Assembly of a Ternary Complex by the Predicted Minimal Coiled-coil-forming Domains of Syntaxin, SNAP-25, and Synaptobrevin

A CIRCULAR DICHROISM STUDY

Jaume M. Cànaves and Mauricio Montal‡

From the Department of Biology, University of California San Diego, La Jolla, California 92093-0366

The assembly of target (t-SNARE) and vesicle-associated SNAP receptor (v-SNARE) proteins is a critical step for the docking of synaptic vesicles to the plasma membrane. Syntaxin-1A, SNAP-25, and synaptobrevin-2 (also known as vesicle-associated membrane protein, or VAMP) bind to each other with high affinity, and their binding regions are predicted to form a trimeric coiled-coil. Here, we have designed three peptides, which correspond to sequences located in the syntaxin-1A H3 domain, the C-terminal domain of SNAP-25, and a conserved central domain of synaptobrevin-2, that exhibit a high propensity to form a minimal trimeric coiled-coil. The peptides were synthesized by solid phase methods, and their interactions were studied by CD spectroscopy. In aqueous solution, the peptides were unstructured and showed no interactions with each other. In contrast, upon the addition of moderate amounts of trifluoroethanol (30%), the peptides adopted an α-helical structure and displayed both homomeric and heteromeric interactions. The observations in ternary mixtures induce a stabilization of peptide structure that is greater than that predicted from individual binary interactions, suggesting the formation of a higher order structure compatible with the assembly of a trimeric coiled-coil.

Understanding the interactions between the proteins of the trimeric complex in a simplified model may outline new ways to control its assembly and dissociation or to modulate the conformational changes that are presumably necessary for the progression from the docking step to the subsequent phases in the secretory process. The structural domains that appear to be implicated in the protein-protein interactions between SNAP-25, synaptobrevin, and syntaxin show a high propensity for the formation of α-helices (11–15). Secondary structure analysis shows that the periodic distribution of hydrophobic amino acids is consistent with a coiled-coil organization (2, 11, 12, 14, 15). Fluorescence energy transfer experiments (12) and electron microscopy (15) further indicate that synaptobrevin and syntaxin are aligned in parallel in the context of a ternary coiled-coil.

To investigate the postulated coiled-coil interactions between the proteins that constitute the docking complex in a minimal model, we have applied the principles involved in the formation of stable coiled-coils (16) to design three peptides corresponding to predicted coiled-coil-forming domains in SNAP-25, synaptobrevin-2, and syntaxin-1A. We have used CD spectroscopy to determine the secondary structure of these peptides and their interactions in binary and tertiary mixtures. Our findings are consistent with the assembly of the predicted ternary complex.

EXPERIMENTAL PROCEDURES

Reagents—HPLC grade trifluoroacetic acid, trifluoroethanol (TFE), ethanedithiol, thiaoisole, phenol, and acetonitrile were purchased from Aldrich. Methyl tert-butyl ether was from Fisher. HPLC columns were from Vyded (Hesperia, CA). t-Amino acids and protected derivatives used for peptide synthesis were made by Calbiochem. Benzoyl anhydride was obtained from Sigma. All other reagents for peptide synthesis and resins were from Applied Biosystems (Foster City, CA).

Peptide Synthesis and Purification—Peptides SN (human brain SNAP-25-(1–216), SB (human brain synaptobrevin-2-(24–56), ST (human brain syntaxin-1A-(191–218)), and SN(α), (scrambled SN peptide sequence: ESNDTFAIKTQAGS MKRMGLNAAK) were produced using solid phase peptide synthesis. Synthesis started with a tert-butyl ester was removed under high vacuum at 0 °C for 3 h. Samples of crude peptides precipitated from the trifluoroacetic acid mixture in cold methyl tert-butyl ether and centrifuged, the supernatant discarded, and the remaining methyl tert-butyl ether was removed under high vacuum at 0 °C for 3 h. Samples of crude peptide (10–20 mg) were dissolved in 0.1% trifluoroacetic acid, applied to a semipreparatory column (Vydac, C-18), and eluted at a flow rate of 3 ml/min with a linear gradient of 90% acetonitrile in 0.1% trifluoroacetic acid. Eluted peaks were monitored by absorbance measurements at 214 nm, pooled, and lyophilized. Peptide purity was assessed by RP-HPLC in an analytical column (Vydac, C-18).

