Biochimica et Biophysica Acta, 1102 (1992) 266–268
© 1992 Elsevier Science Publishers B.V. All rights reserved 0005-2728/92/$05.00

BBABIO 40265

Rapid Report

The pH dependence of the redox midpoint potential of the 2Fe2S cluster from cytochrome b_6f complex (the 'Rieske centre')

Wolfgang Nitschke [a], Pierre Joliot [a], Ursula Liebl [b], A. William Rutherford [b], Günter Hauska [c], Adolf Müller [d] and Astrid Riedel [d]

[a] *Institut de Biologie Physico-Chimique, Paris (France),* [b] *Section de Bioénergétique, Département de Biologie Cellulaire et Moléculaire, CE Saclay, Gif-sur-Yvette (France),* [c] *Institut für Zellbiologie und Pflanzenphysiologie, Universität Regensburg, Regensburg (Germany) and* [d] *Institut für Biophysik und Physikalische Biochemie, Universität Regensburg, Regensburg (Germany)*

(Received 23 July 1992)

Key words: Cytochrome b_6f complex; pH dependence; Iron-sulfur cluster; EPR; Redox potential

The pH dependence of the redox midpoint potential (E_m) of the Rieske centre from spinach cytochrome b_6f complex was studied by EPR. The E_m was found to be independent of pH up to pH 8 and to decrease at higher pH values. The slope of the decrease above the pK value was roughly consistent with the involvement of a dissociable proton on the oxidized form of the cluster. The E_m in the pH-independent region (i.e., < pH 8) was determined to be +320 mV. This is in contrast to the original value (E_m = +290 mV) reported by Malkin and Aparicio (Biochem. Biophys. Res. Commun. 63 (1975) 1157–1160) and confirms the results reported more recently (E_m = 310–320 mV) by Malkin (FEBS Lett. 131 (1981) 169–172) and Nitschke et al. (Biochim. Biophys. Acta 974 (1989) 223–226).

In 1976, Prince and Dutton [1] demonstrated that the redox midpoint potential (E_m) of the 2Fe2S cluster contained in cytochrome bc_1 complexes from the photosynthetic bacterium *Rhodobacter sphaeroides* and from beef heart mitochondria, often called the Rieske centre, was pH-independent up to values of about pH 8. Above pH 8, the potential decreased with a slope of −60 mV/pH unit. This experimental curve was fitted to an expression with a pK on the oxidized form of the cluster as follows:

$$2Fe2S^{ox}(H^+) + e^- \leftrightarrow 2Fe2S^{red}(H^+) \text{ below pH 8}$$

$$2Fe2S^{ox} + (H^+) + e^- \leftrightarrow 2Fe2S^{red}(H^+) \text{ above pH 8.}$$

In the pH range examined, this showed that the reduced form of the cluster exists in the protonated form, whereas in the oxidized form the proton involved can dissociate from the cluster with an apparent pK of 8.0.

Later studies, which found the same pK in titrations of a menaquinone (MK) oxidizing Rieske centre from *Thermus thermophilus* [2], led to the hypothesis that the proton involved could be located on one of the histidine residues which are proposed to be ligands to one of the Fe atoms of the cluster [3]. Recently, a similar pK value was found for the MK oxidizing Rieske centres from *Bacillus* PS3 [4] and *Heliobacterium chlorum* (Nitschke, W. and Liebl, U., unpublished data), suggesting that it might be a common feature of all Rieske centres from cytochrome *bc* complexes.

On the basis of data obtained on *Chlorobium limicola* and *Bacillus alcalophilus*, however, several authors have concluded that much lower pK values might be possible, resulting in pH dependence of the redox midpoint potential in the neutral pH range [1,5,6].

For the plant Rieske centre, Malkin and Aparicio [7] determined an E_m that was independent of pH in the range of pH 6.5 to pH 8.0. No data, however, were available for pH values above pH 8, so that it was not possible to decide whether the pH dependence of the midpoint redox potential of the Rieske centre in chloroplasts was similar to that in mitochondria and purple bacteria. Furthermore, simulations of kinetic parameters during cytochrome b_6f turnover and subsequent electron flow to PS I via plastocyanin were difficult to reconcile with the reported equilibrium E_m of the Rieske centre at pH 7 [8–10].

