INSTITUTO POLITÉCNICO NACIONAL

ESCUELA NACIONAL DE MEDICINA Y HOMEOPATÍA
Sección de Estudios de Posgrado e Investigación

“Estabilidad térmica de la proteína Bcl-2 humana: Estudios experimentales y simulaciones computacionales”

TESIS

QUE PARA OBTENER EL GRADO DE:

Doctor en Ciencias en Biotecnología

Presenta:

M. en C. IAN ILIZALITURRI FLORES

Directores:

Dra. Claudia Guadalupe Benítez Cardoza
Dr. José Correa Basurto

MÉXICO, D.F. ENERO 2013

SIP-14-BIS

INSTITUTO POLITÉCNICO NACIONAL
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En la Ciudad de México siendo las 14:00 horas del día 16 del mes de Enero del 2013 se reunieron los miembros de la Comisión Revisora de la Tesis, designada por el Colegio de Profesores de Estudios de Posgrado e Investigación de ENMH para examinar la tesis titulada:

Estabilidad térmica de la proteína Bcl-2 humana: Estudios experimentales y simulaciones computacionales.

Presentada por el alumno:

Ilizaliturri	Flores	Ian
Apellido paterno	Apellido materno	Nombre(s)

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aspirante de:

Doctorado en Ciencias en Biotecnología

Después de intercambiar opiniones los miembros de la Comisión manifestaron ***APROBAR LA TESIS***, en virtud de que satisface los requisitos señalados por las disposiciones reglamentarias vigentes.

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CARTA CESIÓN DE DERECHOS

En la Ciudad de México, el día 16 del mes de enero del año 2013, el que suscribe, Ian Ilizaliturri Flores alumno del Programa de DOCTORADO EN CIENCIAS E BIOTECNOLOGÍA con número de registro B08-1103, adscrito a la Escuela Nacional de Medicina y Homeopatía, manifiesta que es autor intelectual del presente trabajo de Tesis bajo la dirección del Dra. Claudia Guadalupe Benítez Cardoza y del Dr. José Correa Basurto y cede los derechos del trabajo intitulado " Estabilidad térmica de la proteína Bcl-2 humana: Estudios experimentales y simulaciones computacionales", al Instituto Politécnico Nacional para su difusión, con fines académicos y de investigación.

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M. en C. Ian Ilizaliturri Flores

AGRADECIMIENTOS

Un profundo agradecimiento a mis asesores, la Dra. Claudia Benítez Cardoza, el Dr. José Correa Basurto y el Dr. Absalóm Zamorano Carrillo por su orientación y apoyo.

A todos mis compañeros de la ENMH que siempre me apoyaron a en esta maravillosa área.

Al IPN, LA ENMH y al doctorado en Biotecnología en red del IPN a los cuales e tenido el privilegio de pertenecer.

A la ESM-IPN por el uso de los laboratorios de Modelado Molecular y Bioinformática.

Al CONACYT (101229, CB132353), ICYT PICSA 1019-6968, SIP proyecto multidisciplinario 1480, SIP20120196, SIP20130466, ICYTDF (PIRIVE09-9), SIP-COFAA/IPN, BECA INTITUCIONAL-IPN, PIFI por las becas otorgadas y el financiamiento para la realización de la presente investigación.

Índice

Abstract

The anti-apoptotic B-cell lymphoma 2 (Bcl-2) protein interacts with several proteins that regulate the apoptotic properties of cells. In this research, we conducted several all-atom molecular dynamics (MD) simulations under high-temperature unfolding conditions, from 400 K to 800 K, for 25 ns. These simulations were performed using a model of an engineered Bcl-2 human protein (Bcl-2-Δ22Σ3), which lacks 22 C-terminal residues of the transmembrane domain. The aim of this study is to gain insight into the structural behavior of Bcl-2-Δ22Σ3 by mapping the conformational movements involved in Bcl-2 stability and its biological function. To build a Bcl-2-Δ22Σ3 three-dimensional model, the protein core was modeled by homology and the flexible loop domain (FLD, residues 33-91) by *ab initio* methods. Further, the entire protein model was refined by MD simulations. The MD simulations showed that the FLD at 400 and 500 K has several conformations reaching into the protein core, whereas at 600 K some of the alpha-helices were lost. At 800 K, the Bcl-2 core is destabilized suggesting a possible mechanism for protein unfolding, where the alpha helices 1 and 6 were the most stable, and a reduction in the number of hydrogen bonds initially occurs. In conclusion, the structural changes and the internal protein interactions suggest that the core and the FLD are crucial components of Bcl-2 in its function of regulating access to the recognition sites of kinases and caspases. Also, we have carried out MD simulations to explore the intrinsically disordered region (IDR) of the FLD of Bcl-2 protein. This theoretical study allows explaining the biological functions of FLD which lacks stable structure under physiological conditions; however, could adopt regular structures under particular interacting conditions, which are associated to regulate the Bcl-2 anti-apoptotic functions. The FLD structure does not appear in any experimental procedure; however, we have proposed the existence of disordered-to-helix transitions in a region of the FLD. Then, we analyzed the IDR properties of a fragment (amino acids 60-77) in the FLD of the human Bcl-2 protein (Bcl-2-60-77) through 25 ns (whole protein) followed 1 μs (Bcl-2-60-77) of atomistic MD simulations in explicit solvent at 310 K to explain the activity of Bcl-2-60-77 on the antiapoptotic properties the Bcl-2 protein. Bcl-2-60-77 contains some phosphorylation sites (Thr69, Ser70, and Thr74) that are essential for the regulation of Bcl-2 activity. There is still controversy over the significance of these sites of phosphorylation because these post-translational modifications can determine the extent of these disordered regions and critical for regulation Bcl-2 protein. Then, MD simulations results provided insights into the molecular mechanisms on the properties of FLD. In addition, by employing a standard principal component analysis (PCA), a dihedral angles phi and psi analysis as well as time course measurements of the MD evolution was possible to learn about the largest structural movements of Bcl-2-60-77. These simulations reveal that in their free states, Bcl-2-60-77 and FLD tend to adopt helical conformation in diferent extents.

