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A chirality change in XPC- and Sfi1-derived peptides affects their affinity for centrin

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ABSTRACT

The Ca^{2+}-binding protein centrin binds to a hydrophobic motif ($W^1xxL^4xxxL^8$) included in the sequence of several cellular targets: XPC (xeroderma pigmentosum group C protein), Sfi1 (suppressor of fermentation-induced loss of stress resistance protein1), and Sac3 [the central component of the transcription and mRNA export (TREX-2) complex]. However, centrin binding occurs in a reversed orientation ($L^8xxxL^4xxW^1$) for Sfi1 and Sac3 compared with XPC. Because D-peptides have been investigated for future therapeutic use, we analyzed their centrin-binding properties. Their affinity for centrin was measured using isothermal titration calorimetry. The chirality change in the target-derived peptides affected their ability to bind centrin in a specific manner depending on the sequence orientation of the centrin-binding motif. In contrast to L-XPC-P10, D-XPC-P10 bound C-HsCen1 in a Ca^{2+}-dependent manner and to a lesser extent. D-XPC-P10 exhibited a reduced affinity for C-HsCen1 ($K_a = 0.064 \times 10^6\ M^{-1}$) by a factor of 2000 compared with L-XPC-P10 ($K_a = 132 \times 10^6\ M^{-1}$). D-peptides have a lower affinity than L-peptides for centrin, and the strength of this affinity depends on the sequence orientation of the target-derived peptides. The residual affinity observed for D-XPC suggests that the use of D-peptides represents a promising strategy for inhibiting centrin binding to its targets.

1. Introduction

Centrins are small Ca^{2+}-binding proteins that bind several cellular targets: XPC, Sfi1, Sac3, and transducin β. Because of their binding activity, centrins are involved in several cellular processes, including centrosome division (Sfi1), DNA repair (the recognition of UV-induced damage by XPC), mRNA export (Sac3) and transduction (transducin β), that are regulated by Ca^{2+}. Centrins are mainly found in the cytoplasm and nucleus (90%), the remaining pool is found at the centrosome [1]. Sfi1 contains at least 21 centrin binding sites although all other targets contain only 1 binding site. XPC is located on nucleus [2] and Sac3 is located in nuclear membrane [3]. Sfi1 is located in the half bridge and its N terminus is embedded in the SPB's central plaque, C terminus marks the distal end of the half bridge [4]. Sfi1 and centrin that constitute the SPB are essential for SPB duplication. Interaction of centrin with Sfi1 occurs throughout the cell cycle [5].

Centrin are phosphorylated by MPS1 (T138), CK2(T138) and Aorora-A (S170). The localization of centrin 2 at the centrosome is dependent on the availability of a phosphorylatable T118 and functional calcium binding EF-hands [6]. Phosphorylation of human centrins 1 and 2 regulate the interaction with Sfi1 and transducin β [7]. Centrins 1 and 2 are substrates of SUMO conjugation and SUMOylation of centrin 2 is required for its efficient localization to the nucleus. XPC also undergoes the SUMOylation but the post-translational modifications of XPC and centrin 2 are dependent

Abbreviations: C-HsCen2, human centrin 2C-terminal domain; Sfi1, suppressor of fermentation-induced loss of stress resistance protein1; XPC, xeroderma pigmentosum group C protein; Sac3, is the central component of the transcription and mRNA export (TREX-2) complex; ITC, isothermal titration calorimetry; CD, circular dichroism.

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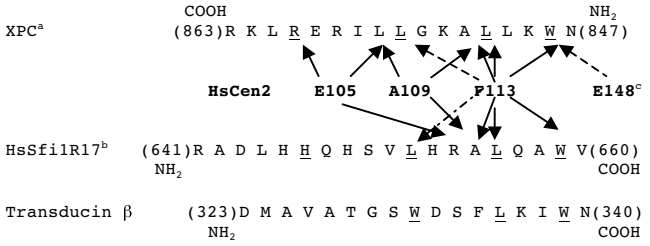

Fig. 1. Binding of crucial human centrin 2 residues (E105, A109, F113 and E148) to centrin-binding motif of XPC or of human Sfi1.

on different factors [8]. SUMOylation of centrin 2 is also required for efficient interaction with XPC. SUMOylation and ubiquitination of XPC drive the DNA repair [9]. Sfi1C-terminal domain harbors phosphorylations sites for Cdk1 and the polo-like kinase Cdc5. Phosphoregulation of Sfi1 by Cdk1 has the dual function of promoting SPB separation for spindle formation and preventing premature SPB duplication [5]. The protein phosphatase Cdc14 has the converse role via dephosphorylation of Sfi1 [10].

Centrins bind targets through the hydrophobic triad $W^1xxL^4xxxL^8$, which was first identified in XPC. However, the sequence of this motif is in the reversed amino-acid sequence $L^8xxxL^4xxW^1$ in centrin's other targets (Sfi1, Sac3 and transducin β) (Fig. 1). Crystal [11–14] and NMR structures [15,16] of centrins in complex with truncated targets or target-derived peptides (i.e., XPC, Sfi1, or Sac3) have been solved. In all cases, the target-binding pocket is located in the C-terminal domain of centrin, and the F113 residue of human centrin is the main residue involved in target binding.

