# Rieske Iron–Sulfur Proteins From Extremophilic Organisms

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Proteins located on the outside of the membranes of organisms thriving under extreme conditions like high or low pH, or high salinity face special challenges maintaining their structural integrity. This review is focused on the Rieske iron–sulfur proteins from these organisms. Rieske proteins are essential subunits of the cytochrome *bc*-complexes, which are often of crucial importance for the energy metabolism of the cells. On the basis of the available data we propose strategies by which these proteins are able to stabilize their noncovalent bound cofactor and adapt to the function under extreme conditions.

**KEY WORDS:** Rieske protein; iron–sulfur cluster; stability; redox potential; structure.

## INTRODUCTION

Extremophilic organisms have received extensive interest as sources of stable enzymes for industrial production as well as for laboratory research. Numerous research programs have been launched to study the nature of the stabilizing effects that enable the proteins from these organisms to function under extreme conditions. The high growth temperatures of thermo- and hyperthermophilies represent more or less comparable stability problems to all cellular components, Whereas, the proteins located on the outside of the membranes, or within the periplasmatic spaces of acido-, alcalo-, or halophiles are exposed to even more challenging conditions. Prominent members of the later group are many transport proteins as well as the proteins of the respiratory and photosynthetic electron transfer chains. Central components of many of these electron transfer chains are cytochrome $bc_1$- and the related $b_6 f$-complexes. The consensus minimal structure of these complexes consists of three ($bc_1$) to four ($b_6 f$) subunits: A membrane intrinsic low potential *di* heme *b*-type cytochrome which is split into two subunits, cytochrome $b_6$ and subunit IV in the $b_6 f$-complexes, a high potential mono heme cytochrome consisting of a single trans membrane helix acting as a membrane anchor and of a globular domain containing the heme group and the Rieske iron–sulfur protein. The Rieske protein dis-plays the same general architecture as the high-potential cytochrome. The globular, cofactor containing domains of the later two subunits are located on the outside of the plasma membrane, or the equivalent side of the mito-chondrial or the chloroplast membranes. The mechanism and thermodynamics of the cytochrome *bc*-complexes have been extensively studied (Bartoschek *et al.*, 2001; Brandt, 1996; Darrouzet *et al.*, 2001). They function as quinol:acceptor oxidoreductases and contribute to the formation of the proton motive force across the membranes. Essential for the mechanism of the Q-cycle is the existence of two quinone-binding sites. The quinone reduction site $Q_i$ is located close to the inside (or the equivalent) of the cells and is exclusively formed by the transmembrane subunits cytochrome *b*, or cytochrome $b_6$ and subunit IV in the case of the $b_6 f$-complexes. The quinol oxidation site $Q_o$ is formed by the *b*-type cytochrome and the Rieske iron–sulfur protein. It is located in close proximity to the outside of the membrane. Critical steps for the function of the enzyme are the deprotonation and the initial oxidation of the quinol by the Rieske iron–sulfur cluster as well as the subsequent large scale movement of the globular domain of the Rieske protein from a position close to the *b*-type cytochrome (*b* position) to a position close to the hem group of the high-potential cytochrome ($c_1$ position) (Darrouzet *et al.*, 2001). The majority of the studies on the cytochrome $bc_1$ and related complexes have been performed with enzymes from mesophilic organisms. Previous studies focused on the phylogeny of the Rieske proteins (Schmidt and Shaw 2001) and

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other subunits of cytochrome *bc*-complexes (Hiller *et al.*, 2003; Schütz *et al.*, 2000) have provided clear evidence that cytochrome *bc*-related complexes are widely spread among extremophilic organisms from the bacterial as well as the archaeal kingdom. Aim of this review is to compile the available data about the structures and function of the Rieske iron–sulfur subunits of cytochrome *bc*-complexes from extremophilic organisms and to discuss the adaptations of these proteins to their function under extreme environmental conditions.
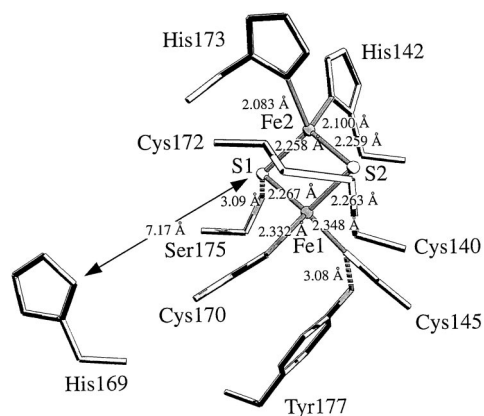
### THE IRON–SULFUR CLUSTER