To provide an experimental basis for these considerations, we determined the redox midpoint potential of the Rieske cluster at several pH values in the range of

Correspondence to (present address): W. Nitschke, Institut für Botanik II, Universität Freiburg, 7800 Freiburg, Germany.

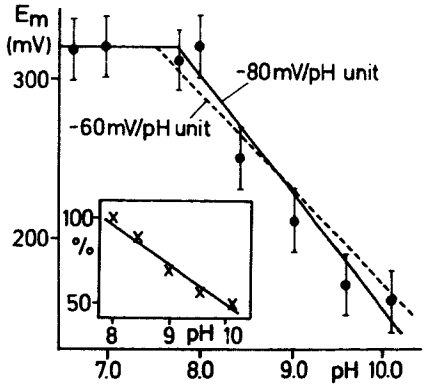

Fig. 1. E_m vs. pH dependence for the Rieske 2Fe2S cluster in isolated cytochrome b_6f complex from spinach. The individual redox titrations at each pH value were evaluated at the g_y-line. Inset: pH dependence of the maximal signal size at g_y. EPR conditions were as described in Ref. 17.

pH 6.6 to pH 10.1 in isolated cytochrome b_6f complex from spinach.

The cytochrome b_6f complex was purified according to the method described in [11]. To obtain higher concentrations, the final sucrose density gradient was replaced by a second ammonium sulphate precipitation in the presence of 0.25% cholate [9].

Redox titrations were carried out according to Ref. 12 with the following redox mediators used at 50 μM: *N,N*-dimethyl-*p*-phenylenediamine, 1,4-benzoquinone, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD), diaminodurol (DAD), 2,6-dichlorophenolindophenol (DCPIP), variamine blue, 1,2-naphthoquinone, toluylene blue, phenazine methosulphate (PMS), phenazine ethosulphate (PES), methylene blue, duroquinone. For the reductive titrations, sodium dithionite was used, oxidative titrations were done using ferricyanide. EPR spectra were taken in X-band on a Bruker ER200 spectrometer fitted with an Oxford Instruments helium cryostat.

Fig. 1 shows the E_m vs. pH relationship obtained for the Rieske centre from purified plant cytochrome b_6f complex. Above pH 8.0, the redox midpoint potential decreased with pH. Linear regression of the datapoints assuming a pK of ~ 7.8 [1,2,4], yielded a slope of −80 mV/pH unit. This is slightly higher than the value of −60 mV/pH unit, as found in purple bacteria and mitochondria [1]. Within the estimated error limits, our datapoints could possibly also accommodate a −60 mV/pH unit dependence (see Fig. 1, dotted line). It is of note, however, that recently the involvement of a second proton with a higher pK value, resulting in a slope steeper than −60 mV/pH unit has been invoked based on data obtained for the 2Fe2S cluster of the cytochrome bc_1 complex from beef heart mitochondria [13] and *Bacillus* PS3 [4].

Unexpectedly, the maximal signal size at pH 10.1 was considerably below (~ 50%) that seen at pH 8.0 (Fig. 1, inset). This effect was not due to pH-induced denaturation of a part of the sample, since the higher signal could be regained on taking the same sample back to lower pH values. A general broadening of the g_y line which would explain the decreased amplitude was not detected. Other possible mechanisms that may account for the observed changes in signal amplitude include (a) reversible pH-induced conformational changes or (b) antiferromagnetic interactions of the 2Fe2S cluster with another paramagnet, e.g., one of the mediators employed for redox potentiometry. Further work will be required in order to determine the source of this effect; however, this is beyond the scope of the present report.

