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Exploring the structure and conformational landscape of human leptin. A molecular dynamics approach

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ABSTRACT

Leptin is a hormone that regulates energy homeostasis, inflammation, hematopoiesis and immune response, among other functions (Houseknecht et al., 1998; Zhang et al., 1995; Paz-Filho et al., 2010). To obtain its crystallographic structure, it was necessary to substitute a tryptophan for a glutamic acid at position 100, thus creating a mutant leptin that has been reported to have biological activity comparable to the activity of the wild type but that crystallizes more readily. Here, we report a comparative study of the conformational space of WT and W100E leptin using molecular dynamics simulations performed at 300, 400, and 500 K. We detected differences between the interactions of the two proteins with local and distal effects, resulting in changes in the conformation, accessible surface area, compactness, electrostatic potential and dynamic behavior. Additionally, the series of unfolding events that occur when leptin is subjected to high temperature differs for the two constructs. We observed that both proteins are mostly unstructured after 20 ns of MD simulation at 500 K. However, WT leptin maintains a significant amount of secondary structure in helix α2, while the most stable region of W100E leptin is helix α3. Furthermore, we found that the region between residues 25 and 42 might adopt interconverting secondary structures ranging from α-helices and random coils to β-strand structures. Thus, this region can be considered an intrinsically disordered region. This atomistic description supports our understanding of leptin signaling and consequently might facilitate the use of leptin in treatments for the pathophysiologies in which it is implicated.

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1. Introduction

Leptin is one of the most important hormones; it participates in energy homeostasis in mammals as well as in many other physiological processes, including glucose homeostasis (Paz-Filho et al., 2010; Seufert et al., 1999), fatty acid homeostasis in non-adipocytes (Unger et al., 1999), reproduction (Brenner and Makc, 2009) and sexual development (Moschos et al., 2002), immune response (Lord et al., 1998; Martí et al., 2001), angiogenesis (Sierra-Honigmann et al., 1998), wound healing (Ring et al., 2000) and bone remodeling (Ducy et al., 2000). Encoded by the *lep* or *ob* gene, leptin is mainly produced in white adipose tissue, followed by brown adipose tissue and several other tissues, including skeletal muscle, bone marrow, stomach, pituitary gland, and liver (Prins, 2002; Masuzaki et al., 1997).

The role of leptin in energy balance was noticed upon the discovery that humans and rodents lacking a functional leptin protein or receptor exhibit hyperphagia and obesity that can be solved by leptin administration (Zhang et al., 1995). However, obesity is typically associated with high leptin levels rather than leptin deficiency (Flier, 1998; Heymsfield et al., 1999).

Consequently, the use of leptin as a therapeutic option in obese patients has, in many instances, been unsatisfactory (Bence et al., 2006). Additionally, certain characteristics of leptin, such as its short circulating half-life, low potency, and poor solubility at physiological pH, have been considered limitations of leptin treatments (Lo et al., 2005). Several efforts have focused on improving leptin solubility at physiological pH (Lo et al., 2005). One of the first examples was the mutant construct W100E leptin, which shows dramatically improved solubility and a propensity to crystallize, but with comparable

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biological activity to the activity of the wild-type protein (Zhang et al., 1997). It has been shown that the treatment of W100E leptin implanted subcutaneously in obese mice receiving high-calorie diet resulted in reduced food intake, body weight and triglycerides levels, but increased HDL-cholesterol levels (Chimal et al., 2014).

The crystal structure of leptin revealed a four-helix bundle and two relatively long interconnected loops; this structure is similar to the conformation of the long-chain helical cytokine family. Interestingly, leptin shows a unique slipknot structure created by a disulfide bridge between the C-terminus and residue 96 to make a 50-residue covalent loop. The N-terminal region threads through the covalent loop. This structure is the simplest slipknot topology described to date. Additionally, it has been demonstrated that a lack of the disulfide bridge leads to a loss of leptin functionality, resulting in morbid obesity (Boute et al., 2004). The most plausible explanation for this observation is the improper folding and knotting of the N-terminal region of leptin through the loop (Haglund et al., 2012).

Nevertheless, no thorough analysis and comparison of the conformation and dynamical properties of WT and W100E human leptin have been performed since the report of the crystal structure of W100E leptin.

