PAGE4 and Conformational Switching: Insights from Molecular Dynamics Simulations and Implications for Prostate Cancer

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https://doi.org/10.1016/j.jmb.2018.05.011
Edited by Jianhan Chen

Abstract

Prostate-associated gene 4 (PAGE4) is an intrinsically disordered protein implicated in prostate cancer. Thestress-response kinase homeodomain-interacting protein kinase 1 (HIPK1) phosphorylates two residues in PAGE4, serine 9 and threonine 51. Phosphorylation of these two residues facilitates the interaction of PAGE4 with activator protein-1 (AP-1) transcription factor complex to potentiate AP-1's activity. In contrast, hyperphosphorylation of PAGE4 by CDC-like kinase 2 (CLK2) attenuates this interaction with AP-1. Small-angleX-ray scattering and single-molecule fluorescence resonance energy transfer measurements have shown that PAGE4 expands upon hyperphosphorylation and that this expansion is localized to its N-terminal half. To understand the interactions underlying this structural transition, we performed molecular dynamics simulations using Atomistic AWSEM, a multi-scale molecular model that combines atomistic and coarse-grained simulation approaches. Our simulations show that electrostatic interactions drive transient formation of an N-terminal loop, the destabilization of which accounts for the dramatic change in size upon hyperphosphorylation. Phosphorylation also changes the preference of secondary structure formation of the PAGE4 ensemble, which leads to a transition between states that display different degrees of disorder. Finally, we construct a mechanism-based mathematical model that allows us to capture the interactions of different phosphoforms of PAGE4 with AP-1 and its downstream target, the androgen receptor (AR)—a key therapeutic target in prostate cancer. Our model predicts intracellular oscillatory dynamics of HIPK1-PAGE4, CLK2-PAGE4, and AR activity, indicating phenotypic heterogeneity in an isogenic cell population. Thus, conformational switching of PAGE4 may potentially affect the efficiency of therapeutically targeting AR activity.

Please cite this article as: X. Lin, et al., PAGE4 and Conformational Switching: Insights from Molecular Dynamics Simulations and Implications for Prostate Cancer, J. Mol. Biol. (2018), https://doi.org/10.1016/j.jmb.2018.05.011

Introduction

A significant fraction (≈ 15%–30%) of proteins lack a stable structure, at least when they are not bound to an interaction partner or ligand [1–5]. Such unstructured proteins populate a diverse ensemble of interconverting conformations in order to function and, thus, they are referred to as intrinsically disordered proteins (IDPs). Despite their lack of rigid structure, IDPs are involved in regulation, signaling, and control of information flow in the cell. Binding to multiple partners and high-specificity/low-affinity interactions play crucial roles in the functions of IDPs [6,7]. Disorder enables IDPs to participate in both one-to-many and many-to-one signaling [3,8]. However, when overexpressed or aberrantly expressed, IDPs are prone to engage in promiscuous interactions that lead to various pathological conditions [9,10].

Complete structural characterization of IDPs has, in general, remained intractable to classical biophysical methods. Therefore, mechanistic insight into how IDPs function is currently limited to only a few examples. Prostate-associated gene 4 (PAGE4) is an IDP that has been relatively well characterized both structurally and functionally. PAGE4 has the hallmarks of a proto-oncogene: while it is highly expressed during its development in the fetal prostate [11,12], it is aberrantly expressed in the diseased gland where it plays an important role in tumorigenesis [11].

PAGE4 is also a stress-response factor[11] and functions as transcriptional coactivator in prostate cancer (PCa) cells where it can potentiate transactivation by c-Jun[12]. The proto-oncogenec-Jun heterodimerizes with c-Fos to form the activator protein-1 (AP-1) transcription factor complex that can negatively regulate the activity of androgen receptor (AR) in PCa cells [13,14]. Furthermore, in PCa cells, PAGE4 is phosphorylated by the stress-response kinase homeodomain-interacting protein kinase 1 (HIPK1). Phosphorylation of PAGE4 by HIPK1 occurs predominantly at Thr51 and, to a significantly lower level, at Ser9 [15]. PAGE4 can also be hyperphosphorylated by another kinase named CDC-like kinase 2 (CLK2) [16]. Cell-based studies have revealed that phosphorylation of PAGE4 by the two kinases leads to opposing functions. Thus, while HIPK1-phosphorylated PAGE4 (HIPK1-PAGE4) potentiates c-Jun, CLK2-phosphorylated PAGE4 (CLK2-PAGE4) attenuates c-Jun activity [16]. Biophysical measurements employing small-angleX-ray scattering (SAXS), single-moleculefluorescence resonance energy transfer (smFRET), and NMR indicate that HIPK1-PAGE4 exhibits a relatively compact conformational ensemble that binds AP-1, whereas CLK2-PAGE4 is more expanded and resembles a random coil with reduced affinity for AP-1 [16]. Although these experiments indicate that different phosphoforms of PAGE4 have different sizes and functions, the molecular details of the critical interactions within PAGE4 that are responsible for such conformational changes have, until now, remained unclear.

To investigate the structural details during the change in the size of PAGE4 upon phosphorylation and to overcome the temporal and spatial resolution limits associated with SAXS and smFRET experiments [17–19], we have used molecular dynamics (MD) simulations with an optimized protein folding landscape model. The Associative memory, Water mediated, Structure and Energy Model (AWSEM)[20] is based on the principles of the energy landscape theory of protein folding and is optimized using a quantitative formulation of the principle of minimal frustration [21–23]. AWSEM has been effective in moderate resolution protein structure prediction and accurate identification of protein–protein binding interfaces [20,24]. Moreover, when combined with energy landscape analyses, AWSEM can elucidate the molecular mechanisms underlying the regulation of cellular responses [25]. An important component of the AWSEM force field, called the associative memory term, models local structural preferences by searching for protein fragments in the Protein Data Bank (PDB) [26] that have sequences that are locally similar to those in the protein being simulated.

Recently, we developed a multi-scale atomistic AWSEM model (AAWSEM) [27], which harnesses the power of all-atomexplicit-solvent simulations to directly generate atomistic structures that can be used as fragments by the associative memory term of AWSEM. AAWSEM can reliably fold both α-exclusive proteins [27] and α/β proteins [28]. AAWSEM is especially useful for investigating disordered proteins such as PAGE4 because the PDB often lacks adequate protein fragments for modeling the local structural preferences of IDPs. In addition, the coarse-grained nature of AAWSEM makes it suitable for fully sampling the part of the conformational space corresponding to the functional states of IDPs, which is significantly larger for disordered proteins than it is for folded proteins.

Here, we performed simulations with AAWSEM to investigate the interactions underlying the structural transitions between the PAGE4 conformational ensembles. Consistent with the experimental findings, our simulations show a change in the size of PAGE4 upon phosphorylation. By analyzing contact formation in all phosphoforms of PAGE4, we see that the formation and destabilization of an N-terminal loop is the predominant source of this variation in size. After gaining insights into the differences between the conformational ensembles of different forms of PAGE4 (wild type, i.e., non-phosphorylated WT-PAGE4, double-phosphorylated HIPK1-PAGE4, and hyper-phosphorylated CLK2-PAGE4), we developed a mechanism-based mathematical model to describe how switching from one form to another

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