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Steered molecular dynamics study of inhibitor binding in the internal binding site in dehaloperoxidase-hemoglobin

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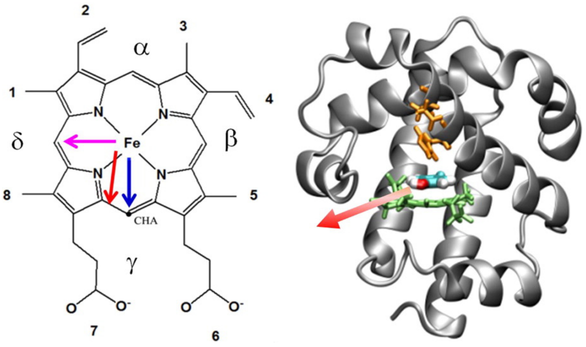
HIGHLIGHTS

- Use of steered molecular dynamics to determine molecular binding trajectory in a protein
- Comparison of umbrella sampling and jarzynski equality methods for validation
- Systematic test of effect of restraints on calculated binding free energy
- Comparison of calculated binding free energy with experiment in dehaloperoxidase
- General method applicable to full range of substrates and inhibitors that bind to dehaloperoxidase

GRAPHICAL ABSTRACT



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ABSTRACT

The binding free energy of 4-bromophenol (4-BP), an inhibitor that binds in the internal binding site in dehaloperoxidase-hemoglobin (DHP) was calculated using Molecular Dynamics (MD) methods combined with pulling or umbrella sampling. The effects of systematic changes in the pulling speed, pulling force constant and restraint force constant on the calculated potential of mean force (PMF) are presented in this study. The PMFs calculated using steered molecular dynamics (SMD) were validated by umbrella sampling (US) in the strongly restrained regime. A series of restraint force constants ranging from 1000 down to 5 kJ/(mol nm^2) were used in SMD simulations. This range was validated using US, however noting that weaker restraints give rise to a broader sampling of configurations. This comparison was further tested by a pulling simulation conducted without any restraints, which was observed to have a value closest to the experimentally measured free energy for binding of 4-BP to DHP based on ultraviolet–visible (UV–vis) and resonance Raman spectroscopies. The protein-inhibitor system is well suited for fundamental study of free energy calculations because the DHP protein is relatively small and the inhibitor is quite rigid. Simulation configuration structures are compared to the X-ray crystallography structures of the binding site of 4-BP in the distal pocket above the heme.

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1. Introduction

The binding of an inhibitor, 4-bromophenol (4-BP), in a well-defined binding site in the distal pocket of the multi-functional enzyme dehaloperoxidase-hemoglobin (DHP) from *Amphitrite ornata* presents an interesting set of biological questions while simultaneously providing a well-defined model system to test computational methods for the calculation of binding free energy [1,2]. The nature of the binding trajectory is of interest since 4-BP must displace a number of amino acid side chains in DHP in order to enter or exit the distal pocket. The modeling of the trajectory using molecular dynamics (MD) simulations has the same features observed in the general class of calculations of drug and ligand binding [3,4] and is particularly closely related to the binding of substrates to cytochrome P450 [5–9]. Since 4-BP is relatively rigid, having only the bending and torsions of the hydroxyl group as internal degrees of freedom, the parameters are relatively easily incorporated into the force field for studies of the binding pathway using and the free energy via the potential of mean force (PMF) calculated using steered molecular dynamics (SMD) simulations. This makes the problem similar to the binding of small molecules such as diatomic ligands (CO, NO, O_2), indazole or nicotinic acid, which have also been studied using similar methods [10–14]. However, the inhibitor, 4-BP, is significantly larger than a diatomic molecule and therefore the results have more relevance for binding free energy calculations in drug-target or ligand-receptor systems. The calculation of the free energy of binding using non-equilibrium methods has been demonstrated using a variety of systems, many of them with far greater complexity that the DHP + 4-BP combination [5,6,11,15–20]. When the ligand has significant internal degrees of freedom, such as in peptide-protein or protein–protein binding, correction terms must be applied to thermodynamic integration to extract the free energy from the simulated trajectory [21–24]. By contrast the DHP + 4-BP system consists of a small rigid molecule that nonetheless has a well-defined internal binding pocket based on X-ray crystal structures (Fig. 1) [1,25–33]. In this study the restraints applied to the DHP protein are systematically reduced and ultimately compared to a restraint-free calculation. The systematic approach reveals a trade-off in the large magnitude of fluctuations in the calculated free energy and the increase in tractability as DHP is more restrained[34].