Secondary and Tertiary Structure Predictions—Propensities of peptides to adopt a coiled-coil structure were estimated using two different methods, and their interactions were studied by CD spectroscopy to determine the secondary structure of these peptides and their interactions in binary and tertiary mixtures. Our findings are consistent with the assembly of the predicted ternary complex.

The assembly of the synaptic core complex is essential for Ca²⁺-dependent neuroexocytosis. This early event in the secretory cascade is then followed by the priming and vesicle fusion steps (1–6). According to the SNARE model, docking of synaptic vesicles to the plasma membrane is a critical step that involves the formation of a ternary complex by the v-SNARE synaptobrevin (also known as vesicle-associated membrane protein, or VAMP), and two t-SNAREs: SNAP-25 and syntaxin (7–9). Reconstitution of the v-SNARE synaptobrevin into lipid vesicles and the two t-SNAREs, SNAP-25 and syntaxin, into a distinct vesicle pool has provided evidence that the formation of a ternary complex is sufficient to join the independent vesicle pools and lead to fusion of the apposed bilayer membranes (10).
programs: Coils and Paircoil. The Coils program uses the Lupas algorithm (18, 19). Sequences were compared with an unweighted MTIDK matrix (18) using 14- and 28-residue scanning windows. The second program uses the Berger algorithm (20), which is more stringent. Both methods are based on the relative frequency of occurrence of amino acids at each position (α-φ) of the coiled-coil heptad repeat. Secondary structure predictions were performed using the SOPMA method (21, 22) and the AGADIR program (23).

**CD Measurements**—CD measurements were carried out on a modified Cary 61 (24) or an AVIV model 202 spectropolarimeter. The original Pockel cell and Cary linear polarizer in the Cary 61 were replaced with a 50-kHz photoelastic modulator (Hinds International, PS-5/PEM-80) and a MgF₂ linear polarizer (AVIV Inc.). The phase-detected output of the original end-on photomultiplier and preamplifier were integrated using an Egg Princeton Applied Research model 128A lock-in amplifier. System automation and multiple scan averaging were accomplished with an IBM PC-compatible computer interfaced directly to both the Cary 61 and the 128A amplifier. Constant N₂ flushing was employed. Spectra were measured at 195–250 nm using a 0.05-cm cell, a 1-nm bandwidth, a 0.3-ms time constant, and a cell temperature of 25 °C. All recordings were performed in 10 mM sodium phosphate buffer, pH 7.4, 100 mM NaCl, with or without TFE, unless otherwise indicated. Twenty scans were averaged for every spectrum. Base line subtraction, conversion of measured rotations to mean residue ellipticity [θ] (degcm²dmol⁻¹) (25), and filtering of the spectra using a fast fourier transform filter were performed using the Microcal Origin 3.5 program. The percentage of α-helical content was estimated directly from the molar residue ellipticity at 222 nm as described by Chen et al. (26). Percentages of secondary structures were also estimated using the neural network-based K2 algorithm (27). To evaluate the spectral changes induced by peptide-peptide interactions in mixtures, the non-interacting spectra were calculated from the individual spectra using the equation,

\[ \theta_{\text{mix}} = \Sigma c_i \cdot n_i \cdot [\theta] \Sigma c_i \cdot n_i \]  
(Eq. 1)

where \( c \) denotes the molar peptide concentrations, \( n \) represents the peptide lengths in number of residues, and \([\theta]\) values are observed mean residue ellipticities.

**RESULTS AND DISCUSSION**

**Peptide Design**

**Basic Criteria for the Design of the Minimal Predicted Coiled-coil-forming Peptides**—The sequences of the peptides synthesized from selected regions from human SNAP-25 (peptide SN), synaptobrevin-2 (peptide SB), and syntaxin-1A (peptide ST) are shown in Fig. 1A. Sequence selection was based on six criteria: 1) information about the minimal domains of SNAP-25, synaptobrevin, and syntaxin involved in protein-protein interactions in the core complex (1–5, 28); 2) botulinum neurotoxins (BoNTs) cleavage sites and their effects on neurotransmitter release (29–37); 3) prediction of secondary structure formation of coiled-coil structures; and 6) a minimum length for a stable parallel coil peptide of ~28 residues, or 4 heptad repeats (48, 49).

