Hydrolysis of Phosphohistidine in Water and in Prostatic Acid Phosphatase

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Supplementary Information

Methods

All the classical MD simulations were done with GROMACS 3.3.[1] The force field parameters of phosphohistidine were obtained by comparison to standard residue types in the topology. The partial charges were assigned by a careful inspection of those obtained based on CHELPG method[2] implemented in the Gaussian 03 program[3] at the level of HF/6-31G* and GROMACS atom types. The dihedral parameters were taken form PRODRG.[4] The obtained force field parameters were tested by carrying out 3x2ns MD simulations of phosphohistidine in explicit water. The final simulation was done for 5ns. To equilibrate HIP in water, first the residue was capped with Ace and NH2 on the N- and C- termini. The system was then placed in dodecahedron box of size 67.67 nm^3 and solvated with 2223 SPC water molecules.[5]

In the case of enzyme, the initial structural model of phosphohistidine enzyme intermediate was obtained from our previous study on the first step of the reaction catalyzed by PAP (Ref. 6 in Main Communication). The obtained product geometry from the previous ONIOM calculations, with the leaving phenol removed, was fitted back into the MD minimized whole protein. To relax the active site residues and waters in response to the removed phenol, MD simulation of whole protein was carried out in a SPC water box (15386 waters in 518 nm^3 box). Following the conjugate gradient minimization until a tolerance of 100 kJ mol^{-1}, a short (50 ps) position restrained simulation was done. The restraints were applied on all heavy atoms (force constant of 1000 KJ mol^{-1} nm^{-2}). The restraints were kept for the backbone of loop with residues 63-66 and 90-110 during the 5 ns production simulations to avoid the artifacts of simulating a monomer. This 90-110 loop region shows a bending motion when a monomer of the enzyme is simulated, which is not seen in the dimer.

For all production runs, the time step for the integration of motion was 2 fs. The simulations were run at constant temperature and pressure by coupling to an external bath with coupling constant of 1.0 ps.[6] The fast particle mesh Ewald summation technique (PME) was employed to correctly compute long-ranged Coulomb forces.[7] The bond lengths were constrained using the LINCS algorithm.[8] The SETTLE algorithm[9] was employed to constrain internal degrees of freedom of the water molecules.

One of the time points from the MD simulation in explicit water was selected and all the atoms within 12 Å of P atom of HIP were taken to build a two-layered ONIOM model. The side chain of HIP (including Cβ atom) and seven waters were included in the QM region. The active site ONIOM model employed a 15 Å shell centered at P atom of HIP. The atoms in the outer 3 Å layer were frozen to their

cartesian coordinates to keep the active site intact. The QM motif included side chains up to Cδ of Arg11, Arg15 and Arg79; Cβ of HIP12; Cβ of Asp258 and the nucleophilic water. The hydrogen link atoms were used to cap the QM part. The QM region was treated at B3LYP/6-31+G* level of theory using Gaussian 03 and Gaussian 09.[3,10] Rest all the atoms were treated with AMBER force field parameters. The optimizations were performed for each point along the reaction coordinate to locate putative stationary points. The scan had a resolution of 0.01 Å in the regions of interest. Finally, the single point energies were obtained at 6-311+G* basis set for the QM system.

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Additional Figures:

