FURTHER STUDIES ON THE RIESKE IRON–SULFUR CENTER IN MITOCHONDRIAL AND PHOTOSYNTHETIC SYSTEMS: A p*K* ON THE OXIDIZED FORM

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1. Introduction

The Rieske iron–sulfur center, characterized by an electron paramagnetic spin resonance spectroscopic signal at g 1.90 when in the reduced form, is a widely distributed component of electron transfer systems, having been found in animal [1], yeast [2], and avian [3] mitochondria, chloroplasts [4], the purple sulfur bacterium *Chromatium vinosum* [5,6] the purple non-sulfur bacteria *Rhodopseudomonas sphaeroides* [7] and *Rps. capsulata* [8] and the green sulfur bacterium *Chlorobium limicola* f. *thiosulfatophilum* (D. B. Knaff and R. Malkin, personal communication).

In a recent paper in this journal [9] we examined the pH-dependency of the oxidation–reduction midpoint potential of the g 1.90 iron–sulfur center in pigeon heart mitochondria and chromatophores from *Rps. sphaeroides* and *Rps. capsulata,* and found that it was independent of pH between approx. pH 6 and pH 8. A similar pH independency over this range was subsequently found for the Rieske center in spinach chloroplasts [4] although in the green sulfur bacterium *Chl. limicola* f. *thiosulfatophilum* (D. B. Knaff and R. Malkin, personal communication) it was pH dependent (−60 mV/pH unit) from pH 6.8 to 8.4. More recently [10] we have found that chromatophores are stable to a much wider range of pH than we had previously examined, and this has prompted us to re-examine the pH dependency of the midpoint potential of the Rieske center. The new experiments have revealed a p*K* on the oxidized form of the iron–sulfur center at pH 8 in both *Rps. sphaeroides* chromatophores and pigeon heart mitochondria.

2. Materials and methods

The experimental methods were identical to those used in the previous paper [9].

3. Results

Figs.1 and 2 show the results of our further studies, and include the data presented in our previous paper [9]. Chromatophores from *Rps. sphaeroides* are stable from pH 4.7 to 11.0 [10] and the results in fig.1 reflect this. Pigeon heart mitochondria were not as stable, and we have only been able to use pH values within the range pH 6 to pH 9; beyond these values the mitochondrial preparations precipitated. However, this range was wide enough to demonstrate the existence of a p*K* at approx. pH 8, and a similar p*K* can be seen in *Rps. sphaeroides*.

Membranes prepared from aerobically grown

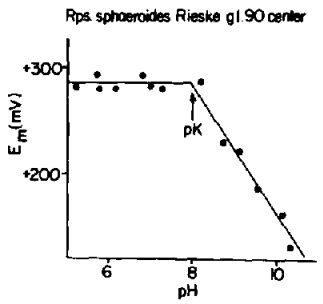

Fig.1. The midpoint potential of the Rieske g 1.90 signal in *Rps. sphaeroides* as a function of pH.

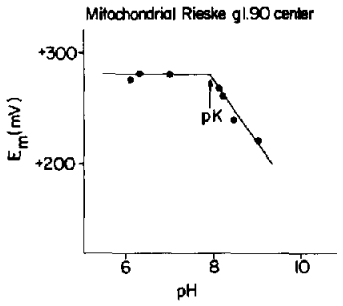

Fig.2. The midpoint potential of the Rieske g 1.90 signal in pigeon heart mitochondria as a function of pH.

Rps. sphaeroides, grown with saturating oxygen concentrations to repress the synthesis of the photosynthetic pigments, also possess a g 1.90 iron–sulfur center (O. T. G. Jones and R. C. Prince, unpublished observations), and the midpoint potential of this at pH 7.2 is also +280 mV.

4. Discussion

In our earlier paper [9] we showed that the oxidation and reduction of the Rieske center in mitochondria and chromatophores of *Rps. sphaeroides* and *Rps. capsulata* did not involve a net change in protonation between pH 6 and 8. However, figs.1 and 2 show that beyond pH 8, in both *Rps. sphaeroides* and pigeon heart mitochondria, the midpoint potential of the Rieske center becomes pH-dependent by −60 mV per pH unit, indicating that at equilibrium a proton, in addition to an electron is necessary to reduce the iron–sulfur center at alkaline pH. The course of the E_m/pH dependency lines identify p*K*s on the *oxidized forms* of the centers, so that at pH values above pH 8 the reduction of the center can be represented by,

$$FeS_{oxidized} + e^- + H^+ \rightleftharpoons FeS(H)_{reduced}$$

while below pH 8 the reaction is,

$$FeS(H^+)_{oxidized} + e^- \rightleftharpoons FeS(H)_{reduced}$$

This is the first recognition of a p*K* apparent on an iron–sulfur center. If it is a general property of g 1.90 iron–sulfur centers, and if the midpoint potential for the couple below the p*K* of the oxidized form is always approximately + 280 mV, then the data of Knaff and Malkin (personal communication) on the g 1.90 signal in *Chl. limicola* f. *thiosulfatophilum* (E_{m7} = 160 mV, pH dependent between pH 6.8 and 8.4 by −60 mV/pH unit) would suggest that the p*K* of the oxidized form of this g 1.90 center is at approximately pH 5.

The first question to be asked is whether the Rieske center is involved in electron transport, and secondly whether the p*K* has any function on the timescale of electron flow. In mitochondria, Leigh and Chance [11] have reported that the reduced Rieske center is rapidly oxidized, even at temperatures between −50 and −20°C, when carbon monoxide is flash photolyzed from the terminal oxidase in the presence of oxygen. The rate of oxidation of the Rieske center was essentially similar to the rates of oxidation of cytochromes *c* and *a* [11]. The involvement of the Rieske center is less well established in photosynthetic electron flow. In both *C. vinosum* [6] and *Rps. sphaeroides* [9] the center was oxidized if chromatophores were illuminated prior to freezing, but this involved steady state illumination, which could allow relatively slow, non-physiological redox equilibration; no experiments have been reported using single turnover activation to rigorously test its involvement.

From our current knowledge we can say that at higher pH values in mitochondria and *Rps. sphaeroides*, the Rieske center could act as a proton carrier. For a similar function at neutral and acid pH values the p*K* of the group would have to be shifted to more acid values. This could come from alterations of the electrical environment of the group under functioning conditions.

Acknowledgements

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