THE STUDY OF STEREOSELECTIVE INTERACTION OF TRYPTOPHANE ALKYL ESTERS WITH HUMAN APO-TRANSFERRIN USING STEERED MOLECULAR DYNAMICS METHODS

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Abstract

The significance of the stereoselective interactions for biological and biochemical phenomena is well known, and in this paper we wish to approach this subject from a new and interesting perspective by employing non-equilibrium molecular dynamics methods. Starting from previous results obtained using classical molecular dynamics methods, we applied Steered Molecular Dynamics paradigm to observe the mechanism of interaction between small molecule ligands and human apo-transferrin. The pulling energies calculated this way were predictive for capillary electrophoresis migration speeds, and the lack of structure of the pulling force graph indicates that there aren't any stable intermediate states of this interaction. Thus we have established the usefulness of non-equilibrium molecular dynamics methods to the study of stereoselective interactions.

Keywords: stereoselective interactions, steered molecular dynamics, transferrin, in-silico modelling.

STUDIUL INTERACŢIUNII STEREOSELECTIVE DINTRE ESTERI ALCHILICI AI TRIPTOFANULUI ŞI APO-TRANSFERINA UMANĂ FOLOSIND METODE DE DINAMICĂ MOLECULARĂ DIRIJATĂ

Rezumat

Semnificaţia deosebită a interacţiunilor stereoselective în cadrul fenomenelor biologice şi biochimice este binecunoscută, iar lucrarea de faţă doreşte să abordeze acest domeniu dintr-o perspectivă nouă şi incitantă făcând apel la metodele de dinamică moleculară de non-echilibru. Pornind de la rezultate anterioare obţinute prin metode clasice de dinamică moleculară am aplicat paradigma Dinamicii Moleculare Dirijate pentru a observa mecanismul de interacţiune dintre anumiţi liganzi cu masă moleculară mică şi apo-transferina umană. Energiile de deplasare astfel calculate au fost predictive pentru ordinea de migrare în experimente de electroforeză capilară, iar lipsa palierelor pe graficul forţei de deplasare indică lipsa unor structuri intermediare stabile în cadrul acestei interacţiuni. Astfel am reuşit să demonstrăm utilitatea metodelor de dinamică moleculară de non-echilibru la studiul fenomenelor de interacţiune stereoselectivă.

Cuvinte cheie: interacţiuni stereoselective, dinamică moleculară dirijată, transferină, modelare in-silico.

1.INTRODUCTION

In previous work we have shed some light on the mechanism of stereoselective separation of a series of tryptophane alkyl esters through capillary electrophoresis (CE), using human apo-transferrin as a chiral selector [4]. The most probable bound conformations of (S)-2-amino-3-indolyl-methyl propionate (S-TME) and (R)-2-amino-3-indolyl-methyl propionate (R-TME) respectively, on the surface of the binding site for iron of the N-terminal lobe

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of human apo-transferrin, were established by molecular docking techniques [2]. Further on kinetic aspects of the interaction were established using Molecular Dynamics methods (MD) [1], while in this present work we are trying to improve the sampling of the system phase space around the stereoselective interaction of interest by applying non-equilibrium simulations known under the generic term of Steered Molecular Dynamics (SMD).

2. MATERIALS AND METHODS

Simulations were performed on an IBM computer having an Intel Pentium 4 and Windows XP (Microsoft) operating system. Following applications were used: NAMD 2.6 (Theoretical Biophysics Group, University of Illinois and Beckmann Institute) [7] for the actual MD experiments and VMD 1.8.6 (idem) [3] for preparing and visualizing the molecular system. The electrophoretic experiments, to which we compare our simulations results, were described in [4]. Briefly, stereoselective separation of a series of tryptophane alkyl esters was achieved by CE using apo-transferrin as a chiral selector. Peak assignment was done using pure enantiomers and it was confirmed that for 2 molecules (TME, TEE) the R enantiomer is the one migrating slower, hence it is interacting stronger with the apo-transferrin [4]. For TBE pure enantiomers were not available. Further on we will concentrate our study on the interaction of R/S-TME with apo-transferrin.

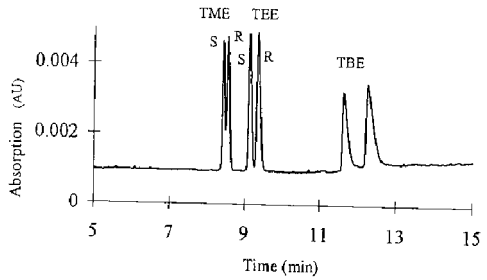

Figure 1. Typical electropherogramm showing the stereoselective separation of R/S-TME, R/S-TEE, R/S-TBE [4].