Typical for a Rieske iron–sulfur cluster is the mixed coordination of one of the iron ions (Fig. 1). The replacement of two of the sulfur ligands by nitrogens form the histidine side chains is responsible for the distinctive spectroscopic properties of these clusters as well as for the relatively high redox potential compared to the exclusively sulfur coordinated [2Fe–2S] ferredoxins (Link 1999). Structural data at atomic resolution are currently available for the Rieske proteins from the thermophilic bacterium *Thermus thermophilus* (Hunsicker-Wang *et al.*, 2003), the hyper thermoacidophilic archaeon *Sulfolobus acidocaldarius* (Bönisch *et al.*, 2002) as well as for the proteins from mitochondria with the protein from bovine heart (Iwata *et al.*, 1996) representing the prototype and chloroplasts (Carrell *et al.*, 1997) from mesophilic organisms. The structure of the [2Fe–2S] is almost identical in all studied proteins (Hunsicker-Wang *et al.*, 2003), despite the dif-
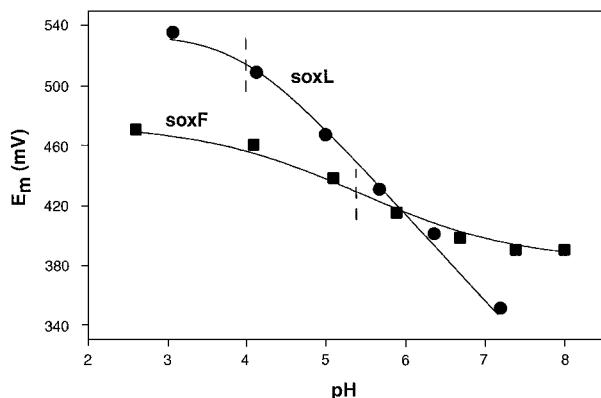


**Fig. 1.** Iron–sulfur cluster of a Rieske protein shown on the example of the soxF protein from *S. acidocaldarius*. The numbers indicate the bond length within the iron–sulfur cluster. The hydrogen bonds between Tyr177 and Cys145 as well as Ser175 and S1 of the FeS cluster are shown as discontinuous lines.

ferent electrochemical properties, quinone-substrates and the different growth conditions of the organisms (Table I).

