ELSEVIER

Biochimica et Biophysica Acta 1185 (1994) 81–84

BBA Biochimica et Biophysica Acta

Two pK values of the oxidised 'Rieske' [2Fe-2S] cluster observed by CD spectroscopy

Thomas A. Link *

Universitätsklinikum Frankfurt, ZBC, Therapeutische Biochemie, D-60590 Frankfurt / Main, Germany

(Received 27 October 1993)

Abstract

The pH dependence of the CD spectrum of the oxidised 'Rieske' [2Fe-2S] cluster was measured over pH = 6.1 to 10.6. The spectral changes could be fitted with two pK values of 7.7 and 9.1. No pH dependent spectral changes were observed on the reduced protein. The far UV CD spectrum of the protein was the same at pH = 6.0 and pH = 10.7; therefore, secondary structure changes are not responsible for the changes in the CD spectrum. It is concluded that the pK values belong to groups directly associated with the [2Fe-2S] cluster, most likely to its histidine ligands.

Key words: pK value; 'Rieske' cluster; Mitochondrial bc_1 complex; Redox potential; CD spectroscopy

1. Introduction

In addition to cytochrome b and c_1, the mitochondrial bc_1 complex contains an iron sulphur protein with a high potential [2Fe-2S] cluster. Ligands of this 'Rieske' cluster are two cysteines and two non-cysteine residues, most likely two conserved histidines [1–4].

The 'Rieske' protein contains a group with a redox dependent pK value. In 1976, Prince and Dutton performed an EPR monitored redox titration of pigeon heart mitochondria and of chromatophores from *Rhodobacter sphaeroides* and found that the midpoint potential of the cluster was pH dependent at pH values greater than pH = 8 [5]. They concluded that the oxidised cluster has a pK value of approximately 8 while the reduced cluster has a pK greater than 10. Kuila and Fee confirmed these results by an optical redox titration of *Thermus thermophilus* 'Rieske' protein and by optical titration of the oxidised form [6].

Recently, we have measured the redox potential of the water soluble fragment of the 'Rieske' protein containing the intact cluster by cyclic voltammetry [7]. The pH dependence of the midpoint potential could only be fitted assuming two groups with redox dependent pK values of 7.6 and 9.2 [7]. However, optical titration of the oxidised fragment showed only a single pK value of 7.5 [7]. The optical spectrum of the oxidised fragment was pH independent above pH = 9. By EPR monitored redox titration of the 'Rieske' clusters of the bacterium PS3 and of spinach b_6f complex, Nitschke and coworkers have measured a slope of −80 mV/pH at pH above 8, indicating a stoichiometry of the redox dependent protonation greater than one H^+/e^- [8,9].

In order to study the pH dependence of the optical transitions of the 'Rieske' cluster and to characterise the redox dependent pK values, I have monitored the pH titration of the cluster by CD spectroscopy.

2. Materials and methods

The water soluble fragment of the 'Rieske' iron sulphur protein of the bc_1 complex was prepared as described previously [7]. This fragment contains the intact [2Fe-2S] cluster. The fragment was oxidised with $K_3[Fe(CN)_6]$ and desalted over a Sephadex G-25 column equilibrated with a buffer containing 10 mM each of the following: MES (pK = 6.1), MOPS (pK = 7.2), EPPS (pK = 8.0), *N*-[cyclohexyl]amino-ethanesulfonic acid (CHES; pK = 9.3), and *N*-[cyclohexyl]amino-pro-

* Corresponding author. Fax: +49 69 63016970.

Abbreviations: CD, circular dichroism; Im, imidazole. Enzyme: bc_1 complex (EC 1.10.2.2).

