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Solution structure of a peptide derived from the oncogenic protein β-Catenin in its phosphorylated and nonphosphorylated states

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Abstract

β-Catenin plays an essential role in the Wingless/Wnt signaling cascade. Phosphorylation of β-Catenin in its N-terminal region by the kinase GSK-3β is required for the interaction with the SCF-β-TrCP protein complex that targets β-Catenin for proteasome degradation. In the present work, we used two peptides of 32 amino acids referred to β-Cat$^{17–48}$ and P-β-Cat$^{17–48}$ for the phosphorylated peptide at the two sites Ser33 and Ser37. Circular dichroism and NMR techniques were used to assess the influence of the phosphorylation. The spectra of the peptides at pH 7.2 were completely assigned. Analysis of the medium-range NOE connectivities indicated that β-Cat$^{17–48}$ seems to be only poorly folded. These data are in agreement with the result of structure calculations. P-β-Cat$^{17–48}$ possesses two helical segments around the DpSGXXpS motif, which forms a large bent with the phosphate groups pointing out of the structure. On the contrary, β-Cat$^{17–48}$ shows less well-defined secondary structures and appears as a more flexible peptide, but adopts in the motif DSGXXS a more compact conformation than P-β-Cat$^{17–48}$. Differences in this molecular region suggest that conformational changes of phosphorylated β-Catenin play an important role for the interaction with the SCF-β-TrCP protein complex.
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Keywords: β-Catenin; β-TrCP; Molecular modeling; NMR spectroscopy; Phosphorylated peptide structure; Wingless/Wnt signals

1. Introduction

β-Catenin is a multifunctional protein that plays an essential role in the transduction of Wnt (*Wingless int-1*) signals [1,21,22]. It is also a component of the cadherin cell adhesion complex. In the absence of Wnt signaling, the cytoplasmic level of β-Catenin is kept low through interaction with a protein complex composed of APC, axin, protein phosphatase 2A, and glycogen synthase kinase-3β (GSK-3β). In this complex, β-Catenin is phosphorylated by GSK-3β. Phosphorylation of β-Catenin results in its ubiquitination, mediated by β-TrCP in combination with Skp1 and Cullin1 (composing the SCF-β-TrCP complex), and subsequent degradation through a proteasome-dependent pathway [8,9,19] (Fig. 1A). If β-Catenin phosphorylation or its subsequent ubiquitination are blocked, β-Catenin is thus diverted from the proteasome, accumulates, and enters the nucleus where it activates expression genes, leading to the formation of tumors [22]. Evidence has been provided that β-Catenin/β-TrCP interaction depends on the phosphorylation of β-Catenin on a motif DSGXXS which is found in several cellular signaling proteins known to be subjected to degradation by the proteasome, cellular signaling proteins, such as IκBα, the inhibitor of the master transcription factor NFκB, or Vpu, a viral protein from HIV-1. Hence, all substrates of β-TrCP, either viral like Vpu, or cellular like IκBα, or β-Catenin, share a similar motif of the type DSGXXS, the phosphorylation of which on the two

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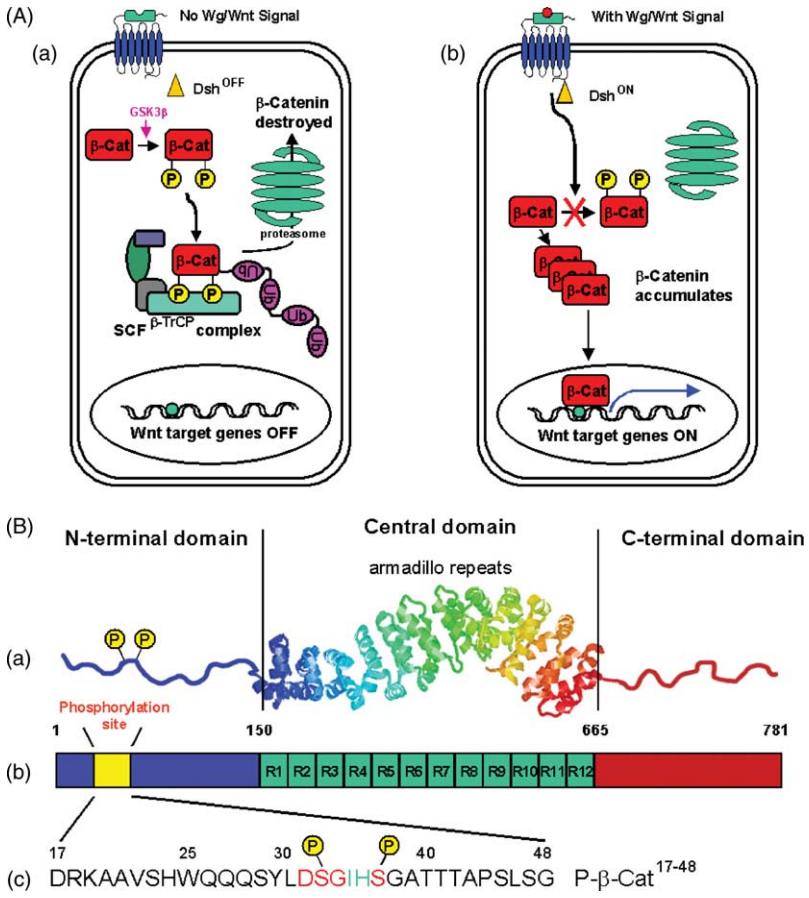