3. RESULTS AND DISCUSSIONS

The details regarding docking of the ligand conformations, system assembly and classical molecular dynamics are given in [1,2], but a short overview would be as follows. Ligand molecules – S-TME and R-TME - were docked on the surface of the 'receptor' – apo-transferrin - using previous knowledge locating the site of stereoselective interaction in the iron-binding cleft of the apo-transferrin [4]. Multiple docked conformations were generated using FlexX algorithm under Sybyl for each ligand which were then classified according to energetic and geometrical criteria. The most probable conformations for each ligand were chosen for further processing through molecular dynamics [1]. A protein-ligand complex was immersed in a 63 x 73 x 89 Å cuboid containing about 10.500 water molecules parameterized according to the TIP3P model. In order to simulate the effect of the solution ionic strength Na^+ and Cl^- ions were placed randomly in the system. Following molecular dynamics parameters were used: force field CHARMM 22 for proteins [6] and a classical MD algorithm using the integration scheme Verlet 1 (r-RESPA). Integration step was 2 fs (2 x 10^{-15} s), hydrogen atoms were restrained using SETTLE algorithm, cut-off for van der Waals forces was at employed at 12 Å, while electrostatic interactions were calculated using Particle Mesh Ewald method. The simulations were performed in a cuboid cell with periodic boundary conditions applied, in a canonical ensemble (isothermic-isobaric). Target temperature was 310 K and it was maintained by Langevin dynamics. Isobaric conditions were achieved by applying a Nosé-Hoover barostat at a target pressure of 1,01325 bar (1 atmosphere). The simulation protocol contained an initial minimization of the system for 500 steps followed by 12.000 molecular dynamics steps for a total simulation time of 24 ps [1]. Equilibration was checked by ploting thermodynamic values like: temperature, volume, total energy, kinetic energy. Also fit of Maxwell-Boltzmann distribution for kinetic energy was performed, RMSD (Root mean square deviation) of molecular structures from the equilibrium conformations and Ramachandran plots for the proteic part of the system were checked [5].

Starting from the equilibrated docked conformation of ligand and protein, an atom of the ligand is chosen and labelled as an SMD atom. To this atom a "dummy" (non-existing) atom is bound through an elastic spring with a know stiffnes (potential force constant k). The dummy will be subject to a constant-force or constant-velocity pulling which will be transmited through the SMD atom to the whole ligand molecule [7]. As a consequence the molecule experiences the additional potential ΔU defind as:

$$\Delta U = \frac{1}{2} k \left(\boldsymbol{v} - \left(\left(\vec{R}_t \right) - \left(\vec{R}_0 \right) \right) * \vec{n} \right)^2$$

where, k is spring constant (stiffness), $\vec{R}$ represents the position of the SMD atom at time t and time 0 respectively and $\vec{n}$ is the unit vector.

In our experiments we have used constant-velocity pulling using a spring constant of 5 kcal/mol*Å^2 (which is equal to 347,6 pN/Å) and a pulling velocity of 0,0006 Å/timestep which for a 2 fs time step means 0,3 Å /ps. Size of the spring constant was chosen in accordance with recommendations for systems of this size, not very large in order to reduce the effect of simulation noise but still high enough to cover efficiently the phase space considering the available computational power [7]. Pulling direction was specified by three vectorial components pointing away from the bulk of the protein in order to probe the reversed

path of the interaction between protein and ligand (ligand unbinding event). Total SMD simulation time was 20.000 steps (40 ps).

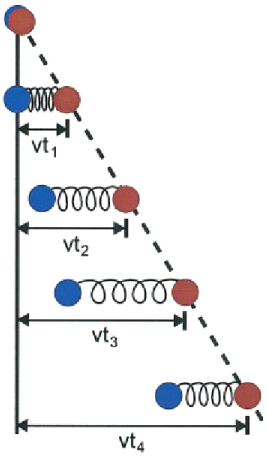

Figure 2. Conceptual SMD simulation (in red "dummy" atom, in blue SMD atom, in-between the atoms the pulling spring, v constant velocity of pulling).

During the simulation the SMD algorithm is outputting to a log file the components of the forces acting on the SMD atom which by scalar multiplication with the vectorial components of the pulling direction will give the magnitude of the instantaneous force acting on the SMD atom. Following pulling-force graphs were obtained for the two ligand of interest.