Keywords: Bcl-2, intrinsically disordered region, Molecular Dynamic, Apoptosis, Unfolding.

Resumen

La proteína antiapoptotica Bcl-2 interactúa con varias proteínas que regulan las propiedades apoptoticas de las celulas. En esta investigación, realizamos varias simulaciones de dinámica molecular (MD) all-atom, bajo condiciones de alta temperatura, a partir de 400 K a 800 K, de 25 ns. Estas simulaciones se realizaron en base al modelo de ingeniería de la proteína Bcl-2 humana (Bcl-2-Δ22Σ3), que carece de 22 residuos del extremo C-terminal del dominio transmembranal. El objetivo de este estudio es obtener información sobre el comportamiento estructural de Bcl-2-Δ22Σ3 mediante el sondeo de los movimientos conformacionales implicados en Bcl-2 y la estabilidad de su función biológica. Para construir un modelo de Bcl-2-Δ22Σ3 tridimensional, el globulo de la proteína fue modelado por homología y el dominio del loop flexible (FLD, del residuo 33 al 91) por el método *ab initio*. Además, el modelo de la proteína completado se refinó mediante simulaciones MD. Estas simulaciones mostraron que el FLD a 400 y 500 K tiene varias conformaciones que llegan hasta el globulo de la proteína, mientras que a 600 K algunas de las hélices alfa se perdieron. A 800 K, el glóbulo de la Bcl-2 se desestabiliza sugiriendo un posible mecanismo para el desplegamiento de la proteina, donde la hélices alfa 1 y 6 fueron las más estables, y una reducción en el número de enlaces de hidrógeno se lleva acabo inicialmente. En conclusión, los cambios estructurales y las interacciones internas de la proteína sugieren que el globulo y el FLD son componentes cruciales de Bcl-2 regulando el acceso a los sitios de reconocimiento de cinasas y caspasas. Además, hemos también llevado a cabo también simulaciones de MD para explorar la región intrínsecamente desordenada (IDR) del FLD de la proteína Bcl-2. Este estudio teórico permite explicar las funciones biológicas de FLD que carece de estructura estable en condiciones fisiológicas, sin embargo, podría adoptar estructuras regulares en determinadas condiciones de interaccion, que podrían estar asociadas a la regulación de las funciones anti-apoptóticas de Bcl-2. La estructura FLD no aparece en cualquier procedimiento experimental, sin embargo, se han propuesto la existencia de estructura-desorden en una región del FLD. Posteriormente, se analizaron las propiedades de un fragmento de IDR (aminoácidos 60-77) en el FLD de la proteína Bcl-2 humana (Bcl-2-60-77) a través de 25 ns (proteína total), seguido de 1 μs (Bcl-2-60-77) de simulaciones de MD all-atom en solvente explícito a 310 K para explicar la actividad de Bcl-2-60-77 sobre las propiedades antiapoptóticas de la proteína Bcl-2. Bcl-2-60-77 contiene algunos sitios de fosforilación (Thr69, Ser70 y Thr74) que son esenciales para la regulación de Bcl-2. Aún existe controversia sobre el significado de estos sitios de fosforilación por estos cambios después de las modificaciones postranduccionales, las cuales pueden determinar la extensión de estas regiones desordenadas y críticas para la regulación de la proteína Bcl-2. Los resultados de estas simulaciones de MD proporcionan una visión de los mecanismos moleculares sobre las propiedades del FLD. Además, utilizando un análisis de componentes principales (PCA), análisis de ángulos diedro phi, psi, y mediciones en tiempo de evolución de la simulacion fue posible constatar los movimientos estructurales más importantes. Estas simulaciones revelan que en sus estados libres, Bcl-2-60-77 y FLD tienen propension para formar una estructura helicoidal, pero en diferentes grados.

Palabras Clave: Bcl-2, Región desordenada intrínseca, Dinamica Molecular, Apoptosis, desplegamiento.

Objetivos

Objetivo general

Analizar el comportamiento molecular de la proteína Bcl-2 humana en cuanto a su estabilidad global y de sus sitios funcionales.

Objetivos específicos

1. Construir un modelo de la proteína Bcl-2 que incluya su loop largo flexible.
2. Verificar la consistencia del modelo de la proteína Bcl-2 mediante el análisis de su estereoquímica.
3. Generar las dinámicas de desplegamiento térmico de la proteína Bcl-2 mediante dinámica molecular y analizar el comportamiento de los dominios funcionales de la proteína.
4. Estudiar mediante un análisis de componentes principales fragmentos desordenados de proteínas, en particular el loop largo flexible de la proteína Bcl-2.

Justificación

La actividad de la proteína bcl-2 se encuentra relacionada con el control de la muerte celular, por lo que estudios de su estabilidad junto con la información estructural pueden dar claridad sobre el funcionamiento de esta molécula, Esta información será útil para proponer el diseño racional de fármacos dirigidos contra procesos patológicos relacionados con la apoptosis. Además, es de interés describir las posibles conformaciones incorrectas que la proteína pudiera adoptar y tener consecuencias patológicas.

