Molecular Dynamics Simulations of a Low Oxygen Affinity Mutant Hemoglobin

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A recombinant(r) hemoglobin(Hb), α96Val $\rightarrow$ Trp, has been produced by using *Escherichia coli* expression plasmid in which synthetic human α and β -globin genes are coexpressed with the *Escherichia coli* methionine aminopeptidase gene.[1] The artificial hemoglobin has low oxygen affinity and high cooperativity in oxygen binding with the Hill constant of 2.2-2.6, which compares to the value of 3.0 for normal Hb A. The carbonmonoxy form of rHb(α96Val $\rightarrow$ Trp) can be easily converted to a ligated T-like quaternary structure.

These functional properties provide an opportunity to develop a potential candidate for hemoglobin-based blood substitutes, and there have been extensive studies to characterize the mutant hemoglobin by NMR spectroscopy, X-ray diffraction and molecular dynamics (MD) simulations.[1,2] In rHb(α96Val $\rightarrow$ Trp), a valine located in the α_1-β_2 (and α_2-β_1) subunit interface is replaced by a bulky tryptophan. ^{1}H NMR spectroscopy data indicated that the mutant hemoglobin had the tertiary and quaternary structures similar to those of Hb A in both the deoxy and carbonmonoxy forms.

Prior to the X-ray crystallographic structure determination, preliminary MD simulations of the deoxy T-state rHb (α96Val $\rightarrow$ Trp) were undertaken using CHARMM22 with the standard polar hydrogen protein potential parameter set (PARAM19).[3] The rHb model was based on the crystal structure of deoxy-Hb A with both 96Val transformed to Trp. All side chain angles of the tryptophan residue with more than 10% incidence in the rotamer library were considered as the initial conformation.[4] The side chain angles, χ_1 and χ_2. are listed in Table 1. The origin of the coordinate system was set to the middle of the C^{β} of the two α96 side chains. The protein model was hydrated with 103 TIP3P water molecules. The system is partitioned by 10 Å and 15 Å radius spheres into reaction, buffer and reservoir regions for stochastic boundary (SB) MD simulations. A stochastic solvent boundary potential was applied to the 15 Å spherical surface and the Langevin equations of motion were integrated for the buffer region atoms. The final conformation derived from the second rotamer was chosen as the mutant structure by considering bulkiness and symmetric arrangement of the tryptophan residues. The result showed that the α96Trp residues were sufficiently separated and directed into the $\alpha\beta$ interface so as to introduce an additional hydrogen bond between the indole nitrogen of α96Trp and the carboxylate side chain of β99Asp. The unique oxygen binding properties of rHb(α96Val $\rightarrow$ Trp) were explained with the additional hydrogen bond.

The crystallographic data of rHb(α96Val $\rightarrow$ Trp) was obtained later at the 1.9 Å resolution,[2] which revealed that α96Trp was in a conformation different from that predicted by the previous MD simulation. The indole side chain of the tryptophan is directed away from the interface and into the central cavity. There is no evidence for the hydrogen bond between α96Trp and β99Asp in the crystallographic structure. In the structure, the indole nitrogen makes water mediated hydrogen bonds with β101Glu instead. The water mediated hydrogen bonds are proposed as the structural basis for the low oxygen affinity of the mutant hemoglobin.

We have extended the molecular model of rHb(α96Val $\rightarrow$ Trp) and performed SBMD simulations. Here, we use CHARMM27 with the all hydrogen protein potential parameter set.[5] Our model is based on the deoxy-Hb coordinates obtained from Protein Data Bank (pdb2hhb).[6] Considering

Table 1. α96Trp side chain conformations of the SBMD simulation structures[a]

No.	Rotamer Library		15 Å spherical SBMD[b]				20 Å spherical SBMD[c]			
			α_1 Trp96		α_2 Trp96		α_1 Trp96		α_2 Trp96	
	χ_1	χ_2	χ_1	χ_2	χ_1	χ_2	χ_1	χ_2	χ_1	χ_2
I	-70.4 ± 7.0	100.5± 18.2	-63.3	-176.3	-88.5	128.9	-178.6	-108.8	-160.0	-104.0
II	64.91± 3.0	-88.9± 5.3	92.3	-71.0	106.2	-78.7	-179.3	-132.5	-157.3	-68.0
III	-177.3 ± 7.9	-95.1± 7.6	-86.5	86.9	-174.2	-116.3	178.6	-119.5	178.7	-115.3
IV	-179.5 ± 3.4	87.5± 3.8	-118.0	89.5	-177.1	71.8	-153.0	-106.7	-178.7	-118.0

[a]Data are given in degree. [b]From Ref. 1. These were based on the dynamics average structures. [c]Angles are averaged for the dynamics frames from 90ps to 100ps with the 0.1ps interval.

hydrogen bonding feasibility and exposure into the solvent, we determine the protonation state of titratable residues. All aspartic acids and glutamic acids are in the carboxylate form and all lysines have ε-NH_3^+. α20, α50, α58, α87, α89, β2, β63, β77, β92, β97 and β143 His have δ-NH and α45, α72, α103, α112, α122, α116 and α117 His have ε-NH. β146 Histidines are doubly protonated. Four heme molecules are included. 221 TIP3P water molecules are placed at the crystal water positions in the 2hhb structure. Hydrogens are placed by using the CHARMM HBUILD function.

