Communication

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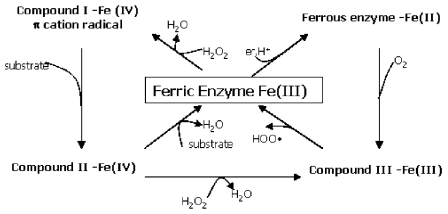
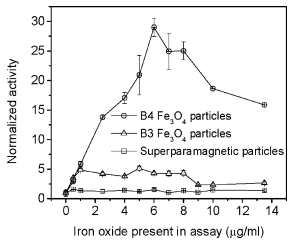
J. Am. Chem. Soc., **2008**, 130 (10), 2910-2911 • DOI: 10.1021/ja7102263





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Published on Web 02/15/2008

Activity Increase of Horseradish Peroxidase in the Presence of Magnetic Particles

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Received November 11, 2007; E-mail: nc68@cornell.edu

Magnetic field effects (MFE) on enzymatic reactions have been investigated as early as the 1960s, and most of the experiments carried out initially reported no effect on the reaction rates.[1] In the mid-1980s focus was turned to radical pairs;[2,3] in the context of the radical pair recombination theory,[3] the effect of magnetic fields on biological reactions with paramagnetic species was studied.[4] Recently, a number of publications examined the effect of an external magnetic field and that of spin orbit coupling (SOC) on the kinetics of enzymatic reactions.[5] In particular, evidence was found for the existence of a magnetosensitive step in the catalytic cycle of horseradish peroxidase (HRP).[6,7]

Here, for the first time, we report evidence for the effect of very low magnetic fields on HRP. The presence of magnetite particles (Fe_3O_4) in the enzyme assay had a 30-fold increase on the rate of reaction of horseradish peroxidase.

During the catalytic cycle, native HRP, its' intermediate compounds I and II, and the oxidized organic substrate radicals are all paramagnetic species.[7] A combination of any two can form a paramagnetic (radical) pair that can undergo spin selective processes.[7] The presence of magnetic particles in the assay is hypothesized to have an effect on the spin states of geminate pairs (G-pairs) and on random-encounter diffusing radicals (F-pairs) via an accelerated intersystem crossing (ISC) mechanism that can alter the overall kinetics of the enzyme reaction.[3,8] To test the effect of the magnetite particles on enzymatic activity we used iron oxide particles with varying magnetic strengths. Specifically we used a series of iron oxide particles with permanent magnetic moments of 1, 0.48, and 0 emu/g denoted B4, B3, and superparamagnetic (SPM), respectively. Given the average diameter of the B4 particles (~300 nm) and their magnetic strength, an estimated magnetic field was calculated for the vicinity of such a particle. The magnetic field ranged from 3 G, 200 nm from the particle's surface, to 0.1 G for 1 μm from the surface (details in the Supporting Information).

The catalytic activity of horseradish peroxidase increased 30-fold, when magnetite particles were present in the assay at low concentrations. These results were reproduced using different batches of enzyme and different vendors (Fischer, Pierce). In Figure 1 the activity of HRP is shown with varying particle concentrations in the assay (the phenol-aminoantipyrine (AAP) chromogen was used).[9] In contrast, the enzyme activity did not change when commercial super-paramagnetic particles with no permanent magnetic moment were present in the assay.

We examined various other redox enzymes with and without a heme group (cytochrome *c*, chloroperoxidase, catalase, and glucose oxidase) under the same limiting conditions, but we were unable to observe any positive or negative effect on the activity when magnetic particles were present in the assay.

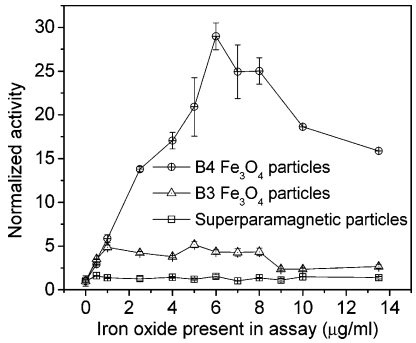

Figure 1. Activity of HRP in the presence of magnetic and superparamagnetic iron oxide particles. Values normalized with the activity of neat HRP. The error bars are the standard deviation of four repetitions.

The iron oxide particles appeared to have no reducing or oxidizing effect on the substrates, and no activity was found when the protocol was tested in the absence of HRP. The protocol was also tested in the presence of Fe^{3+} ions; likewise no effect on the measured activity of HRP was observed.

In addition, the possibility of a redox process between the enzyme's active site and the particles was examined, and the Soret band of the enzyme was probed. The characteristic peak of the heme group at 403 nm was not shifted, and the intensity of the peak did not change in the presence of iron oxide particles.

We also found that the effect of the particles was reversible; namely, the enzymatic activity returned to the original value once the particles were removed with a strong Nd magnet. Furthermore, the activity increase was not observed when smaller particles (<80 nm) of the B3 batch were tested in similar concentrations. The above result ruled out the possibility of a surface effect responsible for the observed activity increase. Thus, the observed activity increase appears to be related to the magnetic properties of the iron oxide particles.