### ELECTROCHEMICAL PROPERTIES

A typical feature of the Rieske protein subunits of the cytochrome $bc_1$-/$b_6 f$-complexes is their pH-dependent midpoint potential ( Brugna *et al.*, 1999; Link *et al.*, 1996; Zu *et al.*, 2001). At least one, in many cases two $pK_{ox}$ values associated with the reduction of the FeS-cluster have been determined (Table I). These $pK_{ox}$ values have been assigned to the protonation of the N$\varepsilon$2 atoms of the histidine ligands of the FeS cluster (Covián and Moreno-Sánchez 2001; Link 1997). The first $pK_{ox}$ is in the range of 7.6–8.1 for all proteins except from those isolated from acidophilic organisms. The second $pK_{ox}$ is significantly higher usually in the range of 8.6–9.7. Additional effects of protonizable groups not directly connected to the FeS cluster with p$K$ values in the range of 5–6 have been detected in the cases of the *Aquifex* petA Rieske protein (Schütz *et al.*, 2003) and the protein from *Thermus* (Zu *et al.*, 2001). The most outstanding properties could be observed for the two Rieske proteins from *S. acidocaldarius* (Fig. 2). The midpoint potential of the soxL protein displays a normal pH dependence. However, the $pK_{ox}$ is shifted to 4.0. In contrast, the pH-dependence of the midpoint potential of the soxF protein is untypical for a Rieske protein. It can be described as a pH-dependent conversion between a high (+475 mV) and a low potential form (+385 mV) with an apparent p$K$ of 5.4. These data could be explained assuming an upshift of the first $pK_{ox}$ to a value above 8 in combination with the effect of a protonizable group not directly connected to the FeS cluster as discussed for the *Aquifex* and *Thermus* proteins. A likely candidate for this group is His169, which is located at a distance of 7.2 Å from the FeS cluster (Fig. 1). The protonation of the imidazol ring creates a positive charge in the proximity of the FeS cluster that would lead to an upshift of the redox potential due to electrostatic interactions with the cluster. A corresponding histidine residue (His134) is also present in the *Aquifex* petA protein and could be responsible for one of the additional p$K$ values observed for this protein. However, no corresponding histidine residue could be identified in the *Thermus* Rieske protein. The downshift of the redox p$K$ values as observed for the soxL protein and the protein from *Acidithiobacillus* was previously discussed as one of the factors contributing to the stability of the iron–sulfur cluster of the Rieske proteins from acidophiles (Brugna *et al.*, 1999). Thus, other stabilizing factors may be involved in the stabilization of the FeS cluster of the soxF protein (compare below).

**Table I.** Properties of Rieske Proteins From Extremophilic Organisms and Their Quinone Substrates

| Organism | | $E_m$ (low pH) [mV] | $pK_{ox1}$ | $pK_{ox2}$ | Quinone/$E_m$ (mV) | Solvent accessible surface of the His N$\epsilon$2 atoms (Å²) | | References |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Loop 1 | Loop 2 | |
| SoxF *Sulfolobus acidocaldarius* | Thermo acidophilic | +475 | 5.4 | — | Qcal/+103$_{pH6.5}$ [a] +343 at pH 2.5 | His142: 18.7 | His173: 20.2 | This study |
| SoxF *Sulfolobus acidocaldarius* | Thermo acidophilic | +573 | 4.0 | — | Qcal/+103$_{pH6.5}$ [a] +343 at pH 2.5 | | | This study |
| Sulredoxin *Sulfolobus* strain 7 | Thermo acidophilic | +188 | 6.2 | 8.6 | Qcal/+103$_{pH6.5}$ [a] +343 at pH 2.5 | | | Iwasaki *et al.* (1996) |
| *Pyrobaculum aerophilum* | Thermophilic | +224 | 8.1 | 9.8 | MQ/−74 [b] | | | Henninger *et al.* (1999) |
| *Halobacterium salinarum* | Halophilic | +175$_{pH7.4}$ | | | MQ/−74 [b] | | | Personal communication, Dr. S. Anemüller, Lübeck, Germany |
| PetA *Aquifex aeolicus* | Thermophilic | +250 | 7.6 | 9.3 | MQ/−74 [b] | | | Schütz *et al.* (2003) |
| *Aquifex aeolicus* (low potential RFes) | Thermophilic | +95 | 7.6 | — | MQ/−74 [b] | | | Schütz *et al.* (2003) |
| *Thermus thermophilus* | Thermophilic | +140 | 7.9 | 9.7 | MQ/−74 [b] | His134: 15.9 | His154: 25.0 | Zu *et al.* (2001) |
| *Acidothiobacillus ferrooxidans* | Acidophilic | +490 | 6.2 | — | UQ/+84$_{pH7.5}$ [c] +384 at pH 2.0 | | | Brugna *et al.* (1999) |
| *Bacillus alcalophilus* | Alcalophilic | +150$_{pH7.0}$, +75$_{pH9.9}$ | 7.6 | — | UQ/+84[c] − 96 at pH 10.0 | | | Lewis *et al.* (1981) |
| *Bos taurus* | Mesophilic | +311 | 7.6 | 9.2 | UQ/+84[c] | His141: 19.2 | His161: 25.6 | Link *et al.* (1992) |
| *Spinacia oleracea* | Mesophilic | +359 | 6.5/8 | — | PQ/+100[d] | His109: 20.7 | His128: 27.9 | Zhang *et al.* (1996)/ Brugna *et al.* (1999) |

