High Resolution Structure, Stability, and Synaptotagmin Binding of a Truncated Neuronal SNARE Complex*

Received for publication, November 21, 2002, and in revised form, December 19, 2002
Published, JBC Papers in Press, December 20, 2002, DOI 10.1074/jbc.M211889200

James A. Ernst and Axel T. Brunger‡

From the Howard Hughes Medical Institute and Departments of Molecular and Cellular Physiology, Neurology and Neurological Sciences, and Stanford Synchrotron Radiation Laboratory, Stanford University, Stanford, California 94305

The crystal structure of the neuronal SNARE complex revealed a conserved buried ionic layer at the center of the four-helix bundle (9) whose function is still uncertain (16). Most probably, it plays a role during N-ethylmaleimide-sensitive factor (NSF) driven disassembly of the SNARE complex, because mutations of this central layer can disrupt this process (17).

Here we present the crystal structure of the neuronal SNARE complex at a 1.4-Å resolution. To obtain this high resolution crystal structure, the individual SNAREs were truncated in comparison with the corresponding constructs used in the previously published crystal structure solved at a 2.4-Å resolution (9). This high resolution structure reveals new sites of hydration and stabilizing intermolecular interactions. We further characterize the thermal stability of this SNARE complex by CD and SDS melts, its oligomerization state, and its binding properties to synaptotagmin in the presence of Ca2+ and EDTA.

MATERIALS AND METHODS

Constructs

Constructs encoding sequences for the “minimal” complex (Fig. 1), rat syntaxin 1a residues 180–262 (SXa), synaptobrevin II residues 1–96 (SBa), SNAP-25 B residues 1–83 (SN1a), and SNAP-25 B residues 120–206 (SN2a) were described elsewhere (18). The cDNA-encoding sequences for the N-terminally truncated minimal complex and the microcomplex (Fig. 1), rat syntaxin 1a residues 188–262 (SXb) and residues 191–256 (SXc), synaptobrevin II residues 25–96 (SBb) and residues 28–89 (SBC), SNAP-25 B residues 7–83 (SN1b), and SNAP-25 B residues 132–204 (SN2b) and 141–204 (SN2c) were subcloned from these constructs into the expression plasmid pET28a (Novagen) or pGEX-2T (Amersham Biosciences). The SN2b only. The cDNA encoding the sequence for rat synaptotagmin I-(139–421) were subcloned from synaptotagmin I cDNA into the pGEX-2T expression vector. The G374 sequence variant of synaptotagmin (19) was generated using the sequence for rat synaptotagmin I-(139–421) were subcloned from synaptotagmin I cDNA into the pGEX-2T expression vector. The G374 sequence variant of synaptotagmin (19) was generated using the

The crystal structure of the neuronal SNARE complex revealed a conserved buried ionic layer at the center of the four-helix bundle (9) whose function is still uncertain (16). Most probably, it plays a role during N-ethylmaleimide-sensitive factor (NSF) driven disassembly of the SNARE complex, because mutations of this central layer can disrupt this process (17).

Here we present the crystal structure of the neuronal SNARE complex at a 1.4-Å resolution. To obtain this high resolution crystal structure, the individual SNAREs were truncated in comparison with the corresponding constructs used in the previously published crystal structure solved at a 2.4-Å resolution (9). This high resolution structure reveals new sites of hydration and stabilizing intermolecular interactions. We further characterize the thermal stability of this SNARE complex by CD and SDS melts, its oligomerization state, and its binding properties to synaptotagmin in the presence of Ca2+ and EDTA.

