

Abstract

The focus of this work was the characterization of the PACSIN1 and PAST2 proteins after their cloning, eucaryotic expression and purification. The proteins are known to participate in intracellular transport-systems. Polyclonal antibodies against the PAST2 protein were generated and used to confirm the PACSIN1 / PAST2 interaction, that had been earlier detected in two hybrid assays and by affinity precipitation. In pull-down assays additional binding partners, involved in different intracellular transport pathways were identified. A broad tissue distribution for PAST2 was shown by immunochemical methods. Additionally, A/GTPase activity could be predicted from the sequence and was confirmed by using recombinantly expressed and purified protein in A/GTPase assays.

An oligomerisation of the PACSIN1 adapter protein had been detected in two-hybrid screens and in assays using the GST-PACSIN1 expressed in bacteria. This homo-oligomerisation was now characterised using the PACSIN1 expressed in mammalian cells. By several different methods the protein was shown to form stable tetramers in solution. Additionally, it was possible to verify the presence of aldehyd groups on the protein after periodate-oxidation, indicating glycosylation of PACSIN1.