Self-association of the H3 Region of Syntaxin 1A

IMPLICATIONS FOR INTERMEDIATES IN SNARE COMPLEX ASSEMBLY*

Received for publication, October 23, 2000, and in revised form, December 7, 2000
Published, JBC Papers in Press, December 15, 2000, DOI 10.1074/jbc.M009636200

Kira M. S. Misura‡§, Richard H. Scheller§, and William I. Weis§**

From the Departments of ‡Structural Biology and of §Molecular and Cellular Physiology and ¶The Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, California 94305

Intracellular membrane fusion requires SNARE proteins found on the vesicle and target membranes. SNAREs associate by formation of a parallel four-helix bundle, and it has been suggested that formation of this complex promotes membrane fusion. The membrane proximal region of the cytoplasmic domain of the SNARE syntaxin 1A, designated H3, contributes one of the four helices to the SNARE complex. In the crystal structure of syntaxin 1A H3, four molecules associate as a homotetramer composed of two pairs of parallel helices that are anti-parallel to each other. The H3 oligomer observed in the crystals is also found in solution, as assessed by gel filtration and chemical cross-linking studies. The crystal structure reveals that the highly conserved Phe-216 packs against conserved Gln-226 residues present on the anti-parallel pair of helices. Modeling indicates that Phe-216 prevents parallel tetramer formation. Mutation of Phe-216 to Ala destabilizes the protein. These results indicate that Phe-216 has a role in preventing formation of stable parallel tetramers, thus favoring the interaction of the H3 region of syntaxin 1A with other proteins involved in membrane fusion.

Eukaryotic cells transport cargo between different intracellular compartments, and release selective cargo into the extracellular space. This task requires that transport vesicles bud from the membranes of organelles and fuse specifically with target membranes. An extensively studied example of this process is synaptic vesicle exocytosis, in which neurotransmitter-filled vesicles fuse with the plasma membrane of a presynaptic neuron to release neurotransmitter into the synaptic cleft

* This work was supported by Grants MH58570 (to W. I. W.) and MH38710 (to R. H. S.) from the National Institute of Mental Health. Part of this work is based upon research conducted at the Stanford Synchrotron Radiation Laboratory, which is funded by the Department of Energy, Office of Basic Energy Sciences, and Biological and Environmental Research; the National Center for Research Resources, Biomedical Technology Program, NIH, and the National Institute of General Medical Sciences. The Advanced Light Source is supported by the Director, Office of Science, Office of Basic Energy Sciences, Materials Science Division, of the U. S. Department of Energy under contract DE-AC03-76SF00098 at Lawrence Berkeley National Laboratory. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
** To whom correspondence should be addressed: Dept. of Structural Biology, Stanford University School of Medicine, 299 Campus Dr. West, Stanford, CA 94305. Tel.: 650-725-4623; Fax: 650-723-8464; E-mail: bill.weis@stanford.edu.

1 The abbreviations used are: NSF, N-ethylmaleimide-sensitive factor; SNAP, SNAP receptor; v-SNARE, vesicle SNARE; t-SNARE, target SNARE; β-Me, β-mercaptoethanol; MAD, multilwavelength anomalous dispersion; PAGE, polyacrylamide gel electrophoresis; CD, circular dichroism; GST, glutathione S-transferase; r.m.s.d., root mean square deviation; SNAP, soluble NSF attachment protein.

This paper is available on line at http://www.jbc.org
Self-association of the H3 Region of Syntaxin 1a

(13–15), and it is possible that other conformations of the protein exist but have not yet been observed.

To better understand the role of syntaxin 1a in membrane fusion, we have determined the x-ray crystal structure of the H3 region of syntaxin 1a. The structure reveals that H3 forms a homotetramer, and suggests that a conserved phenylalanine residue may determine the orientation of the four helices with respect to each other. Mutation of this phenylalanine to alanine produces an oligomeric assembly, as assessed through gel filtration chromatography, chemical cross-linking, and circular dichroism spectroscopy. The structure also suggests mechanisms for SNARE complex assembly and may provide a model for SNARE precursor complexes.

