Mitochondrial cytochrome *c* oxidases and many bacterial ones are structurally related and grouped as A-type, while many oxidases in extremophiles compose another subgroup and are classified as B-type. We have found a B-type oxidase, cytochrome bo_3, in a transformable Gram-positive thermophile *Geobacillus thermodenitrificans* K1041 and have studied its functional properties using basic cytochrome *c*-551 (pI = 8.46) from a close relative Bacillus PS3 as the substrate [1–3]. Recent whole genome project on *G. thermodenitrificans* revealed that its cytochrome *c*-551 is really quite similar to the PS3 counterpart in amino acid sequence, however, a few residues are substituted, which surprisingly result in a significant shift of the calculated pI value to 4.99. Since most cytochromes *c* ever studied are basic and electrostatic bonds are crucial in their docking to A-type oxidases, it is interesting to see how an acidic substrate interacts with a B-type partner oxidase. Here, we investigated the interaction between this acidic cytochrome *c*-551 and cytochrome bo_3-type oxidase, either the wild-type enzyme or site-directed mutant ones. The gene for the acidic cytochrome *c* was cloned using genomic DNA from *G. thermodenitrificans* as PCR template, ligated to pSTE12 a shuttle vector between thermophilic Bacillus and *E. coli*, and over-expressed in the authentic host cells. The oxidase activity of the wild type enzyme increased as the salt concentration increased and approached maximum around 200 mM. The optimal pH was 5.0, which is much lower than that obtained with using the basic substrate, 6.7. The substrate-binding site of bo_3-type oxidase might be on the hydrophilic CuA-cupredoxin domain, which contains several acidic amino acid residues and the calculated pI value for which is 4.82. Therefore, the total charge of the acidic cytochrome *c* and the CuA-domain are both neutral at their optimal pH, while the basic substrate is positively charged and the CuA-domain is charged to the opposite direction with a similar extent at their own optimum. Several mutants are constructed for bo_3-type oxidase, in which acidic residues are substituted to neutral ones, and the study using them is underway.

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11P.24 Assessment of Michaelis–Menten parameters by analysis of single time courses of enzyme-catalyzed reactions

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Michaelis–Menten K_M and turnover k_{cat} constants are usually determined by applying the Michaelis–Menten rate equation to a set of initial reaction rates v_0 of product formation at different starting concentrations of substrate. Our theoretical analysis of the quasi-steady state approximation of the Michaelis–Menten model shows that the values of v_0 are inherently underestimated at their experimental determination. We propose a new method for assessment of K_M and k_{cat} without measuring of v_0. The method is based on an analysis of a single time course of product formation or substrate decay by non-linear regression. The non-linear regression procedure uses an explicit solution of the Michaelis–Menten rate equation in terms of the Lambert-W function [1, 2] with transformed variables. Previous attempts to use the integrated Michaelis–Menten equation to assess K_M and k_{cat} have so far met with very little success, because of bad convergence. We significantly improve the convergence by transformation of coordinate system used for the non-linear regression. Test of the new method in experimental conditions of enzyme kinetics of cytochrome *c* oxidase from *Rhodobacter sphaeroides*, as well as tests with computer-simulated data yield that our single curve method is more precise in assessment of K_M and k_{cat} than conventional linear and nonlinear methods using v_0. We infer from our study, that the single trace method can replace usual time- and sample-consuming assays of enzymatic activity, especially where a rapid and reliable control is required.

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11P.25 Heme-copper oxygen reductases superfamily revisited

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Heme-Copper Oxygen reductases (HCOs) are the principal enzymes responsible for reduction of dioxygen in respiratory chains being present in Bacteria, Archaea and mitochondria. Besides catalysing the last reaction of respiratory chains, the reduction of dioxygen to water, HCOs directly contribute to energy conservation first by charge separation (protons and electrons needed for the reaction come from opposite sides of the membrane), and second by proton translocation, part of the energy released during the chemical reaction is used to promote unfavourable proton translocation across the membrane. A primary structural analysis of several HCOs including examples from organisms of different taxonomic groups leads to the identification of distinct patterns for the proton channels. These became the basis for the classification of those enzymes into families, A, B and C, the first, further divided in two, A1 and A2 [1]. Recently, and also based on sequences comparisons, an extension of this classification (family D to H) was proposed, [2]. With the increasing number of available sequences and the new high-throughput methods of sequencing, the number and the taxonomic distribution of known HCO sequences expanded considerable. With this more comprehensive dataset, a re-evaluation of the accepted classification was a natural requirement. We calculated phylogenetic profiles for each of the sub-types of HCOs, and will discuss several evolutionary considerations.

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