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CK2 phosphorylation of human centrins 1 and 2 regulates their binding to the DNA repair protein XPC, the centrosomal protein Sfi1 and the phototransduction protein transducin β

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ABSTRACT

Centrins are calcium-binding proteins that can interact with several cellular targets (Sfi1, XPC, Sac3 and transducin β) through the same hydrophobic triad. However, two different orientations of the centrin-binding motif have been observed: $W^1xxL^4xxxL^8$ for XPC (xeroderma pigmentosum group C protein) and the opposite orientation $L^8xxxL^4xxW^1$ for Sfi1 (suppressor of fermentation-induced loss of stress resistance protein 1), Sac3 and transducin β. Centrins are also phosphorylated by several protein kinases, among which is CK2. The purpose of this study was to determine the binding mechanism of human centrins to three targets (transducin β, Sfi1 and XPC), and the effects of in vitro phosphorylation by CK2 of centrins 1 and 2 with regard to this binding mechanism. We identified the centrin-binding motif at the COOH extremity of transducin β. Human centrin 1 binds to transducin β only in the presence of calcium with a binding constant lower than the binding constant observed for Sfi1 and for XPC. The affinity constants of centrin 1 were 0.10 10^6 M^{-1}, 249 10^6 M^{-1} and 52.5 10^6 M^{-1} for Trd, R17-Sfi1 and P17-XPC respectively. CK2 phosphorylates human centrin 1 at residue T138 and human centrin 2 at residues T138 and S158. Consequently CK2 phosphorylation abolished the binding of centrin 1 to transducin β and reduced the binding to Sfi1 and XPC. CK2 phosphorylation of centrin 2 at T138 and S158 abolished the binding to Sfi1 as assessed using a C-HsCen2 T138D-S158D phosphomimetic form of centrin 2.

1. Introduction

The EF-hand superfamily is primarily composed of calmodulin, and centrins, which are small Ca^{2+}-binding proteins. Centrins were first identified in the unicellular green algae, *Tetraselmis striata* [1] and *Chlamydomonas reinhardtii* [2] and are conserved proteins. However, although lower eukaryotes such as unicellular algae and yeast, possess only one centrin, higher eukaryotes possess several isoforms that are generated by either gene duplication or retrotransposition from messenger RNA. These isoforms can integrate into the chromosome as demonstrated by murine Cen1 [3] and Cen4; in some cases, isoforms have been reduced to pseudogenes, such as in humans [4,5]. Centrins are composed of two relatively independent EF-hand domains with each domain containing two EF-hand motifs that bind Ca^{2+}. Although the two Ca^{2+}-binding sites located within the C-terminal domain are functional, the Ca^{2+}-binding sites in the N-terminal domain may not be functional, depending on the organism.

Centrins are localized to the centrosomes [6] and spindle pole bodies in *Saccharomyces cerevisiae* [7]. Centrin 2 is ubiquitously expressed, but centrin 1 expression is restricted to ciliated cells, such as retinal photoreceptor cells [8]. Currently at least five centrin-targets are known: XPC (xeroderma pigmentosum group C protein), which has 1 centrin-binding site and, is involved in DNA repair [9]; Sfi1 (suppressor of fermentation-induced loss of stress resistance protein 1), which is a helicoid protein, has 25 centrin-binding motifs and is involved in centrosome duplication and cellular division [10]; Sac3 which is involved in RNA transfer from

Abbreviations: C-HsCen, C-terminal domain of centrin; CK2, casein protein kinase; HPLC, high pressure liquid chromatography; HsCen, human centrin; ITC, isothermal titration calorimetry; MALDI-TOF, matrix-assisted laser desorption ionization time of flight; Sfi1, suppressor of fermentation-induced loss of stress resistance protein 1; Trd, transducin; XPC, xeroderma pigmentosum group C protein

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the nucleus to the cytoplasm [11]; Kar1 which is located on the spindle pole bodies [12]; and transducin which is a G-protein involved in the phototransduction cascade [13,14]. In all cases, centrin binds its targets through a hydrophobic triad $W^1xxL^4xxxL^8$ that was originally identified in XPC. However, in other centrin targets, such Sac3 and Sfi1, the triad is in the reverse orientation $L^8xxxL^4xxW^1$.

Two crystal structures of integral human centrin 2 in complex with a 17-residue peptide derived from the human XPC [15,16] and one crystal structure of yeast centrin Cdc31 in complex with two or three repeats of *S. cerevisiae* Sfi1 [17] have been solved by X-ray crystallography. From these structures, the residues involved in the interaction between centrin and its targets have been determined, and the F113 residue of human centrin was identified as the critical residue necessary to bind its targets (XPC and Sfi1) through several contacts, especially with the W^1 residue of the centrin-targets.

Solution structures of centrin in apo form [18] as well as in complex with either XPC [19] or Sfi1 [20] have also been resolved using NMR. Because the helix dipoles of XPC and Sfi1 are in opposing orientations, the consequences of helix dipole orientation were analyzed after the superimposition of the two NMR structures of human centrin 2 in complex with either XPC or with Sfi1. For both structures, the W residue of the triad is similarly embedded in the cavity where the centrin F113 residue is situated. However, for Sfi1, this implies a rotation of the W residue, which is accompanied by a slight translation of the movement along the peptide helix axis toward centrin 2. Owing to this movement the E148 residue, which contacts XPC, is pushed out and is no longer able to contact Sfi1.