EXPERIMENTAL PROCEDURES

Protein Expression and Purification—The H3 region of syntaxin 1a (residues 191–267) was cloned into the vector pGEX-KG (Amersham Pharmacia Biotech) as described previously (16), and the vector was transformed into the AB1199 strain of Escherichia coli. The cells were grown in LB broth at 37°C for 1 h in the presence of 1 mM ampicillin and then induced with 1 mM isopropyl-1-thio-β-D-galactopyranoside. Cells were harvested by centrifugation and lysed by French press in the presence of phenylmethylsulfonyl fluoride. The lysate was mixed with glutathione-agarose beads (2 ml of 50% beads for each liter of cells) and incubated at 1 h at 4°C. The beads were washed with buffer containing 250 mM NaCl, 2 mM CaCl2, 2 mM β-mercaptoethanol (β-Me), 50 mM Tris, pH 8.0, and the fusion protein was eluted with Thrombin (Sigma Chemical Co.). The supernatant was applied to a Mono Q HR10/30 (Amersham Pharmacia Biotech) column at 4 °C (10 mM or 1 M NaCl, 15 mM Tris, pH 8.2) with mercury compounds, and then apply the proteins to a gel filtration column (Sephadex G15 resin) to remove unbound mercury compounds. Covalent attachment of the mercury atom was assayed by mass spectrometry (data not shown). The only successful heavy atom derivative was obtained by reacting 2,6-bischloromercury-4-nitrophenol with the Y257C mutant protein.

Phasing—A difference Patterson synthesis calculated between the 2,6-bischloromercury-4-nitrophenol derivative of theY257C mutant protein and native H3 data sets revealed a single mercury site. Refinement of this site using the program SOLVE2 gave SIRAS phases to 3.3 Å Bragg spacings. The resulting electron density maps revealed the helical backbone but were not of sufficient quality to assign the sequence.

Gel Filtration—Assembly and may provide a model for SNARE precursor complexes.
and mutant F216A and F216L syntaxin 1a H3 proteins, fractions containing ~95% pure protein (as determined by SDS-PAGE, data not shown) were applied to a Superdex 200 HR10/30 or Superdex 75 HR 10/30 column (Amersham Pharmacia Biotech) in a buffer containing 150 mM NaCl, 2 mM β-Me, and 20 mM Tris, pH 8.0, or 20 mM sodium acetate, pH 5.5. The peak elution volume was compared with protein standards of known molecular weight (Sigma).

Circular Dichroism Measurements—Circular dichroism (CD) spectroscopy and melting temperature experiments were performed on fast protein liquid chromatography-purified dialyzed against a buffer containing 150 mM NaCl and 20 mM Tris, pH 8.0, or 150 mM NaCl and 20 mM sodium acetate, pH 5.5. The protein concentration for all samples was 126 μM (1.1 mg/ml). Data were collected using an AVIV 62 A DS CD spectrometer. The higher molar ellipticity values for some of the samples result from differences in helical content and not differences in protein concentration. To determine thermal stabilities (Tm values), a-helical signals were monitored by the ellipticity at 222 nm, the sample was heated from 4 °C to 90 °C. All apparent Tm values were taken as the maximum of the first derivative of the melting curve. Wavelength spectra and temperature melt data were acquired using a 1-mm path length quartz cuvette. For thermal unfolding experiments, a 1° step size and 30-s equilibration time were used. The F216L mutant 1-mm path length quartz cuvette. For thermal unfolding experiments, a 1° step size and 30-s equilibration time were used. The F216L mutant consists of anti-parallel pairs of parallel helices. Another difference is that the H3 region in the core complex structure is structured further toward the C terminus. The last ordered syntaxin 1a residue seen in the crystal structure of the core SNARE complex is 259 or 261 (seen in different copies in the asymmetric unit), whereas in the H3 tetramer the last visible residue is either 253 or 256. Both crystal structures show more order at the C terminus than NMR solution studies of full-length syntaxin like proteins (11, 24), in which the H3 region is disordered from residues 226–267. This discrepancy may arise from the fact that in full-length syntaxin, the interaction with Habc stabilizes the first half of the H3 peptide, leaving the rest disordered. Alternatively, the high local concentration of the H3 peptide in the crystal, the nature of the crystallization conditions, or preferential incorporation of the more helical population into the crystal lattice may make the present structure more ordered than the ensemble present in solution. Electron density maps calculated from data measured from crystals diffracting to a lower resolution reveal that the C terminus of each copy of H3 is less well ordered from residues 247 to 256 than in the structure presented here, again suggesting that these residues are ordered only under limited conditions.

