A Hydroperoxylation Mechanism for Hydroxyethylphosphonate Dioxygenase

SUPPORTING INFORMATION

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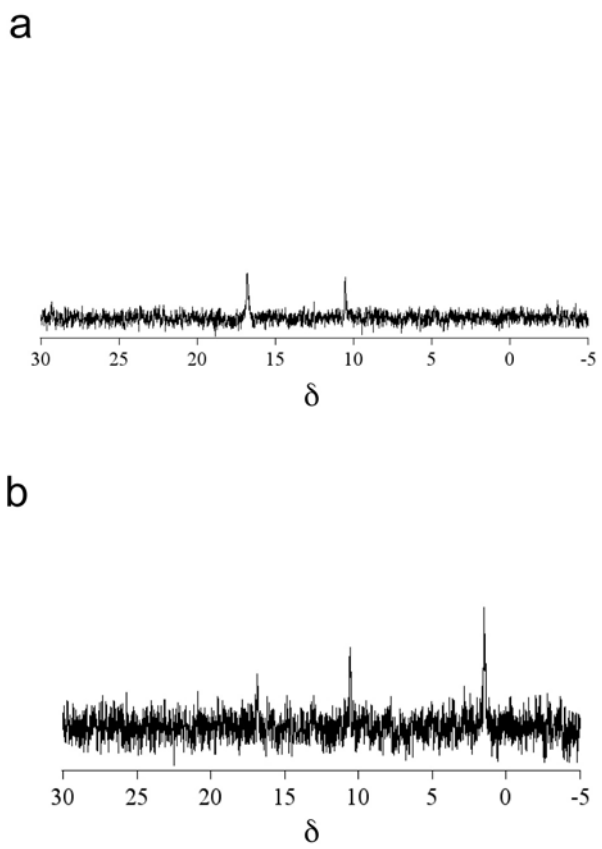
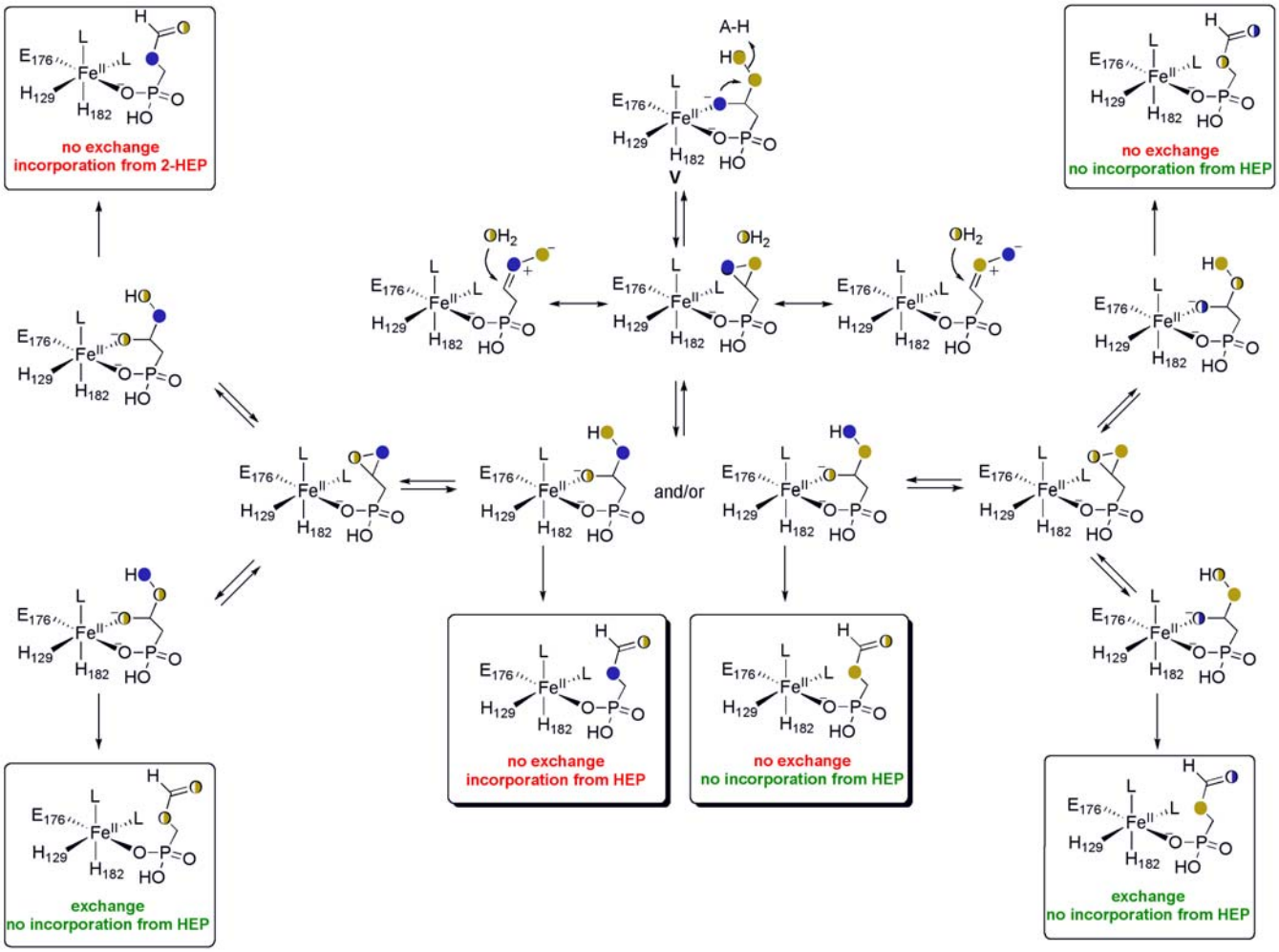


Figure S1: A mixture of OFHMP:HMP (1:1) was incubated in HEPES buffer for 2 h a) in absence of HEPD and b) in presence of HEPD.



Scheme S1. More complete dioxirane exchange mechanism showing all products after two rounds of exchange. Oxygen derived from 2-HEP is depicted in blue, oxygen derived from molecular oxygen is depicted in orange and any exchange that takes place with solvent is shown in half-circles. Products from one round of exchange are shown in shaded boxes with products with two rounds of exchange shown in regular boxes. The mechanism involving an equilibrium between a peroxyacetal and dioxirane shown in the main text depicts the products after one round of exchange. Shown in this scheme are the products after two rounds of exchange. Depicted in red are outcomes that are inconsistent with the experimental results (incorporation of oxygen from solvent into HMP and no incorporation of the hydroxyl oxygen from 2-HEP into HMP) whereas in green are depicted outcomes that are consistent with the experimental results. Only two pathways provide products that are consistent with the experimental observations, but there is no obvious explanation why the pathways leading to these products would be preferred over the other pathways. Whereas the asymmetric environment of the active site in principle could favor exchange of one of the two dioxirane oxygens, both pathways that provide the observed products require exchange of the alkoxide oxygen of the peroxyacetal in one round of exchange and then exchange of the proximal peroxy oxygen in the other round of exchange. This requirement makes this mechanism highly unlikely. In addition, both pathways result in exchange at both positions of formyl-HMP, adding other constraints. These include complete absence in HMP of the oxygen derived from the hydroxyl group of 2-HEP but only exchange of 40% of the oxygen derived from molecular oxygen, as well as the observation that none of the isolated formate product contains two oxygen atoms derived from solvent. Collectively, we feel that the dioxirane mechanism does not agree with all experimental observations.