[a] Caldariella quinone (Schäfer *et al.*, 1999).
[b] Menaquinone (Kröger and Unden, 1985).
[c] Ubiquinone (de Vries *et al.*, 1982).
[d] Plastoquinone (Vener *et al.*, 1997), values in the range from +70 +112 mV for ubiquinone and +80 to +120 mV for plastoquinone can be found in the literature.

A further adaptation to the function of the Rieske proteins at extreme pH values is evident from the midpoint potentials listed in Table I. The potentials of proteins from the acidophiles *Sulfolobus* and *Acidithiobacillus* are significantly more positive than those from chloroplasts and mitochondria, whereas the potential of the alcalophile *Bacillus alcalophilus* is untypical low for an ubiquinol containing organism (Brugna *et al.*, 1999). However, the comparison of the pH dependencies of the midpoint potentials of the Rieske proteins with the potential of the ubiquinone/ubiquinol couple demonstrates that *B. alcalophilus* protein is capable to oxidize ubiquinol at high pH (Fig. 3). Since a sufficient difference between the redox potentials of the Rieske protein and the quinol is a prerequisite for the function of a cytochrome $bc_1$-complex (Denke *et al.*, 1998), it is obvious from Fig. 3 that the *B. alcalophilus* cytochrome $bc_1$-complex will be inactive at neutral pH. The incompatibility of the cytochrome $bc_1$-complex with the growth under neutral conditions and a consequential down regulation of the synthesis of its subunits provides a further explanation for the absence of the Rieske protein from the membranes of the nonalcalophilic *B. alcalophilus* mutant strain KM23 (Lewis *et al.*, 1981).

## RIESKE PROTEINS NOT ASSOCIATED WITH CYTOCHROME *bc*-COMPLEXES

A large number of Rieske-type proteins, which are not subunits of cytochrome *bc*-complexes, are known from mesophilic organisms (Schmidt and Shaw 2001). These proteins display the same mixed coordination of the FeS cluster as the Rieske proteins as well as the characteristic EPR spectra (Link 1999). They differ from the Rieske pro-
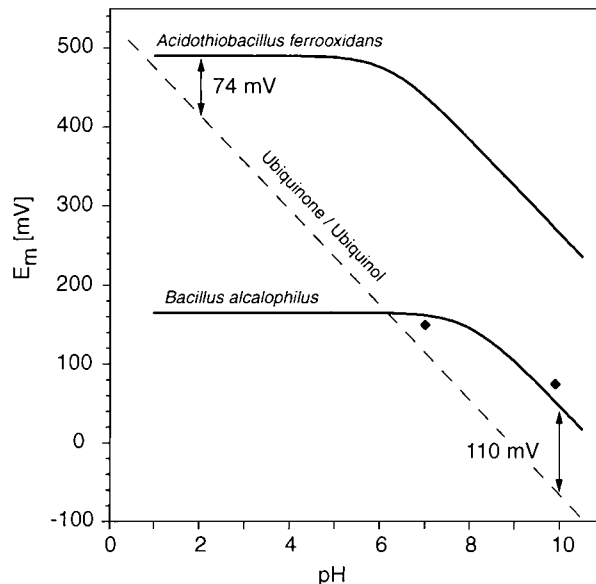


**Fig. 3.** Comparison of the pH dependencies of the Rieske proteins from *A. ferrooxidans, B. alcalophilus*, and ubiquinol. The diamonds indicate the midpoint potentials of the *B. alcalophilus* protein according to Lewis *et al.*, (1981). The pH dependence was extrapolated from these points assuming a single $pK_{ox}$ of 8.0 and an $E_{m,lowpH}$ of +165 mV. The arrows indicate the differences between the midpoint potentials of ubiquinol and the Rieske proteins at the pH of the growth media.

teins by their lower ($-100$ to $-150$ mV) and usually pH-independent midpoint potentials (Link 1999). Whereas the majority of these proteins are associated with bacterial oxigenases, several eucaryal members of this group of proteins are known as well (Schmidt and Shaw 2001).