**Design of the SN Peptide**—The region of SNAP-25 interacting with synaptobrevin-2 has been localized between residue 41 and the C-terminal residue (2, 4). The segment from residue 181 to the C terminus is necessary for the SNAP-25-synaptobrevin interaction (3). Peptides corresponding to the 20 and 26 C-terminal residues, the latter analogous to the SNAP-25 segment released after cleavage by BoNT E, inhibit neurotransmitter release with IC₅₀ values of 10 and 0.25 μM respectively by preventing the docking of synaptic vesicles (40, 43). The C-terminal region of SNAP-25 delimited by residues 169 and 206 displays a high propensity (99%) to form coiled-coil structures. Two distinct domains are predicted: one from position 166 to 187 (62%), and the second from position 189 to the C terminus (55%). Accordingly, the 26-residue peptide corresponding to the C-terminal segment of SNAP-25, hereafter designated as SN, was selected based on the fact that it is nearly 4 heptads long, it exhibits high propensity to form coiled-coils, and it is an efficient inhibitor of neurotransmitter release (Fig. 1A, Peptide SN).

**Design of the ST Peptide**—Similar considerations were used in the design of a potentially coiled-coil-forming peptide from syntaxin-1A. The region between positions 194 and 261 is necessary for the interaction with synaptobrevin-2 and SNAP-25 (2, 3, 5). The segment necessary for interaction with SNAP-25 has been located between residues 199 and 267 (1) and further delimited to residues 199–220 (4). All of these studies define the SNAP-25 binding region on syntaxin to the N-terminal portion of the H₃ domain (residues 191–266) (2, 4), and a putative minimal SNAP-25 binding domain (residues 189–220) has been identified (11, 44). Coiled-coil predictions using the program of human syntaxin revealed a region between positions 199 and 214 with high probability (78%) of coiled-coil formation.

Syntaxin-1A mutants containing point mutations (4, 11, 44) at the a and d repeats of a predicted coiled-coil show reduced SNAP-25 binding, supporting the involvement of this region in the interaction with SNAP-25 (Fig. 1B). Peptides corresponding to the predicted coiled-coil-forming region of syntaxin-1A have also been shown to inhibit neurotransmitter release (11, 42, 44). Given these considerations, the selected 4-heptad synthetic peptide corresponding to human syntaxin spanned from residue 191 to 218 (Fig. 1A, Peptide ST).

**Design of the SB Peptide**—The region of synaptobrevin-2 between positions 27 and 96 interacts with both SNAP-25 and syntaxin-1A in the core complex (3, 28). Synaptobrevin-2 contains a conserved domain between residues 57 and 88, with high propensity (95%) to form coiled-coils, and two distinct subdomains (28–42 and 52–72). Deletion of the region spanning from residue 41 to 50 abolishes endocytosis (45), and mutants lacking the segments 41–50 or 51–60 do not bind to SNAP-25 and syntaxin-1A. The mutants with deletion of segment 31–38 show weak binding to t-SNAREs, whereas the deletion of segments 61–70 or 71–80 results in poor binding to syntaxin while maintaining the interactions with SNAP-25 (47). Moreover, a single mutation (M46A) inhibits endocytosis by 80% and reduces binding to syntaxin-1A and SNAP-25 (45, 46). Taken together, this information suggests that the region delimited by positions 40 and 60 is involved in the ternary interactions that result in the assembly of the docking complex; therefore, the peptide synthesized encompassed 4 heptads from position 40 (Fig. 1A, Peptide ST).

**Design of the SN₃₉₀ Control Peptide**—A control peptide corresponding to the scrambled sequence of the selected SNAP-25 peptide was also synthesized. Randomized sequences were generated, their secondary structures were predicted using the SOPMA method, and the sequences with an α-helical content similar to the original sequence were run against the Prosite data base. A peptide with the same functional sites but without the heptad periodicity was synthesized (sequence shown under “Experimental Procedures”), and it was shown to be pharmacologically inactive. The SN₃₉₀ peptide, at variance to SN, did not affect Ca²⁺-dependent release in chromaffin cells.

