Structure-function studies of iron-sulfur clusters and semiquinones in the NADH-Q oxidoreductase segment of the respiratory chain. Biochim Biophys Acta

ARTICLE *in* BIOCHIMICA ET BIOPHYSICA ACTA · JULY 1998

Impact Factor: 4.66 · DOI: 10.1016/S0005-2728(98)00082-6

ELSEVIER

Biochimica et Biophysica Acta, 1365 (1998) 301–308

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Structure–function studies of iron–sulfur clusters and semiquinones in the NADH–Q oxidoreductase segment of the respiratory chain

Tomoko Ohnishi[a,*], Vladimir D. Sled[1,a], Takahiro Yano[b], Takao Yagi[b], Dosymzhan S. Burbaev[c], Andrei D. Vinogradov[d]

[a]*The Johnson Research Foundation and Department of Biochemistry and Biophysics, 606 Goddard Labs., University of Pennsylvania, 37th Hamilton Walk, Philadelphia, PA 19104, USA*

[b]*The Division of Biochemistry, Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA 92037, USA*

[c]*Institute of Chemical Physics, Russian Academy of Science, 117977 Moscow, Russian Federation*

[d]*Department of Biochemistry, School of Biology, Moscow State University, 119899 Moscow, Russian Federation*

Received 19 February 1998; received in revised form 11 March 1998; accepted 12 March 1998

Abstract

Our recent experimental data on iron–sulfur clusters and semiquinones in the complex I segment of the respiratory chain is presented, focusing on the *Paracoccus* (*P.*) *denitrificans* and bovine heart studies. The iron–sulfur cluster N2 has attracted the attention of investigators in the research field of complex I, since the mid-point redox potential of this cluster is the highest among all clusters in complex I, and is pH dependent (60 mV/pH). It is known that this cluster is located either in the NQO6 (NuoB in *E. coli*/PSST in bovine heart nomenclature) or in the NQO9 (NuoI/TYKY) subunit in the amphipathic domain of complex I. Our preliminary data indicate that the cluster N2 is located in the NuoB rather than the long-advocated NuoI subunit, and may have a unique ligand structure. We previously reported spin–spin interactions between cluster N2 and two distinct species of semiquinone (designated SQ_{Nf} and SQ_{Ns}) associated with complex I. A parallel intensity change was observed between the SQ_{Nf} ($g=2.00$) signal and the N2 split $g_{\|}$ signal, further supporting our proposed interaction between SQ_{Nf} and N2 spins. © 1998 Elsevier Science B.V.

Keywords: NADH-Q oxidoreductase; Complex I; Iron-sulfur cluster; Fast relaxing semiquinone; Slow relaxing semiquinone

1. Introduction

Recently, the genes of bacterial complex I (this is also designated as NDH-1 [1,2]) have been cloned and sequenced from several strains including *Escherichia* (*E.*) *coli* [3], *Paracoccus* (*P.*) *denitrificans* [2], *Rhodobacter* (*R.*) *capsulatus* [4] and *Thermus* (*T.*) *thermophilus* HB-8 [5]. These bacterial enzymes contain 13 or 14 subunits; it is a much simpler subunit composition than bovine heart complex I containing 43 subunits [6,7]. Important clues concerning the subunit location of the substrate binding site and of intrinsic redox centers have been obtained based on their fully conserved amino acid sequence

*Corresponding author. Fax: +1 215 8988024; E-mail: ohnishi@mail.med.upenn.edu

[1]Vladimir D. Sled died tragically on October 31, 1996. He dedicated most of his scientific career to the study of bioenergetics, specifically of complex I.

PII: S0005-2728(98)00082-6

motifs [2–5,8]. Characterization of redox components in the well-defined three subfractions of the isolated *E. coli* complex I [9] and in the individually over-expressed and purified *P. denitrificans* single subunits [10–12] showed that the NADH-binding site, flavin, and most of the iron–sulfur clusters are located in the electron entry hydrophilic domain of complex I (Fig. 1). The cluster N2 has been located either in the NQO6 (corresponding to the NuoB/PSST subunit in the nomenclature of the *E. coli* and the bovine heart complex I, respectively) or in NQO9 (NuoI/TYKY) subunit in the amphipathic domain of complex I [9]. The NQO9 subunit contains the stereotypical ligation motif of {CxxCxxCxxxCP} for two tetranuclear [4Fe–4S] clusters similar to the bacterial 8Fe-ferredoxin (Fd) [13,14], which is considered to be evolutionarily the most primordial structure [15]. In contrast, NQO6 (NuoB/PSST) subunit seems to contain a primary sequence motif for an atypical mixed ligand of a single tetranuclear iron–sulfur cluster, which is also conserved among primitive iron–sulfur proteins in hydrogenases [16,17]. The discovery of $g=2.00$ EPR signals arising from two distinct species of semiquinone (SQ) in the activated bovine heart submitochondrial particles (SMP) is another line of recent progress. The apparent signal amplitude of SQ signals is sensitive to $\Delta\mu_{H^+}$ and is diminished by specific inhibitors of complex I. To date, semiquinones similar to those reported for the bovine heart mitochondrial complex I have not been discovered in bacterial systems yet.