Two structures of full-length and truncated HsCen2 in complex with an XPC peptide have been solved by X-ray crystallography [17,18], and one structure of the C-terminal domain of HsCen2 in complex with XPC peptide in solution has been solved by NMR [19]. The structure from [17] shows that only residues in the C-terminal domain of HsCen2 are involved in the interaction with the XPC peptide. Nine non-polar residues of HsCen2 (L133, L112, M145, F113, M166, L126, A109, E105 and V129) form contacts with the residues of the XPC peptide. The F113 and M145 residues form the pocket where the W residue of XPC binds.

The structure of CID region (proximal CTD-Interacting Domain) of Sac3 in complex with Sus1 and Cdc31 has been solved by X-ray crystallography [20,21]. In the complex, the W802 residue of Sac3 appears to play a central role in the interaction with the hydrophobic cavity of the centrin Cdc31. The F105 residue of Cdc31 in turn plays an important role in the association with Sac3.

A crystal structure of centrin Cdc31 in complex with truncated Sfi1 has been solved by X-ray crystallography [22]. Both the C-terminal and the N-terminal domains of Cdc31 make contact with Sfi1. The structure also reveals several centrin-centrin interactions. These interactions suggest that a filament of several centrin molecules is formed by centrin-centrin interactions and that this filament is stabilized by Sfi1 through Sfi1 repeat-centrin interactions.

An NMR structure of human centrin 2 in complex with the R17-Sfi1 peptide has been solved [16]. Comparison of this structure with the NMR structure of the C-terminal domain of human centrin 2 in complex with P17-XPC peptide [19] led to the proposal that the centrin 2 E148 residue discriminates between XPC and Sfi1 [16].

The reversed amino-acid sequence of the centrin binding motif has several consequences. The Ca^{2+}-dependence of the binding reaction is affected by its sequence orientation. Thus, the binding of centrin to transducin β occurs in a Ca^{2+}-dependent manner [7]. Conversely, the binding of centrin to XPC occurs in a Ca^{2+}-independent manner. The HsCen2 residue E148 discriminates between XPC and Sfi1; therefore, HsCen2 E148 binds to XPC but not to Sfi1 (Fig. 1). We demonstrated previously that the mutation E144A in *Scherffelia dubia* centrin led to a lower affinity for XPC compared with wild type centrin [23]. Furthermore, the specific role of the E148 residue was elucidated using target-derived peptides the charged the extremities of centrin, which can affect its binding properties. However, the full-length target must be investigated to confirm the role of the E148 residue in target discrimination. We reported that centrin affinity is affected upon CK2 phosphorylation and that this effect is more important for binding to transducin β and Sfi1 than for binding to XPC [7].

Among the various roles that centrin plays, its putative role in cell division is interesting because centrin could be used as a target for cancer therapy. Indeed, structural and numerical centrosome aberrations have been implicated in cancer [24–26]. From this perspective, one might block cell division by focusing on the centrin target Sfi1, which is involved in centrosome division. One way to block the centrin-target interaction involves the use of target-derived peptides—in particular, target-derived D-peptides—that are resistant to in vivo proteolysis.

Homochirality is a rule of living cells. Thus, proteins are composed of L-amino acids, whereas RNA and DNA are composed of D-sugars. However, D-amino acids exist in vivo and serve as a component of bacterial cell walls [27,28]. In eukaryotes, D-amino acids have been found in various tissues [29], including cartilage [30], hair [31], and tooth enamel [32]. In these examples, amino acid racemization is age-dependent [33]. However, racemization can also be functional, as in the case of the age-dependent aspartate racemization of mammalian histone H2B [34]. Amino acids racemization occurs also in reverse; indeed, bacteria use external D-amino acids as a source of nitrogen via enzymatic racemization [35].

D-Peptides have been used successfully in many applications. L-Peptides that have an affinity for D-protein have been identified using mirror image phage display [36]. The interaction between L-protein and target-derived D-peptide have been analyzed, and X-ray crystal structures of human Pin1 bound to d-peptide have been reported [37]. d-Peptides have been used successfully as inhibitors to the entry of HIV-1 into the cell [38] and for interaction with p53-MDM2 [39].

The properties of retro-inverso peptides have also been investigated. A retro-inverso peptide is comprised of D-amino acids in a reversed amino-acid sequence. Interaction between the tumor suppressor protein p53 and its negative regulator MDM2, the retro-inverso isomer of p53, retained the same binding activity as the wild type [40]. However, studies have been reported in which retro-inverso peptides are not functional. Li et al. [41] published that the retro-inverso strategy performs poorly in the molecular mimicry of biologically active helical peptides due to inherent differences at the secondary and tertiary structure levels between an L-peptide and its retro-inverso isomer. Conversely, D-peptides have been used for the treatment of Alzheimer's disease in mice [42]. These successes have encouraged us to analyze the binding of D-peptides to centrin.

Because the amino-acid sequence of the centrin binding motif for Sfi1 is in the opposite orientation, D-Sfi1 must be a retro-inverso of XPC. We investigated the binding reaction of centrin to the Sfi1-derived D-peptide and to the true retro-inverso XPC-derived peptide. Here, we analyzed the affinity of C-HsCen1 and C-HsCen2 for several target-derived peptides composed of either L-residues or D-residues using isothermal titration calorimetry (ITC). We also analyzed the effect of the retro-inverso peptide and observed that binding of the retro-inverso to centrin depended on its sequence. Thus, the XPC-derived RD-peptide bound to centrin with a reduced binding constant compared with the L-peptide. However, the Sfi1-derived D-peptide, which represents a retro form of XPC but is not strictly in sequence, did not bind to centrin even in the presence