Bioinorganic Chemistry

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Supramolecular Bioinorganic Chemistry: Model Complexes of Cytochrome *c* Oxidase Meet Functional Surfaces

*Martin Bröring**

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A long-standing and still very vital branch of bioinorganic chemistry is the design and investigation of model substances mimicking functional and/or structural properties of enzyme active sites.[1] This approach appears particularly successful for active sites containing a molecular cofactor such as a heme group or other bioporphyrinoids. Indeed, four of the binding positions of the metal ion in question are blocked already by the macrocycle in a kinetically inert fashion, so that the chemical reactivity is directed exclusively towards the desired distal or proximal position. Furthermore, porphyrins allow simple functional engineering of the second coordination sphere by covalently attaching selected substituents and groups to the ligand backbone. Simple as well as complex mimetics of protein active site pockets are thus accessible and can be used to gain control of the investigated reaction.[2] The first examples emerging from the application of this strategy were the picket-fence porphyrins, which reversibly bind O_2. These coordination compounds have found their way into many textbooks, and their advent marks the onset of field of bioinorganic chemistry.[3] The strategy has also been applied to compounds other than porphyrins.[1]

The functional mimicking of biological redox processes by molecular models is particularly challenging, especially when dioxygen or partially reduced oxygen species (PROS) play a role in the process. Biomimetic chemistry of dioxygen poses two major problems for the experimentalist: Firstly, clean analytical proof of short-lived species and particularly of oxygen radicals is difficult but essential for the determination of the number of electrons turned over by a redox catalyst. Secondly, molecular model compounds often react nonspecifically with PROS, which ultimately leads to the oxidative decay of the enzyme mimic. Whereas peroxidase and oxygenase functions are usually two-electron processes for which modelling by molecular mimics is generally rather simple, the difficulty in preparing and studying functional models for higher redox processes increases enormously with the number of electrons. The class of membrane-bound cytochrome *c* oxidases (C*c*O) that are used by cells in a four-electron process to transform dioxygen into water and biologically exploitable energy and their mimetics are good examples for the necessity of extremely elaborate systems in both nature and laboratory.[4] In particular, the leaking of toxic and membrane-attacking PROS such as $HOO^{\bullet}$ and $HO^{\bullet}$ has to be suppressed at any price.

The oxygen-reducing site of C*c*O consists of a heme a_3 group with a proximal histidine ligand and a distal Cu_B center at a distance of about 5 Å with a posttranslational modified tyrosine residue Tyr244 (Figure 1). Formally, this special O_2-binding pocket is able to hold all four necessary reduction equivalents and seems to be optimized for the task of clean oxygen reduction. Electrocatalytic studies on model complexes containing these three functionalities in a biomimetic orientation (Fe/Cu/ArOH models[6]) have in fact demonstrat-

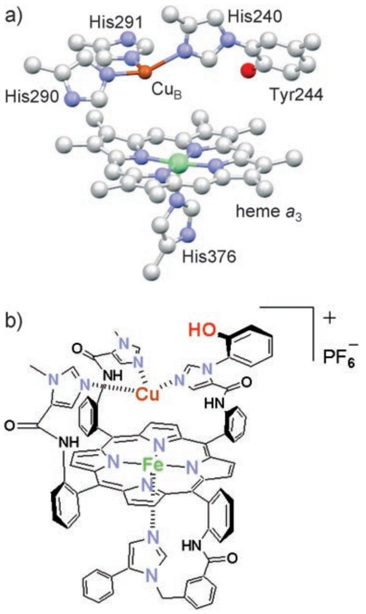

Figure 1. a) Detailed view of the oxygen-reducing site in bovine heart C*c*O.[5] b) Synthetic Fe/Cu/ArOH model complex.[6]

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[*] Prof. Dr. M. Bröring
Fachbereich Chemie
Philipps-Universität Marburg
Hans-Meerwein-Strasse, 35032 Marburg (Germany)
Fax: (+49) 6421-282-5653
E-mail: martin.broering@chemie.uni-marburg.de