Two Rieske, or Rieske-type proteins, not associated with cytochrome *bc*-complexes have been described from extremophilic organisms. These are a soluble protein named Sulredoxin isolated from Sulfolobus strain 7 (Iwasaki *et al.*, 1995, 1996) and a Rieske protein detected in the membranes of *Aquifex aeolicus* (Schütz *et al.*, 2003). Both proteins display relatively high, pH-dependent midpoint potentials (Table I) and may represent an intermediate from between the Rieske and Rieske-type proteins. Their physiological function remains to be established.

## STABILITY

Figure 4 compares the temperature and pH stability of the iron–sulfur clusters from the isolated, recombinant *S. acidocaldarius* and *P. aerophilum* Rieske proteins. parR and soxF display the same thermostability, whereas soxL loses about 70% of its iron–sulfur cluster within 10 min at 80°C (Fig. 4(a)). Thus, soxL may be stabilized in vivo by interactions with other subunits of the soxLN-
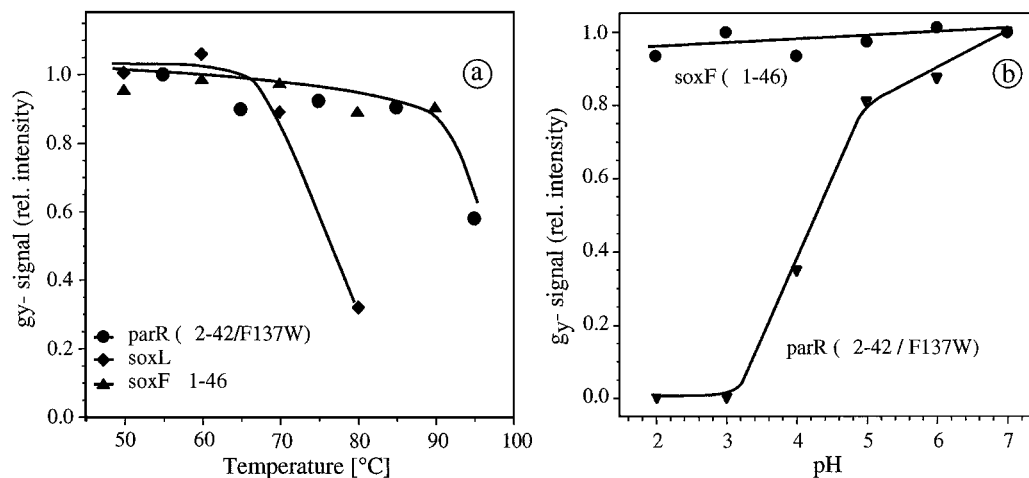
**Fig. 4.** (a) Temperature stability of the FeS clusters of the Rieske proteins soxF and soxL from *S. acidocaldarius* and parR from *P. aerophilum*. The proteins were incubated in 50 mM Tris HCl, pH 7.5, for 10 min at the indicated temperatures. Subsequently ascorbate was added to a final concentration of 5 mM and the samples analyzed by EPR spectroscopy, using the $g = 1.89$ to quantify the concentration of the FeS cluster as described by Schmidt *et al.*, (1995). (b) pH stability of the FeS clusters of soxF and parR. The proteins were incubated for 15 min at 45°C in a solution containing 50 mM acetic acid and 50 mM sodium phosphate adjusted to the indicated pH with NaOH and analyzed for the concentration of the FeS cluster as above.

complex (Hiller *et al.*, 2003). soxF and parR differ dramatically with respect to the acid stability of their FeS clusters (Fig. 4(b)). The parR FeS cluster displays the same acid sensitivity as it was described for Rieske proteins from mesophilic organisms (Brugna *et al.*, 1999). In contrast, the FeS cluster of soxF withstands pH values as low as 2. This acid stability is well in line with the proposed location of the FeS cluster on the outside of the *Solfolobus* membrane (Brugna *et al.*, 1999). As mentioned above, the structure of the soxF FeS cluster itself provides no explanation for its increased stability. Especially there is no indication for the previously (Brugna *et al.*, 1999) proposed shielding of the N$\varepsilon$2 atoms of the histidine ligands from the solvent (Table I). Thus, we conclude that the stability of the FeS cluster within the soxF protein is a mere consequence of the stability of the protein structure itself.