2. The subunit location of the cluster N2 and its possible ligand residues

Over-expression and purification of the amphipathic NQO9 (NuoI/TYKY) subunit of *P. denitrificans* were much harder than in the case of hydrophilic subunits, because this subunit readily formed inclusion bodies. However, the NQO9 subunit has recently been overexpressed in, and purified from

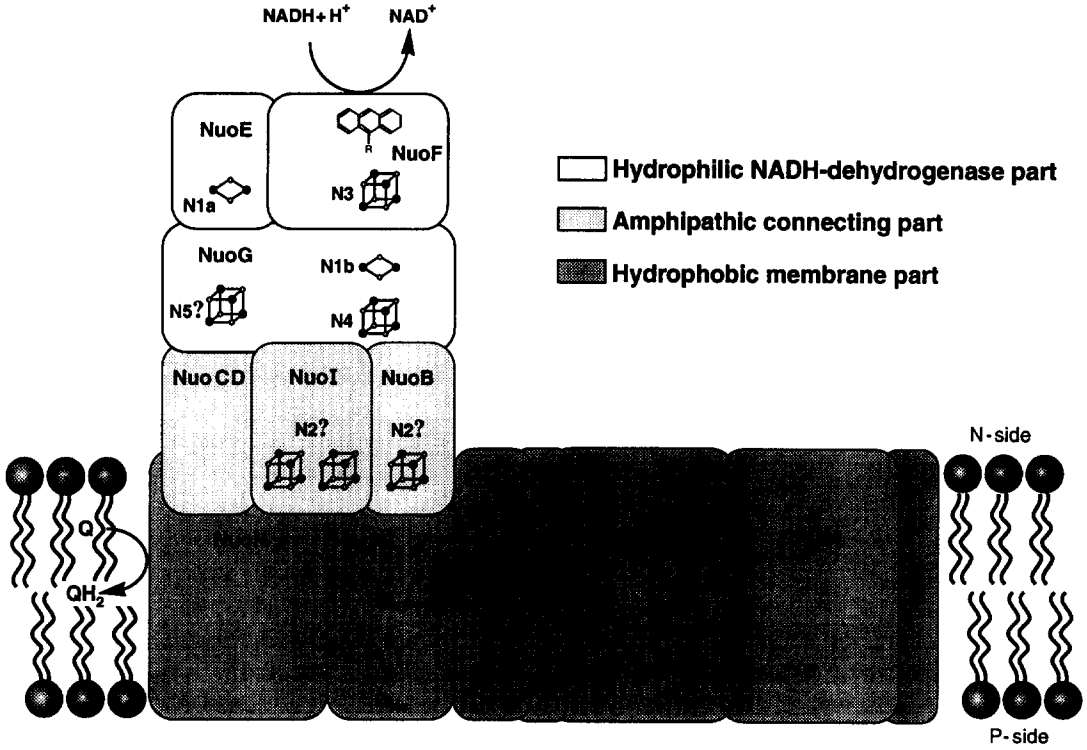


Fig. 1. Schematic outline of the minimal complex I structure and a summary of our present knowledge on the proposed subunit distribution of different redox centers and NADH and Q binding sites. The unusual L-shape image of complex I is from a low-resolution electron microscopic analysis of *N. crassa* and *E. coli* complex I [53,54]. The promontory arm protrudes from the matrix side of the membrane by 80 Å, and the larger hydrophobic arm extends 180 Å within the membrane. The three subfractions of *E. coli* complex I and the corresponding genes are indicated in gray scale. The cluster N1c is not included in this figure, because this cluster is not present in the bovine heart, *P. denitrificans*, and *R. capsulatus* complex I [2,4,8].