The α96Val side chain is replaced by the Trp indole ring. Without consulting the newly available crystallographic data, the conformation of the indole ring is built into the structure based on the rotamer library as in the previous MD simulation. Four possible rotamer side chain angles of Table 1 are used to generate the initial coordinates. Each rHb(α96Val $\rightarrow$ Trp) model is briefly minimized in energy to remove abnormal strain due to the addition of the tryptophan rings. The origin of the molecular coordinate is set to the middle of the C^{β} of the two α96 side chains. The system is solvated with a water sphere of 20 Å in radius and is partitioned with 16 Å and 20 Å radius spheres to set the stochastic boundary conditions.[7] 112 or 113 TIP3P water molecules are introduced in the solvation process. Coordinates of hydrogen atoms and the bulk water molecules are optimized in the solvated system. The inside of the 16 Å sphere is the reaction region, in which atoms move following the normal molecular dynamics. Atoms in the buffer region between 16 and 20 Å spheres run under the Langevin stochastic dynamics. The outside of the 20 Å radius sphere is the reservoir region, where all atoms are fixed in position.

The leap-frog integrator of CHARMM is used to integrate both Langevin and normal equations of motion with the time increment of 1.0fs. All hydrogen connected bond lengths are fixed with the SHAKE method.[8] The SBMD simulation is performed for 100ps with each rotamer system at 300 K. The coordinate trajectories saved at every 100fs are analyzed to yield the conformation of the indole side chain of α96Trp as summarized in Table 1. In contrast to the previous MD simulation with the 15 Å SB, our simulations converge into the conformation with $\chi_1 = 180^o$ and $\chi_2 = -110^o$ with the fluctuation of 10^o approximately. The dimers tend to be symmetric. The crystallographic data are $\alpha_1\chi_1$=171.6°, $\alpha_1\chi_2$ = −114.9°, $\alpha_2\chi_1$ =162.3° and $\alpha_2\chi_2$ = −114.9, which are most closely reproduced in the simulation III. The 100ps snapshot of the simulation III is shown in Figure 1. The sidechains of the mutant tryptophan residues extrude into the central cavity. In all simulation trajectories, we find one to three crystallographic or bulk water molecules located less than 4.0 Å away from the 97Trp indole nitrogen atom. In the 100ps trajectories, crystallographic and bulk water molecules move about 3.5 Å and 6 Å in average, respectively. Some water molecules are displaced as much as 18 Å. The current simulation reproduces the conformation of the mutant residue and indicates the water mediated hydrogen bonds of the crystallographic structure.

Although the previous MD simulation failed to predict the conformation of the mutant residue, it was a pristine attempt to explore the modified structure resulting from the site-directed mutagenesis. As we review the previous work, we find that the system size is not large enough to provide appropriate conformational space for the relatively large tryptophan residues and too many water molecules are added into the void as the bulk water. Here, we have demonstrated that careful SBMD simulations with explicit solvent water molecules are able to predict the structural detail of proteins produced by the site-directed mutagenesis.

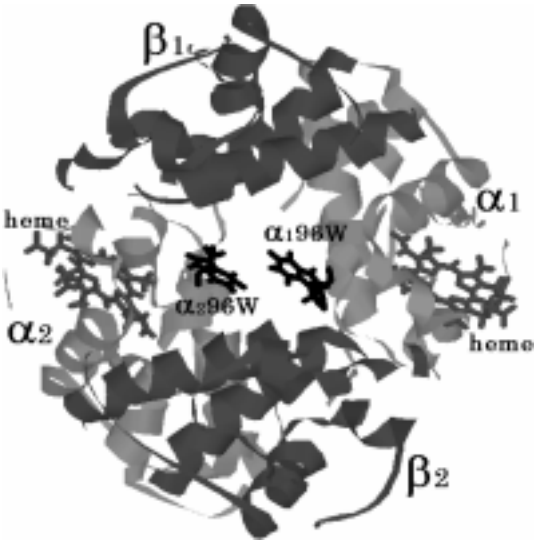

Figure 1. The central cavity of the mutant hemoglobin rHb (α96Val $\rightarrow$ Trp) is shown in the 20 Å radius spherical stochastic boundary. The structure is the 100ps snapshot of the simulation III, which is very close to the crystallographic data. Hemes of subunits and α96W residues are also depicted in the figure. The tryptophan sidechains extrude into the central cavity.

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