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ORIGINAL ARTICLE

How does stress affect human being—a molecular dynamic simulation study on cortisol and its glucocorticoid receptor

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Abstract Stress can be either positive or negative to human beings. Under stressful conditions, the mental and physical conditions of human can be affected. There exists certain relation between stress and illness. The cortisol and other glucocorticoids bind to the same receptor, which is called glucocorticoid receptor. Some evidences indicated that cortisol molecule binding to its glucocorticoid receptor was necessary for the stress response. Up to now, the structure–function relationships between cortisol molecule and its glucocorticoid receptor have not been deliberated from the atomic-level. In order to get a detailed understanding of the structure–function relationships between the cortisol molecule and glucocorticoids receptor, we have carried out molecular dynamic (MD) simulations on glucocorticoid receptor (Apo system) and cortisol with its glucocorticoid receptor complex (HCY system). On the basis of molecular dynamic simulations, a couple of key residues were identified, which were crucial for the binding of cortisol molecule. The results of binding free energy calculations are in good agreement with the experiment data. Our research gives clear insights from atomic-level into the structural–functional aspects of cortisol molecule and its glucocorticoid receptor, and also provides valuable information for the design of drug which can treat stress related illnesses.

1. Introduction

As the pace of life grows faster and faster and when we feel everything has become too much, we generally use the word "stress". We are overloaded and wondering whether we really can cope with the pressures. That is how we feel the stress in daily life. From the physiological or biological point of view, the stress can be described in such way as when there is a stressor, like an environmental condition or stimulus, the

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organisms themselves can respond to it. Using this method, the body can react to stress. Regarding stressful event, the body's response is to activate sympathetic nervous system. As it is impossible for the body to keep this state for a long time, the body will return to a normal physiological condition with the help of parasympathetic system. For human beings, stress can be either a positive or a negative condition. Under such conditions, the mental and physical properties of human can be affected. There exists certain connection between stress and illness. Several studies (Schneiderman et al., 2005) pointed out that both acute and chronic stresses can cause illness. Stress can also make human more susceptible to physical illness such as common cold (Cohen et al., 1997). It is of great importance to develop certain drugs which can relieve stress.

The cortisol and other glucocorticoids bind to the same receptor, which is called glucocorticoid receptor. Like other steroid receptors (Kumar and Thompson, 1999), the glucocorticoid receptor represents as a modular structure (Kumar and Thompson, 2005) and consists of the following domains (marked A–F) including A/B-N-terminal regulatory domain; C-DNA-binding domain; D-hinge region; E-ligand-binding domain and F-C-terminal domain. The ligand binding domain is the region where cortisol binds. It has been reported that cortisol molecule binding to its glucocorticoid receptor is necessary for the stress response (Kolodkin et al., 2013). Unfortunately, the detailed information about the cortisol molecule binding to its glucocorticoid receptor has not been studied yet. To this end, we have investigated the atomic-level structural characterization of glucocorticoid receptor (Apo system) and cortisol with its glucocorticoid receptor complex (HCY system). Molecular dynamics (MD) simulations can be used as an effective way to study the conformational changes on atomic level (Wang et al., 2013). In this study, MD simulations for Apo and HCY systems were carried out. The aims of this work are to figure out the details about cortisol molecule binding to glucocorticoid receptor and to identify the key residues which are responsible for the cortisol binding. Our work provides detailed atomistic insights into the structure–function relationships between cortisol molecule and its glucocorticoid receptor, and also provides valuable information for the design of drug which can treat stress related illnesses.

2. Computational methods

2.1. Initial structures

The crystal complex structure of cortisol and its glucocorticoid receptor was retrieved from the RCSB Brookhaven Protein Data Bank (PDB entry: 4P6X (He et al., 2014), which served as the starting structure for the following molecular dynamic (MD) simulations. Only the chain A of the crystal complex remained. The protonation states of ionizable residues were determined at pH = 7.0 using H++ server (Gordon et al., 2005), which can predict the p*K*a value of protein residues at a given pH. The prepared complex structure (HCY system) was used as the starting structure of the subsequent MD simulations. The cortisol molecule was removed from this prepared structure to create the Apo form of glucocorticoid receptor.

2.2. Molecular dynamic (MD) simulations

MD simulations for both HCY and Apo systems were implemented in AmberTools15 by using sander.MPI module. The 99SB force field (Hornak et al., 2006) was chosen to be the force field for the protein. The force field parameters for cortisol molecule were supplied by general AMBER force eld (Wang et al., 2004). Two sodium ions (Na^+) were added to each of the two systems using coulomb potential grid in order to keep the whole system neutral. TIP3P water model (Jorgensen et al., 1983) was selected to solvate both systems using a truncated octahedron box. The size of the water box was set to 10 A distance around the solute molecule. The two systems were first carried out for 2000 steps minimization by employing the decent method and then for 3000 steps conjugate minimization of the entire systems. Then the two systems were heated from 0 to 300 K. The time scale for this process was 1000 ps. The ensemble for the heating process was the canonical ensemble (NVT ensemble).

During this process, a force constant of 10.0 kcal mol^{-1} and a harmonic restraint were applied on the protein and small cortisol molecules. The Langvie thermostat was employed to maintain the temperature, and then the two systems were equilibrated for 2000 ps. During this process, the NPT ensemble was adopted and the constant pressure was set to 1.0 bar. The total relaxation time for the barostat bath was set to 2.0 ps. In the end, the Apo and HCY systems were both simulated for 100 ns. The periodic boundary conditions were employed in this research. The long range electrostatics was handled by the particle-mesh Ewald (PME) method (Darden et al., 1993). The cut-off value for short range interactions was set to 10.0 A. Shake algorithm was employed to hold fixed bonds involving hydrogen. The time step for all the simulations was all set to 2.0 f s.

2.3. MD trajectories analysis

The MD simulations were carried out for both Apo and HCY systems for 100 ns. To obtain thoughtful insights into the motion behavior during the 100 ns simulation time, the trajectory obtained by MD simulation was analyzed. These trajectories were processed with AmberTools1.5 module. Root-mean-square deviation (RMSD) was employed to quantify the conformational changes of the same protein. This value was an important criterion in judging the structures of protein. In this study, C-RMSD was calculated for all systems with the first frame as reference structure. Root-mean-square fluctuations (RMSF) were used to evaluate the fluctuation of each residue during the simulation time. Hydrogen bonds are of great importance to biological molecules. We employed the following criteria for the hydrogen bonds analysis: The cut-off value of distance between the two heavy atoms was set to 3.0 A; the angle between acceptor and donor atom for hydrogen bonds employed a 120 cut-off value. The cluster analysis was also employed for the trajectories analysis. In order to visualize the trajectory and to present the structures, VMD (Humphrey et al., 1996), Chimera (Pettersen et al., 2004) and PyMOL (DeLano, 2002) softwares were used.

2.4. MM-GB/SA calculations

The MM-GB/SA methods (Wang et al., 2013) were applied to estimate the binding free energies between the ligand and its