E. coli, in a relatively native form (Yano et al., unpublished data), by co-expressing it with *E. coli* thioredoxin [18]. The iron–sulfur clusters were chemically reconstituted in vitro with a reconstitution efficiency of about 30% as judged from the chemically determined nonheme iron content. As shown in Fig. 2A, our preliminary EPR experiments clearly showed multiple signals from two [4Fe–4S] clusters at $g=2.08$, 2.05, 1.94, 1.93, 1.90, and 1.88. The 2.01 peak indicates that a small portion of the original $[4Fe–4S]^{1+(2+,1+)}$ cluster may be converted to the $[3Fe–4S]^{1+(1+,0)}$ cluster by contact with air oxygen, but its concentration is very low relative to the other two clusters (Yano et. al., unpublished data). This spectral profile is reminiscent of the two [4Fe–4S] clusters analogous to the F_A and F_B clusters harbored in the PsaC subunit of photosystem I, which contains two stereotypical [4Fe–4S] cysteine sequence motifs [19,20]. Potentiometric redox titrations of these multiple signals show tri-phasic $n=1$ Nernst curves. Two major components exhibit E_m values of about −510 and −570 mV at approximately the same concentrations, which is also similar to the E_m values of the F_A and F_B clusters, while a minor component with an E_m value of about −370 mV (10–15%) may represent a partially modified cluster (data is not shown). The NQO6 (NuoB/PSST) subunit on the other hand contains four conserved cysteines $\{C^{53}C^{54}xxE^{57}—C^{118}—C^{148}P\}$ in the *P. denitrificans* sequence number. Two adjacent cysteines (C^{53} and C^{54}) cannot function as the cluster ligands concurrently, therefore, NuoB possesses only three candidate Cys ligands. Because the C-terminal end C^{148} is next to the proline, a [4Fe–4S] cluster structure was suggested. It is likely to have a mixed ligand residue where a candidate ligand is the conserved Glu (E^{57}). In a very preliminary experiment, we observed weak EPR signals in the cytoplasmic membranes of the *E. coli* cells harboring overexpressed NQO6 subunits at temperatures below 10 K, indicating the presence of a [4Fe–4S] cluster (see Fig. 2B). We are currently working to obtain a higher level of the subunit over-expression and of the in vitro cluster reconstitution. The involvement of a non-Cys ligand, either oxygen or nitrogen in addition to the sulfur ligands tend to shift the E_m to higher values than seen in the bacterial Fd-type iron–sulfur clusters with 4-Cys ligands. A good example is the Rieske binuclear

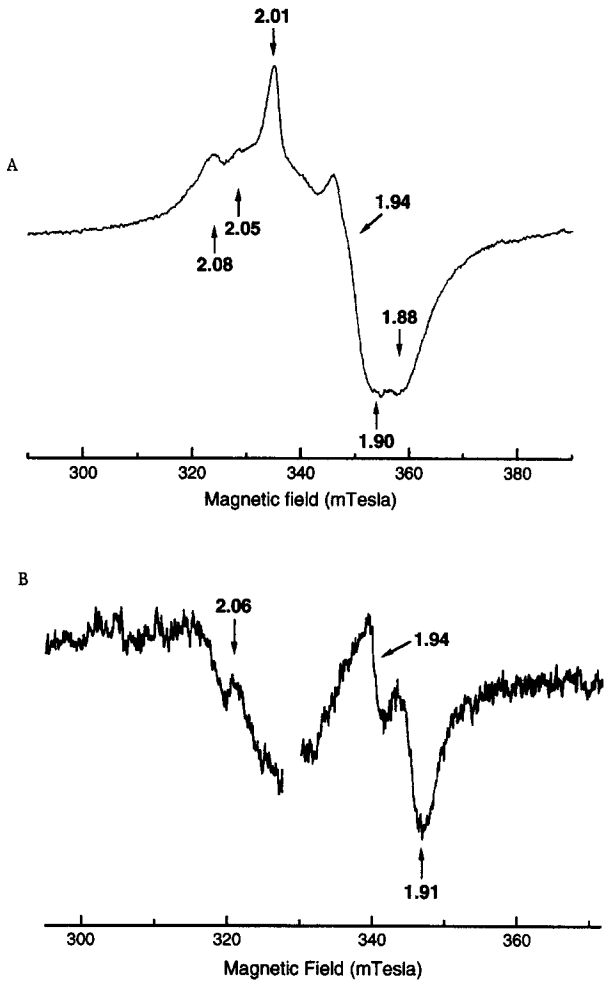


Fig. 2. EPR spectra of *P.denitrificans* NQO9 (NuoI/TYKY) and NQO6 (NuoB/PSST) subunits. Spectrum A was obtained with the dithionite-reduced NQO9 subunit at 350 μM (7.0 mg protein/ml) and 850 μM nonheme iron/mg protein, which corresponds to about 30% reconstitution of iron–sulfur clusters. The subunit was overexpressed in *E. coli* cells, purified and chemically reconstituted with Na_2S $(NH_4)_2Fe(SO_4)_2$, and dithiothreitol in vitro under anaerobic conditions. EPR conditions: microwave power, 20 mW; sample temperature, 10 K. Spectrum B was obtained as the difference spectrum of the dithionite-reduced cytoplasmic membrane preparation containing overexpressed NQO6 (NuoB/PSST) subunit and that of the wild-type. The nonheme iron concentration of these two samples were 141 μM (34.2 mg protein/ml and 4.1 natom Fe/mg protein) and 27 μM (13.4 mg protein/ml and 2.0 natom Fe/mg protein), respectively. Spectral gains were normalized to their protein concentrations for taking the difference spectrum. EPR conditions: microwave power, 10 mW; sample temperature, 10 K.