MATERIALS AND METHODS

Constructs

Constructs encoding sequences for the “minimal” complex (Fig. 1), rat syntaxin 1a residues 180–262 (SXa), synaptobrevin II residues 1–96 (SBa), SNAP-25 B residues 1–83 (SN1a), and SNAP-25 B residues 120–206 (SN2a) were described elsewhere (18). The cDNA-encoding sequences for the N-terminally truncated minimal complex and the microcomplex (Fig. 1), rat syntaxin 1a residues 188–262 (SXb) and residues 191–256 (SXc), synaptobrevin II residues 25–96 (SBb) and residues 28–89 (SBC), SNAP-25 B residues 7–83 (SN1b), and SNAP-25 B residues 132–204 (SN2b) and 141–204 (SN2c) were subcloned from these constructs into the expression plasmid pET28a (Novagen) or pGEX-2T (Amersham Biosciences) (SN2b only). The cDNA encoding the sequence for rat synaptotagmin I-(139–421) were subcloned from synaptotagmin I cDNA into the pGEX-2T expression vector. The G374 sequence variant of synaptotagmin (19) was generated using the QuikChange mutagenesis kit (Stratagene) using the oligomers S-TG-TAACCAACGGAAGACTTTGCCGATGGCGTCGTTCTTGCC-3′ and S-GCGAAGACCGCATCGCGAAAAGTTCTGGTTGTTACA-3′. The correct sequences of all of the constructs were verified by DNA sequencing (Biocore Inc., Palo Alto, CA, or Keck facility, Yale University, New Haven, CT).

The pET28a expression plasmids were transformed into E. coli BL21 (DE3) competent cells using standard protocols (20). Cells were grown at 37 °C in terrific broth supplemented with 100 μg/ml ampicillin media in 4-liter flasks. At an A600 of ~1, the cells were immediately frozen in liquid nitrogen and stored at ~80 °C.

Members of the conserved family of SNARE1 proteins play an important role in protein-assisted vesicle membrane fusion (1–7). SNARE complex formation juxtaposes synaptic vesicle and plasma membranes and thus may set the stage for vesicle membrane fusion. In the final stages of fusion, neurotransmitter release is probably regulated by the Ca2+-binding protein synaptotagmin (8). Each SNARE protein contains at least one core domain that binds to other SNARE proteins to form a four-helix bundle (8). This four-helix bundle is composed of 16 layers transverse to the helical axes including a buried ionic layer at the center of the four-helix bundle (9).

The neuronal SNARE complex consists of three SNAREs: synaptobrevin, syntaxin, and SNAP-25 (Synaptosome-associated protein, 25 kDa) (Fig. 1). Synaptobrevin (also referred to as vesicle-associated membrane protein) is a 12-kDa protein with a SNARE binding domain and a single spanning transmembrane domain (10, 11). Syntaxin is a 35-kDa protein with a three-helix bundle regulatory domain, a SNARE binding domain, and a single spanning transmembrane domain (1, 3, 12, 13). SNAP-25 is a 25-kDa protein with two SNARE binding domains and a linker domain of ~45 amino acids. SNAP-25 is targeted to the plasma membrane by its association with syntaxin via palmitoylation of three cysteine residues in the linker domain (14, 15).

* 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.

1 The abbreviations used are: SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor; SNAP-25, Synaptosome-associated protein, 25 kDa; GST, glutathione S-transferase; DTT, dithiothreitol; MALLS, multi-angle laser light scattering; MDPI, (2-methyl-2,4-pentanediole; MES, 4-morpholineethanesulfonic acid.

‡ To whom correspondence should be addressed. Tel.: 650-736-1031; Fax: 650-745-1463; E-mail: brunger@stanford.edu.

1 The abbreviations used are: SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor; SNAP-25, Synaptosome-associated protein, 25 kDa; GST, glutathione S-transferase; DTT, dithiothreitol; MALLS, multi-angle laser light scattering; MDPI, (2-methyl-2,4-pentanediole; MES, 4-morpholineethanesulfonic acid.
Results

FIG. 1. Sequence alignment of neuronal SNAREs. The sequence for the constructs used for the 2.4-Å crystal structure of the minimized complex (9) is shown in italics. In text, they are referred to as constructs SBa, SXa, SN1a, and SN2a. The N-terminal terminus of these constructs are underlined. In text, they are referred to as constructs SBb, SXb, SN1b, and SN2b. The constructs of the microcomplex are indicated by boxes. In the text, these constructs are referred to as SBc, SXc, SN1b, and SN2c. The N-terminal layer of the SNARE complex are shown in blue, the ionic central (zero) layer is shown in red. Sequences displayed are rat synaptotobrevin II (gi:6981613), rat syntaxin-1A (gi:2071262), rat SNAP-25 B (gi:2116627).

