CHAPTER 4

DETAILS IN SIMULATIONS

4.1 Method

The crystal structure of the hDHFR (1DRF in Protein Data Bank code) [14], refined at a resolution of 2.0 Å was used as the starting geometry where atomic coordinates of folate molecules and the crystal water were deleted.

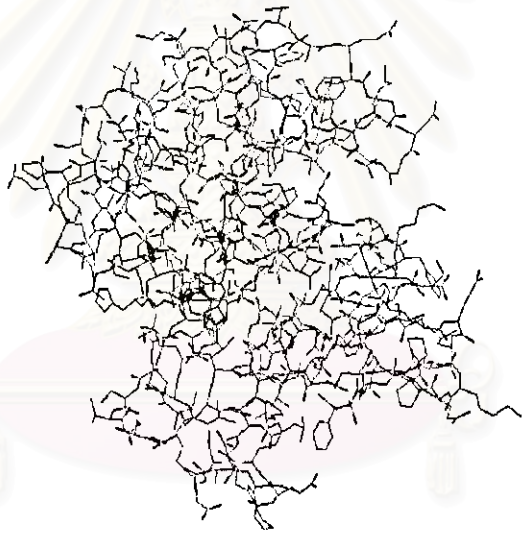

Figure 4.1 X-ray structure of hDHFR (1DRF).

Still there were missing coordinates due to the unresolvable atoms of the enzyme in the x-ray structure. These missing coordinates were then added by using the coordinates of the individual amino acids provided by the AMBER force field library. Hydrogen atoms were added to the x-ray crystallographic coordinates not only hydrogen in amino acids but also in the ionization states of side chains for Glu, Asp, Arg and Lys as well as at N-terminal amine and C-terminal carboxylic groups. In order to improve the

poorly defined regions present in the protein structure as a consequence of the geometrically inappropriate construction of the atoms added and to relax the structure to a minimum of the potential energy prior to the MD simulation, the crystal structure was then minimized using repeated cycles of 60,000 steps of minimization in vacuum (5,000 steps using steepest descents and 55,000 steps using conjugate gradient). The convergence criterion of 1.0×10^{-4} kcalmol^{-1}Å^{-1} was chosen for the energy gradient. The periodic boundary conditions and the dielectric constant of 1 were used throughout the simulations in explicit solvent, water in this case. The treatment of long-range electrostatic interactions was employed by a residue-based cutoff value of 10.0Å.

The optimized crystal structure was placed afterwards in a rectangular box with the dimension of 64.8 Å x 57.6 Å x 52.8 Å (65.0 Å x 57.8 Å x 53.0 Å for the system at 310.5K) with explicit water molecules filled. Thus the box was consisted of 3,013 protein atoms and 5,640 generated water molecules, a total of 19,933 atoms. This corresponds to a concentration of ca. 1.6×10^{-2} mol l^{-1}. The interactions involving the solvent molecules were modeled by the well-known three-center charge TIP3P model [33]. Another 60,000 steps of minimization was then performed in explicit water (5,000 steps using steepest descents and 55,000 steps using conjugate gradient).

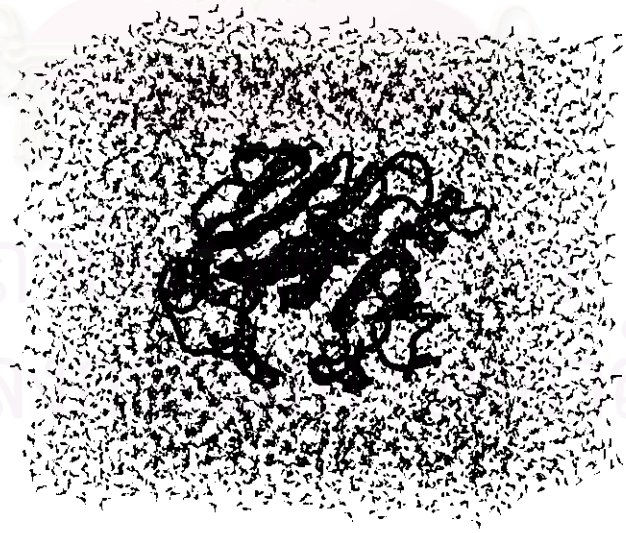

Figure 4.2 hDHFR and 5640 generated water molecules in periodic box of dimension 64.8 Å x 57.6 Å x 52.8 Å.

An unrestrained molecular dynamics simulation was then initiated from the minimized structure, using the Leapfrog algorithm for solving the classical equation of motion. The isobaric-isothermal MD simulations of an enzyme consisting of 186 amino acid residues, hDHFR, in water were carried out using the program AMBER at pressure of 1 atm and temperature of 300K and 310.5K. The AMBER parameter set [34] was used for the protein and TIP3P model for water. Within the first 50 ps of the MD simulation, an equilibration of the protein in solution was reached after warming up the system for 15 ps. Bonds with hydrogen atoms were constrained using SHAKE algorithm allowing an integration time step of 0.001 ps to be used. During the thermalization period, the temperature was adjusted using the Berendsen coupling algorithm [32] where a single scaling factor is used for all atoms at 300 and 310.5 K. Velocities were assigned to each atom from a Maxwellian distribution corresponding to both desired temperatures. The equilibration run of 50 ps was followed by 250 ps MD simulation.

Coordinate sets so called MD trajectory were saved every 0.1 ps for 300 ps. The last 250 ps were analyzed and used to monitor dynamics behavior of the system. The overall behavior of the MD simulation were analyzed by assessments of the fluctuation of the total energy, the kinetic energy, the potential energy, the pressure, the volume, the temperature of the solution and the deviation of the atomic positions as a function of time. The change in atomic positions and dihedral angles were analyzed by estimating root-mean-square differences (RMSD). By neglecting the effect of the N- and C-terminal, RMSD values were calculated for residue Ser3-Glu183 using

$$RMSD = \sqrt{\left\langle \left(x_i - \bar{x} \right)^2 \right\rangle}$$

where <x> denotes the average value of the property x.

Relevant simulation parameters have been summarized in Table 4.1. All MD simulation and analysis of MD trajectory were mainly performed using AMBER5 [34;35]. Protein structure was visualized, plotted and checked for its quality using the program INSIGHT II [36], RASMOL [37], WEBLAB VIEWER [38], MOLSCRIPT [39]

and PROCHECK [40]. The simulations were performed on an SGI Power Challenge XL 8 x R10000.

Table 4.1 Relevant simulation parameter for the 300 ps MD simulation of human dihydrofolate reductase in water.

Protein-solvent simulation conditions	Simulation data
Energy minimization	
- in vacuo	60,000 steps (5,000 steps steepest descent and 55,000 conjugated gradient)
- in water	60,000 steps (5,000 steps steepest descent and 55,000 conjugated gradient)
Molecular dynamics	
Number of atoms	19,933 (atoms) 3,013 protein atoms and 5,640 TIP3P water molecules
Thermodynamic ensemble	Isobaric-isothermal ensemble (NPT)
Integration algorithm	Leapfrog
Integration time step	0.001 ps
Long-range interactions	10 Å residue cut off distance
Periodic boundary conditions	Box dimension 64.8×57.6×52.8 $Å^3$ (65.0 Å x 57.8 Å x 53.0 Å for the system at 310.5K)
Thermalization	15 ps
Equilibration	35 ps
Dynamics trajectory for structural evaluation	250 ps
Collection of coordinate sets	2500 substructures (every 0.1 ps)