Modelling copper-containing proteins

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SUMMARY

The main subject of this thesis is the development of a force field for copper that can be used in molecular dynamics simulations of copper proteins. The force field has been used in simulations of a number of copper proteins. A molecular dynamics simulation starts with an initial structure, $\{\mathbf{r}_i(0)\}$, that usually derives from an XRD or NMR structure. After assigning initial velocities, $\{\mathbf{v}_i(-1/2\Delta t)\}$, to all atoms, new atomic positions are calculated by: $\{\mathbf{r}_i(t)\}=\{\mathbf{r}_i(0)\}+\{\mathbf{v}_i(-1/2\Delta t)\}\Delta t$. A so-called force field is used to mimic the bonding and non-bonding forces, $\{\mathbf{F}_i(t)\}$, that act on each atom. These forces are subsequently used to determine the new atomic velocities: $\{\mathbf{v}_i(+1/2\Delta t)\}=\{\mathbf{v}_i(-1/2\Delta t)\}+\{\mathbf{F}_i(0)\}\Delta t/m_i$. This procedure is repeated over and over again and so the internal dynamics of the molecule is simulated. To apply this technique to copper-containing proteins, the copper atom needs to be parameterised in the force field of the protein. Transition metals are relatively hard to model compared to organic systems since they delocalise their charge and often exhibit a variety of ligand sphere co-ordination geometries. This makes it impossible to describe the (non-) bonding forces for a transition metal by a single set of parameters. Different force field parameter sets have therefore been developed for the diverse Cu-geometries over the years. They are discussed in **chapter 1**.

Chapter 2 describes the development of an empirical, non-bonded force field to describe the interaction between the Cu-ion and its ligands in the redox protein azurin. The energy of the system is evaluated for different copper positions while systematically varying the force field parameters of the Cu-site. The position where the energy is mimimal for the various force fields is compared with crystal structure. The parameter set that gave the best results was selected and used for MD-simulations of proteins containing a similar Cu-co-ordination sphere as azurin (type-I Cu-containing proteins or

cupredoxins), proteins containing a type-II Cu-site (nitrite reductase) and azurin variants where the Cu-ligand Met 121 was replaced by several other amino acids.

Chapter 3 describes how a model of the Cu-site of an azurin double mutant, Asn42→Cys His117→Gly, was obtained. Experimental results have led to the tentative conclusion that Cys 42 on the surface of the protein co-ordinates the Cu-atom together with one of the original ligands, Cys 112. By gradually shortening a restraint between Cys 42 and the Cu-atom, the loop containing residue 42 was pulled inward into the protein so that, finally, both Cys residues co-ordinated the Cu-atom. This was done in three different ways by defining a various constraints between the copper atom and the protein. The stability of the three end structures was investigated using the non-bonded force field as developed in chapter 2. It appeared that the Met 121 ligand is unlikely to remain bound to the Cu-atom in the double mutant, but that the His 46 is necessary to obtain a stable Cu-site.

Azurin contains a tryptophan residue in the hydrophobic core of the protein that exhibits fluorescent and phosphorescent features. When introducing mutants nearby this residue, Ile7→Ser and Phe110→Ser, these spectroscopic features are affected. **Chapter 4** shows the results of the MD-simulations where the differences in flexibility around the tryptophane residue in the different azurin variants were analysed in relation to the differences in spectroscopic features. Furthermore, the diffusion of solvent molecules into the cavity created by the mutations was analysed. Hereby, the different effects of externally added agents on the spectroscopic features could be explained.

Chapter 5 describes an attempt to use MD-simulations for calculating the redox potential for azurin at different pH values. Azurin was used as a test-case since extensive experimental information is available on the redox properties of azurin at different pH values and of azurin variants. In collaboration with the Theoretical Chemistry group at the Groningen University, Density Functional Theory was used to obtain the charge distribution of the Cu-site in the reduced and oxidised state. MD-simulations were performed at maximal 21 intermediate states where the charge of the Cu-site was interpolated between the reduced and oxidised charge distribution. The thermodynamic

integration method was used to calculate the change in free energy going from the reduced to the oxidised state by different routes. The calculation was performed at high pH (His 35 protonated) and low pH (His 35 deprotonated). Two other sets of calculations were performed to study the effect of (de)protonation of His 35 on the free energy of the reduced and the oxidised states. The different calculations can be arranged in a thermodynamic cycle. The precision of the free energy calculations was evaluated as function of the number and sequence of intermediate states, the sampling time and the initial conditions. It was found that the precision is critically dependent on the relaxation of hydrogen bonding networks inside the protein. The errors in the free energies range from 1 to 10 k_BT. Only qualitative estimates of the differences in redox potential between protein variants can be obtained.

The homo-dimer quercetinase, or 2,3 quercetin dioxygenase, catalyses the oxygenation of flavonols like kaempferol. It is known from X-ray diffraction studies that a loop connecting the C-terminal and N-terminal domains in the monomer is partly disordered and forms a flexible lid at the entrance of the substrate cavity. Upon substrate binding, this loop becomes more ordered and closes the catalytic Cu-site. In **Chapter 6**, MD-simulations are described of the enzyme-substrate complex and of the enzyme after removal of the substrate, to investigate the dynamics of this loop. The loop increased flexibility after removal of the substrate. The number of hydrogen bonds between the loop and the rest of the protein decreased while the number of hydrogen bonds between the loop and the solvent increased gradually, implying an unfolding of the loop. The newly created cavity is filled with solvent molecules within a nanosecond.