Coexpression and protein-protein complexing of DIX domains of human Dvl1 and Axin1 protein

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INTRODUCTION

Wnt signaling is a key regulatory pathway in animal development and is critical for the homeostasis of adult tissues (1, 2). In the canonical Wnt pathway, Dvl proteins are cytoplasmic and are recruited to the plasma membrane by binding to Fz receptors. Upon activation, Dvl proteins suppress the three component proteins (GSK3β, APC and Axin) of the Axin complex, resulting in regulation of the phosphorylation and stability of β-catenin (1, 2). Three Dvl genes (Dvl1, 2 and 3) along with two Axin genes (Axin1 and 2) have been isolated from mammals. β-catenin acts as a transcriptional coactivator in the Wnt signaling pathway, and its inappropriate activation causes tumor-related diseases such as colorectal cancer (3-5). Therefore, studies on the interaction between Dvl and Axin protein are important to understanding signal transduction in the Wnt pathway. However, the interaction mechanism between the two proteins remains unclear.

Dvl proteins contain three highly conserved domains: an N-terminal DIX domain, a central PDZ domain and a DEP domain (6-9) (Fig. 1). Disruption of the PDZ domain abolishes Dvl activity during Wnt signaling (10, 11), and the DEP domain is critical for the upregulation of β-catenin activity and stimulation of LEF-1, which mediates transcription in mammalian cells (12). Interestingly, the C-terminal region of Axin is homologous to the DIX domain of Dvl. Moreover, the activity of Axin protein is inhibited upon the interaction of its DIX domain with that of Dvl (6-8, 13, 14). However, the 3D structure of the Dvl DIX domain and DIXDvl-DIXAxin complex remain undetermined. In addition to their heterogeneous interaction, the DIX domains of Dvl and Axin have been reported to mediate self-association both in vitro and in vivo (6, 15, 16).

According to X-ray crystallography, the Axin DIX domain can form head-to-tail filaments by self-association (17). In this study, we demonstrated that the DIX domains of human Dvl and Axin were not expressed in E. coli when using a mono-cistronic vector and highly expressed when using a multi-cistronic vector. Interaction of the DIX domains mediated the formation of a DIXDvl-DIXAxin complex in solution. Moreover, we reported the initial crystallization conditions of the protein-protein complex for x-ray crystallography.

RESULTS AND DISCUSSION

Coexpression of Each DIX domain gene from hDvl1 and hAxin1

To express the DIX domains of Dvl1 and Axin1 with high yield in E. coli, we inserted the DIX domains of hAxin1 and hDvl1 into multiple cloning sites (MCS) 1 and 2 of the multi-cistronic vector pACYCDuet-1, respectively (Fig. 1). The inserted Axin1 DIX domain was designed to be expressed with a N-terminal hexahistidine tag while the Dvl1 DIX domain expressed no tag. Dvl1 DIX and Axin1 DIX recombinant proteins had predicted sizes of 230 and 308 amino acid residues, respectively, with theoretical molecular masses of 25.5 and 34.1 kDa. E. coli BL21 (DE3) host cells harboring pACYCDuet-1 were induced by IPTG for the production of recombinant proteins, and a distinct band of ~25 kDa and ~35 kDa was observed by SDS-PAGE (Fig. 2A). However, there was no detectable expression of the DIX domains of Dvl1 or Axin1 using mono-cis-
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Fig. 1. Domain structures of human Dvl1 and Axin1 proteins, and a schematic diagram of the coexpression vector. Human Axin1 is schematically represented with its ligand proteins, which bind to known structural domains. Pro, promoter; MCS, multiple cloning site; His, histidine tag.

tronic pET28a vector (Fig. 2A), which implies that the separately-expressed protein in E. coli was unstable. Due to this noticeable in expression difference between the multi- and mono-cistronic vectors, instability was avoided by complexing the DIX domains with other proteins.

Affinity purification of recombinant proteins
After the harvested cells were resuspended in ice-cold lysis buffer, homogenization was carried out with an ultrasonic processor. The crude lysate was centrifuged and only the supernatant fraction was loaded onto a Ni-NTA affinity column. Nonspecific proteins were washed away with buffer A containing 50 mM Tris-HCl (8.0) and 100 mM NaCl. Elution of bound proteins was performed in a linear salt gradient, which was facilitated by mixing with buffer B containing 500 mM imidazole.

Both Dvl1 and Axin1 DIX were eluted simultaneously in the range of 100-300 mM imidazole and confirmed as homogeneous by SDS-PAGE (Fig. 2B). Since only Axin1 DIX protein contained a hexahistidine tag, co-elution of Dvl1 DIX protein at an increased imidazole concentration indicated interaction with Axin1 DIX protein. After affinity purification, dialysis was performed against buffer A to remove imidazole salt from the pooled sample.

Formation of DIXDvl1-DIXAxin1 complex
To investigate the formation of the DIXDvl1-DIXAxin1 complex, we analyzed the molecular weight of purified protein using size-exclusion chromatography. Based on the molecular weight of each protein, the DIXDvl1-DIXAxin1 complex was expected to be about 60.4 kDa. A standard curve was prepared as described above. As shown in the SEC profile, the highest peak, which was eluted at ~12-14 ml, corresponded to a molecular weight ranging from 67 kDa to 440 kDa (Fig. 3A). This reveals that the eluted protein particles were definitely larger than Dvl1 DIX (25.5 kDa) and Axin1 DIX (34.1 kDa). SDS-PAGE showed that the two proteins were simultaneously eluted (Fig. 3B), although both differed considerably in molecular weight. Together with co-elution by affinity column chromatography, these results reveal that the DIX domains formed a heterogeneous protein-protein complex with another DIX domain.

In addition to heterogeneous interaction, another research group reported the homo-oligomerization of Dvl1 DIX and Axin1 DIX by self-association, with homo-oligomers resembling a trimer or tetramer (6). Interestingly, the band widths of Axin1 DIX and Dvl1 DIX did not show 1:1 stoichiometry in SDS-PAGE. Thus, the co-eluted fraction in the SEC profile was a mixture of several species containing a homo-oligomer and hetero-dimer of Dvl1 DIX and Axin1 DIX. However, it was not possible to determine the molecular weight of the hetero-dimer due to the presence of a trimer or tetramer of the homo-oligomer.

Crystallization screening of DIXDvl1-DIXAxin1 Complex
Among the three domains involved in Dvl protein folding, the structures of PDZ and DEP have been determined. However, the structure of DIX domain remains unsolved. The structural