[2Fe–2S] iron–sulfur cluster ($E_{m7.0}=150$–300 mV) in the cytochrome (cyt.) bc_1 complex with an average g value of 1.91 [21,22]. This cluster was demonstrated to ligate two His (to the ferrous site) and two Cys (to the ferric site) ligands [22–25]. Another binuclear cluster S1 in the succinate–Q oxidoreduc-

tase is more relevant to cluster N2, because this cluster has an E_{m7} value in the range of −80 to +80 mV close to that of cluster N2, and shows EPR spectrum with an average g value of ~1.96 as in the case of cluster N2 as well as the 4-Cys-ligated Fd-type clusters [26,27]. Nevertheless, its third Cys in the stereotypical conserved primary sequence {CxxxxCxxC (or D)–C} is not conserved in three different bacteria so far known [28], indicating an atypical mixed cluster-ligation. Based on site-directed mutagenesis studies, a water molecule was suggested to function as the third ligand which may be hydrogen bonded to either an Asp or Cys residue [29]. Perhaps a non-Cys residue in this case is ligated to the ferric site of the binuclear cluster [30]. Several [4Fe–4S] clusters with mixed-ligand systems are also known in nature. One example is a hyper-thermophile *Pyrococcus (P.) furiosus* [4Fe–4S] ferredoxin which is ligated by one Asp (D) and three Cys residues with a cluster ligation motif of {CxxD^{14}xxC—C}. This cluster shows an EPR spectrum with $g_{z,y,x}$ values of 2.10, 1.86, 1.80 (g_{av}=1.92) detectable at temperatures below 15 K [31]. The D^{14} cluster ligand in *P. furiosus* [4Fe–4S] was identified based on the 1D and 2D ^{1}H NMR analysis [32]. On the other hand, the aconitase [4Fe–4S]$^{1+(2+,1+)}$ cluster was shown to be coordinated by three Cys and one H_2O ligands by high-resolution X-ray structural analysis [33]. The EPR spectrum of this [4Fe–4S]$^{1+}$ cluster shows the $g_{z,y,x}$=2.07, 1.95, 1.86 with a g_{av} of 1.95 in the absence of the added substrate [34], which is similar to the usual 4-Cys-ligated iron–sulfur clusters [13]. Here again, these examples suggest that even in the case of the tetranuclear cluster ligated by mixed ligands (sulfur with either oxygen or nitrogen), its EPR spectrum can be similar to the all-sulfur-ligated cluster [32]. This information is consistent with the notion that the electron density is localized also in the tetranuclear cluster [35], and provides some support to our speculative proposal of assigning cluster N2 to the NQO6 (NuoB/PSST) subunit and its possible atypical ligand structure.

3. Two distinct species of semiquinones and cluster N2

The NADH binding site, flavin, and iron–sulfur clusters are located in either the hydrophilic or connecting amphipathic domains of complex I (see Fig. 1). Therefore, to identify hydrogen and electron carriers, which participate in the vectorial proton translocation at the first energy coupling site within the membrane, is the most challenging problem. Ubiquinone has been proposed to translocate protons in complex I as a natural hydrogen carrier [36]. Suzuki and King [37] first reported EPR g=2.00 signals of ubisemiquinone (SQ) observed at 23°C in the NADH-reduced bovine heart complex I. Based on the Q band EPR analysis, they demonstrated that SQ signals arise from protein-bound SQ species by their anisotropic lineshape with $g_{z,y,x}$=2.0060, 2.0051, 2.0022. They also resolved the spectrum into two overlapping SQ species based on their different sensitivities to a sulfhydryl reagent, PCMB. Subsequently, Vinogradov's group [38,39] discovered an extremely fast-relaxing SQ species in the complex I segment of the respiratory chain in bovine heart SMP, which was found to be $\Delta\mu_{H^+}$ dependent and rotenone sensitive. These investigators proposed a possible spin–spin interaction of the SQ with the cluster N2 spins, based on the fact that SQ spins relax almost as fast as the N2 spins. They presented a model for the proton translocation driven by the electron transfer from the iron–sulfur cluster N2 to the bulk ubiquinone with two intermediate SQ species [40]. De Jong and Albracht confirmed these observations, but they reported that SQ formation was $\Delta\mu_{H^+}$ independent [41]. Subsequently, de Jong et al. recognized a pronounced effect of the membrane energization on the saturation behavior of SQ and on the g_z line shape of the cluster N2, but they interpreted this phenomenon as a reflection of the energy-induced protein conformational change of the complex I. They also concluded that only single species of SQ resided in complex I and its spin relaxation was enhanced by membrane energization [42]. Contrary to their interpretation, Vinogradov et al. demonstrated the presence of at least two distinct species of semiquinone in complex I, and designated them as SQ_{Nf} and SQ_{Ns} because of their fast and slow spin relaxation, reflecting their different strength of spin-coupling with cluster N2 [43]. Fig. 3A presents EPR spectrum of tightly coupled (respiratory control of about 8) bovine heart SMP during the steady-state NADH oxidation, recorded at 16 K. Two prominent features are clearly

