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Topical Perspectives

Homology modeling and molecular dynamics simulation of the HIF2α degradation-related HIF2α-VHL complex

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ABSTRACT

Background: Hypoxia-inducible factor 2 alpha (HIF2α), prolyl hydroxylase domain protein 2 (PHD2), and the von Hippel Lindau tumor suppressor protein (pVHL) are three principal proteins in the oxygen-sensing pathway. Under normoxic conditions, a conserved proline in HIF2α is hydroxylated by PHD2 in an oxygen-dependent manner, and then pVHL binds and promotes the degradation of HIF2α. However, the crystal structure of the HIF2α-pVHL complex has not yet been established, and this has limited research on the interaction between HIF and pVHL. Here, we constructed a structural model of a 23-residue HIF2α peptide (528–550)-pVHL-ElonginB-ElonginC complex by using homology modeling and molecular dynamics simulations. We also applied these methods to HIF2α mutants (HYP531PRO, F540L, A530V, A530T, and G537R) to reveal structural defects that explain how these mutations weaken the interaction with pVHL.

Methods: Homology modeling and molecular dynamics simulations were used to construct a three-dimensional (3D) structural model of the HIF2α-VHL complex. Subsequently, MolProbity, an active validation tool, was used to analyze the reliability of the model. Molecular mechanics energies combined with the generalized Born and surface area continuum solvation (MM-GBSA) and solvated interaction energy (SIE) methods were used to calculate the binding free energy between HIF2a and pVHL, and the stability of the simulation system was evaluated by using root mean square deviation (RMSD) analysis. We also determined the secondary structure of the system by using the definition of secondary structure of proteins (DSSP) algorithm. Finally, we investigated the structural significance of specific point mutations known to have clinical implications.

Results: We established a reliable structural model of the HIF2α-pVHL complex, which is similar to the crystal structure of HIF1α in 1LQB. Furthermore, we compared the structural model of the HIF2α-pVHL complex and the HIF2α (HYP531P, F540L, A530V, A530T, and G537R)-pVHL mutants on the basis of RMSD, DSSP, binding free energy, and hydrogen bonding. The experimental data indicate that the stability of the structural model of the HIF2α-pVHL complex is higher than that of the mutants, consistently with clinical observations.

Conclusions: The structural model of the HIF2α-pVHL complex presented in this study enhances understanding of how HIF2α is captured by pVHL. Moreover, the important contact amino acids that we identified may be useful in the development of drugs to treat HIF2a-related diseases.

Abbreviations: HIF2α, hypoxia-inducible factor 2α; VHL, Von Hippel–Lindau; pVHL, von Hippel Lindau tumor suppressor protein; CODD, C-terminal oxygen-dependent degradation domain; 3D, three-dimensional; ARNT, aryl hydrocarbon receptor nuclear translocator; HRE, hypoxia response element; EPO, erythropoietin; VEGF, vascular endothelial growth factor; GLUT1, glucose transporter 1; EDN1, endothelin 1; TFDP3, transcription factor DP family member 3; NMR, nuclear magnetic resonance; Blastp, protein–protein BLAST; PSI-BLAST, position-specific iterated BLAST; PDB, Protein Data Bank; VBC, pVHL-elonginB-elonginC; WT, wild-type.

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1. Introduction

Hypoxia-inducible factor (HIF), composed of two subunits (α and β), is a highly conserved protein [1]. The β subunit is oxygen-insensitive and constitutively expressed, whereas the α subunit is oxygen-sensitive and continually synthesized [1–5]. However, the α subunit is undetectable in the presence of oxygen because of hydroxylation by the prolyl hydroxylase domain proteins (PHDs), a family of enzymes with three members (PHD1, PHD2, and PHD3), of which PHD2 is considered to be the most important human PHD isoform [6,7]. PHDs are activated only in the presence of oxygen hydroxylate-specific prolyl residues in the oxygen-dependent degradation (ODD) domain of the α subunit of HIF [1]. Then, the α subunit can be recognized by the von Hippel Lindau (VHL), a component of an E3 ubiquitin ligase (pVHL–elonginB–elonginC–Cul2–Rbx) [2]. Finally, this subunit is targeted by the proteasome for degradation.

Hypoxia-inducible factor 2 alpha (HIF2α), also called endothelial PAS domain protein 1 (EPAS1), is a transcription factor that translocates to the nucleus under hypoxic conditions. There, it dimerizes with the aryl hydrocarbon receptor nuclear translocator (ARNT), binding to the DNA sequence hypoxia response element (HRE), which contains a conserved RCGTG core sequence [8,9]. HIF2α controls several vital cellular functions by regulating an array of hypoxia-related target genes, including erythropoietin (EPO), vascular endothelial growth factor (VEGF), glucose transporter 1 (GLUT1), endothelin 1 (EDN1), and transcription factor DP family member 3 (TFDP3) [10,11]. By affecting these genes, HIF2α contributes to the regulation of angiogenesis, glycolysis, growth, proliferation, and apoptosis; when dysregulated, it is associated with tumorigenesis, cancer progression, invasion, and refractory treatment behavior [12–14].