Introducción

La apoptosis es un proceso natural, necesario para la eliminación de las células redundantes durante el desarrollo, las células potencialmente peligrosas y las células en la senescencia [1]. Cuando no existe control de la muerte celular se asocia con una variedad de patologías como son las enfermedades autoinmunes [3], el cáncer [2] y trastornos neurodegenerativos [4]. La apoptosis, está regulada por varias proteínas que pertenecen a la familia Bcl-2, las cuales han sido ampliamente estudiadas. Los miembros de esta familia se agrupan de acuerdo con su homología y a la participación en la ruta mitocondrial de la apoptosis (proteínas pro-y anti-apoptóticos-) [5]. La estructura primaria de estas proteínas comparten homología únicamente en un pequeño tramo de secuencias llamadas dominios Bcl-2 Homology (BH), Hay cuatro de estos dominios (BH1-BH4), Las proteínas Bcl-2 anti apoptóticas contienen (BH1-BH4), los miembros proapoptóticos se encuentran divididos en multidominio y BH3-only, las multidominio tienen únicamente BH1-3[6]; sin embargo, alineamientos basados en la estructura de la proteínas del glóbulo de la familia de proteínas Bcl-2 reveló un conservado motif BH4[7] (Fig. 1). Sin embargo a pesar de estas diferencias en las secuencias, todas las proteínas anti-apoptóticas, todas la proteínas proapoptóticas multidominio y al menos un miembro de las BH3-only exhiben plegamientos muy similares de hélices alfa. La topologia global consiste de una helice hidrofobica central rodeada por cinco o seis hélices amfiopaticas [8]. La familia de proteinas Bcl-2 esta influenciada por modificaciones posttransduccionales, estas modificaciones incluyen la fosforilacion, sitios para corte de proteasa, ubiquitinacion y degradacion proteosomal. Estas modificaciones, dependen del contexto celular y las proteinas involucradas, las cuales podrian inactivar la actividad anti-apoptotica o activar la actividad pro-apoptotica. Muchas de estas modificaciones estan governadas por la actividad de enzimas[9]. Los dominios homologos BH se sabe que están implicados en las interacciones entre los miembros de la familia Bcl-2. Sin embargo, estas interacciones a nivel estructural 3D se encuentran poco estudiadas y, en consecuencia, no hay suficiente información sobre el comportamiento estructural de estas proteínas durante los eventos anti o pro-apoptóticos [10, 11]. Hay evidencia experimental de que esta familia de proteínas se somete a cambios estructurales y / o la actividad durante la fosforilación [9, 10] y de cortes de proteasas [12, 13]. El gene bcl-2 fue originalmente descubierto como el oncogen responsable del linfoma folicular humano de células B, producido por la translocación cromosomal t(14;18), que yuxtapone el locus de bcl-2 (B-cell eukemia/lymphoma-2) del cromosoma 18 con la cadena pesada de la inmunoglobulina J del cromosoma 14. Debido a que la región codificante queda bajo el control del promotor de dicha inmunoglobulina, el resultado es una sobreexpresión excesiva de bcl-2 [14]. Además, la proteína Bcl-2 se ha encontrado sobreexpresado en muchas células cancerosas tales como células B derivados linfomas [15] y los adenocarcinomas de colon [15]. Además, la proteína Bcl-2 se ha implicado en la resistencia quimioterapéutica de muchos tipos de cáncer [16, 17]. Por esa razón, algunos grupos de trabajo se han centrado en el diseño de nuevos compuestos para inhibir la actividad de Bcl-2 [18].