A.

B.

C.

D.

E.

F.

G.

H.

I.

J.

K.

L.

M.

N.

O.

P.

Q.

R.

S.

T.

U.

V.

W.

X.

Y.

Z.

* [*] ORCID iDs

** Correspondence:** nh@nih.gov

*** Funding Information:**

This work was supported by the Intramural Research Program of the National Institutes of Health, National Institute of General Medical Sciences (Z01GM076598). The authors declare no competing interests.

Acknowledgments.

Programs.

Data.

Resources.

Chemicals.

Methods.

Overall dimensions of the minimization were 20.0, 22.0, and 24.0 Å. The complex was flash-frozen in liquid nitrogen and stored at 80 °C.

Biochemistry

**CD**—CD experiments were performed on a Aviv 62DS spectrometer at 150 mM NaCl, 20 mM Na3HPO4, pH 7.8, and 10 μM SNAP-25 complex. Temperature scans were performed between 37 and 97 °C at two-degree intervals with 1-min equilibration between temperature changes and 1-min acquisition with data averaging at each temperature point. The concentration was 9 mg/ml in a solution of 200 mM NaCl, 10 mM HEPES (Fluka), pH 7.8, and 5 mM DTT. The elution profile was monitored by UV absorption at 280 nm, light scattering at 690 nm, and differential refractometry. Light scattering and differential refractometry were carried out using the Dawn and OptiLab instruments (Wyatt Technology). Analysis was carried out using the Astra software (22).

**NMR**—NMR was performed on a Varian Unity 500 spectrometer at 25 °C. Samples were prepared using 10 mM HEPES, pH 7.8, and 1 mM EDTA. The concentration was 9 mg/ml in a solution of 200 mM NaCl, 10 mM HEPES, pH 7.8, and 1 mM EDTA. Samples were mixed at room temperature, incubated for 1 h with GST beads, and washed three times with buffer and mixed with SDS sample loading buffer. The samples were analyzed by SDS-PAGE using 10–25% SDS Phast gels.

**MALDITOF**—MALDI Orbitrap was performed using a Bruker autoflex III mass spectrometer at 1-cavity with a laser power of 100 mJ. The mass accuracy was 10 ppm (Fluka), 75–125 mM CaCl2 (Fluka), and 50 mM MES (Fluka) at pH 5.0–6.0. Initial drops consisted of a one-to-one mixture of protein sample and well solution resulting in a total volume of 4 μl. The crystals grew in clusters as thick needles. To obtain single crystals, these clusters of crystals were used for streak seeding into preequilibrated hanging drops. Single crystals were prepared for freezing by serial transfer using nylons loops into mother liquor with increasing amounts of MPD. The differential refractive index increment (dn/dc) is fairly constant for proteins and was set to 0.185.

**Crystallography**

**Crystallization**—Crystallization trials were conducted using the hanging drop vapor diffusion method. The initial SNAP-25 protein concentration was 9 mg/ml in a solution of 200 mM NaCl, 10 mM HEPES (Fluka), pH 7.8, and 5 mM DTT (American BioAnalytical). Crystals appeared at 4 °C in 1–3 days and grew to full size in 3–5 days. The well solution contained 15–20% (v/v)-2-methyl-2,4-pentanediol (MPD) (Fluka), 75–125 mM CaCl2 (Fluka), and 50 mM MES (Fluka) at pH 5.0–6.0. Initial drops consisted of a one-to-one mixture of protein sample and well solution resulting in a total volume of 4 μl. The crystals grew as clusters as thick needles. To obtain single crystals, these clusters of crystals were used for streak seeding into preequilibrated hanging drops. Single crystals were prepared for freezing by serial transfer using nylon loops into mother liquor with increasing amounts of MPD as a cryoprotectant up to 55%. Crystals were then frozen by rapid transfer directly into liquid nitrogen.