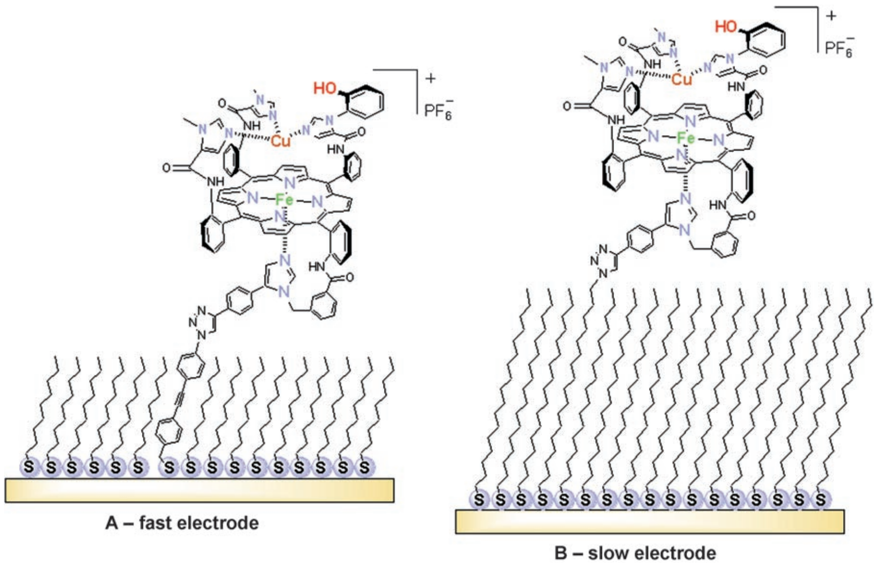
ed the clean four-electron reduction of molecular dioxygen at physiological potential and pH at these engineered catalytic sites. This finding seems to support the assumption stated above that each of the three subunits heme a_3, Cu_B, and Tyr244 provides the required number of electrons to complete the four-electron reduction of dioxygen rapidly enough that the dissociation of toxic PROS intermediates from the active site does not compete. Reference studies performed on partial models lacking the Cu_B and/or Tyr244 mimicking subunits (Fe-only or Fe/Cu models), however, have proven that the four-electron reduction of O_2 can be realized with less-elaborate compounds as well.[7] The limited knowledge of the course of this important reaction in nature, particularly concerning the individual role of each of the three components, is thus evident. As the molecular design of structural and functional C*c*O mimics has been exhausted, these questions are unlikely to be answered by studying classical model complexes, regardless of their complexity.

An obvious difference between the electrocatalytic study of model complexes on a edge-plane graphite electrode and the biological activity of a membrane-bound protein is the velocity with which the reduction equivalents are provided to the catalyst. Whereas on an electrode surface the electrons are available close to the catalytic site in huge excess, the biological system requires stepwise delivery from soluble cytochrome *c* to the membrane protein, where they are then directed to the active site one by one over several additional cofactors. It is possible that the time dioxygen and derivatives are bound to the Fe/Cu/Tyr* active site is thereby significantly increased, as is the probability of release of PROS (which is, however, avoided by nature!).

This point is focused on in the latest work of Collman, Chidsey, and co-workers.[8] By using selectively functionalized gold surfaces with covalently attached C*c*O model complexes, they were able to investigate functional mimics of the active site of four-electron O_2-reducing enzymes with various electron transfer rates. Figure 2 illustrates the composition of such surfaces. The behavior on surface **A** (fast electron transfer through the unsaturated diaryl linker) parallels earlier findings on graphite electrodes. The model complexes gain only a little additional selectivity for the four-electron process with increasing complexity of the system (i.e., Fe-only < Fe/Cu < Fe/Cu/ArOH). However, on the slow surface B, which contains only aliphatic alkylthiolate linkers, a larger variation is observed. Up to 96 % selectivity was found for the Fe/Cu/ArOH system, whereas rapid and complete catalyst degradation was observed for the Fe-only model complex. This result points out in appealing clarity that the interplay of all three substructures provides a finely tuned protective function for the catalyst and the surrounding at slow electron-transfer rates.

The final word on functional modeling of cytochrome *c* oxidase, however, has not yet been spoken, as selectivity as low as 96 % would be fatal in nature. The biological system in fact requires more than 99 % selectivity for the complete four-electron conversion of dioxygen to avoid self-induced degradation.[9] In a postscript the authors describe how the environment (polar gold thiolate/water interface versus non-polar protein/membrane matrix) and the cooperative iron and copper ion reduction found in the enzyme influence the selectivity of the overall process. A solution for the polarity problem is indicated, but mimicking cooperative behavior remains a long-standing and as yet unresolved problem in bioinorganic chemistry. Here one may hope for future developments.

The general applicability of the approach described herein has been ensured by the development of reproducible SAMs located on rotating gold disk electrodes and by the gentle click method used for the attachment of the catalysts.[10] Therefore, further work can easily be foreseen in this area, in particular, in relation to other biological multiple-electron processes that have been difficult to model by molecules. Functional supramolecular assemblies of biomimetic model complexes such as the system presented herein or the artificial



A – fast electrode

B – slow electrode

Figure 2. Fast and slow gold–thiolate electrodes with covalently attached Fe/Cu/ArOH model complexes.

photosynthetic membranes from Gust and co-workers[11] appear to be inspiring and trendsetting constructions for bioinorganic chemists. They promise a better understanding of natural processes and also take into consideration the function(s) of the biogenous matrix in an appropriate, and perhaps more importantly, predictable way.

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