All Rieske proteins share the same principle structure (Bönisch *et al.*, 2002; Carrell *et al.*, 1997; Hunsicker-Wang *et al.*, 2003; Iwata *et al.*, 1996). The globular domain is build of a large subdomain consisting of an irregular $\beta$-barrel and at least one $\alpha$-helix (Bönisch *et al.*, 2002; Carrell *et al.*, 1997; Hunsicker-Wang *et al.*, 2003; Iwata *et al.*, 1996) and a small iron–sulfur cluster binding subdomain consisting of a single antiparallel $\beta$-sheet. Significant differences between the various Rieske proteins exist in the size and the structures of the loops between the individual $\beta$-strands (Bönisch *et al.*, 2002; Hunsicker-Wang *et al.*, 2003). Especially the loops between the strands 3–4, 4–5, and 5–6 are significantly enlarged in the *Sulfolobus*

soxF protein (Bönisch *et al.*, 2002; Hunsicker-Wang *et al.*, 2003). Because of these additional sequence elements the structure of soxF is larger and more compact than those of the other Rieske proteins (Fig. 5(a) and Hunsicker-Wang *et al.*, 2003). The most significant differences between
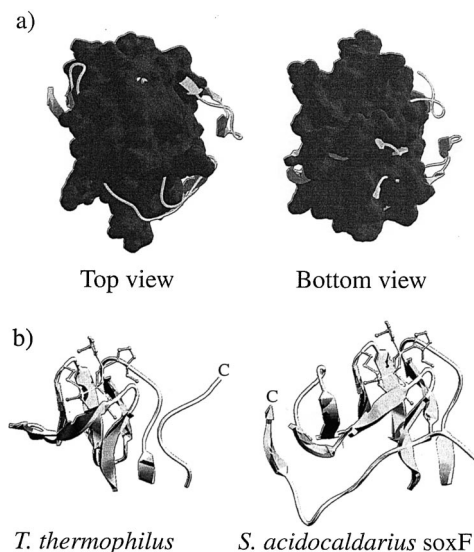


**Fig. 5.** Comparison of the structures of the Rieske proteins from *T. thermophilus* and soxF from *S. acidocaldarius*. (a) Overlay of the structures. The diagram shows the enlarged loops of soxF protein protruding from the calculated surface of the *T. thermophilus* protein. The C- and N-termini of soxF are marked as "C" and "N," respectively. (b) Comparison of the iron–sulfur cluster binding subdomains of the two proteins.

the structures of soxF and all other Rieske proteins are present in the FeS cluster binding subdomain. It is built of 7 $\beta$-strands in soxF, one of which is located at the very C-terminus of the protein, whereas this subdomain consists of only four $\beta$-strands in all other Rieske proteins (Fig. 5(b)) (Bönisch *et al.*, 2002; Hunsicker-Wang *et al.*, 2003). This unique structure may contribute to the acid stability of the soxF FeS cluster. Another unique feature, which also may be related to the acid stability of soxF, is the high abundance of acidic residues at the surface of the protein resulting in a strong negative surface charge at neutral pH (Bönisch *et al.*, 2002) (Fig. 6).