Secondary structure predictions using the SOPMA method (21, 22) indicate that all three peptides may form stable α-helices in the context of a whole protein structure. Predicted helicities for SN, SB, and ST peptides were 62, 96, and 86%, respectively, when considered integrated in the protein, in contrast to 42, 57, and 67% as isolated peptides. The behavior of the isolated peptides in an aqueous environment was predicted by using the AGADIR algorithm (23). This program uses
**Mineral SNARE Peptide Coiled-coil Complex Assembly**

**Fig. 1. Model of a minimal trimeric coiled-coil using selected sequences from SNAP-25, synaptobrevin-2, and syntaxin-A.** A, schematic representation of the relative location of SNAP-25, synaptobrevin-2, and syntaxin-A in the synaptic terminal (black boxes). The white boxes represent the segments corresponding to the synthetic peptides SN, SB, and ST, respectively. The relative size of the proteins and synthetic peptides are not to scale. The amino acid sequences of the SN, SB, and ST peptides are given in the box. B, triple helical coiled-coil model of the segments of SNAP-25, synaptobrevin-2, and syntaxin-A represented by the synthetic peptides SN, SB, and ST, respectively. The sequences have a heptad pattern of residues (designated as a–g), where a and d are usually hydrophobic and e and g are frequently charged. Solid arrows denote hydrophobic interactions in the core of the complex, whereas broken arrows refer to potential ionic interactions, as described in detail in C. Mutations known to disrupt the interactions between proteins (*) are located in the hydrophobic core. Mutations known not to interfere in the assembly of the complex (§) are all located outside the hydrophobic core. Most of the charged residues are located in the outer shell of the complex (positions b, c, and f). Hollow arrows in A and B show the relative location of Botulinum neurotoxin cleavage sites, which are all accessible on the surface of the complex. C, potential interactions between charged residues of peptides SN, SB, and ST. Charged residues in positions e and g or at i + 3- or i + 4-positions can form intramolecular (squares) or intermolecular (circles) salt bridges that contribute to the overall stability of the trimeric complex.

---

Statistical mechanics to consider short range interactions between residues at different pH and temperature. Given the size of our peptides, they are predicted to be unstructured (4% for SN, 3% for SB, and 2% for ST peptide, respectively) under aqueous conditions. Thus, considering both sets of data, we infer that these peptides can adopt α-helical structures only in the context of the intact protein and, therefore, that the presence of helicity-inducing conditions may be necessary to mimic the secondary structure of the peptides in the cytosol. TFE, a hydrophilic and hydrogen-bonding solvent, has been widely used to stabilize marginally stable α-helical structures in potentially α-helical peptides (48, 50–53). TFE is not limited to promoting helix formation, since it has also been shown to stabilize β-turns and even β-strands (54, 55). Notwithstanding, TFE-induced α-helical conformation in fragments of proteins known to be β-sheet in the native context has been documented (56–63). Therefore, caution must be exercised in inferring structure from CD data of peptides in the presence of TFE, particularly with regard to the extent that it represents the native structure in the context of the intact protein from which the peptide sequences were selected.

**Trimeric Coiled-coil Model**

**Theoretical Considerations—**A model of one of the trimeric conformers of the selected peptides forming a coiled-coil structure is shown in Fig. 1B. Given the helical wheel representation, residues at the α- and d-positions stabilize the structure by hydrophobic interchain interactions. According to this model, the synaptobrevin-2 residue Met45, which upon mutation inhibits endocytosis, would be located in the hydrophobic core, where such a change would be predictably disruptive. The mutations in syntaxin that reduce its binding to SNAP-25 would also be located in positions a and d (residues denoted with an asterisk in Fig. 1B). Interestingly, the cleavage sites for.
five of the seven BoNT serotypes (BoNT A, B, D, E, and F) are found in the model peptides, and all are in surface locations potentially accessible to the BoNT proteases.

Interchain interactions of e- and g-positions mediated by charged residues also contribute to the stability of a coiled-coil (64). There are 12 charged residues in positions e and g; accordingly, inter- or intrahelical ionic interactions could synergistically contribute to the stability of the coil (Fig. 1C). Polar residues implanted in the hydrophobic core are potentially disruptive, although strategic placement can facilitate correct oligomerization arrangements (65). In the model, the core contains only two charged residues: Arg198 (peptide ST) could establish an intramolecular salt bridge with either Glu194 or Glu203 (Fig. 1B), and Arg267 (peptide SB) would be at a suitable distance to interact with the glutamate residues in position g of the ST peptide and form an intermolecular linkage (Fig. 1B).