Fig. 1. (A) The Wingless/Wnt signaling pathway. Schematic summary of the response of a cell to Wg/Wnt signal. Wnt signaling is regulated by the presence or absence of the intracellular protein β-Catenin. (a) When Wnt signal is absent, the signal transduction pathway is OFF because β-Catenin is rapidly destroyed. A large multiprotein machine normally facilitates the addition of phosphate groups to β-Catenin by glycogen synthase kinase-3β (GSK-3β). Phosphorylated β-Catenin binds to a protein called β-TrCP, and is then modified by the covalent addition of a small protein called ubiquitin. Proteins tagged with ubiquitin are degraded by the 26S proteasome, the cell's protein-recycling center. (b) When cells are exposed to Wnt signal, it binds to cell surface receptors. Receptor activation blocks β-Catenin phosphorylation and its subsequent ubiquitination by an unknown mechanism that requires Disheveled protein (Dsh). β-Catenin is thus diverted from the proteasome, and it accumulates and enters the nucleus, where it finds a partner, a DNA binding protein of the TCF/LEF family. Together, they activate new gene expression programs. In human colon cancer cells, inappropriate activation of the Wnt pathway drives cell proliferation by turning on genes encoding oncoproteins and cell-cycle regulators [22,35]. (B) Schematic representation of the β-Catenin. (a) The three-dimensional structure of a protease-resistant fragment of β-Catenin containing the armadillo repeat region. The core region of β-Catenin is composed of 12 copies of a 42 amino acid sequence motif known as an armadillo repeat. The 12 repeats form a super helix of helices that features a long, positively charged groove of the proteolyse resistant fragment [10]. The structure of the N and C terminal domains remain unresolved. (b) Primary structure sequence of the full β-Catenin protein. The 12 armadillo repeats are shown in green. The phosphorylation site containing the consensus motif DpSGXXpS is shown in yellow. (c) The sequence (32 residues) of the phosphorylated β-Catenin fragment, which was investigated in the present work.

Ser residues is required for interaction with β-TrCP and the subsequent targeting of these substrates to be degraded by the proteasome. In order to drive rationally new therapeutic approaches, the elucidation of the mechanism involved in β-Catenin recognition by β-TrCP upon phosphorylation of this DSGXXS motif is essential.

β-Catenin structure can be described as different domains (Fig. 1B). The core domain of β-Catenin is composed of 12 copies of a 42 amino acid sequence motif known as an armadillo repeat. The 12 repeats form a super helix of helices that features a long, positively charged groove of the proteolyse resistant fragment [10]. The structure of the N and C terminal domains remain unresolved. The phosphorylation site, containing the consensus motif DSGXXS, is shown in yellow in the N-terminal domain. The sequence (32 residues) of the phosphorylated and nonphosphorylated β-Catenin fragments, which were investigated in the present work are shown in Fig. 1B(c). It is known that the N-terminal domain of β-Catenin including the consensus motif DSGXXS is responsible for direct interaction with β-TrCP that targets β-Catenin for ubiquitination and then proteasome degradation [7,15,31,40]. Phosphorylation of Ser33 and Ser37 by GSK-3β is necessary for interaction with β-TrCP. Residues which are essential for correct phosphorylation and degradation of β-Catenin have been mapped by the identification of specific mutations in β-Catenin that are present in human tumors and cancer lines [24]. Most of them are located between residue 32 and residue 45, and a majority in the DSGIHS motif,

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