The interior of a solid tumor is usually characterized by hypoxia [15,16]. A previous study has shown that HIF2α overexpression in tumors results from tumor hypoxia [17] and that hypoxia may result in reduced HIF2α degradation [5,8,11]. Thus, HIF2α plays an important role in tumor development.

The von Hippel Lindau protein (pVHL) is the product of the VHL tumor suppressor gene and is a component of E3 ubiquitin-protein ligase complex. It plays an important regulatory role in cell growth and differentiation [18]. pVHL forms a stable complex with elongin B and elongin C, two factors that stabilize and activate the transcription elongation factor elongin A [19]. Loss of VHL function in cells leads to increased expression and stabilization of HIF. The VHL/HIF pathway has been implicated in tumorigenesis of hemangioblastomas, renal cell carcinoma, and other VHL tumors [20].

Computational prediction techniques are becoming increasingly advanced. Many accurate 3D models have been constructed by comparing the amino acid sequences of unknown proteins to those of proteins whose crystal structure is known [21–23]. Internal coordinate mechanics (ICM) homology modeling has been shown to be a very powerful modeling tool [24–26] allowing new protein conformations to be created via iterative random modifications of an established protein conformation. Furthermore, the molecular dynamics simulation method provides insight into the movement of atoms and molecules by using Newton's equations of motion to model their trajectories within an interacting particle system.

Protein structure and folding are associated with biological functions. The structure of a protein is determined by its amino acid sequence, which contains an enormous amount of evolutionary information [21]. Protein folding is achieved through various non-covalent interaction forces, including hydrogen bonding, vav der Waals forces, and hydrophobic interactions. X-ray crystallography and NMR spectroscopy are typically used to determine protein structure. However, owing to the difficult nature of crystallography, the structures of many proteins remain unresolved. For example, the crystal structure of the HIF2α-pVHL complex has not yet been solved. Similarly, the HIF2α-related structure degraded *in vivo* by the proteasome associated with VHL remains unclear.

Homology modeling is a common method for creating 3D models of unknown structures based on the structures of related proteins. Molecular dynamics simulation is a powerful computer tool in molecular modeling that can track the physical movement of individual atoms. This technique can also simulate the natural motion of a protein complex, which is useful in biotechnology and drug development [27,28]. We used homology modeling and molecular dynamics simulation methods to construct a reliable structural model of HIF2α-pVHL. The model provides insight into the HIF2α degradation-related structure mediated by VHL. It may also be used to shed light on disease-associated genetic mutations and to design targeted therapies for HIF2α-related diseases.

Table 1
Alignment results of multiple sequence structures in the protein data bank (PDB), with HIF2α query sequences, using BLASTP and PSI-BLAST.

Template[a] (PDB ID _ chain)	Resolution (Å)	E-value	Query cover	Sequence identity
1LQB_D	2.00	0.021	88%	60%
1LM8_H [51]	1.85	0.015	61%	71%
4AJY_H [52]	1.73	0.77	55%	63%

[a] Orange domain from human HIF2α-VHL complex (PDB ID: 1LQB, 1LM8, and 4AJY).

2. Materials and methods

2.1. Sequence analysis and homology modeling

The HIF2α amino acid sequence was obtained from the UniProt Knowledgebase15 (ID: Q99814) (http://www.uniprot.org/); the conserved domain information was acquired from Pfam [29]. The CODD (residue numbers: 516–550) of HIF2α is a domain (Pfam 11413) that is highly conserved across species (Fig. 1) and is associated mainly with protein degradation; however, its structure has not been extensively investigated. To obtain the molecular model for our query sequence, we performed a similarity search using protein–protein BLAST (BLASTP) and position-specific iterated BLAST (PSI-BLAST) [30]. Along with these searches, we performed an alignment of multiple sequence structures in the Protein Data Bank (PDB) [31–33] using a threshold E-value of 10 and an inclusion threshold value of 0.005. From the results, we identified final authentic sequence identification numbers (PDB ID) for the selected proteins on the basis of the specificity of our query sequence (Table 1). The crystal structure of 1LQB (PDB ID) [34], a pVHL-elonginB-elonginC (VBC) complex bound to HIF1α, was selected as a template because of sufficiently high homology and the best query cover between its D chain and the HIF2α CODD domain. ICM modeling with the Monte Carlo method was used to construct the homology model of HIF2α CODD based on HIF1α in 1LQB. This method enabled us to use an established protein conformation to create new conformations based on iterative random modifications. We also used ICM global optimization. During this process, each iteration sampled a molecule's spatial configuration along with a random movement after local energy minimization. On the basis of the energy and the temperature calculations, each iteration was accepted or declined [35,36]. HIF2α models were refined by using the ICM Refine Model macro, which anneals the backbone and optimizes the side chains. Two Monte Carlo fast [36] simulations were conducted.