Interaction of synapsin I with actin and SSVs:
Differential regulation by calmodulin

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The synapsin family of proteins has been implicated in the formation and retention of synapses. The four mammalian synapsins (Ia, polypeptide chain size 74 kDa; Ib, 70 kDa; Ila, 63 kDa; and Ilib, 52 kDa) are derived from two genes (I and II), the a and b isoforms being formed by differential mRNA splicing (Sudhof, 1989). These abundant proteins are peripheral to the membranes of small synaptic vesicles (SSV) and are major targets for multiple protein kinases (including cAMP-dependent kinase and Calmodulin-dependent kinases I and II). Studies on transgenic mice carrying null mutations in one or both of the synapsin genes (Rosahl et al., 1993, Rosahl et al., 1995) have suggested that synapsins are required for normal synaptic organisation and function. Recently, the synapsins in Drosophila have been molecularly cloned (Klagges et al., 1996).

The synapsins cross-link SSVs to the cytoskeletal elements at presynaptic terminals, forming a 'reserve' pool of vesicles. Previous studies have demonstrated that these interactions are regulated by activation of calmodulin-dependent protein kinases (Bahler & Greengard, 1987, Petrucci & Morrow, 1987, Valtorta et al., 1992) and direct calmodulin binding (Goold et al., 1995); these regulatory motifs depress the activities of synapsins, leading to release of the vesicle-cytoskeleton bridges, and release of SSVs for exocytosis.

Here we use an optical biosensor to show that synapsin I directly binds calmodulin (figure 1). This calcium-dependent interaction is of high affinity (19nM) and is typified by a relatively rapid association phase. No binding was observed in the absence of Ca2+. We also show that calmodulin selectively regulates the interaction of synapsin I with the cytoskeleton. Both calmodulin and phosphorylation control the enhancement of actin polymerisation by synapsin I. Calmodulin in concentrations up to 3μM suppressed the enhancement of polymerisation by each phosphorylated form of synapsin I: both synapsin's endogenous nucleating activity, and promotion of polymerisation of actin grown from F-actin seeds were suppressed. By contrast, the addition of Ca2+/calmodulin (1-10μM) to synaptic vesicle preparations did not significantly displace endogenous synapsin I or II from the synaptic vesicle membrane. These data indicate that Ca2+/calmodulin selectively regulates the interaction of synapsin I with the cytoskeleton.

Figure 1. Interaction of ovine synapsin Ia / Ib with immobilised calmodulin using an IAys optical biosensor. The association phase data from a range of concentrations (12nM to 200nM) of synapsin Ia / Ib in Ca2+ containing buffer are shown. A plot of Kon against concentration of synapsin is shown inset.

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References