The NADH:ubiquinone oxidoreductase (EC 1.6.5.3., complex I) couples the electron transfer from NADH to ubiquinone with a proton translocation across the membrane. It consists of two arms, the peripheral and the membrane arm. The structure of the bacterial complex I showed that the NADH binding site is made up by a novel type Rossman-fold providing binding sites for the FMN as well as for NADH. We solved the structure of the NADH binding site of the *Aquifex aeolicus* enzyme with bound substrates at 2 Å resolution. From the structure it is evident, that the Glu 183 (numbering according to *E. coli* complex I) provides one of the most prominent interactions with NADH by building a hydrogen bond to the hydroxyl group of C2'-ribose. The affinity of the *E. coli* complex I to NADH is 50 times higher than that to NADPH. The reason could be a sterical clash between the C2'-PO_3^{2-} of NADPH and the Glu183-side chain. This prediction is examined by testing the activity of protein variants with mutations at this position. The effect of the mutations on the NAD(P)H/ferricyanide, NAD(P)H/HAR and NAD(P)H:decylubiquinone oxidoreductase activity will be examined.

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doi:10.1016/j.bbabio.2010.04.080

1P.33 Characterization of the binding site of Δlac-acetogenin in bovine mitochondrial complex I by photoaffinity labeling

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In the course of wide structural modifications of natural acetogenins, we synthesized "Δlac-acetogenins" by deleting a γ-lactone ring that is a structural feature common to a large number of natural acetogenins. Characterization of the inhibition mechanism of Δlac-acetogenins revealed that they are a new type of complex I inhibitors; for instance, i) the level of superoxide production induced by Δlac-acetogenins is remarkably low and ii) the inhibitory potency for the reverse electron transfer is significantly weaker than that for the forward event [*1, 2*]. Considering the unusual inhibitory effects of Δlac-acetogenins, further exploration of their inhibition manner including identification of the binding site would provide valuable insights into the terminal electron transfer step of complex I. In order to clarify the binding site of Δlac-acetogenins, we here synthesized a photoreactive Δlac-acetogenin ([^{125}I]*dia*zirinylated Δlac-*a*cetogenin, [^{125}I]DAA), which has a small photolabile diazirine group attached to a pharmacophore (bis-THF ring moiety), and carried out photoaffinity labeling with bovine heart submitochondrial particles (SMP). Biochemical characterization revealed that DAA retains very potent inhibitory activity at nM level, whereas its inhibition mechanism differs slightly from that of the original Δlac-acetogenins. Analysis of the SMP photocross-linked by [^{125}I]DAA revealed that [^{125}I]DAA binds to the ND1 subunit in the membrane domain with a high specificity. The Lys-C and Asp-N digest of the cross-linked ND1 and careful analysis of the peptide fragments suggested that [^{125}I]DAA binds to two different sites in the ND1 subunit; one is located between Tyr127 and Phe198 (covering 4th and 5th transmembrane helices) and the other is located between Asp199 and Lys262 (covering 6th and 7th transmembrane helices). Consideration that a photolabile diazirine group is attached at hydrophilic moiety of amphiphilic [^{125}I]DAA, the cross-linked residues may be located in the loop region connecting the 5th and 6th transmembrane helices. In the presence of an excess amount of Δlac-acetogenin, the cross-linking solely of the region Asp199–Lys262 was completely suppressed. This result strongly suggests that Δlac-acetogenin binds this region with a high specificity.

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doi:10.1016/j.bbabio.2010.04.081

1P.34 EPR detection of protein-associated ubiquinone molecules in purified bovine heart NADH–ubiquinone oxidoreductase (complex I)

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It has been known that various energy transducing membrane systems utilize protein-associated quinone molecules. Based upon EPR spin-coupling studies, T. Ohnishi and her collaborators reported that bovine heart NADH–ubiquinone oxidoreductase (complex I) contains two different types of semiquinone. They called them fast relaxing semiquinone (SQ_{Nf}) and slow relaxing semiquinone (SQ_{Ns}) [1–4], and assigned each of them different roles. The former is for gated-proton transport, and the latter, for the converter between one-electron and two-electron transport. In recent years, complex I has been isolated and purified in many laboratories. A problem in the purification of complex I seems to be that quinone is easily lost during the purification. Recently, Yoshikawa's group reported that their highly purified, active bovine heart complex I preparations maintain one protein-associated ubiquinone per complex I molecule [5]. Using these preparations, we detected EPR signals of both semiflavin (SF) and semiquinone (SQ) radicals (S. T. Ohnishi et al. BBA. in press). We now report that we detected the signal from endogeneously-bound semiquinone, and succeeded in separating signals from two types of semiquinone. Possible implications are twofold. The first role: three types of quinone-inhibitors, Type-A (piericidin A), Type-B (rotenone) and Type-C (capsaicin and tridecylstigmatellin) are known [6–7]. This categorization of inhibitors may be explained by the binding specificity of these inhibitors to the Q_{Nf} and Q_{Ns} sites. For example, piericidin A binds to both Q_{Nf} and Q_{Ns}, rotenone binds preferentially to Q_{Nf} and TDS binds to Q_{Ns}. The second role is their involvement in electron–proton coupling. T. Ohnishi now hypothesizes that Q_{Ns} may also play an important role in indirect electron–proton coupling.