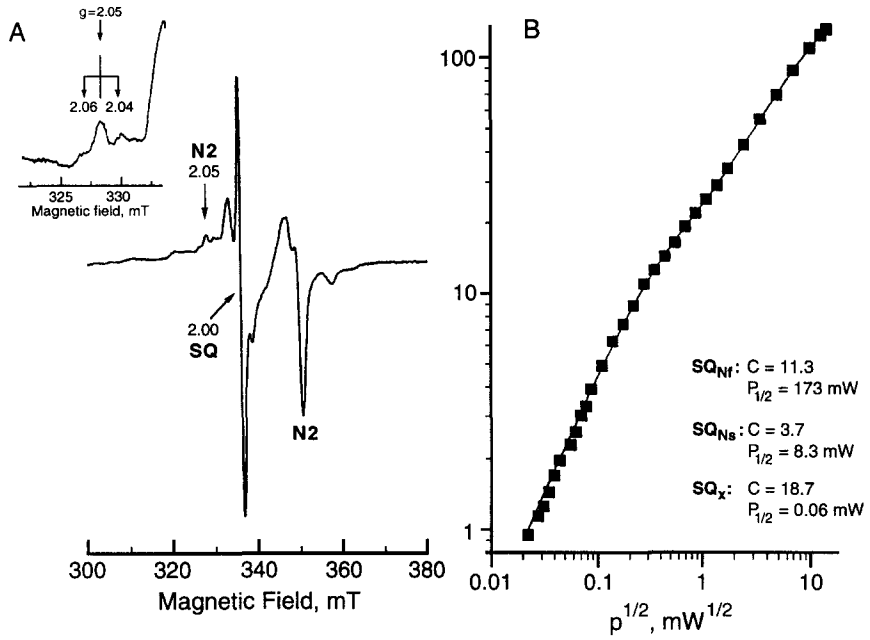


Fig. 3. (A) EPR spectra of tightly coupled (respiratory control RC=8) activated bovine heart submitochondrial particles during steady-state NADH oxidation. EPR conditions: submitochondrial particles (15 mg protein/ml) in 0.25 M sucrose, 50 mM Hepes (pH 8.0) and bovine serum albumin (1 mg/ml) were placed in an EPR tube at room temperature. The steady-state electron transfer was initiated by the addition of NADH (1.5 mM) under an aerated state. Within 5 s, the sample was frozen using a cold freezing mixture (5:1 (v/v) isopentane and methylcyclohexane). Sample temperature, 16 K; microwave power, 2 mW. (B) Power saturation profile of overlapping semiquinone signals. Sample temperature, 40 K. Power saturation data was analyzed by computer fitting to the equation

$$A=\sum_{i=1}^{n} A_i=\sum_{i=1}^{n} C_i\sqrt{P}/(1+P/P_{1/2(i)})^{0.5b_i}$$

A_i is the amplitude of the i-th type free radical; $P_{1/2(i)}$ is the half-saturation power and b_i is the homogeneity parameter [55].

seen in the spectrum, i.e., the presence of an intense SQ $g=2.005$ signal and the two-split peaks at $g=2.044$ and 2.064 on both sides of the $g_{\|}$ peak of the cluster N2 signal. The dipole coupling between two spins leads to a spin-relaxation enhancement effect and/or a broadening or splitting of the signal [44]. Considering the dipole point approximation, the distance between cluster N2 and SQ_{Nf} can be calculated using the equation $\Delta B=\beta\cdot g\cdot r^{-3}|1-3\cos^2\theta|$, where ΔB is the peak separation (in gauss), r is the distance (in Å) between dissimilar interacting spins, g is the g factor of SQ_{Nf} species, and θ is the angle between the inter-dipole vector and the magnetic field. The distance of 8–11 Å was calculated from the peak-to-peak separation of 3.3 metals. If the exchange coupling is significant, the distance between cluster N2 and SQ_{Nf} could be as long as 12 Å [43]. Previously, Salerno et al. [45] demonstrated that the $g_{\|}$ direction is perpendicular to the membrane, using oriented multilayer mitochondrial preparations. Therefore, if the vector connecting SQ_{Nf} and N2 are along the membrane normal, the mutual distance is 11 Å. The power saturation profile of the SQ signal amplitude is presented in Fig. 3B. In order to visualize multiplicity of the SQ species, a power saturation curve of the $g=2.00$ signal (at 40 K) was plotted as a function of $P^{1/2}$ in a log–log plot. The observed tri-phasic power saturation curve was resolved into three individual SQ species, with $P_{1/2}$ values of 173, 8.3 and 0.06 mW, respectively. Based on the enhanced spin relaxation of the SQ_{Nf} by the paramagnetic cluster N2, we obtained a distance (10–13 Å) consistent with that obtained from the splitting (Burbaev et al., unpublished data). Incidentally, 12 Å distance is also in agreement with the distance between two tetranuclear clusters in the individual TYKY subunits proposed by Albracht's group [46], although we do not agree with the idea of two N2 clusters present in the TYKY subunit [47].