The data shown above demonstrate that the redox midpoint potential of the 2Fe2S cluster from the plant cytochrome b_6f complex is pH dependent at high pH values with an apparent pK of about 8.0. Thus the plant Rieske centre shows a behaviour which is rather similar to that of the homologous clusters in mitochondria, purple [1,13] and Gram-positive bacteria (Ref. 4; Riedel, A. and Nitschke, W., unpublished data). The observed pH dependence is consistent with the involvement of roughly one proton which can dissociate from the oxidized form of the cluster. The increasing evidence for the universality of this dissociable H^+ involved in the electrochemistry of the Rieske clusters from cytochrome *bc* complexes suggests that the chemical group responsible (most likely an amino acid) plays an essential role in maintaining the particular properties of these 2Fe2S centres.

It is worthy of note that Malkin and Aparicio [7] initially determined the E_m of the Rieske centre of chloroplasts to be +290 mV at pH 7.0. In a subsequent study, Malkin [14] found a somewhat higher E_m value of +310 mV which comes close to that determined for a partially purified complex [15] and the isolated cytochrome b_6f complex [16]. The present work therefore adds weight to the correctness of the higher value (320 mV) for the isolated complex.

Thus, the difference in redox midpoint potentials between cytochrome *f* and the 2Fe2S cluster is indeed much smaller (~ 20 to 30 mV) than usually cited (~ 50 to 60 mV). According to our data, the 2Fe2S centre would still be slightly more negative than cytochrome *f*. Simulations of electron transfer equilibrium, however, predict that the 2Fe2S cluster is by about 60 mV more positive than cytochrome *f* [9,10]. A reexamination of the redox midpoint potential together with its pH dependence of cytochrome *f* might help to further clarify the remaining controversy.

The help of E. Herold in providing the required quantities of isolated cytochrome b_6f complex is gratefully acknowledged. The authors would furthermore like to thank Dr. W. Haehnel (Freiburg) for useful comments and Dr. T.A. Link (Frankfurt) for communicating results prior to publication.

References

1 Prince, R.C. and Dutton, P.L. (1976) FEBS Lett. 65, 117–119.
2 Kuila, D. and Fee, J.A. (1986) J. Biol. Chem. 261, 2768–2771.
3 Cline, J.F., Hoffman, B.M., Mims, W.B., LaHaie, E., Ballou, D.P. and Fee, J.A. (1985) J. Biol Chem. 260, 3251–3254.
4 Liebl, U., Pezennec, St., Riedel, A., Kellner, E. and Nitschke, W. (1992) J. Biol. Chem., in press.
5 Knaff, D.B. and Malkin, R. (1976) Biochim. Biophys. Acta 430, 244–252.
6 Lewis, R.J., Prince, R.C., Dutton, P.L., Knaff, D.B. and Krulwich, T.A. (1981) J. Biol. Chem. 256, 10543–10549.
7 Malkin, R. and Aparicio, P.J. (1975) Biochem. Biophys. Res. Commun. 63, 1157–1160.
8 Joliot, P. and Joliot, A. (1984) Biochim. Biophys. Acta 765, 219–226.
9 Rich, P.R., Heathcote, P. and Moss, D.A. (1987) Biochim. Biophys. Acta 892, 138–151.
10 Hope, A.B., Huilgol, R.R., Panizza, M., Thompson, M. and Matthews, D.B. (1992) Biochim. Biophys. Acta 1100, 15–26.
11 Hauska, G. (1986) Methods Enzymol. 126, 271–285.
12 Dutton, P.L. (1971) Biochim. Biophys. Acta 226, 63–80.
13 Link, T.A., Hagen, W.R., Pierik, A.J., Assmann, C. and Von Jagow, G. (1992) Eur. J. Biochem., in press.
14 Malkin, R. (1981) FEBS Lett. 131, 169–172.
15 Rich, P.R. and Bendall, D.S. (1981) Biochim. Biophys. Acta 591, 153–161.
16 Nitschke, W., Hauska, G. and Rutherford, A.W. (1989) Biochim. Biophys. Acta 974, 223–226.
17 Riedel, A., Rutherford, A.W., Hauska, G., Müller, A. and Nitschke, W. (1991) J. Biol. Chem. 266, 17838–17844.