Summarizing, it can be concluded that the currently available data provide some insights into the strategies by which the Rieske proteins are adapted to the function under extreme conditions. These strategies include the adjustment of the midpoint potential to compensate for the pH-dependent change of the potential of the quinol and a downshift of the first $pK_{ox}$ value in the case of some acidophilic organisms. The pronounced negative surface charge of soxF and the extended of the structure iron–sulfur-cluster-binding domain may represent further stabilizing factors for proteins from acidophilic organisms. However, these conclusions have to be drawn with caution. Special adaptations of the protein structures to the functional contexts of the proteins, i.e., the other subunits of the cytochrome *bc*-related complexes have to be considered as well. This applies especially to cases like the *Sulfolobus* proteins, which are (soxF) (Komorowski *et al.*, 2002), or may be (soxL) (Hiller *et al.*, 2003) subunits of a terminal oxidase supercomplex instead of a classical cytochrome *bc*-complex.

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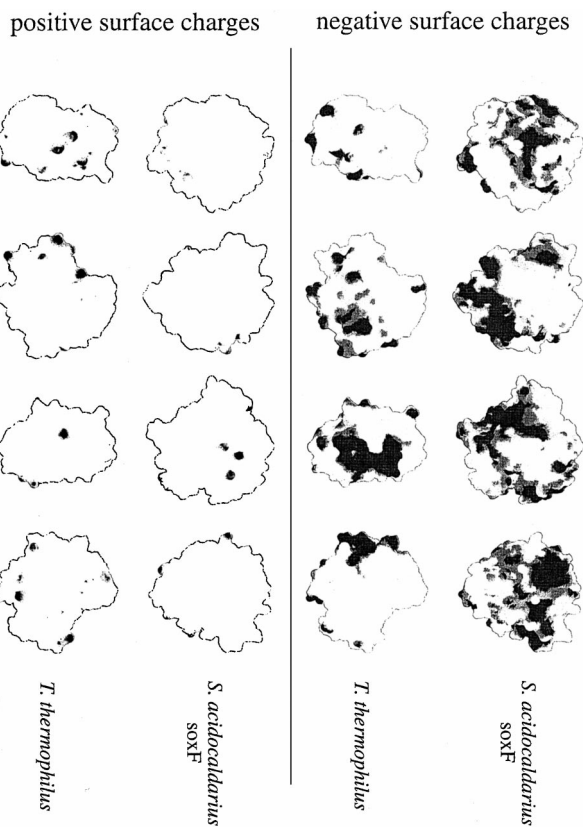
**Fig. 6.** Surface charges of soxF and the Thermus Rieske protein calculated at pH 7.0. The charges were calculated using the program "Deep View/Swiss-PdbViewer" Version 3.7 (Guex and Peitsch, 1997).

## REFERENCES

Bartoschek, S., Johansson, M., Geierstanger, B. H., Okun, J. G., Lancaster, C. R. D., Humpfer, E., Yu, L., Yu, C.-A., Griesinger, C., and Brandt, U. (2001). *J. Biol. Chem.* **276**, 35231–35234.
Bönisch, H., Schmidt, C. L., Schäfer, G., and Ladenstein, R. (2002). *J. Mol. Biol.* **319**, 791–805.
Brandt, U. (1996). *FEBS Lett.* **387**, 1–6.
Brugna, M., Nitschke, W., Asso, M., Guigliarelli, B., Lemesle-Meunier, D., and Schmidt, C. L. (1999). *J. Biol. Chem.* **274**, 16766–16772.
Carrell, C. J., Zhang, H., Cramer, W. A., and Smith, J. L. (1997). *Structure* **5**, 1613–1625.
Covián, P., and Moreno-Sánchez, R. (2001). *Eur. J. Biochem.* **268**, 5783–5790.
Darrouzet, E., Moser, C. C., Dutton, P. L., and Daldal F. (2001). *TIBS* **26**, 445–451.
Denke, E., Merbitz-Zahradnik, T., Hatzfeld, O. M., Snyder, C. H., Link, T. A., and Trumpower, B. L. (1998). *J. Biol. Chem.* **273**, 9085–9093.
de Vries, S., Berden, J. A., and Slater, E. C. (1982). In *Function of Quinones in Energy Conserving Systems: Function and Properties of a Semiquinone Anion Located in the $QH_2$:Cytochrome c Oxidoreductase Segment of the Respiratory Chain* (Trumpower, B. L., ed.) Academic Press, New York, pp. 235–246.
Guex, N., and Peitsch, M. C. (1997). *Electrophoresis* **18**, 2714–2723.
Henninger, T., Anemüller, S., Fitz-Gibbon, S., Miller, J. H., Schäfer, G., and Schmidt, C. L. (1999). *J. Bioenerg. Biomembr.* **31**, 119–128.
Hiller, A., Henninger, T., Schäfer, G., and Schmidt, C. L. (2003). *J. Bioenerg. Biomembr.* **35**, 121–131.
Hunsicker-Wang, L. M., Heine, A., Chen, Y., Luna, E. P., Todaro, T., Zhang, Y. M., Williams, P. A., McRee, D. E., Hirst, J., Stout, C. D., and Fee, J. A. (2003). *Biochemistry* **42**, 7303–7317.
Iwasaki, T., Imai, T., Urushiyama, A., and Oshima, T. (1996). *J. Biol. Chem.* **271**, 27659–27663.
Iwasaki, T., Isogai, Y., Iizuka, T., and Oshima, T. (1995). *J. Bacteriol.* **177**, 2576–2582.