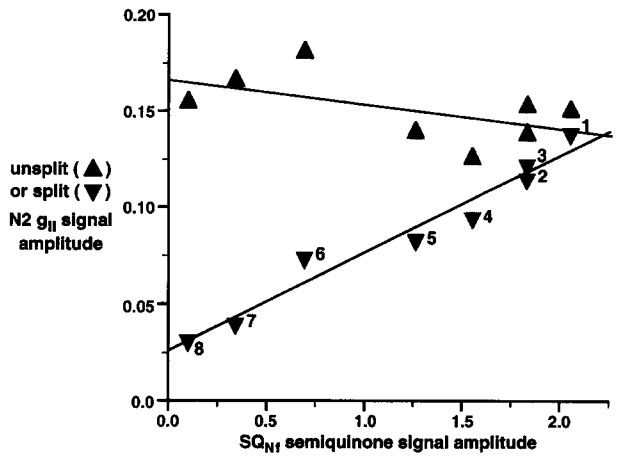
Only the two faster relaxing components of the SQ

signals can be quenched by piericidin A at an equivalent concentration to complex I, leaving signals from one species of SQ with slowest spin-relaxation ($P_{1/2}$=0.06 mW). Since this is insensitive to the complex I inhibitors, this may not be associated with complex I and it was designated SQ_X. We have not yet examined the identity of SQ_x. Van Belzen et al. tentatively suggested rotenone insensitive SQ species as the SQ_{III} residing at the Q_i site in complex III [46].

A weaker magnetic interaction between cluster N2 and SQ_{Ns} is also indicated because the $P_{1/2}$ value of the SQ_{Ns} is clearly higher than that of a semiquinone species magnetically isolated from any other paramagnetic centers. The SQ_{Ns} seems to be further away (>30 Å) from the cluster N2, perhaps close to the cytosolic surface of the membrane, but the accurate distance estimation is difficult to make because of the possible spin relaxation effect to SQ_{Ns} from the cluster N2 via SQ_{Nf}.

Recently, Albracht and his co-workers reported detailed experimental data on this topic [46]. They have demonstrated that $g_{\|}$ splitting of the cluster N2 indeed arises from magnetic interactions between neighboring spins, because the splitting was found to be independent of the applied microwave frequency (X-band and P-band). Their reported splitting ΔB of 2.8 mtesla and our 3.3 mtesla would provide almost the same distance r between interacting spins at our resolution level, because the splitting is proportional to r^{-3}. These investigators, however, interpreted that the strong interaction manifested by the $g_{\|}$ splitting of the cluster N2 arises from 'exchange coupling' between the two N2 clusters residing within each NQO9 (NuoI/TYKY) subunits [46,48].

In order to further examine our proposed strong spin-coupling between the SQ_{Nf} and the cluster N2, we analyzed the correlation between the strength of their spin-coupling (probed by an increase of the split signal at g=2.044 as well as by a decrease of the non-split signal at g=2.05 of cluster N2) and the size of the g=2.00 SQ_{Nf} signal in the bovine heart SMP under various redox equilibrium states of complex I (see Fig. 4). The relative concentration of the SQ_{Nf} is dependent on the $\Delta\mu_{H^+}$ imposed to the mitochondrial inner membrane [39]. The SMP can catalyze forward (NADH→Q) and reversed (succinate→NAD^+) electron transfer with different steady-state levels of SQ_{Nf}



number	description
1	E_{act} + succ., 5 sec
2	E_{act} + NADH, 5 sec
3	E_{act} + La^{3+} + NADH, 5 sec
4	E_{act} + NADH + succ., 5 sec
5	E_{act} + Mg^{2+} + NADH + succ., 5 sec
6	E_{in} + La^{3+} + NADH + succ., 5 sec
7	E_{in} + Mg^{2+} + NADH + succ., 5 sec
8	E_{act} + NADH, 2 min

Fig. 4. Close correlation observed between the relative intensity of the g=2.00 signal of the SQ_{Nf} species and the strength of the spin-coupling between N2 and SQ_{Nf}. The latter parameter was probed by the decrease of non-split g_z=2.055 (▲) as well as the increase of the split g_z=2.044 (▼) signal amplitude of the cluster N2, and were plotted as a function of the SQ_{Nf} signal amplitude. All signals were normalized to the g=1.92 intensity of the cluster N2. Different steady-state levels of the SQ_{Nf} signal were obtained in different preparations of the tightly coupled bovine heart SMP trapped in the different states of SMP were tabulated at the bottom of the figure (see the text for more detail).