In the outer layer (positions b, c, and f), 14 negatively and 3 positively charged residues would be exposed. This arrangement of negative charges mostly in the surface is consistent with observations by Regazzi et al. (47) that substitutions of negatively charged residues of synaptobrevin-2 do not alter function (66) (Fig. 1B).

Circular Dichroism Results—In aqueous media, all peptides (alone or in mixtures) were unstructured, and neither increasing peptide concentration nor changing pH, ionic strength, or divalent cation concentration increased the α-helical content. Typical single-stranded polypeptides generally do not form stable α-helices in aqueous solution and require the additional stabilization of less polar solvents (67, 68); therefore, we resorted to the use of the helix-promoting solvent TFE.

In the presence of increasing concentrations of TFE (Fig. 2, A–C), there was a significant increase in the α-helical content. The minimal concentration at which the peptides underwent a transition from mostly unstructured to partially structured was approximately 30%. At that concentration, the α-helical contents of the SNAP-25, synaptobrevin, and syntaxin peptides were 31, 44, and 32%, respectively. At the maximum concentration of TFE used (75%), the α-helical contents of the peptides were 59, 87, and 85%, respectively. TFE increases the α-helical content, while it disrupts tertiary and quaternary structures stabilized by hydrophobic interactions (69); therefore, it was imperative to use a concentration of TFE low enough to marginally stabilize the secondary structure of monomeric peptides while still allowing the expression of tertiary interactions. Notwithstanding the disrupting effects of TFE on the tertiary structure of oligomeric complexes, peptide-peptide interactions producing stable oligomers have been documented at concentrations of TFE as high as 50% (70). Interestingly, the TFE concentration used in our experiments (30%) has been reported to yield for numerous peptides secondary structures that compare favorably with those of the native systems (71–73).

Equimolar ternary mixtures SN/SB/ST in aqueous solution showed no interaction between the non-α-helical peptides (not shown). In the presence of TFE, the spectrum of the SN/SB/ST mixture (Fig. 3E, solid line) was significantly different from a noninteracting spectrum (Fig. 3E, dashed line) calculated from the three individual CD spectra (Fig. 3A). The expected α-helicity from the calculated spectrum was 35%, whereas the α-helicity from the experimental spectrum was 46%; i.e., a 31% net increase over the predicted value. The ratios between the intensities of the bands at 222 and 208 nm were 0.76 for the calculated and 0.80 for the experimental spectrum, respectively. This larger Ω222/Ω208 ratio is consistent with an increase in coiling.

The 31% net increase in helicity observed in the experimental ternary mixture spectrum with respect to the prediction could arise from the occurrence of distinct binary complexes in the mixture. Equimolar binary mixtures SN/SB, SN/ST, and SB/ST in aqueous solution showed no interaction between the peptides (not shown). In the presence of 30% TFE, the SN and SB peptides did not interact in binary mixtures (Fig. 3B). The helicity of the experimental spectrum was identical to that predicted by the noninteracting calculated spectrum (36%). In contrast, spectra from binary mixtures SB/ST (Fig. 3C) and SN/ST (Fig. 3D) indicated that both pairs of peptides interact under these experimental conditions. In each case, the α-helical content calculated from the experimental spectra was ∼15% greater than expected for a noninteracting mixture. Predicted helicities were 34 and 33%, respectively, for the SB/ST and SN/ST mixtures, whereas the experimental values were 39 and 38%, i.e., 15% higher than expected for noninteracting mixtures.

Increasingly higher peptide concentration in equimolar mixtures of the SN, SB, and ST peptides in the presence of 30% TFE (Fig. 4A) also results in an increase in helicity and therefore a stabilization of the complex. Increasing the individual peptide concentrations from 10 to 30 μM results in an increase in helicity from 46 to 54%. It is noticeable that the three spectra define a unique isodichroic point, consistent with the occur-
rence of a single specific complex. When equimolar ternary mixtures are exposed to higher concentrations of NaCl in the presence of 30% TFE (Fig. 4B), there is a remarkable increase in the helicity (from 55% at 0.1 M NaCl to 72% at 0.5 M and 81% at 1 M). This feature is consistent with hydrophobic peptide-peptide interactions as suggested by the model (Fig. 1B). The increased α-helical content with increasing ionic strength is in accordance with data for coiled-coil peptides and can be explained by the increased strength of the hydrophobic interactions as the polarity of the medium is increased (69).

