Novel sequence patterns specific to VAMP subfamilies

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Introduction

In eukaryotic cells, SNARE proteins of the vesicle or target membrane (v- or t-SNAREs) play a central role in the control of membrane fusion and protein and lipid traffic. SNAREs' coiled-coil domains (CCDs) have probably evolved from a common ancestor with a hydrophobic heptad register, interrupted by a conserved polar residue at the ionic "zero" layer [1]. Depending on the nature of such residue, SNAREs have been reclassified as either Q- or R-SNAREs [2]. R-SNAREs consist of two subfamilies: (i) short VAMPs or brevins (from the latin word "brevis" = short), and (ii) long VAMPs or longins, sharing a conserved N-terminal Longin Domain [3,4]. Distinct amino acid patches are likely to determine specificity of SNARE pairing by reducing structural integrity when mismatched SNAREs interact [2]. When considering pairing of the Q- and R-SNARE CCDs, an asymmetric ''complementarity'' is found in layers -3, -2, and +6, where bulky side chains are packed together with smaller ones, possibly enforcing the correct register between the CCDs of the fusion complex [1]. Sequence variation in the SNARE domains, by altering local charges at the interaction layers, is likely to mediate a fine modulation of the interaction specificity and/or kinetics, regulating intramolecular binding as well as binding to a growing family of SNARE-interacting factors. Although the structure of the SNARE complex is evolutionarily conserved, biological specificity is probably mediated mainly by accessory proteins recognizing different CCD surface patterns of charges, polar and nonpolar side chains different between the endosomal and neuronal complexes. Recently [5], it has been reported that the interaction among acidic surface residues from the SNAREs and basic residues over the concave surface of α-SNAP is crucial to the disassembly of the complex.

Results and Discussion

To date, v-SNAREs are tagged by profiles for the CCD which are not specific to any v-SNARE subfamily, as the only specific SNARE signature (PROSITE, accession PS50859) has been developed for a domain other than the CCD. Concerning patterns, current "synaprobrevin signature" (PROSITE accession PS00417) was created in 1990 and it does not discriminate between brevins and longins. The "Recall" index (i.e., true hits / (true hits + false negatives)) of PS00417 is slightly lower than 60%; thus, it is unable to identify roughly 40% of true R-SNARE sequences. Based either on profiles or patterns, current signatures for the SNARE CCD. Thus, we performed a comparative analysis of VAMP sequences to infer specific patterns capable of classifying R-SNAREs in subfamilies based on the conservation of residues at positions of the CCD other than the "zero" and the hydrophobic layers. Building such patterns by iteration allowed us to obtain two signatures (RD-SNARE and RG-SNARE) with final indexes of 100% precision and 98-100% recall (Table 1). RD-SNAREs are limited to Bilateria and group only short VAMPs or "brevins" [3] such as (i) VAMP1, 2, 3, (ii) *D. melanogaster* SybA/B, and n-syb and (iii) *C. elegans* Snb. Instead, RG-SNAREs consist of a larger group including (i) all longins, (ii) non-neuronal brevins such as yeast Snc1/2, mammalian VAMP5 and VAMP8, and (iii) VAMP4 proteins, which

are neither brevins nor longins. It is noteworthy that metazoan brevins involved in synaptic transmission or fast exocytic processes, in addition to lack the inhibitory LD, share an RD-type SNARE CCD, whose amino acid patches are likely involved in determining the specificity for interactions able to mediate neuronal fast exocytosis. Instead, the RG-SNARE signature is common to yeast brevins, VAMP4 and VAMP8, as well as to "low-fusion" SNAREs such as longins [4]. Evidence that longins are more widely distributed among eukaryotes than brevins [3] and that yeast and "cellular" mammalian brevins also share an RG-SNARE CCD, suggests that this latter is possibly involved in a wider range of transport pathways, based on fusion rates lower than those mediated by the RD-SNARE CCD. When compared to the actual synaptobrevin signature, RD- and RG- patterns show at the same time high "Precision" and "Recall" indexes, hence possibly representing *functional* tags for newly identified R-SNAREs. Evidence that v-SNAREs involved in neurotransmitter release are all RD-SNAREs suggests that RD-SNAREs evolved to specialize into the most rapid fusion reactions that occur in animals.

RD-SNARE signature
[LIVM]-x-[VTND]-[NTHA]-x(3)-[LIVT]-x(2)-[RK]-[DE]-[QSTVKA]-x-[LIS]-x(2)-[LIVM]-x(3)-[ASTIN]-x(5)-[GQ]-x(3)-[FYMNS]-[EQ]-x(3)-[AGRS]
TP = 66, of which: 13 (+ 3 isoforms) from SwissProt[(a)] and 50 from TrEMBL+TrEMBLnew[(b)]
FP = 0; FN = 0; Precision = 100.00 %; Recall = 100.00 %
RG-SNARE signature
[LIVMA]-x-[DENQSTKRAG]-[NTHAID]-x(3)-[LIVTMA]-x(2)-[RKV]-G-[DEQTAV]-x-[LISV]-x(2)-[LIVM]-x(3)-[ASTIN]-x(2)-[LM]-x(2)-{CFHIKPRVW}-x(3)-[FYMNS]-[NSKRHYAQ]-x(2)-[GASTN]-[QNKRTVFYS]
TP = 90, of which: 22 (+ 1 isoform) from SwissProt[(a)] and 67 from TrEMBL+TrEMBLnew[(b)]
FP = 0; FN = 2; Precision = 100.00 %; Recall = 97.82 %

Table 1 RD- and RG-SNARE signatures (in standard PROSITE syntax: http://www.expasy.ch/prosite) were obtained by iteration of a four-steps process: (i) pattern enlargement (starting from residues at positions found to be specific to RD- and RG-SNAREs, further residues are progressively included), (ii) scanning of the SWISS-PROT+TrEMBL databases by the modified patterns, using the "ScanProsite" tool at ExPASy, (iii) analysis of extracted hits to verify the presence of a SNARE CCD and to identify false positives using RPS-BLAST, (iv) calculation of precision and recall indexes (following PROSITE definition). Sequences were extracted from EBI and NCBI non-redudant protein databases by BLAST, using default parameters (but no filters). Multiple alignments were performed using Megalign (DNASTAR). True (TP) or false (FP) positive hits and false negatives (FN) concern the scanning of databases released on 15-Dec-2003: (a) SwissProt 42.7 (141681 entries) and (b) TrEMBL+TrEMBLnew 25.7 (1078339 entries). Indexes of both "Precision" (true hits / (true hits + false positives)) and "Recall" (true hits / (true hits + false negatives)), were calculated following PROSITE definition.

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