Transducin is another centrin target that has been analyzed at the cellular level; however, no structural study of the transducin–centrin complex has yet been conducted. Two transducin β isoforms are found in the retina: transducin β1 is expressed in rods and transducin β3 is expressed in cones. Experiments using immunoelectron microscopy have shown that centrin and transducin co-localize in the connecting cilium of rod photocells in mice, and co-immunoprecipitation studies and centrifugation and size-exclusion chromatography experiments have demonstrated that transducin β and the heterotrimer transducin αβγ bind to centrin 1 [13]. The four isoforms of murine centrin have different subcellular localizations. Cen1, 2, and 3 are found in the connecting cilium although both Cen2 and Cen3 are found in the basal body whereas Cen4 is only located in the basal body [14,21].

The heterotrimeric G proteins (αβγ) transduce and amplify external signals. When GDP is bound to Gα it leads to the association of Gα with the two other subunits βγ. Furthermore, G protein-coupled signaling is regulated by two mechanisms. The first is the intrinsic GTPase activity of $G_t\alpha$, by which $G_t\alpha$ GTP is inactivated and reassociates with $G_t\beta\gamma$. Second, phosducin binds $G_t\beta\gamma$ thereby blocking its association: with $G_t\alpha$. Phosducin was first found in retina extracts but has since been shown to be expressed in various tissues [22,23]. The structure of phosducin in complex with transducin βγ has been solved using X-ray crystallography [24,25]. The binding of phosducin to transducin βγ is regulated by the phosphorylation of the S73 residue of phosducin by PKA. Phosphorylated phosducin then releases transducin β which then reassociates with transducin α [26].

In vertebrate photoreceptor cells, centrins are phosphorylated by the casein kinase CK2 in a light-dependent manner; this allows CK2 to co-localize with the centrins in the inner lumen of the connecting cilium. As a consequence of phosphorylation, the centrins have reduced affinities for transducin [27]. CK2 has been shown to phosphorylate murine centrin at T138, which is increased during the dark adaptation of photoreceptor cells and leads to the reduced binding of centrin to transducin. After illumination, the dephosphorylation of centrins by the PP2C-α and β phosphatases [28] results in increased binding to transducin. The transport of these proteins between the outer and inner segments at the ciliary junctions of photoreceptor cells is also light-dependent. This information, in addition to the large Ca^{2+} variations in these cells, suggests that Ca^{2+} may be involved in the transport of these proteins during the phototransduction cascade.

Because the centrin-binding motifs on XPC and both Sfi1 and transducin β have an opposing orientation, we investigated the effect of this inversion on the binding reaction. We also analyzed the effect of phosphorylation of centrin on its binding to targets based on the binding-motif orientation. The thermodynamics of the binding of human centrin 1 to transducin β was assessed using microcalorimetry and compared to the binding of other centrin targets (XPC, Sfi1). We report here that centrin 1 binds transducin β exclusively in the presence of Ca^{2+}, but with a lower affinity than that of centrin for its other targets XPC and Sfi1. A detailed study using transducin β variants allowed us to determine which residues were responsible for this lower binding affinity. We also report that the phosphorylation of centrin 1 by CK2 regulates its binding to transducin β. Additionally, we observed that CK2 phosphorylates human centrin 2 at T138 and S158 and consequently regulates centrin 2 binding to Sfi1 and XPC.

2. Results

2.1. CK2 phosphorylates centrin 1 on T138 and centrin 2 at T138 and S158

Both human centrins 1 and 2 (C-terminal domain including residues M93 to Y172) have been shown to be phosphorylated by the protein kinase CK2 (catalytic α and regulatory β subunits complex). Consequently, one phosphorylated form of centrin 1 was generated, but two phosphorylated forms of centrin 2 were identified by HPLC chromatography (Fig. S1A). The chromatograms show that the phosphorylated centrin 1 was the only protein observed after 24 h. Diphosphorylated centrin 2 was the primary form observed after 24 h. The determination of the phosphorylated residues was assessed using MALDI-TOF mass spectrometry of trypsinized fragments of both C-HsCentrins 1 and 2. It was observed that CK2 phosphorylated centrin 1 on T138 and centrin 2 on T138 and S158.

The sequences of centrins 1 and 2 differ by only three residues (Fig. 1). The centrin 1 amino acids N131, N158, and E160 are replaced by K131, S158, and Q160 in centrin 2. The T138 residue is present in both centrins and surrounded by following three acidic residues: D139, E140, and E141, which constitute the signature of a CK2 phosphorylation site. The S158 residue is only found in centrin 2 and is also surrounded by two acidic residues E159 and E161.

T138 residue is located within an unstructured loop between the two α-helices F (residues 124–132) and G (residues 139–149), and the three acidic residues (D139, E140, E141) are located at the extremity of the α-helix G. S158 is located at the junction of calcic loop IV and α-helix H (residues 159–166), and the acidic residues (E159, E161) are located at the extremity of the α-helix H (Fig. S1B, C).

2.2. Residue 131 is responsible for the different phosphorylation rates between centrins 1 and 2

The kinetics of CK2 phosphorylation on the C-terminal domains (M93-Y172) of both centrins 1 (C-HsCen1) and 2 (C-HsCen2) were performed in parallel in the presence of either EGTA or Ca^{2+} (Fig. 1A and B). First, the two isoforms exhibited a different phosphorylation rate; after 3 h at 37 °C, 62% of the C-HsCen1 protein was phos-