SSDI 0005-2728(93)E0202-2

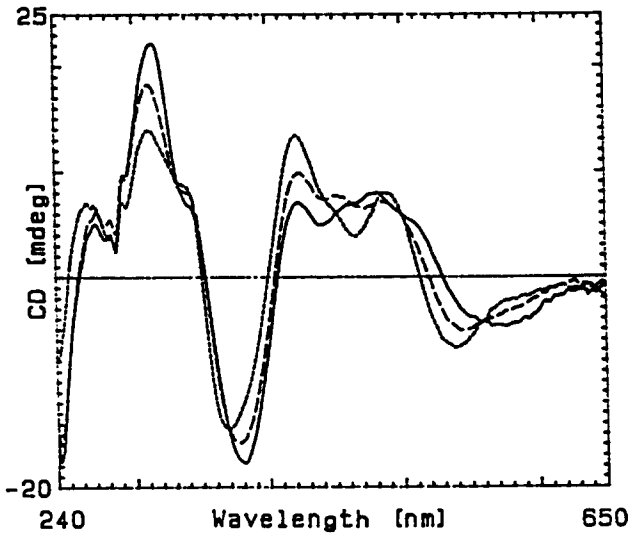

Fig. 1. CD spectrum of the oxidised 'Rieske' fragment (0.6 mg/ml) at three pH values. (———) pH = 6.1; (— — —) pH = 8.3; (· · · · · ·) pH = 10.6. Experimental conditions: bandwidth, 1.0 nm; scan speed, 50 nm/min; response, 4 s; resolution, 0.5 nm; accumulation, 1; pathlength, 10 mm; temperature, 17°C.

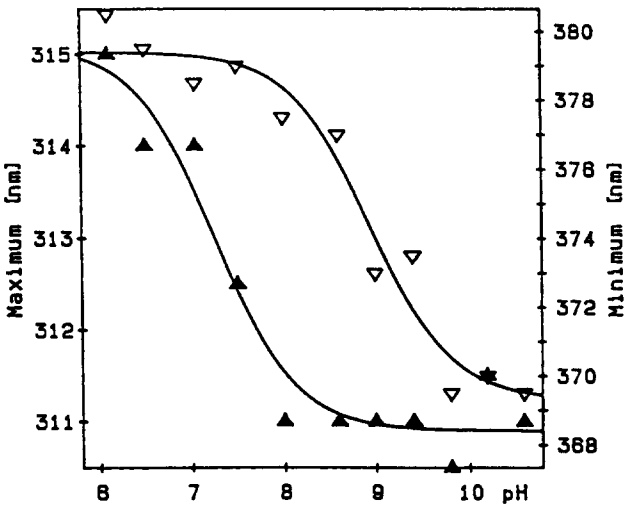

Fig. 2. pH dependence of the maximum (▲) and of the minimum (▽) of the CD spectrum. The lines show fits to the data points using pK values of 7.3 (maxima) and 9.0 (minima), respectively.

panesulfonic acid (CAPS; pK = 10.4), adjusted to pH = 6.1. The final concentration was approximately 0.6 mg/ml. 50 mM NaCl were added and the pH was titrated with 1 M NaOH.

CD spectra were recorded using a Jasco J-720 spectropolarimeter in cylindrical quartz cuvettes thermostatted to T = 17°C.

3. Results

Fig. 1 shows the CD spectrum of the isolated 'Rieske' fragment from 240 to 650 nm at three selected pH values. Above pH = 9, significant reversible changes are observed in the CD spectrum but not in the optical spectrum.

Different regions of the spectrum show different behaviour: (a) the position as well as the ellipticity of the bands around 310 nm and 375 nm changes; (b) the bands between 400 and 500 nm have different relative intensities; and (c) the redox dependent [10] band shifts from 520 to 550 nm and broadens at high pH.

Fig. 2 shows the pH dependence of the maximum around 310 nm and of the minimum around 375 nm. The maximum shifts from 315 nm at pH = 6.1 to 311 nm above pH = 8.0; the data points can be fitted with a pK of 7.3. The minimum shifts from 379 nm at pH ≤ 7.5 to 370 nm at basic pH; these data points can be fitted with a pK of 9.0.

The acidic form of the cluster has a maximum of 315 nm and a minimum of 379 nm. The basic form has a maximum of 311 nm and a minimum of 370 nm. Around pH = 8, an intermediate form exists with a maximum of 311 nm and a minimum of 379 nm.

At 465 nm, the acidic and the intermediate forms have an isosbestic point while the basic form has a trough. All three forms have a positive band at 424 nm. The pH dependence of the 424 nm band was fitted by non-linear regression (Fig. 3). Two pK values of 7.7 and 9.1 were obtained; these values are identical within 0.1 pH unit to those obtained by fitting the pH dependence of the redox potential obtained by cyclic voltammetry [7]. Identical results were obtained by fitting any of the other CD bands.

The CD spectrum of the reduced cluster was pH independent over pH = 6 to pH = 11.

