

New Reactive Intermediates in Enzymatic Oxidations Studied by Means of Spin Chemistry Methods

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Enzymatic processes in their entirety have been a long-standing attraction for those interested not only in the biochemical and biomedical implications of the reaction mechanisms, but also in the understanding of the underlying reasons of the striking selectivity and efficiency of these processes. However, the traditional kinetic methods of monitoring the substrate disappearance and/or enzyme inactivation do not provide necessary information on the transient species involved in the enzymatic processes, thus leaving the fine details of molecular mechanism beyond the limits of the capabilities of these methods. On the other hand, the increasingly spreading viewpoint on the existence of electron transfer stages in the enzymatic oxidations provides for the application of spin chemistry methods capable to clarify the structure and reactivity of paramagnetic intermediates including those formed in biologically relevant processes.

The present work demonstrates the potentialities of the spin chemistry methods, namely, the magnetic field effects, in the investigations of fine details of enzymatic oxidations catalyzed by one of the most common representatives of heme-enzymes family, horseradish peroxidase (HRP).

The enzymatic oxidations of the isobutyraldehyde enol anion have been studied at different external magnetic field strengths. It has been found that magnetic field exerts notable (~30%) influence upon the effective rates of interconversion of the reactive intermediates of the catalytic cycle of HRP, thus confirming the existence of single electron transfer stages which result in paramagnetic intermediates. The analysis in the framework of radical pair theory has allowed to suggest the formation of new radical derivative of HRP's Compound II, and the calculated field dependence is in a good agreement with the experimental one.

In contrast to the above, the magnetic field effects observed in the reaction of HRP with the native substrate, NADH, favor the formation of ferropoxidase (HRP-Fe²⁺) resulting from the initial single electron transfer step between native HRP and NADH. Theoretical modeling of these effects presumably formed in quartet-doublet transitions in the starting paramagnetic pair, by means of two-position model accounting for electron exchange interaction, is in line with the experimental field dependence.

Both results show the new prospects of the catalytic cycles of the heme enzymes as well as of the selectivity of their oxidations.

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