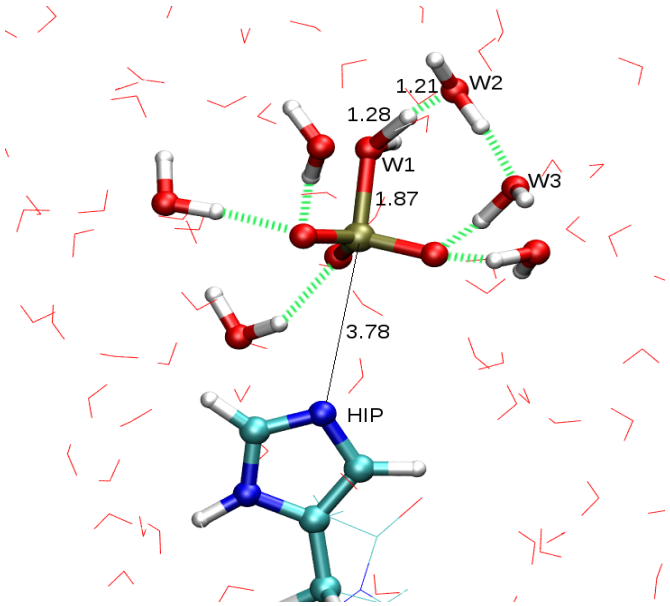

Figure S1. The structure of the second transition state (TS2) in case of phosphohistidine hydrolysis in water cluster.

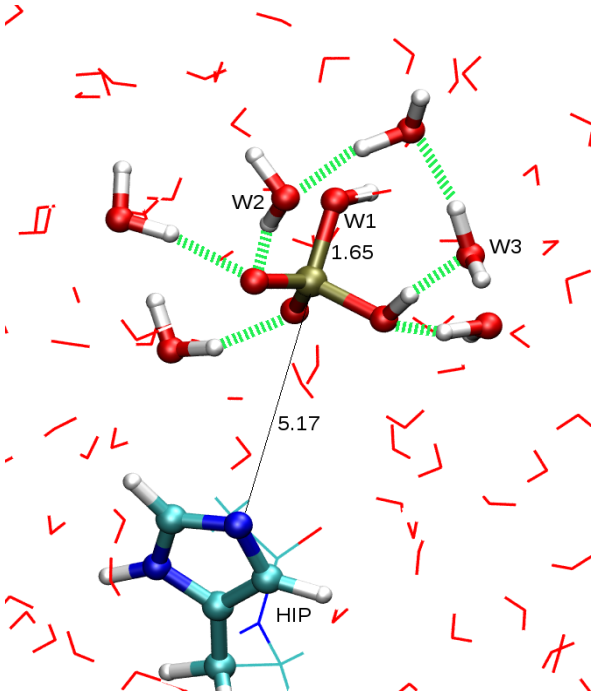

Figure S2. The structure of the products in case of phosphohistidine hydrolysis in water cluster.

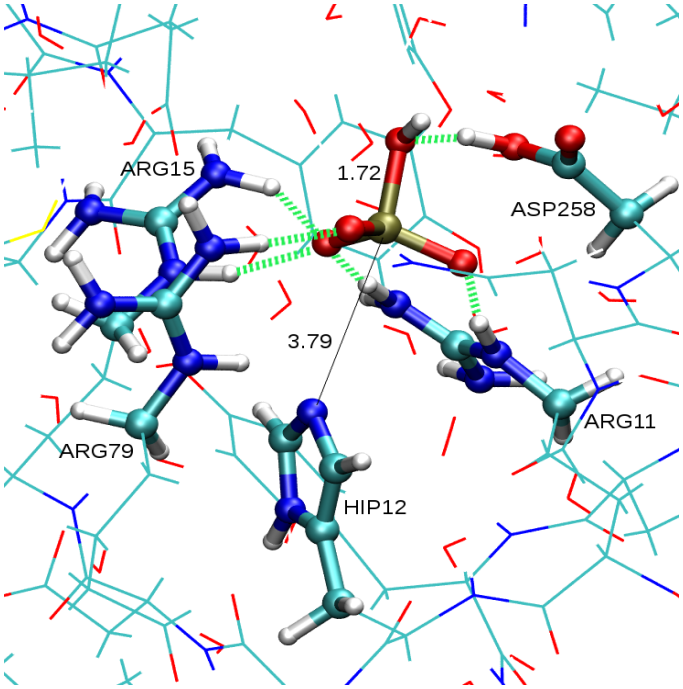

Figure S3. The structure of the products in case of phosphohistidine hydrolysis in Prostatic Acid Phosphatase active site.