**Diffraction Data**—Diffraction data were collected at the Lawrence Berkeley National Laboratory Advanced Light Source beamline BL 8.2.1 from a single crystal in one pass at 100 K using an Area Detector System Quantum 210 2 × 2 CCD detector. The diffraction data were collected to a 4.0 Å resolution. All of the data processing was carried out using the programs Denzo and Scalepack (23). Statistics of the diffraction data are shown in Table 1. The crystals formed with one copy of the SNARE complex per asymmetric unit in space group P212121. **Phases**—The phases for the diffraction data were obtained by molecular replacement using the direct rotation search (24) as implemented in the program CNS (version 1.1) (25) using diffraction data from 15 to 4.0 Å resolution and resulted in an unambiguous solution. The search model consisted of one of the three non-crystallographically related copies of the neuronal SNARE solved at 2.4 Å (9). It was truncated to contain only those residues present in the microcomplex.

**Model Building**—Model building was performed using the program O (26). The initial model was optimized by rigid body refinement followed by simulated annealing with torsion angle dynamics (27), restrained B-value refinement (28), and conjugate gradient minimization using the MLF target function (29). Overall anisotropic scale factors and bulk solvent correction were applied to the diffraction data. The progress of model rebuilding and refinement was monitored by cross-
validation using \( R_{\text{free}} \) (30), which was computed from a randomly chosen test set comprising 10% of the data. The sites of hydration were placed by inspection of peaks larger than three standard deviations above the mean in \( F_o - F_c \), \( \sigma_o \)-weighted electron density maps. Only those sites were kept that exhibited reasonable protein solvent hydrogen-bonding distances without steric conflict and whose \( B \)-value refined to \(< 55 \text{ Å}^2\). MPD and \( \text{Ca}^{2+} \) were identified by inspection of \( F_o - F_c \) and \( 2F_o - F_c \), \( \sigma_o \)-weighted electron density maps. At various points during refinement, \( \sigma_o \)-weighted, annealed \( 2F_o - F_c \) composite omit maps were used to minimize the effects of model bias. All of the refinements were carried out using the program CNS (25). Statistical linear least-squares superposition of the structures was performed using the LSQMAN (31) from the Uppsala software factory suite. Graphical images were prepared using PyMOL (Fig. 5) (32) or GRASP (Fig. 4, a and b) (33).

**RESULTS**

**Oligomeric State of the SNARE Complex**—The neuronal SNARE complex has a tendency to oligomerize as shown by analytical ultracentrifugation and MALLS (18). The minimal SNARE complex obtained by limited proteolysis that was used in the 2.4-Å crystal structure had an apparent molecular mass of 60–90 kDa compared with a calculated molecular mass of 41 kDa (18). The C-terminal truncations of synaptobrevin by botulinum toxin B or tetanus toxin produced a monomeric SNARE complex (34). Furthermore, the C-terminal truncation of endobrevin (vesicle-associated membrane protein 8) in the endosomial SNARE complex produced a monodisperse sample (35). Therefore, we truncated the neuronal synaptobrevin at Trp-89 instead of 32.5 kDa (18). The C-terminal truncations of synaptobrevin by botulinum toxin B or tetanus toxin produced a monomeric SNARE complex (34). Generally, we truncated the neuronal SNARE proteins then expressed, purified, and assembled. This “micro”-SNARE complex has an apparent molecular mass of 32.5 kDa ± 2% as determined by MALLS (data not shown) compared with a calculated molecular mass of 32.5 kDa. Thus, the microcomplex is both monomeric and monodisperse.