The selection of an appropriate free energy calculation method is important for modeling the DHP + 4-BP binding process, and can affect the calculated binding energy. Several methods are present in the literature that permit the calculation of free energy differences from equilibrium MD simulation trajectories, such as thermodynamic integration, free energy perturbation (FEP) [4], and umbrella sampling (US) employing the weighted histogram analysis method (WHAM) [35]. These equilibrium methods can provide an accurate estimation of protein-ligand binding affinities, but they are only applicable if the system is sufficiently rigid so that the various stages of the trajectory have comparable geometries. An alternative non-equilibrium method based on the Jarzynski equality [36,37], the so-called “fast growth” method [38], has been developed for free energy calculations as well. The Jarzynski equality has been employed to extract free energy profiles from repeated non-equilibrium pulling trajectories [39–45]. The non-equilibrium SMD method is considered less accurate than equilibrium US methods [15,46,47]. However, there are two advantages of the non-equilibrium method in free energy calculations compared to equilibrium methods: i) Non-equilibrium binding (or unbinding) simulations can be conducted for any value of the restraint force constant; ii) The method permits a relatively rapid test of different binding trajectories, which is relevant to the mechanism of protein-ligand interactions [12,48–50].

The advantages of the SMD method can be used to study DHP ligand binding. DHP is a multifunctional protein, which has documented functions as a globin (oxygen transport), peroxidase (electron transfer), peroxygenase (oxygen atom transfer) and an oxidase (oxidation by O_2) [51]. The peroxygenase function has similarity with cytochrome P450 since both involve O atom transfer in their mechanism. The difference is that the O atom in P450 comes from bound O_2, while that in DHP

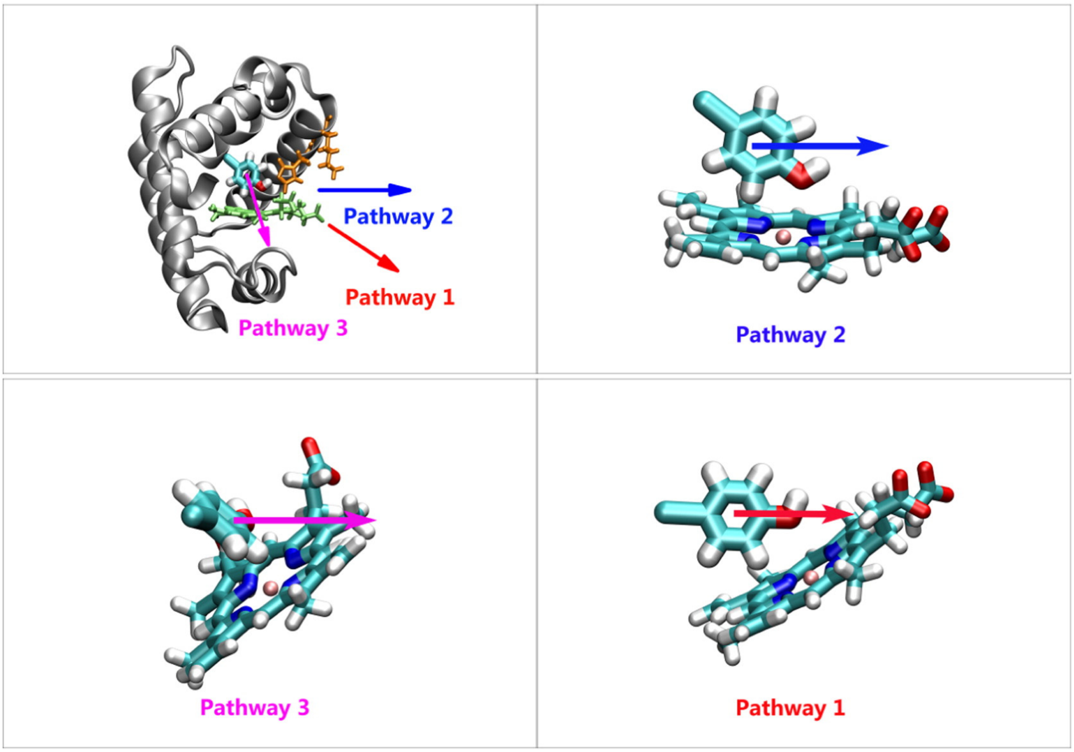

Fig. 1. Schematic representation of the three pathways adopted in the SMD simulations for the DHP-4-BP complex. Pathway 1 is defined by the vector of the Br – O distance. This vector aimed downward towards −y and is ~50° from the x-direction. Pathway 2 is along the x-axis and bisects the two propionate groups on the γ-edge of the heme. Pathway 3 is directed towards the δ-edge of the heme and is along the z-axis and −y-axis.