ABSTRACT

Phosducin (Pdc) is a highly conserved acidic phosphoprotein, which plays an important role in the regulation of G-protein signalization in intact retina. It binds to Gβγ dimer of heterotrimeric G-protein transducin thereby decreases the pool of available transducin resulting in modulation of signal. Function of phosducin is negatively regulated by its phosphorylation followed by interaction with the 14-3-3 protein. Besides this established way of regulation, we were interested in other putative interaction partners of phosducin, like SUG1 and CRX. SUG1 is a subunit of 26S proteasome with a large scale of biological functions, especially a degradation of many transription factors. Its role in regulation of phosducin is still unclear, but is probably involved in targeting of phosducin to 26S proteasome for its degradation. Subsequently, we prepared four different expression constructs of full-length protein in order to find the best expression and purification strategy. These results suggest that all purified fusion proteins of SUG1 form stable and soluble high molecular weight oligomers. This behaviour was confirmed by dynamic light scattering and analytical ultracentrifugation measurements. In addition, this observation is consistent with previous studies of its bacterial counterpart, PAN protein.

Key words: phosducin, SUG1, 26S proteasome, CRX, expression and purification, light scattering