**SNARE Complex Stability**—We performed temperature-dependent SDS and CD melts of the micro-SNARE complex and compared the results to both the minimal SNARE complex (36) and a SNARE complex that was obtained from the minimal complex by truncation at the N terminus (Fig. 2). Our experiments revealed a roughly 20 °C reduction in the stability of the micro-SNARE complex in SDS relative to both the minimal and N-terminally truncated SNARE complexes (Fig. 2a). In light of this reduction in SDS stability, we further investigated the
Fig. 4. Superposition of neuronal and endosomal SNARE complex. A, C, superposition of the micro-SNARE complex and the endosomal SNARE complex (Protein Data Bank code 1GL2). The color code is as follows: synaptobrevin is shown in blue; syntaxin is shown in red; SNAP-25 is shown in green; endobrevin is shown in light blue; vti1b is shown in magenta; and syntaxin-8 and syntaxin-7 are shown in yellow. B, C, superposition of the micro-SNARE complex with the three complexes in the 2.4 Å crystal structure of the minimal SNARE structure (Protein Data Bank code 1SFC). The color code is as follows: the microcomplex is shown in red; the first molecule of the minimal complex crystal structure (chains a–d) (see Ref. 9) is shown in dark blue; the second molecule (chains e–h) is shown in medium blue; and the third molecule (chains i–l) is shown in light blue. C, B-value plot for the C₆ residues of the micro-SNARE complex. The color code is as follows: synaptobrevin is shown in blue; syntaxin is shown in red; SNAP-25 SN1c is shown in light green; and SNAP-25 SN2c is shown in dark green.
endosomal structure is larger than the root mean square difference between the microcomplex structure and the endosomal structure. crystals were obtained in space group P212121 in the presence of MPD and CaCl$_2$ at 4°C. (Table III) It is interesting to note that the root mean square difference between the microcomplex and the endosomal structure of the microcomplex, it is probable that truncated SNARE complexes have a thermal stability of the various SNARE complexes under native conditions by CD. Both the minimal and the N-terminally truncated SNARE complexes have a $T_m$ of 94°C, whereas the micro-SNARE complex has a reduced $T_m$ of 89°C (Fig. 2b).

**Synaptotagmin Binding**—Having demonstrated that the micro-SNARE complex forms a quantitative and stable complex, albeit with somewhat reduced $T_m$, we investigated whether it would retain its ability to interact with the C$_2$ domains of synaptotagmin I. GST pull-down experiments were conducted in the presence of both 1 mM EDTA and 0.5 mM CaCl$_2$. As shown in Fig. 3, synaptotagmin I is capable of binding the micro-SNARE complex in both the presence and absence of Ca$^{2+}$. These findings are consistent with prior reports using the C$_{2}$AB domain of synaptotagmin III and the minimal SNARE complex (37).

**Microcomplex Structure**—We next determined the crystal structure of the microcomplex. Crystals were obtained in space group P2$_1$2$_1$2$_1$ in the presence of MP and CaCl$_2$ at 4°C. These conditions are similar to the previous crystallization conditions used for the minimal SNARE complex (9). The crystal structure contained only one copy of the complex per asymmetric unit in contrast to the minimal SNARE complex that crystallized in a different space group (I222) with three complexes per asymmetric unit. Most importantly, the crystals of the microcomplex diffracted to 1.4 Å, making this the highest resolution crystal structure of a SNARE complex available to date. All of the residues of the microcomplex were visible in the final model, which refined to a $R_{cryst}$ value of 19.8% and a $R_{free}$ value of 22.4%. The statistics of the diffraction data and the final refined model are shown in Tables I and II. The electron density maps are of excellent quality (Fig. 5b) and allowed assignments of nearly all of the side-chain rotamers.

Three Ca$^{2+}$ sites were found that are coordinated by symmetry-related molecules. These sites were visible as 8 $\sigma$ peaks in 2$F_\sigma$ − $F_\sigma$ maps. The coordinating oxygen atoms are located on SNAP-25 Gln-20 and Glu-27 of a SNARE complex and synaptobrevin Asp-80, Lys-83 of SNAP-25, Tyr-88, Trp-89, and syntaxin Lys-256 of a symmetry-related complex. Several water molecules complete the coordination spheres around the Ca$^{2+}$. Because these Ca$^{2+}$ sites are located at the artificially truncated C terminus of the microcomplex, it is probable that these binding sites are the result of crystallization conditions.