Copy A from the H3 tetramer superimposes on VAMP from the core SNARE complex with an r.m.s.d. of 2.3 Å, and copy B from the H3 tetramer superimposes on H3 from the core SNARE complex with an r.m.s.d. of 0.4 Å on Cα positions (Fig. 2A). Either set of two parallel helices in the H3 tetramer does not superimpose with any two helices in the core SNARE complex structure. This is likely due to differences in packing angles between helices, where the values are between 15° and 20° for the H3 tetramer and between 5° and 20° for the core SNARE complex structure. The H3 parallel dimers also do not have as much of a left-handed superhelical twist as the dimers in the core SNARE complex structure (Figs. 2C and 4).

The core of the H3 four-helix bundle is composed primarily of hydrophobic residues, and these same residues face the interior of the core SNARE complex (7). However, each protomer contributes one polar residue (Gln-226) to the hydrophobic core of
Self-association of the H3 Region of Syntaxin 1A

### Table II

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Anomalous diffraction ratios*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\lambda_1$</td>
</tr>
<tr>
<td>$\lambda_1$</td>
<td>0.051</td>
</tr>
<tr>
<td>$\lambda_2$</td>
<td>0.044</td>
</tr>
<tr>
<td>$\lambda_3$</td>
<td>0.034</td>
</tr>
<tr>
<td>$\lambda_4$</td>
<td>0.031</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Resolution</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_1$</td>
<td>29.05–9.52</td>
</tr>
<tr>
<td>$\lambda_2$</td>
<td>9.52–6.08</td>
</tr>
<tr>
<td>$\lambda_3$</td>
<td>6.08–4.77</td>
</tr>
<tr>
<td>$\lambda_4$</td>
<td>4.77–4.05</td>
</tr>
<tr>
<td>$\lambda_5$</td>
<td>4.05–3.59</td>
</tr>
<tr>
<td>$\lambda_6$</td>
<td>3.59–3.25</td>
</tr>
<tr>
<td>$\lambda_7$</td>
<td>3.25–2.89</td>
</tr>
<tr>
<td>$\lambda_8$</td>
<td>2.89–2.70</td>
</tr>
<tr>
<td>Overall</td>
<td>0.006</td>
</tr>
</tbody>
</table>

* Anomalous diffraction ratios = $(\Delta F)/\langle F \rangle$, where $\Delta F$ is the r.m.s. Bijvoet difference at a single wavelength or the r.m.s. dispersive difference between two wavelengths.

### Table III

<table>
<thead>
<tr>
<th>R values and temperature factors</th>
<th>Model geometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. reflections</td>
<td>Working set*</td>
</tr>
<tr>
<td>Test set*</td>
<td>1669</td>
</tr>
<tr>
<td>$R_{\text{free}}^b$</td>
<td>0.238</td>
</tr>
<tr>
<td>$R_{\text{free}}^b$</td>
<td>0.270</td>
</tr>
<tr>
<td>Average B (Å²)</td>
<td>% in additional allowed regions</td>
</tr>
<tr>
<td>Protein</td>
<td>32.9</td>
</tr>
<tr>
<td>Solvent</td>
<td>47.2</td>
</tr>
<tr>
<td>Main chain bond-related B</td>
<td>1.37</td>
</tr>
<tr>
<td>r.m.s.d.</td>
<td>Copy A to Copy B</td>
</tr>
<tr>
<td>Main chain angle-related B</td>
<td>2.52</td>
</tr>
<tr>
<td>r.m.s.d.</td>
<td>Copy A to Copy C</td>
</tr>
<tr>
<td>Side chain bond-related B</td>
<td>2.30</td>
</tr>
<tr>
<td>r.m.s.d.</td>
<td>Copy A to Copy D</td>
</tr>
<tr>
<td>Side chain angle-related B</td>
<td>3.70</td>
</tr>
<tr>
<td>r.m.s.d.</td>
<td></td>
</tr>
</tbody>
</table>

* The Test set comprises a randomly selected subset of the data (8%) that was not included in the refinement of the model. The Working Set contains the remaining reflections from the data set.

Fig. 1. Structure of the anti-parallel H3 four-helix bundle. N- and C termini are indicated, along with the numbers of ordered residues for each copy. The Phe-216 and Gln-226 side chains face the center of the bundle and are drawn as balls and sticks. This figure, Fig. 3, and Fig. 7 were made with MolScript (36) and rendered with Raster3d (37).