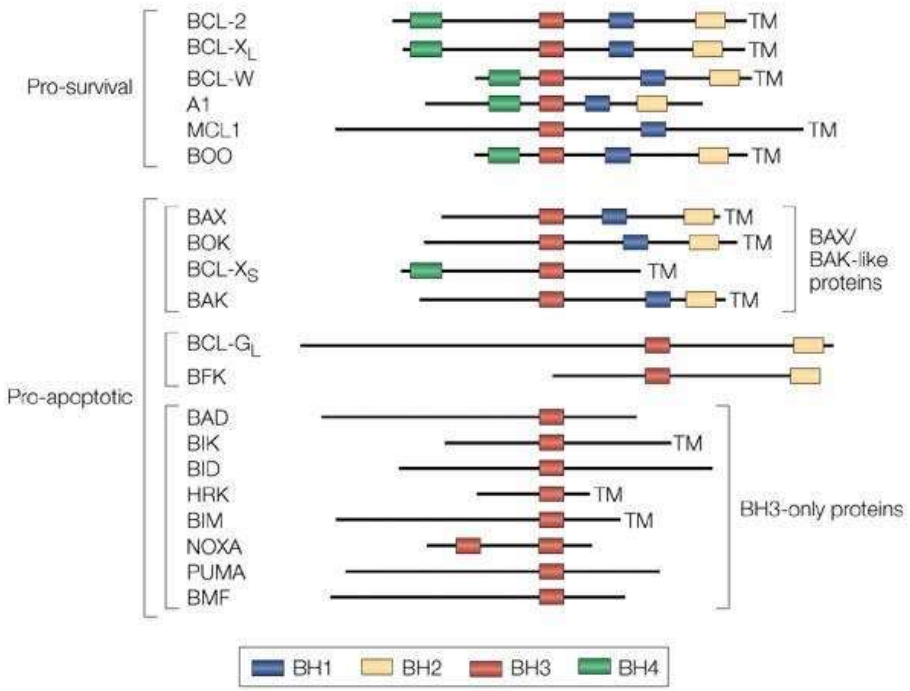


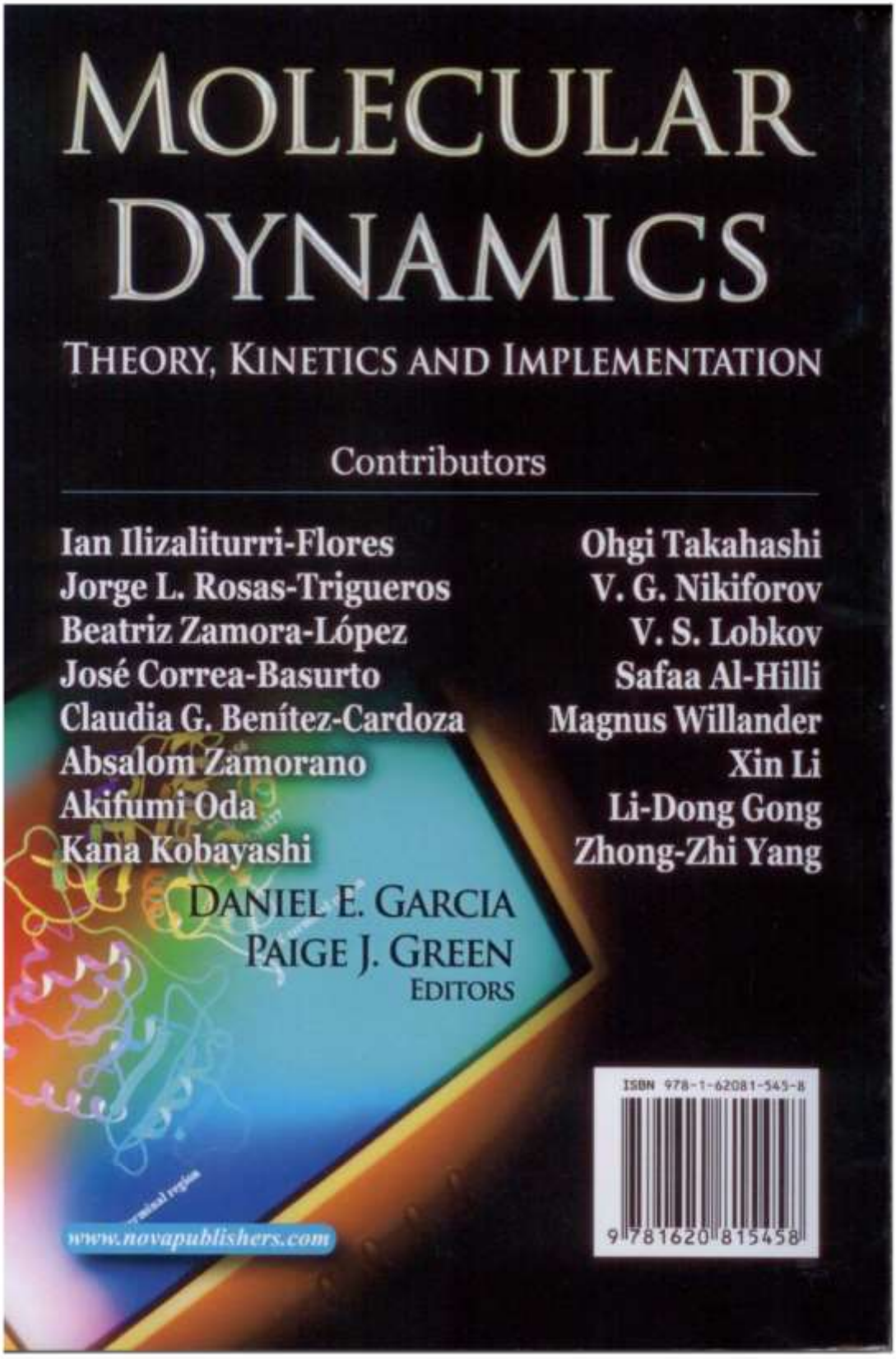
Figura 1. Familia de proteínas Bcl-2 y sus dominios BH (Tomado de: Nature Reviews Immunology 5).

Por otra parte, predicciones bioinformaticas recientes sugieren que más del 40% de las proteínas humanas tienen una región de gran extension (≥ 30 residuos) que en condiciones fisiológicas no se pliega en una estructura ordenada 3D [19]. La falta de estructura estable y bien definida, ofrece una gran variedad de ventajas funcionales en las proteínas llamadas intrínsecamente desordenadas (IDP) [20] y a las regiones (IDR) [21]. Estás regiones se encuentran involucradas en la regulación, señalización, control y la fosforilación de las proteínas, entre muchas otras funciones [22,23]. Por otro lado, un plegamiento incorrecto de las IDP se encuentran asociadas en el desarrollo de las enfermedades con desplegamiento incorrecto de las proteínas o llamadas enfermedades conformacionales [24]. IDP no forman estructuras estables en condiciones fisiológicas. En su lugar, muestras múltiples conformaciones, a menudo mientras conservan algo de la estructura residual [25]. Se ha planteado la hipótesis de que las modificaciones a las proteínas se producen en regiones que son fácilmente accesibles, y muchos de estas regiones se han determinado por estar localizada dentro de las IDR [26]. Los miembros de la familia Bcl-2 o son IDP o contienen IDR que son esenciales para su función [27], Existen modificaciones características en la familia de Bcl-2 que incluyen la fosforilación, ubiquitinación, cortes por caspasa [28]. Estos sitios son en su mayoría confinados a las regiones IDR, lo que indica la importancia de estas regiones en la regulación de la función de la proteína Bcl-2 [27].

