

the less rigid models. Thus, the large frequency shift ascribable to the presence of copper (I) suggests that the copper ion resides in close proximity to the bound CO. The existence of two conformers is discussed. Additional IR studies of oxygen reactivity with these model compounds were also investigated.

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11P.17 Cyanide inhibition and pyruvate-induced recovery of cytochrome c oxidase

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The mechanism of cyanide inhibition of the mitochondrial cytochrome c oxidase (COX) as well as the conditions for its reversal is not yet fully explained. With regard to the inhibition by KCN and its reversal by pyruvate, we investigated three parameters of COX function, namely the transport of electrons in the terms of oxygen consumption, the proton pumping evaluated as mitochondrial membrane potential ($\Delta\psi_m$) and the enzyme affinity to oxygen by means of p_{50} value calculation. We analyzed the function of COX in intact rat liver mitochondria, either within the respiratory chain or as a sole enzyme, using succinate or ascorbate + TMPD to fuel respiration. We found that 250 μ M KCN completely inhibited both electron and proton transport function of COX, and this inhibition was reversible after washing of mitochondria. The addition of 60 mM pyruvate induced the maximal recovery of both parameters to 60–80% of original values. Using KCN in the low concentration range up to 5 μ M, we observed a profound (30-fold) decrease of COX affinity to oxygen. Again, this decrease was completely reversed by washing of the mitochondria while pyruvate induced only a partial yet still significant recovery of oxygen affinity. Our results demonstrate the reversible nature of inhibition of COX by cyanide and reveal the limited potential of pyruvate to act as a cyanide poisoning antidote. Importantly, we also show that the COX affinity to oxygen is the most sensitive indicator for the detection of toxic effect of cyanide.

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11P.18 Evaluation of the mitochondrial metabolism of two invertebrates' species using permeabilized fibres in high-resolution respirometry

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The use of permeabilized fibres instead of mitochondrial isolations allows the estimation of mitochondrial metabolism in an in situ approach. This approach has several advantages compared to the *in*

vitro approach, notably being closer to the real physiological environment (review in Kuznetsov et al., 2008) and has never been used to assess mitochondrial functions in invertebrates. Measurement of O_2 consumption using permeabilized fibres from high energetic muscles flight of *Drosophila simulans* were used for classic assessment of mitochondrial performances at several steps of the ETS. In another example on the whole body musculature of the polychaete *Nereis virens*, we evaluated the normal and the alternative oxidative pathways in order to understand the conditions of maximum efficiency of ETS and the intervention of alternative oxidase as terminal electron acceptor in some invertebrate species. In flies, results showed very good RCR for complex I with high state 3 respiration. The assessment of complex II showed significant contribution of succinate on the electron transport system. It is the first time that respiration from supplying complex II has been quantified in *Drosophila*. When ubiquinol pool was supplied through complex I, complex II and glycerol-3-phosphate dehydrogenase, the activity of the electron transport system reached a maximum state 3 and further uncoupling showed that the OXPHOS capacity was not overwhelmed suggesting that ATP synthase can support the maximum electron flux measured in the electron transport system. In *Nereis*, RCR for complexes I and II showed low values but consistent with previous studies on mitochondrial isolations from *Nereis pelagica* (Tschischka et al., 2000). When inhibiting complex III, O_2 consumptions measurements showed that 28.97% of state 3 respiration are dedicated to supply alternative oxidase in electrons as well as to the backflux of electrons to complex I and/or complex II. SHAM was used to further inhibit alternative oxidase and allowed us to corroborate the significant contribution of the alternative pathway. We demonstrated here that high-resolution respirometry with permeabilized fibres in invertebrates can be used as an accurate tool to evaluate the mitochondrial metabolism at each steps of the ETS and may insure better understanding of the regulation of several processes not detected in vertebrates like the alternative oxidase.

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11P.19 Computer simulations of proton transfer in cytochrome c oxidase and nitric oxide reductase

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Simulation of proton transfer (PT) in proteins through bridging water molecules and ionizable amino acid residues is a challenging task: classical MD simulations cannot, in principle, describe individual PT steps; on the other hand, ab initio QM/MM simulations of biosystems are still limited by many factors (e.g., sampling, simulation time, convergence). One of the most efficient approaches is the empirical valence bond (EVB) method. Recently, we have adopted an EVB-based multi-level modeling strategy for simulations of the coupled ET/PT events in proteins [1, 2]. (1) We will present the results of our recent computational study [1] of cytochrome c oxidase (CcO), a system that has long presented a conceptual challenge in bioenergetics [3]. After its structure has been solved more than a decade ago, CcO was the focus of numerous works, including a number of computational studies with different methods [2, 4]. Although these studies have shed light on many aspects of CcO functioning, the detailed molecular mechanism of proton pumping