Whereas the spectra of all three peptides were independent of the peptide concentration in aqueous solution (Fig. 5, A–C),
in the presence of 30% TFE the increase in peptide concentration resulted in a concomitant increase in the α-helical content, as indicated by the increase in negative ellipticity at 222 nm (Fig. 5, D–F). Helicity increased from 30 to 53% for SN, from 41 to 49% for SB, and from 35 to 51% for ST. This is consistent with the fact that peptides with α-helical structures that are dependent on dimerization or oligomerization show an augmentation of α-helical content as the peptide concentration is increased (74). This presumably arises because the equilibrium between monomeric peptide (in the form of random coil) and coiled-coil dimer is shifted toward the formation of the coiled-coil dimer, which increases the α-helical content of the peptide (65).

Higher peptide concentrations induced a moderate increase in the ratio between the peaks at 222 and 208 nm (θ_222/θ_208): from 0.68 to 0.78 for SN; from 0.75 to 0.77 for SB; and from 0.88 to 0.94 for ST. The ratio between the intensities of the bands at 222 and 208 nm may be regarded as a measure for the extent of coiling of α-helices around each other. The 222-nm CD band is mainly responsive to the α-helical content, whereas the band at 208 nm is sensitive to whether the α-helix is monomeric or is involved in tertiary contacts with other α-helices (75–77). Therefore, this is an additional criterion for the formation of stable coiled-coil structures. Each set of curves defined a unique isodichroic point, consistent with a single monomer-dimer equilibrium, which indicated that the oligomerization observed was sequence-specific and presumably stabilized by a concerted set of ion pairs in a defined spatial arrangement.

The ratio of the 222- to the 208-nm peak is an operational index to detect the presence of pure coiled-coils. For peptides stabilized at low TFE concentrations, an equilibrium between monomeric and multimeric states is anticipated, resulting in a profile intermediate between a pure coiled-coil and a predominantly monomeric situation. Given that the peptides exhibit a relatively low α-helical content and that there is a substantial fraction of peptide in monomeric form, the formation of homomeric or heteromeric arrays arising from interhelical interactions would result in an α-helical content of the mixtures larger than that expected from a spectrum calculated from the individual spectra (Fig. 3A).

Conclusion

Our study identifies a minimal entity that opens a new perspective for the study of the molecular interactions between SNAP-25, synaptobrevin, and syntaxin. Three distinct synthetic peptides patterned after the sequences of the putative coiled-coil-forming domains of the main components of the docking and fusion complex self-assemble into a complex that exhibits spectral characteristics consistent with a coiled-coil structure. A synthetic coiled-coil ternary complex provides a basis for further developments: 1) the ternary complex appears suitable for both crystallization and NMR spectroscopy that, in due turn, may yield a high resolution structure of the fusion core complex; 2) it represents a conceptual framework to assist in the design and test of new peptide inhibitors of neurotransmitter release; 3) it provides leads for the design of small molecule peptidomimetic drugs; and 4) it may be valuable to generate specific antibodies to block neurotransmitter release.
Indeed, the synthetic peptides that, as shown here, participate in the assembly of the ternary complex in fact mimic the action of Clostridial neurotoxins (40, 43). Therefore, our findings may lead to the development of peptide-based agents that may be used as potential therapy in spastic neuromuscular disorders, substituting or complementing the current treatment with BoNTs.

Acknowledgments—We thank Professor Murray Goodman and Dr. Joseph Taulia for the use of the Cary 61 CD spectrometer and Dr. Susan S. Taylor for the use of the AVIV 202 CD spectrometer.