CAPITULO 1

Molecular dynamics of proteins: Towards function identification via stability

Ian Ilizaliturri-Flores, Jorge L. Rosas-Trigueros, Beatriz Zamora-López, José Correa-Basurto, Claudia G. Benítez-Cardoza, Absalom Zamorano. Molecular dynamics of proteins: Towards function identification via stability. Editors: Daniel E Garcia and Paige L Green. In the Book: *Molecular Dynamics: Theory, Kinetics and Implementation.* NOVA Publishers, **2012,** 3rd Quarter. ISBN: 978-1-62081-592-2. Book chapter.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EDITORS
ISBN 978-1-62081-545-8
9 781620 815458
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CONTENTS

Chapter 1

MOLECULAR DYNAMICS OF PROTEINS: TOWARDS FUNCTION IDENTIFICATION VIA STABILITY

Ian Ilizaliturri-Flores,[2*] Jorge L. Rosas-Trigueros,[2,4,*] Beatriz Zamora-López[3], José Correa-Basurto[5], Claudia G. Benítez-Cardoza[1,2] and Absalom Zamorano[1,2,†].

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ABSTRACT

Conformational changes of biomolecules have been proposed as a cause and effect of biological events. Particularly, the content of a specific conformation of a protein in an intracellular localized space has suggested signals addressed to execute specific tasks. Sampling of these structural options, that make of proteins informational molecules, finds in molecular dynamics (MD) a theoretical and computational tool to explain and hypothesize the functionality at atomistic level. Conceptually, if we consider the "final" folded structure as the most functional conformation, possibly we find some obstacle to study the pleiotropicity of proteins and its structural basis. In this order of ideas, the evidence provided by MD in relation to folding and unfolding, where thermodynamic states and kinetic properties characterize conformational changes, sheds light on the pathways towards functionality of a protein. In this chapter, force fields and atomistic interactions considered as relevant parts of a MD simulations are reviewed and a typical protocol of stability is explained. Finally, statistical mechanics as a theoretical frame to explore the conformational space is discussed in the context of available computational methods devoted to discover functional domains in proteins.

1. INTRODUCTION

Proteins perform important tasks including direct their own folding. Exposition to variety of stressful conditions may promote proteins misfolding, as is shown in tumor development wherein mutations interfere with the appropriate role of tumor-suppressor proteins and oncogenes. Alterations of catalytic efficiency, loos of binding sites or of functional form, are included as consequences. Some examples such as Src family kinases, p53, mTOR, and C-terminus of HSC70 interacting protein (CHIPs) are associated with protein misfolding and tumorigenesis. Therefore, a therapeutic strategy could be related to repair or eliminate protein misfolding (Nagaraj et al. 2010). Another interesting instance is the fusion oncoprotein formed by Promyelocytic leukemia (PML) and the retinoic acid receptor (RAR) that is associated to the transformation of acute promyelocytic leukemia (APL). When this protein fusion is misfolded promotes the same in the nuclear receptor corepressor (N-CoR) protein, a corepressor essential for the growth-suppressive function of several tumor-suppressor proteins. This "trans-misfolding" is mediated by inducing an anomalous post-translational modification. (Khan 2010).

CAPITULO 2

IDENTIFICATION OF PHARMACOLOGICAL TARGETS COMBINING DOCKING AND MOLECULAR DYNAMICS SIMULATIONS

Ilizaliturri-Flores I, Rosas-Trigueros JL, Carrillo-Vázquez JP, Vique- Sánchez JL, Carrillo-Ibarra N, Zamora-López B3, Reyes-López CA, Benítez-Cardoza CG, and Zamorano-Carrillo A. Identification of Pharmacological Targets Mixing Docking and Molecular Dynamics Simulation. **2012**, *American Journal of Agricultural and Biological Science, ISSN: 1557-4989, e-ISSN: 1557-4997. Accepted.*

AJABS
IF/SN
27/12/2012
2: 20

IDENTIFICATION OF PHARMACOLOGICAL TARGETS COMBINING DOCKING AND MOLECULAR DYNAMICS SIMULATIONS

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ABSTRACT

Studies that include both experimental data and computational simulations (*in silico*) have increased in number because the techniques are complementary. *In silico* methodologies are currently an essential component of drug design; moreover, identification and optimization of the best ligand based on the structures of biomolecules are common scientific challenges. Geometric structural properties of biomolecules explain their behavior and interactions and when this information is used by a combination of algorithms, a dynamic model based on atomic details can be produced. Docking studies enable researchers to determine the best position for a ligand to bind on a macromolecule, whereas Molecular Dynamics (MD) simulations describe the relevant interactions that maintain this binding. MD simulations have the advantage of illustrating the macromolecule movements in more detail. In the case of a protein, the side chain, backbone and domain movements can explain how ligands are trapped during different conformational states. Additionally, MD simulations can depict several binding sites of ligands that can be explored by docking studies, sampling many protein conformations. Following the previously mentioned strategy, it is possible to identify each binding site that might be able to accommodate different ligands through atomic motion. Another important advantage of MD is to explore the movement of side chains of key catalytic residues, which could provide information about the formation of transition states of a protein. All this information can be used to propose ligands and their most probable site of interaction, which are daily tasks of drug design. In this review, the most frequent criteria that are considered when determining pharmacological targets are gathered, particularly when docking and MD are combined.

