VAT-1 from *Torpedo* Synaptic Vesicles Is a Calcium Binding Protein: A Study in Bacterial Expression Systems

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**SUMMARY**

1. Calcium binding properties were examined in VAT-1, an abundant 41-kDa membrane protein expressed in the cholinergic synaptic vesicles of *Torpedo*.

2. An overlay assay, using $^{45}$Ca$^{2+}$ as a tracer, demonstrated the ability of a recombinant VAT-1 produced from the IPTG-inducible pKK223-3 expression vector to bind calcium.

3. A high yield of recombinant VAT-1 was obtained from the glutathione S-transferase (GST) expression system. The fusion product enabled VAT-1 purification via affinity chromatography. Subsequent cleavage by thrombin resulted in its separation from the GST carrier protein.

4. A direct Ca$^{2+}$-binding study was performed with purified VAT-1 by a quick-spin column technique, in the presence of $^{45}$Ca$^{2+}$. Quantitative analysis revealed a 1:1 molar stoichiometry for binding of Ca$^{2+}$ to VAT-1, with a dissociation constant of 130 μM.

5. A GST-linked truncated protein consisting of 13 kDa from the VAT-1 carboxy-terminal domain was found to retain the capacity to bind Ca$^{2+}$.

6. A data search for homologies between VAT-1 and known Ca$^{2+}$-binding proteins revealed considerable similarity to members of the annexin family in a 140-amino acid region from the carboxy terminal of VAT-1, which overlaps two tandem Ca$^{2+}$-binding domains of the annexin proteins.

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INTRODUCTION

Synaptic vesicles play an important role in uptake and storage of neurotransmitters, exocytosis, and recycling processes within the nerve terminal (Trimble et al., 1991). These processes should be mediated by membrane proteins of the presynaptic zone and those of synaptic vesicles, many of which have been molecularly cloned and identified. However, little is known as yet regarding the specific biological function(s) of most of these proteins (Sudhof and Jahn, 1991). This refers particularly to interactions with Ca\(^{2+}\) ions, which serve as a signal for principal vesicular functions such as neurotransmitter release (Augustine, 1987), activation of protein kinases, and modulation of synapse plasticity (Malenka et al., 1991). Tight control over many of these processes is achieved by efficient mechanisms maintaining low resting cytosolic Ca\(^{2+}\) levels (Tsien and Zucker, 1986). Most of the Ca\(^{2+}\) load that enters the nerve terminal is either buffered by binding to proteins and membranes or sequestered by intraterminal organelles (Nachshen, 1985). Ultimately, the recovery of Ca\(^{2+}\) to its resting level depends on its extrusion from the nerve terminal (Blaustein and Ector, 1976). However, a relatively high transient Ca\(^{2+}\) concentration \([100 \mu M\) (Yamada and Zucker, 1992)] is believed to exist within microdomains in the immediate vicinity of voltage-dependent Ca\(^{2+}\) channels in neurotransmitter release sites, known as active zones. Moreover, discrete and localized domains of high Ca\(^{2+}\) concentration (between 200 and 300 \(\mu M\)) were monitored upon activation of rat cerebral nerve terminals using a fluorescent dye (Llinas et al., 1992). The above observations are consistent with the notion of abrupt Ca\(^{2+}\) concentration changes occurring at or near the active zones. Thus, low-affinity Ca\(^{2+}\) sensors may mediate some of the Ca\(^{2+}\)-dependent processes in the terminal. As the elevation in internal Ca\(^{2+}\) concentration is thought to be confined to the release site, these proteins may reside in the vesicular membrane or in the active zone itself. Ca\(^{2+}\)-binding proteins were hence postulated both in controlling intracellular Ca\(^{2+}\) levels and as Ca\(^{2+}\) sensors in nerve terminals (reviewed by Baimbridge et al., 1992). The growing family of Ca\(^{2+}\)-binding proteins includes members of the annexins, calmodulin, and proteins from the synaptic vesicle membrane as well as proteins of the plasma membrane (reviewed by Augustine, 1987; Trimble et al., 1991; Sudhof and Jahn, 1991).

VAT-1 is an abundant 41-kDa protein from Torpedo, which was purified from membranes of cholinergic synaptic vesicles from the electric organ (Linial et al., 1989). The exclusiveness of VAT-1 expression in the Torpedo electric lobe was the first indication that it might be involved in presynaptic processes occurring in cholinergic neurons. Biochemical characterization of VAT-1 therefore became an essential prerequisite toward understanding its biological function. To this end, we took the initiative to produce the Torpedo VAT-1 protein in bacterial expression systems, purify it, and examine its Ca\(^{2+}\)-binding properties in solution and in solid-phase interactions. Our findings demonstrate that VAT-1 is a low-affinity Ca\(^{2+}\)-binding protein with an apparent \(K_d\) of 130 \(\mu M\) and attribute a Ca\(^{2+}\)-sensory function to this vesicular protein within the nerve terminal.