[39]. Complex I in SMP can also exist in the 'active' or 'inactive' state [49], analogous to the 'pulsed' or 'resting' states of the cytochrome c oxidase [50]. The rate of the activation from the 'inactive' to 'active' states of SMP can be varied by different divalent or trivalent ions [51] (Fig. 4). The relative concentrations of the SQ_{Nf} in SMP were altered by combining these various parameters. Fig. 4 demonstrates that the degree of spin coupling increases with an increase of the SQ_{Nf} signal amplitude (correlation coefficient r=0.97). This correlation supports our proposed spin–spin interaction between N2 and SQ_{Nf}, rather than spin-coupling between two N2 clusters residing within the single NQO9 (NuoI/TYKY) subunit proposed by Albracht and his collaborators [46]. We are currently working to determine the location of cluster N2 within the membrane and to define its spatial relationship relative to the distinct quinone binding

sites. This information will facilitate our understanding of the proton/electron transfer mechanism in the complex I segment of the respiratory chain [[52] and refs. cited therein].

Acknowledgements

The Complex I research cited in this manuscript is supported by the NIH grant GM 30736 to T.O. and NIH Fogarty International Collaborative grant TW00140 to T.O. and A.V., Russian Foundation for Fundamental Research (grant 96-04-48185) to A.V., and NIH grant GM33712 to T.Y.