Keywords: Docking; MD Simulations; *in Silico*, Theoretical Studies, Drug Design

1. Introduction

Experimental techniques of molecular biology can be used to explore the intrinsic mechanisms of storage and transmission of information within the cell. In particular, the cloning and purification of a protein can permit its study by Nuclear Magnetic Resonance (NMR) or X-ray studies. A useful result of these techniques is the structural chemistry of the protein (tridimensional

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CAPITULO 3

COMPUTATIONAL MODELING AND SIMULATION OF THE BCL-2 FAMILY: PAVING THE WAY FOR RATIONAL DRUG DESIGN

Rosas Trigueros JL, **Ilizaliturri-Flores I**, Correa Basurto J, Benítez Cardoza CG and Zamorano-Carrillo A. Computational Modeling and Simulation of the Bcl-2 Family: Paving the Way for Rational Drug Design. *Current Medicinal Chemistry, 19(36) 6081-6094,* **2012***,* ISSN: 0929-8673 (Print), ISSN: 1875-533X (Online).

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Current Medicinal Chemistry, 2012, 19, 6081-6094

Computational Modeling and Simulation of the Bcl-2 Family: Paving the Way for Rational Drug Design

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Abstract: Bcl-2 (B-cell lymphoma 2) family proteins have been studied intensively due to their association with cancer and other human diseases. These proteins were originally associated with the regulation of outer mitochondrial membrane integrity and apoptosis. However, there is experimental evidence that suggests that several members of this family play instrumental roles in other cellular pathways including autophagy, endoplasmic reticulum signaling, mitochondrial morphology and synaptic activity among others. Bcl-2 family proteins have been explored using diverse experimental and theoretical methods to obtain structural information that can provide valuable insight for drug development. This review is focused on computational studies related to Bcl-2 family proteins. Different strategies are described and evaluated, such as Molecular Dynamics simulations, docking, and rational drug design with the aim of demonstrating the importance of structural details of either ligands or proteins. The relevance of the knowledge obtained using these tools to drug design is discussed.

Keywords: Apoptosis, Bcl-2, bioinformatics, cancer, molecular modeling, protein–protein interaction, structure-based drug design.

1. INTRODUCTION

Cell proliferation and cell death must be continuously and exquisitely balanced to maintain homeostasis in healthy tissues [1]. Three main forms of cell death have been proposed: apoptosis, necrosis, and autophagic cell death [2]. Among these categories, apoptosis has been recognized as the genetically controlled and evolutionarily conserved mechanism for killing cells. If apoptosis fails, it will cause either the survival of damaged, infected, superfluous or potentially dangerous cells or the inappropriate killing of vital cells [3]. The consequences of this failure include a variety of health disorders including autoimmunity, degenerative diseases and cancers [4, 5]. Members of the B-cell lymphoma-2 (Bcl-2) protein family are critical regulators of apoptosis and are also essential for the initiation of autophagy [6]. This family is commonly divided into three subgroups of proteins. One of these subgroups promotes the survival of cells (antiapoptotic proteins), another activates the effector pathways of apoptosis (proapoptotic proteins) and a third features members that function as sensitizers or derepressors of the other two groups. In summary, the Bcl-2 family members interact with one another to regulate apoptosis [7, 8]. Furthermore, inhibiting the interaction between pro- and antiapoptotic members is sufficient to promote or detain apoptosis in mammalian cells. Apoptotic pathways are regulated by protein-protein interactions [9]; as a consequence, small molecules, (antagonists or BH3 peptidomimetics) can inhibit this heterodimerization, representing therapeutic prototypes for regulating the apoptotic cascade via the mitochondrial apoptotic pathway. Considerable evidence indicates that such Bcl-2 protein antagonists could be useful drugs in inducing apoptosis preferentially in neoplastic cells [8].

Computational models are useful not only to formally represent and simulate systems of interest but also to predict their response to new perturbations and thereby lead to testable hypotheses [10]. Thus, computational modeling and analysis can suggest new experiments that challenge and help revise our understanding of biological systems [11]. The appreciation of the value of computational modeling and simulation in studying biological systems has increased in recent times because these theoretical strategies have continued to provide valuable insight for various fields, including pharmacology and biochemistry [12, 13].

This review provides an overview of reported computational models and simulations involving the Bcl-2 family, focusing on the important structural details thus elucidated, which have been found to be relevant for drug design.

2. BCL-2 FAMILY: CLASSIFICATION AND RELEVANCE

The normal development and optimization of tissues for inhibiting aberrant cellular proliferation are controlled by a process termed apoptosis. The evasion of this programmed cell death is a hallmark of cancer; however, its uncontrolled promotion leads to chronic degenerative diseases [14-16]. Several proteins participate, constituting a regulatory network whose collective action is involved in tissue homeostasis [17, 18]. The Bcl-2 family is distinguished by its capability to group members with opposite functions sharing some patterns denominated Bcl-2 homology (BH) domains. Based on the content of BH domains in family members, these can be assigned to one of three groups. The antiapoptotic Bcl-2 proteins (Bcl-2, Bcl-x_L, Bcl-w, Mcl-1 and A1/Bfl-1) present four BH domains (BH1-BH4), whereas the proapoptotic proteins (Bax, Bak and Bok) exhibit three (BH1-BH3). As their name suggests, the third group of BH3-only proteins (Bid, Bim/Bod, Bad, Bmf, Bik/Nbk, Blk, Noxa, Puma/Bbc3, Bnip3 and Hrk/DP5) includes only the BH3 domain and plays an activating role in inducing cell death [19-23].