Several noteworthy features are present on the surface of the H3 tetramer. The H3 peptide is slightly acidic (calculated pI = 6.1), and displays a large area of electrostatically negative surface potential (Fig. 4). The H3 tetramer contains only two small regions of positive charge in the central part of the structure, attributable to Arg-246 from copy A and Arg-232 from copy B. Interestingly, both the H3 tetramer and the core SNARE complex have predominantly electrostatically negative surface potentials, and both form long narrow structures (Fig. 4). In addition, both bind α-SNAP and NSF to form a 20 S particle (Ref. 15 and references therein). α-SNAP and NSF are general factors that function in multiple transport pathways and are thus capable of associating with many different SNARE proteins. α-SNAP and NSF may recognize SNARE protein assemblies based on surface complementarity and electrostatic surface potential rather than specific sequences, thus accounting for the versatility of these proteins.

Inspection of the H3 tetramer surface also reveals a potential docking site for a small molecule. A tartrate ion is bound in a pocket formed between Gly-227 of copy A and His-212 of copy C. Interestingly, this is the site of the helix interruption in copy C. This structure cannot be attributed to lattice interactions, because the site is well away from any neighbors in the crystal lattice. Also, the mercury compound (2,6-bis-chloromercuri-4-
nitrophenol), used as the heavy atom derivative, binds in this site. The mercurial is covalently bound to a disordered part of the polypeptide chain of a neighboring molecule in the crystal lattice but was found ordered in the Gly-227/His-212 surface cavity. The cavity seems to be somewhat selective, because several other mercurial compounds that could be covalently attached to Cys-257 solution crystallized but did not show any ordered mercury atoms.

**H3 Forms Oligomers in Solution**—To assess the significance of the crystallographically observed H3 tetramer, the oligomeric state of H3 in solution was investigated by gel filtration and chemical cross-linking. The predicted molecular mass for an H3 tetramer is ~40 kDa; however, the H3 peptide runs at an apparent molecular mass of 60 kDa on a gel filtration column (Fig. 5A). The crystal structure reveals that the H3 tetramer is a long and narrow assembly, so the high molecular weight estimate obtained from the sizing column may be due in part to the elongated shape of the oligomer. Experiments using three different homobifunctional lysine-reactive cross-linking agents also support the existence of H3 oligomers in solution (data for BS3 in Fig. 6A; others not shown). At low concentrations of cross-linker, dimeric species predominate. As the concentration of Sulfo-EGS (16.1 Å spacer, data not shown) or BS3 (11.4 Å spacer) is raised, the molecular mass of the predominant species as estimated by SDS-PAGE is ~28 kDa, which is consistent with a trimer. Only small amounts of tetramer can be detected. The cross-linker with the shortest spacer arm, Sulfo-DST (6.4 Å), produced only dimeric species even at high ratios of cross-linker to protein (data not shown).

The crystal structure of the tetramer is consistent with the observed cross-linking patterns. The three ordered lysine residues in one copy of the H3 polypeptide are at position 204 near the N-terminal end and positions 252 and 253 near the C-terminal end (Fig. 7). The dimers are likely a result of cross-linking of pairs of lysines from parallel helices. For example, the terminal nitrogen atoms of lysines 204 from copies C and D are 13.8 Å apart in the crystal structure, and the terminal nitrogen atoms of lysines 252 and 253 from adjacent parallel helices A and B are 10.1 Å apart in the structure. Modeling indicates that small changes in rotamers would place the 252/253 pair within cross-linking range of even the shortest cross-linker used in these experiments, Sulfo-DST. In contrast, the trimeric species formed with the longer cross-linking agents are likely due to reactions between Lys-204, Lys-252, and Lys-253 (Fig. 7). At each end of the H3 tetramer, Lys-252 and Lys-253 from parallel helices are within cross-linking distance of each other, but only one of the Lys-204 side chains from the anti-parallel helices is in close enough proximity to form cross-links to either of the Lys-252 or Lys-253 side chains.