"Since the pioneer paper entitled 'The Biological Functions of Low-Frequency Phonons' (Chen et al., 1977) was published in 1977, a series of investigations into biomacromolecules from dynamic point of view have been stimulated. These studies have suggested that low-frequency (or terahertz frequency) collective motions do exist in proteins and DNA (Forsen et al., 1981; Chou, 1985; Maggiora et al., 1989; Martel, 1992; Zhou, 1989; Bax et al., 2001; Sinkala, 2006). Furthermore, many important biological functions in proteins and DNA and their dynamic mechanisms, such as switch between active and inactive states (Wang et al., 2009a), cooperative effects (Chou, 1989), intercalation of drugs into DNA (Mao et al., 1988), and assembly of microtubules (Maggiora et al., 1994), can be revealed by studying the low-frequency internal motions as summarized in a comprehensive review (Chou, 1988). Some scientists even applied this kind of low-frequency internal motion for medical treatments (Gordon, 2007, 2008; Madkan et al., 2009). Actually, investigation into the internal motion in biomacromolecules and its biological functions is deemed as a "genuinely new frontier in biological physics", as announced by the Vermont Photonics in an article at http://www.vermontphotonics.com/NewFrontierBiophysics.pdf. In view of this, to really understand the action mechanisms of biomacromolecules, we should consider not only the static structural information but also the dynamical information acquired by studying their internal motions. Molecular dynamics (MD) simulations are a powerful theoretical approach that can successfully complement and extend the experimental results and reproduce them with reasonable accuracy (Daggett, 2006; Day et al., 2010). Recently, MD simulations have been used to study the switch mechanism of human Rab5a (Wang, 2009), the inhibition mechanism of PTP1B (Wang et al., 2009b), the gating and inhibition mechanism of the M2 proton channel from influenza A viruses (Wang and Wei, 2009) based on the NMR structure (Schnell and Chou, 2008; Pielak et al., 2009), the personalized drug design (Wang et al., 2008b, 2007a), the enzyme-ligand binding interaction (Wang et al., 2008b, 2007a), the binding mechanism of H5N1 influenza virus neuraminidase with ligands (Gong et al., 2009), the metabolic mechanism (Wang et al., 2009a), and the binding mechanism of calmodulin with chrysin (Li et al., 2007).

In this work, we obtained the full-length protein structures of the WT and W100E leptin constructs using the widely used protein-structure predictor I-TASSER, which combines threading alignments with *ab initio* procedures. Additionally, we used molecular dynamics (MD) simulations at different temperatures to thoroughly analyze and compare, for the first time, the conformational spaces of wild-type human leptin and its soluble mutant W100E. Differences in the secondary structure propensities, electrostatic and hydrophobic interactions, dynamical properties and conformational stability of the constructs could be identified. The structure and dynamical properties of the obese hormone protein must be described on an atomistic basis to shed light on its biological activity and physicochemical properties. This information will be useful in overcoming the inherent limitations that impede the use of leptin in treatments for obesity and other pathophysiological disorders in which leptin is implicated.

2. Materials and methods

2.1. Theoretical procedure-three-dimensional structure of WT and W100E leptin models

To obtain the initial coordinates of WT and W100E human leptin, we constructed 3D models using the I-TASSER server (Zhang, 2008). The crystallographic structure of W100E leptin that is reported in the protein data bank with the PDB ID 1AX8 was used as a template (Zhang et al., 1997). I-TASSER predicted the full-length leptin structure by combining threading and *ab initio* modeling. The sequence of each construct was submitted independently to the I-TASSER server, which provided five modes for each protein. For the sake of comparison, we also used the programs Modeler (⟨https://salilab.org/modeller/⟩; Eswar et al., 2006; Webb and Sali, 2014) and MOE (Molecular Operating Environmet versión 2008.10.) for structure prediction. The best model was selected after an analysis of the structures using Ramachandran plots (Laskowski et al., 1993, 1996), root mean square deviation (RMSD), and the programs VERIFY3D (Lüthy et al., 1992), PROCHECK (Laskowski et al., 1993, 1996) and Solvx (Holm and Sander, 1992). The best models were used to provide the initial coordinates for the remaining the MD simulations. The electrostatic potential at the surface of the molecules was calculated by solving the Poisson Boltzmann equation using a plugin for the PyMOL molecular graphics program (⟨http://www.pymol.org⟩).

2.2. Normal mode analysis

The normal low-frequency vibrational modes of both leptin constructs were analyzed using the elastic network model, which is implemented with the “rotation-translation-block” approximation in the web interface ElNemo. This analysis identifies potential conformational changes in proteins (Suhre and Sanejouand, 2004; ⟨http://igs-server.cnrs.mrs.fr/elnemo/index.html⟩; Tama et al., 2000; Delarue and Sanejouand, 2002). The following key parameters were used: DQMIN = −100, DQMAX = 100, DQSTEP = 20 and NRBL = auto. We analyzed the structural characteristics, the collectivity of the atomic movements of a total of 106 residues and the low-frequency normal modes.

2.3. Molecular dynamics simulations

MD simulations were performed using GROMACS 4 (Hess et al., 2008) with the OPLS-AA force field (Jorgensen and Tirado-Rives, 1998). The leapfrog algorithm for integrating Newton’s equations was used, and periodic boundary conditions were applied. The protein was solvated in a rectangular box of SPC water (Berendsen et al., 1981) with a minimum distance of 1 nm from the protein to the edge of the box. To obtain a neutral total charge in the system, four Na^+ counterions were added for W100E and three for WT. The total sizes of the systems were 23,073 and 23,160 atoms, including 6933 and 6536 water molecules, for W100E and WT, respectively. All bonds were constrained using LINCS (Hess et al., 1997). During energy minimization, the steepest descent algorithm was used, and convergence was reached in 200 steps. Further equilibration of the system was

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