Bcl-2 protein, which derives its name from the family of proteins, was identified originally at the breakpoint of a t(14:18) translocation in a cell line derived from lymphocytes [24]. Bcl-2 is the most widely studied member of the Bcl-2 family and has been found to be overexpressed in tumors such as B cell-derivative lymphomas, colorectal adenocarcinomas and others [25, 26]. Moreover, Bcl-2 has also been associated with the chemotherapeutic resistance of some cancers [27, 28].

Interactions among Bcl-2 family proteins determine the balance between inducing and inhibiting cell death. Multi-domain members of the family have a hydrophobic groove formed by the BH1-BH3 domains where the BH3 domain of another member can bind. Via the BH3-groove, homo- and hetero-dimers of these proteins initiate or block a death signal to mitochondria [29-31].

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1875-533X/12 $58.00+.00

CAPITULO 4

BACKBONE CONFORMATIONAL PREFERENCES OF AN INTRINSICALLY DISORDERED PROTEIN IN SOLUTION

Backbone conformational preferences of an intrinsically disordered protein in solution. Espinoza-Fonseca LM**, Ilizaliturri-Flores I**, Correa-Basurto J. Mol Biosyst. 2012 Jun;8(6):1798-805. doi: 10.1039/c2mb00004k. Epub 2012 Apr 13.

Molecular BioSystems

Dynamic Article Links

Cite this: DOI: 10.1039/c2mb00004k

www.rsc.org/molecularbiosystems

PAPER

Backbone conformational preferences of an intrinsically disordered protein in solution†

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Received 4th January 2012, Accepted 23rd March 2012
DOI: 10.1039/c2mb00004k

We have performed a 4 μs molecular dynamics simulation to investigate the native conformational preferences of the intrinsically disordered kinase-inducible domain (KID) of the transcription factor CREB in solution. There is solid experimental evidence showing that KID does not possess a bound-like structure in solution; however, it has been proposed that coil-to-helix transitions upon binding to its binding partner (CBP) are template-driven. While these studies indicate that IDPs possess a bias towards the bound structure, they do not provide direct evidence on the time-dependent conformational preferences of IDPs in atomic detail. Our simulation captured intrinsic conformational characteristics of KID that are in good agreement with experimental data such as a very small percentage of helical structure in its segment α_B and structural disorder in solution. We used dihedral principal component analysis dPCA to map the conformations of KID in the microsecond timescale. By using principal components as reaction coordinates, we further constructed dPCA-based free energy landscapes of KID. Analysis of the free energy landscapes showed that KID is best characterized as a conformational ensemble of rapidly interconverting conformations. Interestingly, we found that despite the conformational heterogeneity of the backbone and the absence of substantial secondary structure, KID does not randomly sample the conformational space in solution: analysis of the (Φ, Ψ) dihedral angles showed that several individual residues of KID possess a strong bias toward the helical region of the Ramachandran plot. We suggest that the intrinsic conformational preferences of KID provide a bias toward the folded state without having to populate bound-like conformations before binding. Furthermore, we argue that these conformational preferences do not represent actual structural constraints which drive binding through a single pathway, which allows for specific interactions with multiple binding partners. Based on this evidence, we propose that the backbone conformational preferences of KID provide a thermodynamic advantage for folding and binding without negatively affecting the kinetics of binding. We further discuss the relation of our results to previous studies to rationalize the functional implications of the conformational preferences of IDPs, such as the optimization of structural disorder in protein–protein interactions. This study illustrates the importance in obtaining atomistic information of intrinsically disordered proteins in real time to reveal functional features arising from their complex conformational space.

Introduction

Proteins can spontaneously fold into unique native states on timescales from microseconds to seconds. This efficiency is attributed to the inherent conformational preferences of the polypeptide backbone, which significantly limits the local conformational space and provides a bias toward the folded, functional state.[1–3] Recent studies have unveiled the existence of a class of functional proteins that do not possess a well-defined structure in solution, and therefore their native states cannot be represented by an ensemble of closely related conformations.[4] However, these intrinsically disordered proteins (IDPs) often fold into well-defined structures upon forming intermolecular interactions with their binding partners,[4,5] although in other cases they retain structural disorder in the bound state, which is necessary to optimize their function.[6,7] More important, there is strong experimental evidence indicating that a large number IDPs do not possess a

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† Electronic supplementary information (ESI) available: Fig. S1–S5. See DOI: 10.1039/c2mb00004k

CAPITULO 5

A STUDY OF THE STRUCTURAL PROPERTIES AND THERMAL STABILITY OF HUMAN BCL-2 BY MOLECULAR DYNAMICS SIMULATIONS.

Enviado a Journal of Biomolecular Structure & Dynamics, 2013. Actualmente se encuentra contestándo los comentarios de los referees.

A STUDY OF THE STRUCTURAL PROPERTIES AND THERMAL STABILITY OF HUMAN BCL-2 BY MOLECULAR DYNAMICS SIMULATIONS

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Keywords: Apoptosis, Bcl-2, Molecular Dynamics, Unfolding

Abstract

The anti-apoptotic B-cell lymphoma 2 (Bcl-2) protein interacts with several proteins that regulate the apoptotic properties of cells. In this research, we conduct several all-atom molecular dynamics (MD) simulations under high-temperature unfolding conditions, from 400 K to 800 K, for 25 ns. These simulations were performed using a model of an engineered Bcl-2 human protein (Bcl-2-∆22Σ3), which lacks 22 C-terminal residues of the transmembrane domain. The aim of this study is to gain insight into the structural behavior of Bcl-2-∆22Σ3 by mapping the conformational movements involved in Bcl-2 stability and its biological function. To build a Bcl-2-∆22Σ3 three-dimensional model, the protein core was modeled by homology and the flexible loop domain (FLD, residues 33-91) by ab initio methods. Further, the entire protein model was refined by MD simulations. The MD simulations showed that the FLD at 400 and 500 K has several conformations reaching into the protein core, whereas at 600 K some of the alpha-helices were lost. At 800 K, the Bcl-2 core is destabilized suggesting a possible mechanism for protein unfolding, where the alpha helices 1 and 6 were the most stable, and a reduction in the number of hydrogen bonds initially occurs. In conclusion, the structural changes and the internal protein interactions suggest that the core and the FLD are crucial components of Bcl-2 in its function of regulating access to the recognition sites of kinases and caspases.