References

[1] K. Matsushita, T. Ohnishi, H.R. Kaback, Biochemistry 26 (1987) 7732–7737.
[2] T. Yagi, Biochim. Biophys. Acta 1141 (1993) 1–17.
[3] U. Weidner, S. Geier, A. Ptock, T. Friedrich, H. Leif, H. Weiss, J. Mol. Biol. 233 (1993) 109–122.
[4] A. Dupuis, A. Peinnequin, M. Chevallet, J. Lunardi, E. Darrouzet, B. Pierrard, V. Procaccio, J.P. Issartel, Gene 167 (1995) 99–104.
[5] T. Yano, S.S. Chu, V.D. Sled, T. Ohnishi, T. Yagi, J. Biol. Chem. 272 (1997) 4201–4211.
[6] J.E. Walker, Q. Rev. Biophys. 25 (1992) 253–324.
[7] S.K. Buchanan, J.E. Walker, Biochem. J. 318 (1996) 343–349.
[8] I.M. Fearnley, J.E. Walker, Biochim. Biophys. Acta 1140 (1992) 105–134.
[9] H. Leif, V.D. Sled, T. Ohnishi, H. Weiss, T. Friedrich, Eur. J. Biochem. 230 (1995) 538–548.
[10] T. Yano, V.D. Sled, T. Ohnishi, T. Yagi, Biochemistry 33 (1994) 494–499.
[11] T. Yano, T. Yagi, V.D. Sled, T. Ohnishi, J. Biol. Chem. 270 (1995) 18264–18270.
[12] T. Yano, V.D. Sled, T. Ohnishi, T. Yagi, J. Biol. Chem. 271 (1996) 5907–5913.
[13] H. Matsubara, K. Saeki, Advances Inorg. Chem. 38 (1992) 223–280.
[14] K. Fukuyama, Y. Nagahara, T. Tsukihara, Y. Katsube, T. Hase, H. Matsubara, J. Mol. Biol. 199 (1988) 183–193.
[15] T. Friedrich, H. Weiss, J. Theor. Biol. 187 (1997) 529–540.
[16] S.P. Albracht, Biochim. Biophys. Acta 1188 (1994) 167–204.
[17] A. Volbeda, M.-H. Charon, C. Piras, E.C. Hatchikian, M. Frey, J.C. Fontecilla-Camps, Nature 373 (1995) 580–587.
[18] T. Yasukawa, C. Kanei-Ishii, T. Maekawa, J. Fujimoto, T. Yamamoto, S. Ishii, J. Biol. Chem. 270 (1995) 25328–25331.
[19] N. Hayashida, T. Matsubayashi, K. Shinozaki, M. Sugiura, K. Inoue, T. Hiyama, Curr. Genet. 12 (1987) 247–250.
[20] P.L. Herman, K. Adiwilaga, J.H. Golbeck, D.P. Weeks, Plant Physiol. 104 (1994) 1459–1461.
[21] R.C. Prince, J.G. Lindsay, P.L. Dutton, FEBS Lett. 51 (1975) 108–111.
[22] E. Davidson, T. Ohnishi, E. Atta-Asafo-Adjei, F. Daldal, Biochemistry 31 (1992) 3342–3351.
[23] R.J. Gubriel, T. Ohnishi, D. Robertson, F. Daldal, B.M. Hoffman, Biochemistry 30 (1991) 11579–11584.
[24] R.D. Britt, K. Sauer, M.P. Klein, D.B. Knaff, A. Kriauciunas, C.A. Yu, L. Yu, R. Malkin, Biochemistry 30 (1991) 1892–1901.
[25] S. Iwata, M. Saynovits, T.A. Link, H. Michel, Structure 4 (1996) 567–579.
[26] B.A.C. Ackrell, M.K. Johnson, R.P. Gunsalus, G. Cecchini, in: F. Müller (Ed.), Chemistry and Biochemistry of Flavoenzymes, vol. III, CRC Press, Boca Raton, FL, 1992, pp. 229–297.
[27] L. Hederstedt, T. Ohnishi, in: L. Ernster (Ed.), Molecular Mechanisms in Bioenergetics, Elsevier, Amsterdam, 1992, pp. 163–198.
[28] C. Hägerhäll, Biochim. Biophys. Acta 1320 (1997) 107–141.
[29] M.T. Werth, H. Sices, G. Cecchini, I. Schroder, S. Lasage, R.P. Gunsalus, M.K. Johnson, FEBS Lett. 299 (1992) 1–4.
[30] W.E. Blumberg, J. Peisach, Arch. Biochem. Biophys. 162 (1974) 502–512.
[31] J.B. Park, C.L. Fan, B.M. Hoffman, M.W. Adams, J. Biol. Chem. 266 (1991) 19351–19356.
[32] L. Calzolai, C.M. Gorst, Z.H. Zhao, Q. Teng, M.W. Adams, G.N. La Mar, Biochemistry 34 (1995) 11373–11384.
[33] A.H. Robbins, C.D. Stout, Proteins 5 (1989) 289–312.
[34] M.C. Kennedy, L. Mende-Mueller, G.A. Blondin, H. Beinert, Proc. Natl. Acad. Sci. USA 89 (1992) 11730–11734.
[35] I. Bertini, F. Briganti, C. Luchinat, L. Messori, R. Monnanni, A. Scozzafava, G. Vallini, Eur. J. Biochem. 204 (1992) 831–839.
[36] P. Mitchell, Science 206 (1979) 1148–1159.
[37] H. Suzuki, T.E. King, J. Biol. Chem. 258 (1983) 352–358.
[38] D.S. Burbaev, I.A. Moroz, A.B. Kotlyar, V.D. Sled, A.D. Vinogradov, FEBS Lett. 254 (1989) 47–51.
[39] A.B. Kotlyar, V.D. Sled, D.S. Burbaev, I.A. Moroz, A.D. Vinogradov, FEBS Lett. 264 (1990) 17–20.
[40] A.D. Vinogradov, J. Bioener. Biomemb. 25 (1993) 367–375.
[41] A.M.P. de Jong, S.P.J. Albracht, Eur. J. Biochem. 222 (1994) 975–982.
[42] A.M. de Jong, A.B. Kotlyar, S.P. Albracht, Biochim. Biophys. Acta 1186 (1994) 163–171.
[43] A.D. Vinogradov, V.D. Sled, D.S. Burbaev, V.G. Grivennikova, I.A. Moroz, T. Ohnishi, FEBS Lett. 370 (1995) 83–87.
[44] A. Abragam, B. Bleaney, Electron Paramagnetic Resonance of Transition Ions, Calendar Press, Oxford, 1970.

[45] J.C. Salerno, H. Blum, T. Ohnishi, Biochim. Biophys. Acta 547 (1979) 270–281.
[46] R. van Belzen, A.B. Kotlyar, N. Moon, W.R. Dunham, S.P.J. Albracht, Biochemistry 36 (1997) 886–893.
[47] T. Ohnishi, Biochim. Biophys. Acta 1364 (1998) 186–206.
[48] S.P.J. Albracht, A.M.P. de Jong, Biochim. Biophys. Acta 1318 (1997) 92–106.
[49] A.B. Kotlyar, A.D. Vinogradov, Biochim. Biophys. Acta 1019 (1990) 151–158.
[50] G. Palmer, J. Bioenerg Biomembr. 25 (1993) 145–151.
[51] A.B. Kotlyar, V.D. Sled, A.D. Vinogradov, Biochim. Biophys. Acta 1098 (1992) 144–150.
[52] P.L. Dutton, C.C. Moser, V.D. Sled, F. Daldal, T. Ohnishi, Biochim. Biophys. Acta 1364 (1998) 245–257.
[53] G. Hofhaus, H. Weiss, K. Leonard, J. Mol. Biol. 221 (1991) 1027–1043.
[54] V. Guenebaut, A. Schlitt, H. Weiss, K.R. Leonard, T. Friedrich, J. Mol. Biol. 276 (1998) 105–112.
[55] G. Palmer, Biochem. Soc. Transact. 13 (1985) 548–560.