The stability of the H3 oligomer was assessed by using circular dichroism (CD) spectroscopy to measure melting temper-
Self-association of the H3 Region of Syntaxin 1A

The F216L protein was very unstable as judged by CD temperature melting profiles at pH 8.0, where the peptide seems to have little α-helical secondary structure and the \( T_m = 30 \) °C (Fig. 8D). This is consistent with observations that the F216A...
protein had a tendency to degrade during purification. Surprisingly, acidic conditions had a more dramatic effect on the stability of the F216A protein in comparison to the wild type or F216L mutant proteins; where the $T_m$ was 48 °C (Fig. 8D).

Given this result, the gel filtration profiles for the three proteins were examined at pH 5.5 (Fig. 5B). The elution profiles of the wild type and F216L protein remained at the presumed tetramer position seen at pH 8.0, but the F126A protein clearly shifted from the $\sim 32$-kDa position to the $\sim 60$-kDa position (Fig. 5, B and C). This indicates that the F216A protein forms tetramers at pH 5.5, and this change in oligomeric state may explain the enhanced stability of the F216A mutant under acidic conditions.

Collectively, these results suggest that Phe-216 of the wild type sequence sterically prevents parallel tetramer formation and that the presence of Leu at position 216 allows parallel tetramer formation. The more facile cross-linking properties and the biphasic melting curve of the F216L mutant may reflect a mixed population of all-parallel and anti-parallel tetramers, with the more stable melting phase representing the all-parallel arrangement. Surprisingly, the presence of Ala at position 216 prevents formation of extensive secondary structure at pH 8.0. Presumably, the tetrameric state and enhanced stability of F216A at acid pH reflects compensatory stabilization by hydrogen bonds, as discussed above for the wild type protein.

**DISCUSSION**

The H3 region of syntaxin 1a can form binary complexes with its partner t-SNARE SNAP-25, and this interaction may be an obligatory intermediate in the formation of the SNARE complex (4, 12). *In vitro*, syntaxin 1a and SNAP-25 interact to form a binary complex that significantly enhances their affinity for VAMP over either component alone (2, 24, 34), and this interaction is the rate-determining step in forming the full SNARE heterotrimer (12). The stoichiometry of the binary complex is unclear, but EPR experiments indicate that the complex is a parallel four-helix bundle. CD data obtained using Sso1p and Sec9p (the yeast syntaxin 1a and SNAP-25 homologs) indicate that these proteins form a 1:1 complex, whereas data obtained from the neuronal syntaxin 1a and SNAP-25 proteins support the presence of a 1:1 or 2:1 complex (2). Data obtained from yeast and neuronal systems support the participation of SNAP-25 N-terminal regions in the binary complex (4, 12), but it is unclear if the C-terminal regions are also involved.

---

4 M. Poirier, personal communication.
5 L. Gonzalez, personal communication.
Assuming that the SNAP-25-syntaxin 1a binary complex forms a four-helix bundle structure, it is possible that the parallel syntaxin helices observed in the H3 tetramer structure represent two copies of syntaxin 1a in a complex with SNAP-25. The preformed syntaxin dimer may assist SNAP-25 in adopting 0-helical structure, thus providing a binding site for VAMP and allowing SNARE complex formation. If syntaxin 1a and SNAP-25 are present in a 2:1 molar ratio, then it is likely that both the N- and C-terminal regions of SNAP-25 bind to the syntaxin dimer. If the stoichiometry of the two proteins is 1:1 (overall composition of 2:2), however, then it is likely that only the N-terminal region of SNAP-25 forms the binary complex with syntaxin 1a H3. Molecular modeling indicates no obvious steric clashes to prevent formation of either type of parallel binary SNAP-25-H3 complexes, but further biophysical and structural characterization will be required to determine the nature and relevance of the syntaxin 1a-SNAP-25 complex.

Analytical ultracentrifugation analysis has revealed that the small amount of tetramer in solution (6). This oligomerization was attributed to the H3 region. It seems likely that the assembly observed in the H3 crystal structure represents the behavior of H3 alone as well as the assembly of full-length syntaxin 1a. The H3 construct used here extends to residue 267, but electron density is not seen for residues beyond 253. This observation is curious, because syntaxin 1–265 oligomers appear to be more stable than those of syntaxin 1–253 (6). The dimerization constant for syntaxin 1–265 is 5.8 μM, and the dimer-tetramer constant is 12 μM (6). The CD and cross-linking experiments reported by Lerman et al. (6) were performed at high concentrations (>75 μM), similar to those used in the experiments reported here. Therefore, we might have expected to see some dimers in the H3 gel filtration experiments. However, only one peak was observed, suggesting that the H3 region may oligomerize more easily in the absence of the Habc domain. This may be due to the competing interaction of H3 with the Habc region in the full-length cytoplasmic domain of syntaxin.