CAPITULO 6

ANALYSIS OF INTRINSICALLY DISORDERED REGION AT FLEXIBLE LONG LOOP FROM BCL-2 BY MOLECULAR DYNAMICS SIMULATIONS.

Artículo en preparación, se incluye manuscrito.

Analysis of intrinsically disordered region at flexible long loop from Bcl-2 by molecular dynamics simulations

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Abbreviations

FLL- Flexible long loop.
Bcl-2-60-77 - Long loop fragment of Bcl-2 protein (A60-A77).
IDR - Intrinsically disordered region.
IDP - Intrinsically disordered protein.
MD - Molecular Dynamics.
RMSD - Root mean square deviation Cα.
Rg - Radius of gyration.
RMSF - Root mean square fluctuation.
OMM - Outer Mitochondrial Membrane.
PCA - Principal Component Analysis.
VMD - Visual Molecular Dynamics.
NAMD - NAnoscale Molecular Dynamics.
Bcl-2 - B-cell lymphoma 2.
1GJH - Bcl-2 isoforma 2 structure (from NMR).
2XA0 - Model of complex of Bcl-2 and Bax peptide (from X-ray diffraction).
3D - Three-Dimensional.
PC - Principal component
dPCA - Dihedral Principal Component Analysis.
1G5M- Bcl-2 isoforma 1 structure (from NMR).
NMR - Nuclear Magnetic Resonance.

ABSTRACT

We have carried out molecular dynamics (MD) simulations to explore the intrinsically disordered region (IDR) of the flexible long loop (FLL) of Bcl-2 protein. This theoretical study allows explaining the biological functions of FLL which lacks stable structure under physiological conditions; however, could adopt regular structures under particular interacting conditions, which are associated to regulate the Bcl-2 anti-apoptotic functions. FLL structure not appears in any experimental procedure; however, we have been proposed the existence of disordered-to-helix transitions in a region of the FLL. Then, we analyze the IDR properties of a fragment (amino acids 60-77) in the FLL of the human Bcl-2 protein (Bcl-2-60-77) through 25 ns (whole protein) followed 1 μs (Bcl-2-60-77) of atomistic MD simulations in explicit solvent at 310 K to explain the activity of Bcl-2-60-77 on the antiapoptotic properties the Bcl-2 protein. Bcl-2-60-77 contains some phosphorylation sites (Thr69, Ser70, and Thr74) that are essential for the regulation of Bcl-2 activity. There is still controversy over the significance of these sites of phosphorylation because these post-translational modifications can determine the extent of these disordered regions and critical for regulation Bcl-2 protein. Then, MD simulations results provide insights into the molecular mechanisms on the properties of FLL. In addition, employing a standard principal component analysis (PCA), analysis dihedral angles phi, psi, and measurements in time evolution was possible to evidence the greater structural movements. These simulations reveal that in their free states, Bcl-2-60-77 and FLL have propensities to form a bound-state-like helix structures but to different extents.

Keywords: Bcl-2, intrinsically disordered, Molecular Dynamic, apoptosis.

Conclusiones generales

Se obtuvo un modelo de alta calidad de Bcl-2 incluyendo el FLD mediante modelado por homología y los métodos ab initio. Además, el espacio conformacional y la estabilidad de la proteína Bcl-2 fueron exploradas por simulaciones MD en condiciones de alta temperatura, y diversos parámetros estructurales se analizaron. Bcl-2 muestra una alta estabilidad global, ya que mantiene su forma globular hasta 600 K. Curiosamente, nuestros resultados indican que a 400 y 500 K, el FLD se acerca al glóbulo de la proteína e interactua con los dominios BH3, evitando la exposición a posibles interacciones con otros miembros de la familia Bcl-2. La cara hidrofóbica de la hélice αL también está orientado hacia el núcleo de Bcl-2. Por último, las estructuras secundarias a 600 y 800 K muestran que la hélice alfa 1 y 6 son los elementos más estables. Estos hallazgos mejorar nuestra comprensión de la estabilidad de Bcl-2 y la conmutación entre las formas activa / inactiva de esta proteína para el diseño de medicamentos que regulan la muerte celular. Por otro lado, para entender los procesos de transducción de señales y funciones regulatorias a nivel estructural y mecánico, el análisis detallado de las proteínas intrínsecamente desordenadas es inevitable. Creemos que este trabajo tiene un significado práctico en que se demuestra la existencia de una estructura secundaria, en parte, controlada por el mecanismo de fosforilación. Es evidente que las técnicas complementarias, tanto experimentales y computacionales son necesarios para estudiar las proteínas que no se caracterizan por una estructura bien definida. Anteriormente, hemos proporcionado un argumento que, incluso para muchas de estas proteínas no existe realmente una conformación singular a baja energía. Además, los métodos computacionales se pueden utilizar para proporcionar nueva comprensión de cómo la fosforilación conduce el cambio conformacional.

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