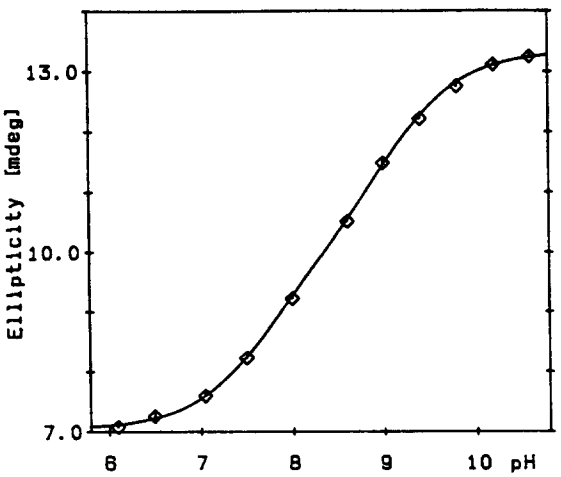

Fig. 3. pH dependence of the ellipticity at 424 nm. The line was fitted to the data points using two pK values of 7.7 and 9.1.

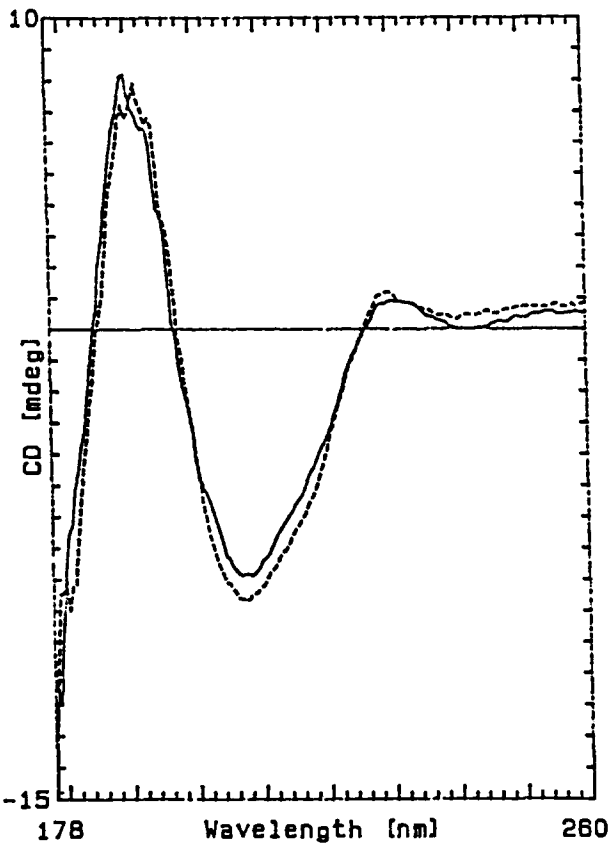

Fig. 4. Far UV CD spectrum of the oxidised 'Rieske' fragment. The fragment was measured in buffer containing 100 mM NaF, 10 mM NaH_2PO_4, and 10 mM $NaBO_3$. (———) pH = 6.0; (· · · · · ·) pH = 10.7. Experimental conditions: bandwidth, 1.0 nm; scan speed, 20 nm/min; response, 4 s; resolution, 0.5 nm; accumulation, 1; path-length, 0.1 mm; temperature, 17°C.

4. Discussion

The data give significant independent evidence for the existence of two redox dependent pK values of 7.7 and 9.1 on the oxidised form; the pK values on the reduced cluster are greater than 11.

In spinach ferredoxin, reversible secondary structure changes have been observed at alkaline pH [11]. In the 'Rieske' protein, no structural changes are observed in the far UV CD spectrum between pH = 6.0 and 10.7 (Fig. 4). Therefore, the pK values must belong to groups closely associated with the redox cluster. The most likely candidates are the two histidine ligands of the [2Fe-2S] cluster.

In the four cysteine coordinated [2Fe-2S] clusters, bands between 350 and 525 nm have been assigned to S → Fe(III) charge transfer bands [12,13]. Although a definite assignment of the bands of the 'Rieske' cluster has not been performed, it is reasonable to assume that the bands of the 'Rieske' cluster will also belong to charge transfer bands within the cluster. The electronic transitions at different pH values will then monitor the shifts of the electronic energy levels induced by protonation/deprotonation of the imidazole rings. The fact that the bands at 310 and 375 nm show independent behaviour with pH indicates that the pH effect is not a general electrostatic effect on the cluster. This assignment would also explain the strong redox dependence of the pK values shown by the shift of more than three pH units upon reduction (from 7.7 to more than 11). Large structural effects during the redox reaction can be excluded, since the entropy of the reaction was found to be similar to that of cytochrome c [7].

A similar conclusion has been drawn for the oxidised *Thermus* 'Rieske' protein where ionisation led to changes of the Mößbauer isomer shifts and of the quadrupole splittings of the site-1 iron [6] bound to the non-cysteine ligands [14].