Iwata, S., Saynovits, M., Link, T. A., and Michel, H. (1996). *Structure* **4**, 567–579.

Komorowski, L., Verheyen, W., and Schäfer, G. (2002). *Biol. Chem.* **383**, 1791–1799.

Kröger, A., and Unden, G. (1985). In *Coenzyme Q*: *The Function of Menaquinone in Bacterial Electron Transport* (Lenaz, G., ed.), Wiley, Chichester, UK, pp. 285–256.

Lewis, R. J., Prince, R. C., Dutton, P. L., Knaff, D. B., and Krulwich, T. A. (1981). *J. Biol. Chem.* **256**, 10543–10549.

Link, T. A. (1997). *FEBS Lett.* **412**, 257–264.

Link, T. A. (1999). In *Advances in Inorganic Chemistry: The Structures of Rieske and Rieske-Type Proteins* (Sykes, A. G., and Cammack, R., eds.), Academic Press, San Diego, CA, Vol. 47, pp. 83–157.

Link, T. A., Hagen, W. R., Pierik, A. J., Assmann, C., and von Jagow, G. (1992). *Eur. J. Biochem.* **208**, 685–691.

Link, T. A., Hatzfeld, O. M., Unalkat, P., Shergill, J. K., Cammack, R., and Mason, J. R. (1996). *Biochemistry* **35**, 7546–7552.

Schäfer, G., Engelhard, M., and Müller, V. (1999). *Microbiol. Mol. Biol. Rev.* **63**, 570–620.

Schmidt, C. L., Anemüller, S., Teixeira, M., and Schäfer, G. (1995). *FEBS Lett.* **359**, 239–243.

Schmidt, C. L., and Shaw, L. (2001). *J. Bioenerg. Biomembr.* **33**, 9–26.

Schütz, M., Brugna, M., Lebrun, E., Baymann, F., Huber, R., Stetter, K. O., Hauska, G., Toci, R., Lemesle-Meunier, D., Tron, P., Schmidt, C., and Nitschke, W. (2000). *J. Mol. Biol.* **300**, 663–675.

Schütz, M., Schoepp-Cothenet, B., Lojou, E., Woodstra, M., Lexa, D., Tron, P., Dolla, A., Durand, M.-C., Stetter, K. O., and Baymann, F. (2003). *Biochemistry.* **42**, 10800–10808.

Vener, A. V., van Kan, P. J. M., Rich, P. R., Ohad, I., and Andersson, B. (1997). *Proc. Natl. Acad. Sci. U.S.A.* **94**, 1585–1590.

Zhang, H., Carrell, C. J., Huang, D., Sled, V., Ohnishi, T., Smith, J. L., and Cramer, W. A. (1996). *J. Biol. Chem.* **271**, 31360–31366.

Zu, Y., Fee, J. A., and Hirst, J. (2001). *J. Am. Chem. Soc.* **123**, 9906–9907.