To obtain a better understanding of the enzyme behavior in the presence of magnetic particles, we examined the apparent Michaelis−Menten kinetics parameters of the enzyme with and without magnetic particles. The neat enzyme had an activity maximum at 0.3 mM hydrogen peroxide. Further increase of substrate in the assay resulted in a decrease of the catalytic activity. This last observation has been well documented for HRP, where at relatively high concentrations of peroxide the enzyme undergoes substrate inhibition.[10,11] The activity of HRP with B4 particles reached a plateau at high peroxide concentrations, and substrate inhibition was registered only at concentrations close to 50 mM (compare to 0.3 mM in the absence of particles).

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10.1021/ja7102263 CCC: $40.75

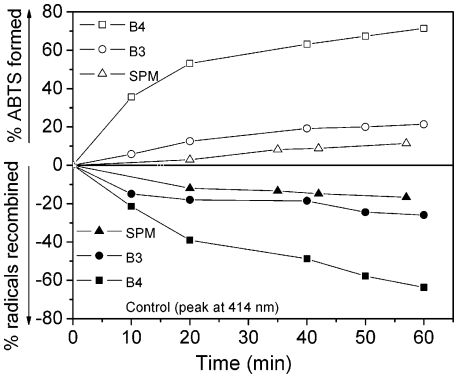

Figure 2. Extent of ABTS radicals recombination in the presence of iron oxide particles with increasing permanent magnetic moment.

For HRP, a modified Michaelis−Menten equation is used that takes into account substrate inhibition.[12]

$$V = \frac{V_{\max} \cdot [S]}{K_m + [S] + K_i \cdot [S]^2}$$

The presence of 6 μg/mL of magnetic particles in the assay significantly decreased the substrate inhibition term K_i (close to 10-fold) and increased the turnover rate (k_{cat}) for HRP. The turnover rate increased almost three times in the presence of B3 and five times in the presence of B4 particles. The tables of the calculated V_{max}, K_m, and K_i are included in the Supporting Information.

Identical experiments were also conducted with 2,2′-azino-di-(3-ethyl-benzthiazoline-6-sulfonic acid) (ABTS) as the chromogen substrate. The Michaelis−Menten kinetics parameters with this chromogen were also estimated (for values see Supporting Information). The k_{cat} of the enzyme once again increased significantly in the presence of magnetic particles. However, in contrast to the results obtained with the phenol/AAP chromogen system, similar substrate inhibition was observed when magnetic particles were present in the assay. The differences between the apparent kinetic parameters obtained with the two chromogen assays are possibly due to the different oxidation mechanisms for each substrate.[11,13] The phenol/AAP depends on the combination of two radicals to generate a colored product[13] whereas ABTS does not. Secondary side reactions exist for phenol radicals resulting in formation of biphenyl products.[13,14] Nevertheless, in both protocols an increase in k_{cat} was observed.

From the data obtained using ABTS, B3 particles appeared to have a larger effect on the turnover rate than the more magnetic B4 particles. This observation was somewhat puzzling, as we expected the more magnetic particles to have a larger effect (as in the case of phenol/AAP). One possibility might be an increase in the recombination rate of the generated ABTS radicals that partially disproportionate back into ABTS in the presence of magnetic particles (for a reaction scheme, see Supporting Information) To test the above hypothesis, we designed an experiment that would track the evolution of a disproportionation reaction between two radicals, when considered as Random Encounter Radical Pairs (RERP or F-pairs).

In Figure 2, the percentage of ABTS radicals recombining back to ABTS is shown, and particles with an increasing permanent magnetic moment had an increasingly beneficial role vis-à-vis the extent of disproportionation. A control solution with the same concentration of ABTS radicals but without iron oxide particles showed no significant disproportionation in the time scale of the experiment (the complete data of the full spectra scans of the samples as a function of time are included in the Supporting Information).

This increase in the disproportionation rate appears to correlate with the increasing magnetic strength of the particles. In particular, the results suggest that the weak magnetic field of the randomly distributed particles increased the recombination rate of the diffusing ABTS radicals.

The above observations suggest that magnetic particles could play a significant role in reactions with spin-correlated radical pairs and possibly affect paramagnetic species, similar to the species formed during the HRP catalytic cycle (geminate pairs or diffusing RERPs).

Acknowledgment. The authors acknowledge financial support from Cornell's Nanobiotechnology Center (NBTC). The authors would like to thank Prof. Anthony Hay, Prof. Tadhg Begley, Dr. Magnus Bergkvist, and Dr. Alejandro Becerra-Arteaga for their comments.

Supporting Information Available: Tables with the values of all kinetic parameters. A reaction scheme of the catalytic cycle of HRP. Characterization data for B4, B3, and SPM particles. Full spectra scans of radicals (oxidized) and unoxidized ABTS as well as detailed experimental descriptions. Reaction schemes for ABTS and ABTS radicals as well as phenol and AAP. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA7102263