crystals of the wild-type enzyme with reagents such that specific states of the enzyme are formed. So far crystals of various qualities have been obtained using the different approaches and datasets from crystals diffracting to high resolution are being processed.

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### 11P.8 Novel structure–function relationships of plant alternative oxidase

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Alternative oxidase (AOX) is a terminal oxidase of mitochondrial respiratory chain, which catalyzes oxidation of ubiquinol and reduction of molecular oxygen in a coupling manner. Although no data from X-ray crystallography is available for structural analysis to date, structure–function relationships of the enzyme have been well characterized with respect to its highly-conserved elements for catalytic and regulatory mechanisms. On the other hand, such relationships involving less-conserved ones, which may confer unique enzymatic characteristics to respective AOX isozymes, still remain uncharacterized. Here, we show that novel structure–function relationships exist in *Arum concinnatum* AOX1a (AcoAOX1a) and that they involve at least three less-conserved structural elements, all of which have not been reported with functional significance except for E83. E83K was previously reported to deprive AOX activity in chimera mutants carrying segmented structures derived from AcoAOX1a and its inactive homologue AcoAOX1b. Nevertheless, our recent data from site-directed mutagenesis of E83K in wild-type AcoAOX1a did not show any significant alteration in the activity, suggesting that interactive effects exist between E83 and the other structural elements held in AcoAOX1a. Careful interpretation of these data predicts some candidate amino acid residues of AcoAOX1a that coordinately function with E83 in retaining its catalytic activity, and furthermore, leads to new proposal for a hypothetical model of their intramolecular interactions. Of particular interest is that these candidate residues lie globally in the primary structure, implying that structural complexity exists beyond a currently supported model of AOX. In this poster, we present our recent findings about structure–function relationships of AcoAOX1a and discuss possible structural interactions involving their responsive residues.

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### 11P.9 Pecularities of cyanide binding to $ba_3$-type cytochrome oxidase from a thermophilic bacterium *Thermus thermophilus*

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Cyanide complexes of the reduced heme $a_3$ in cytochrome $ba_3$ and mitochondrial $aa_3$-type cytochrome oxidase are similar spectroscopically. However, affinity of the reduced heme $a_3^{2+}$ for cyanide may be strikingly different for the two enzymes. In dithionite-reduced bovine oxidase, $K_d$ for cyanide binding with heme $a_3^{2+}$ is 0.4–0.5 mM, in agreement with the data in literature [1]. With $ba_3$ cytochrome oxidase, the affinity of ferrous heme $a_3$ for cyanide is found to depend on the reduction procedure. In the presence of mild reductants (e.g., ascorbate + diaminodurene) or when reduced by dithionite without redox mediators, heme $a_3^{2+}$ binding with cyanide is extremely tight: $K_d$ value for cyanide complex of cytochrome $ba_3$ of the enzyme does not exceed $10^{-8}$ M. The high affinity of cytochrome $ba_3$ for cyanide is associated mainly with extremely low rate of ligand dissociation ($k_{off} < 10^{-6}\ s^{-1}$), while the rate of binding ($k_{on}$ about $10^2\ M^{-1}\ s^{-1}$) is similar to that observed for the mitochondrial cytochrome oxidase [2]. However, if the $ba_3$-type oxidase is reduced by dithionite in the presence of redox mediators which greatly accelerate interaction of the enzyme with the reductant, the $K_d$ for cyanide binding increases to about 0.5 mM, close to the value observed with the mitochondrial oxidase. It is proposed that cyanide affinity for the ferrous heme $a_3$ in $ba_3$ oxidase is controlled by the redox state of $Cu_B$. Binding of a second $CN^-$ to the oxidized $Cu_B$ may lock heme $a_3^{2+}$-bound cyanide in the binuclear site.

**References**

[1] B.C. Hill, et al., Biochem. J. 279 (1991) 355–360.
[2] A.V. Kalinovich, et al., Biochemistry (Moscow) 75 (2010) 342–352.

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### 11P.10 Nitric oxide dependent electron transfer and proton uptake in bacterial nitric oxide reductase

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Nitric oxide reductase (NOR) from *Paracoccus denitrificans* is a deviant heme copper oxidase (HCuO) and catalyzes the two electron reduction of NO to $N_2O$ ($2NO + 2H^+ + 2e^- \rightarrow N_2O + H_2O$). Under aerobic conditions the four electron reduction of $O_2$ to $H_2O$ is catalyzed as a side reaction. In contrast to observations in other HCuOs, neither of the reactions contributes to the proton motive force. NOR purifies as a two subunit complex (NorBC) harboring four redox-active co-factors. A high-spin heme $b_3$ and $Fe_B$, an antiferromagnetically coupled non-heme iron analogous to $Cu_B$ in HCuOs, comprise the binuclear active site in NorB. A low-spin heme *b* in NorB accepts electrons from NorC, a membrane-anchored cytochrome *c*. Using the flow-flash technique we have studied the [NO] dependency of four electron reduced NOR capable of two turnovers: Initially, NO binds to heme $b_3$ with a time constant in the µs range at 1.5 mM NO. This binding is concentration-dependent, which is inconsistent with a previously proposed obligatory binding of NO to $Fe_B$ prior to ligating to heme $b_3$. In the first turnover, NO is reduced by two electrons from the active site. Further oxidation of NOR involves biphasic electron transfer from the low-spin hemes with rates of 50 and 3 $s^{-1}$ at pH 7.5 and 1.5 mM NO. At lower (0.075 mM) [NO] these phases accelerate 3–4-fold. This acceleration is more pronounced (17-fold) at pH 6 due to proton uptake from bulk solution, which is coupled to oxidation of the low-spin hemes. We could demonstrate that substrate inhibition, observed in steady-state measurements, occurs already during the oxidation of the fully reduced enzyme. NO binds to its inhibitory site before electrons can redistribute to the active site from the low-spin hemes *b* and *c*.

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