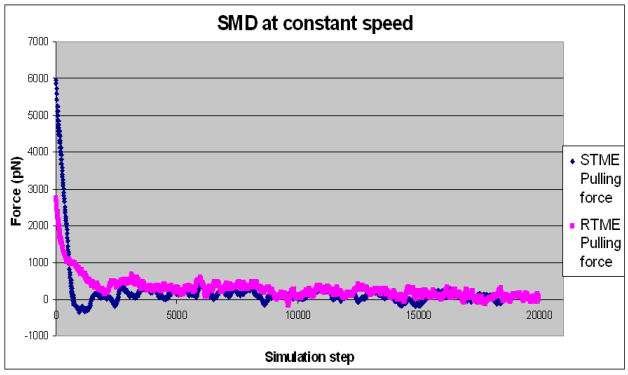

Figure 3. Constant velocity SMD graph for R-TME/apo-transferrin and S-TME/apo-transferrin complex.

Just by inspecting these graphs it is possible to observe that the initial peak in pulling force is higher for S-TME then for R-TME (approx. 6000 versus approx. 2750 pN) which at first sight seem to be at odds with the electrophoretic experimental results which show a more significant interaction of R-TME with apo-transferrin. Subsequently the pulling force graphs is flat and oscillates with an average force value of 101 pN for S-TME while for R-TME it decreases more slowly having an average of 217 pN. Comparative linear regression analysis of the flat area of the graph shows that there is a certain linearly decreasing trend in the graph for R-TME (R^2 is only 0,012 for S-TME but 0,518 for R-TME) although this part of the graph contains significant simulation noise for both molecules which precludes further correlation analysis. Also it's interesting to note that for S-TME the first null force occurs after 250 steps while for R-TME it appears much later, after 7000 simulation steps, pointing to a stronger interaction in this later case.

In order to investigate the overall energetics of the interaction the total area under the force curve is calculated by numerical integration and then multiplied with pulling speed, in order to obtain the energy consumed during pulling simulation, according to the formula:

$$\Delta W = \sum_{i=1}^{n} F_i * t * v$$

where ΔW is pulling energy, t is simulation time step and v is pulling velocity.

Pulling energy calculated in this way it's not a direct estimate of the binding free energy between ligand and protein as, due to the nature of the SMD simulation, it includes also non-reversible work performed on the system. Nevertheless the magnitude of ΔW for S-TME was 0,114 aJ (atto Joules) while for R-TME ΔW was 0,250 aJ, so an approx. 120 % increase in magnitude for the enantiomer which is more strongly bound by the apo-transferrin, as observed during classical MD simulations [1]. This result indicates that the size of the initial peak force has limited predictive significance as long as the tail of the force graph dominates the overall energy calculation, and in this is respect R-TME is clearly superior to S-TME as expected from previous simulations [1].

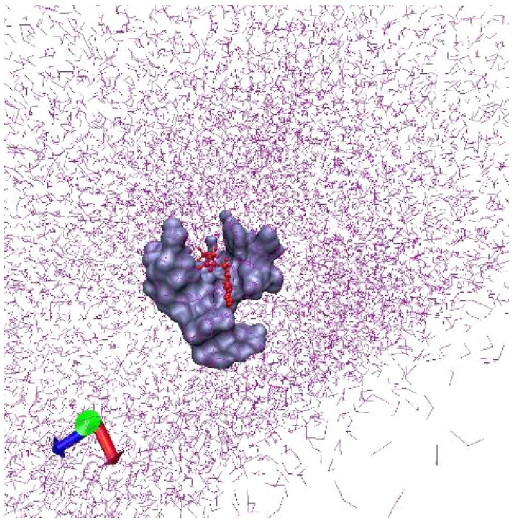

Figure 4. R-TME in the iron binding cleft of apo-transferrin immersed in solvent.

Also total traveled distance during simulation was 23 Å for S-TME but only 15,4 Å for R-TME which means that the later enantiomer is more strongly bound, also in accordance with experimental results, which show a higher retention factor for the R enantiomer [4]. First negative force occurred for both molecules after 11 – 14 Å of traveled distance which is of the same size with the employed van der Waals cutt-off (12Å) thus pointing to the significance of the interaction through this type of forces. Overall there is a lack of significant structure of the pulling force graph which means there aren't any discernable intermediate stable states of the stereoselective interaction. Figure no. 4 shows a typical image of R-TME docked in iron-binding site of transferrin and immersed in water.

4. CONCLUSIONS

In this paper we have shown how non-equilibrium molecular dynamics methods (SMD) can be used for the purpose of gaining a deeper understanding of the stereoselective separation phenomena. Pulling energies calculated by numerical integration are "smoothed out" of simulation noise and hence are predictive for binding behavior as observed in CE experiments. Also other observables (total traveled distance, first peak of negative force) support the conclusion reached by energy comparisons. Another important conclusion was that the existence of intermediate kinetic states of the binding phenomenon can be safely excluded due to lack of observable steady states in the pulling-force graphs. In order to calculate true free binding energies the application of Jarzynski principle [7] will be required in further simulation studies.

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