_o - \frac{0.59}{n} pH$$

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