In v-SNARE-deleted yeast strains a low level of homotypic vacuole fusion is observed (35), suggesting that complexes of syntaxin tetramers might mediate homotypic fusion (6). The results presented here, however, suggest that syntaxin-like proteins are unable to form all-parallel homotetramers. Therefore, if some level of homotypic fusion is mediated by syntaxin-like proteins alone, this mechanism of fusion is different from syntaxin-VAMP-SNAP-25-mediated fusion involving parallel four-helix bundles. It is possible that anti-parallel syntaxin tetramers could form between molecules anchored in opposing membranes, and such an arrangement would bring the membranes close enough to allow fusion to occur. However, data presented here indicate that such structures would be less stable than all-parallel SNARE complex assemblies.

The highly conserved Phe-216 residue seems to prevent formation of stable parallel H3 tetramers. This may be important to ensure that productive complexes, such as the binary SNAP-25 complex or the core SNARE complex, can be formed readily without competition from a self-association reaction. It is likely that substitution of leucine for phenylalanine at position 216 allows parallel four-helix bundle assembly due to relief of steric constraints imposed by the large phenylalanine side chain. It is also possible that the Phe-216 → Ala mutant protein forms parallel four-helix bundles under acidic conditions. The thermal stability of this mutant protein (Tm = 48 °C) is lower than expected for a very stable parallel four-helix bundle such as the SNARE complex (Tm = 90 °C), but this may be due in

![FIG. 6. Representative cross-linking results as analyzed by SDS-PAGE.](Image) Only results obtained with the BS3 cross-linker (11.4-Å spacer arm) are shown, and estimated molecular weights are indicated. For each protein the lanes from left to right are: control, no cross-linker added, 0.66 μg/ml protein (0.56 mg/ml); 1, 15:1 ratio protein:cross-linker; 2, 5:1 ratio protein:cross-linker; 3, 1:2 ratio protein:cross-linker; 4, 1:5 ratio protein:cross-linker; 5, 1:10 ratio protein:cross-linker; 6, 1:20 ratio protein:cross-linker; 7, 1:40 ratio protein:cross-linker; 8, 1:60 ratio protein:cross-linker; and 10-kDa ladder markers are shown in the far right lane. A, wild type protein; B, F216L protein; C, F216A protein. Note that the monomeric H3 peptide runs at 8 kDa rather than the expected 10 kDa.

![FIG. 7. Enlarged view of one end of the H3 tetramer.](Image) Lysine side chains are drawn as balls and sticks, and distances between selected pairs are shown to indicate likely cross-linking sites.
part to suboptimal packing of the hydrophobic core caused by the small alanine side chain. The effect of Phe-216 mutations on the assembly of syntaxin H3 demonstrates that steric complementarity has important effects on the assembly of helical oligomers. The conservation of Phe-216 may be important both in insuring correct register of SNARE helices (33), and also to prevent unproductive modes of syntaxin self-association.

Acknowledgments—We thank K. Ervin, R. Hollomon, and T. Hong for technical assistance; T. Earnest and the staff at the Advanced Light Source for beamline support; M. Soltis and H. Bellamy at the Stanford Synchrotron Radiation Laboratory for beamline support and advice with heavy atom derivatization; A. Kolatkar for assistance with data collection; and A. Kolatkar, L. Gonzalez, R. Samudrala, A. May, and J. Wedekind for helpful discussions.

REFERENCES

FIG. 8. Representative wavelength scans for the wild type, F216A, and F216L proteins at 25 ºC and pH 8.0 (A) or pH 5.5 (B). Data measured at pH 8.0 are shown as filled circles, and data collected at pH 5.5 are shown as open circles. Red, green, and purple curves represent data from the wild type, F216A mutant, and F216L mutant proteins, respectively. C, D, and E show representative temperature melting curves for wild type, F216A, and F216L H3 proteins, respectively. For the temperature melting experiments, the helical signal was monitored at 222 nm as the temperature was varied from 10 ºC to 90 ºC. The black curve in E shows data measured from the F216L mutant protein at pH 5.5 after annealing (see text).
Self-association of the H3 Region of Syntaxin 1A