Three regions can be formally defined in the titration curve:

$$\text{Low pH: } [Fe_2S_2]^0_{ox}\begin{matrix}\text{Im}'\overline{\text{H}}\rceil^0\\ \text{Im}''\text{H}\end{matrix} + e^- \xrightarrow{pH<6.5} [Fe_2S_2]^-_{red}\begin{matrix}\text{Im}'\overline{\text{H}}\rceil^-\\ \text{Im}''\text{H}\end{matrix}$$

$$\text{Intermediate pH: } [Fe_2S_2]^0_{ox}\begin{matrix}\text{Im}'^{-}\rceil^-\\ \text{Im}''\text{H}\end{matrix} + e^- + H^+ \xrightarrow{pH\approx 8} [Fe_2S_2]^-_{red}\begin{matrix}\text{Im}'\overline{\text{H}}\rceil^-\\ \text{Im}''\text{H}\end{matrix}$$

$$\text{High pH: } [Fe_2S_2]^0_{ox}\begin{matrix}\text{Im}'^{-}\rceil^{2-}\\ \text{Im}''^{-}\end{matrix} + e^- + 2H^+ \xrightarrow{pH>9} [Fe_2S_2]^-_{red}\begin{matrix}\text{Im}'\overline{\text{H}}\rceil^-\\ \text{Im}''\text{H}\end{matrix}$$

Low pH refers to pH < 6.7 where both imidazoles are fully protonated; high pH refers to pH > 10.1 where both are fully deprotonated. At intermediate pH around 8.4, only one imidazole is protonated. The three forms of the oxidised protein correspond roughly to the spectra shown in Fig. 1, although the intermediate spectrum contains approximately 10% each of the acidic and of the basic forms.

5. Acknowledgements

I thank G. Beyer for excellent technical assistance and Prof. Dr. G. von Jagow for his support and for

critically reading the manuscript. This work was supported by a grant from the Deutsche Forschungsgemeinschaft (DFG), Priority Programme 'Transition Metals in Biology and their Coordination Chemistry' (Grants Li 474/2-2 and 2-3).

6. References

[1] 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.
[2] Telser, J., Hoffman, B.M., LoBrutto, R., Ohnishi, T., T'sai, A.-I., Simpkin, D. and Palmer, G. (1987) FEBS Lett. 214, 117–121.
[3] Gurbiel, R.J., Batie, C.J., Sivaraja, M., True, A.E., Fee, J.A., Hoffman, B.M. and Ballou, D.P. (1989) Biochemistry 28, 4861–4871.
[4] Britt, R.D., Sauer, K., Klein, M.P., Knaff, D.B., Kriauciunas, A., Yu, C.-A., Yu, L. and Malkin, R. (1991) Biochemistry 30, 1892–1901.
[5] Dutton, P.L. and Wilson, D.F. (1974) Biochim. Biophys. Acta 346, 165–212.
[5] Prince, R.C. and Dutton, P.L. (1976) FEBS Lett. 65, 117–119.
[6] Kuila, D. and Fee, J.A. (1986) J. Biol. Chem. 261, 2768–2771.
[7] Link, T.A., Hagen, W.R., Pierik, A.J., Assmann, C. and Von Jagow, G. (1992) Eur. J. Biochem. 208, 685–691.
[8] Nitschke, W., Joliot, P., Liebl, U., Rutherford, A.W., Hauska, G., Müller, A. and Riedel, A. (1992) Biochim. Biophys. Acta 1102, 266–268.
[9] Liebl, U., Pezennec, S., Riedel, A., Kellner, E. and Nitschke, W. (1992) J. Biol. Chem. 267, 14068–14072.
[10] Degli Esposti, M., Ballester, F., Solaini, G. and Lenaz, G. (1987) Biochem. J. 241, 285–290.
[11] Hasumi, H. (1986) in Iron-Sulphur Protein Research (Matsubara, H. et al., eds.), pp. 83–90, Japan Sci. Soc. Press, Tokyo.
[12] Fu, W., Drozdzewski, P.M., Davies, M.D., Sligar, S.G. and Johnson, M.K. (1992) J. Biol. Chem. 267, 15502–15510.
[13] Noodleman, L. and Baerends, E.J. (1984) J. Am. Chem. Soc. 106, 2316–2327.
[14] Fee, J.A., Findling, K.L., Yoshida, T., Hille, R., Tarr, G.E., Hearshen, D.O., Dunham, W.R., Day, E.P., Kent, T.A. and Münck, E. (1984) J. Biol. Chem. 259, 124–133.