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doi:10.1016/j.bbabio.2010.04.082

1P.35 The structure of complex I from the hyperthermophilic eubacterium *Aquifex aeolicus*

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Complex I from *Aquifex aeolicus* is highly stable and active. Image analysis and 2D and 3D reconstruction by electron micrographs revealed a complete complex I particle of typical L-shape, and a pronounced invariant angle (90°) between the cytoplasmic arm [1–2] and the membrane arm. It showed many details in its external arm. The isoforms of the complex have been detected by mass spectrometry. So far, the subunits in the hydrophilic domain could be clearly assigned to two isoforms. The partial structure of one isoform of *Aquifex* complex I containing all subunits of hydrophilic domain has been determined by X-ray at a 2.9 Å resolution. Interestingly, *Aquifex* complex I contains one extra iron sulfur cluster, which is not found in that of *E. coli* and *T. thermophilus*. These data allow us to describe and discuss the mechanistic hypotheses and models of bacterium complex I [3–5].

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doi:10.1016/j.bbabio.2010.04.083

1P.36 A systematic approach to membrane-protein reconstitution in liposomes, applied to the M2 protein of Influenza virus A

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We present an improved methodology for production of large unilamellar vesicles and reconstitution of membrane-proteins, using gradual detergent removal. We also present two novel membrane-impermeable pH sensors, the porphyrin-based Glu3 and TCHP (Leiding et al., 2009, Anal. Biochem. 388: 296–305). The solubilization behavior of vesicles in different detergents is reported, and the effect of protein-to-lipid concentration on passive ion permeability of the liposomes. The effects of cholesterol and lipid composition on vesicle integrity are also explored — all for the purpose of understanding and optimizing the protein reconstitution process. As a proof of concept, successful unidirectional reconstitution of the Influenza protein A/M2 is reported. The integrity of the proteoliposomes allowed detailed, quantitative data collection over tens of minutes, providing a wealth of new information on ion flux through the protein (cf. Thom Leiding's poster). This reliable reconstitution method, together with pH sensors that stay within vesicles and a semi-automated titration and data-analysis system, provides a strong platform for investigating proton-translocating bioenergetic complexes.

doi:10.1016/j.bbabio.2010.04.084

1P.37 A novel *c*-type cytochrome transfers electrons between sulfite oxidase and cytochrome c_{552} in the respiratory chain of *Thermus thermophilus*

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We here describe a novel *c*-type cytochrome from the extreme thermophile *Thermus thermophilus*. N-terminal sequencing of the purified protein led to the identification of the corresponding gene TTHA1326. The 23 kDa cytochrome possesses two heme *c* binding sites and demonstrates a high sequence identity to cytochrome c_{552}, the substrate of the ba_3-type cytochrome *c* oxidase. Because of the low yield, we have succeeded in its recombinant production in *E. coli* with the simultaneous expression of the *ccm* genes involved in the maturation of cytochrome *c* in the same organism. We have generated milligram quantities of the holo-protein allowing the investigation of its properties and physiological function. There is no evidence that cytochrome c_{550} acts as an electron shuttle between the *bc* complex and Thermus cytochrome *c* oxidases. We have shown that, surprisingly, cytochrome c_{550} clearly mediates electrons to cytochrome c_{552}. Further analysis of the putative operon encoding the protein led to the identification of a potential electron donor namely sulfite oxidase. In order to assess the subsequent electron transfer, sulfite oxidase (SO) TTHA1325 was produced recombinantly in *E. coli* and was shown to utilize the cytochrome c_{550} as the electron acceptor following oxidation of sulfite. To the best of our knowledge, this is the first characterization of the sulfite respiration system from a thermophilic bacterium.

doi:10.1016/j.bbabio.2010.04.085

1P.38 Functional analysis of respiratory complex I (NADH:ubiquinone oxidoreductase) in the early-branching eukaryote *Trypanosoma brucei*

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The protozoan parasite *Trypanosoma brucei* alternates between a mammalian host and an insect vector, and these environmental changes have resulted in dramatic regulation of the organism's