Note Added in Proof—After submission of this manuscript, two papers reported the structure of the SNARE complex as a parallel four-helix bundle determined by x-ray crystallography (Sutton R. B., Fasshauer, D., Jahn, R., and Brunger, A. T. (1998) Nature 395, 347–353) and by electron paramagnetic resonance spectroscopy (Poirier, M. A., Xiao, W., Mocasco, J. C., Chan, Chin, Y.-K., and Bennett, M. K. (1998) Nat. Struct. Biol. 5, 765–769). The results of our study are consistent with the high resolution structure of the SNARE complex.
54. Blanco, J. F., and Serrano, L. (1995) Eur. J. Biochem. 230, 634–649
55. Narayanan, U., Keiderling, T. A., Bonora, G. M., and Toniolo, C. (1986) J. Am. Chem. Soc. 108, 2431–2437
56. Dong, A., Matsuzura, J., Manning, M. C., and Carpenter, J. F. (1998) Arch. Biochem. Biophys. 355, 275–281
57. Arunkumar, A. I., Kumar, T. K. S., and Yu, C. (1997) Biochim. Biophys. Acta 1338, 69–76
58. Jayaraman, G., Kumar, T. K. S., Arunkumar, A. I., and Yu, C. (1996) Biochim. Biophys. Res. Commun. 222, 33–37
59. Luidens, M. K., Figge, J., Breese, K., and Vajda, S. (1996) Biopolymers 39, 367–376
60. Schonbrunner, N., Wey, J., Engels, J., Georg, H., and Kiefhaber, T. (1996) J. Mol. Biol. 260, 432–445
61. Hamada, D., and Goto, Y. (1997) J. Mol. Biol. 269, 479–487
62. Najbar, L. V., Craik, D. J., Wade, J. D., Salvatore, D., and McLeish, M. J. (1997) Biochemistry 36, 11255–11263
63. MacPhee, C. E., Perugini, M. A., Sawyer, W. H., and Howlett, G. J. (1997) FEBS Lett. 416, 265–268
64. Zhou, N. E., Kay, C. M., and Hodges, R. S. (1994) Protein Eng. 7, 1365–1372
65. Adamson, J. G., Zhou, N. E., and Hodges, R. S. (1993) Curr. Opin. Biotechnol. 4, 428–437
66. Pellizzari, R., Rosetto, O., Lozzi, L., Giovedi, S., Johnson, E., Shone, C. C., and Montecucco, C. (1996) J. Biol. Chem. 271, 20353–20358
67. Bieruzynski, A., Kim, P. S., and Baldwin, R. L. (1992) Proc. Natl. Acad. Sci. U. S. A. 79, 2470–2474
68. Brown, J. E., and Klee, W. A. (1971) Biochemistry 10, 470–476
69. Lau, S. Y. M., Taneja, A. K., and Hodges, R. S. (1984) J. Biol. Chem. 259, 13253–13261
70. Vinogradov, A. A., Mari, F., Humphreys, R. E., and Wright G. E. (1996) Int. J. Pept. Protein Res. 47, 467–476
71. Munoz, V., Serrano, L., Jimenez, M. A., and Rico, M. (1995) J. Mol. Biol. 247, 648–669
72. Blanco, F. J., Ortiz, A. R., and Serrano, L. (1997) Folding Design 2, 123–133
73. Ramirez-Alvarado, M., Serrano, L., and Blanco, F. J. (1997) Protein Sci. 6, 162–174
74. Zhou, N. E., Kay, C. M., and Hodges, R. S. (1992) J. Biol. Chem. 267, 2664–2670
75. Zhou, N. E., Zhu, B. Y., Kay, C. M., and Hodges, R. S. (1992) Biopolymers 32, 419–426
76. Cooper, T. M., and Woody, R. W. (1990) Biopolymers 30, 657–676
77. Greenfield, N. J., and Hitchcock-DeGregori, S. E. (1995) Biochemistry 34, 16797–16805
Assembly of a Ternary Complex by the Predicted Minimal Coiled-coil-forming Domains of Syntaxin, SNAP-25, and Synaptobrevin: A CIRCULAR DICHROISM STUDY
Jaume M. Cànaves and Mauricio Montal

J. Biol. Chem. 1998, 273:34214-34221.
doi: 10.1074/jbc.273.51.34214

Access the most updated version of this article at http://www.jbc.org/content/273/51/34214

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 76 references, 23 of which can be accessed free at http://www.jbc.org/content/273/51/34214.full.html#ref-list-1