As expected, the micro-SNARE complex forms a four-helix bundle. The C$_\alpha$ atoms of the microcomplex were superimposed on that of the minimal complex structure and on all of the homologous residues of the endosomal complex structure. The results of these superpositions are shown in Fig. 4, a and b, and Table III. It is interesting to note that the root mean square (r.m.s) difference between the microcomplex structure and the endosomal structure is larger than the root mean square difference observed when comparing either structure to the minimal complex structure. In contrast to the endosomal structure, the microcomplex displays little variation in $B$-values over most of the four-helix bundle (see Fig. 4c). Only the second $\alpha$-helix of SNAP-25 between layers 2 and 8 and synaptobrevin between layers −7 and 0 display any systematic increase in $C_\alpha$ $B$-values.

To further compare the various SNARE complex crystal structures, we superimposed the residues around the ionic central layer (Fig. 1) with the corresponding residues of the previously solved structures. For the neuronal SNAREs, the layer consists of synaptobrevin Arg-56, syntaxin Gln-226, SNAP-25 Gln-53, and SNAP-25 Gln-174. For the endosomal complex, the corresponding residues are endobrevin Arg-76, syntaxin-7 Gln-199, vti1b Gln-170, and syntaxin-8 Gln-179. The root mean square differences for residues at this layer between the microcomplex and the endosomal complex are

---

**Table III**

<table>
<thead>
<tr>
<th>Structure 1</th>
<th>Structure 2</th>
<th>C$_\alpha$ r.m.s.d. (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcomplex</td>
<td>Endosomal complex</td>
<td>1.619</td>
</tr>
<tr>
<td>Microcomplex</td>
<td>Minimal complex</td>
<td>0.947 (Molecule 1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.724 (Molecule 2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.139 (Molecule 3)</td>
</tr>
<tr>
<td>Endosomal complex</td>
<td>Minimal complex</td>
<td>1.187 (Molecule 1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.270 (Molecule 2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.087 (Molecule 3)</td>
</tr>
</tbody>
</table>

---

*Fig. 5. Ionic central layer. A, alternate conformations of synaptobrevin Arg-56 at the ionic central layer. Residues for the microcomplex structure are shown in yellow, residues for the minimal complex (chains a–d) are shown in cyan, and residues for the endosomal structure are shown in gray. Residues are numbered according to the sequences of the neuronal SNARE complex. B, electron density at the ionic central layer of the micro-SNARE complex. Shown is a 2$F_\sigma$ − $F_\sigma$ $\sigma$-weighted omit map. The electron density is contoured at 1.5 $\sigma$. Synaptobrevin is shown in blue, syntaxin is shown in red, and SNAP-25 is shown in green.*
0.315 and 0.509 Å for Cα and all atoms, respectively. Several of the SNARE crystal structures show the presence of a bifurcated hydrogen bond between synaptobrevin Arg-56 and SNAP-25 Gln-53 and Gln-174 (Fig. 5a). However, in one of the molecules of the minimal complex crystal structure (Fig. 5a, cyan), Arg-56 exhibits a rotamer that allows direct hydrogen bonding from each of the side-chain nitrogen atoms of Arg-56 to each of the buried glutamines. This Arg-56 rotamer is also visible in the structure of the squid neuronal SNARE complex with complexin (38). Thus, Arg-56 exhibits significant conformational variability among the different structures, whereas the three glutamines exhibit very similar conformations (Fig. 5a). The observed conformational variability of the central layer may suggest a possible functional role in the disassembly process (39).

The quality of our diffraction data allowed us to assign numerous sites of hydration that were previously unobservable (Fig. 5b). Of particular interest is a buried water molecule (Fig. 5b, H2O 89) at the ionic central layer. This water molecule is located 3.10 Å from the ε-nitrogen of Arg-56, satisfying the hydrogen bond requirements of this nitrogene. It is possible that this water molecule is not present when Arg-56 adopts alternate conformations observed in some of the other crystal structures.

The formation of salt bridges on the surface of proteins is known to stabilize exposed structural elements. The presence of surface salt bridges positioned to stabilize buried structural elements is less common. However, a carboxylic acid ε-oxygen of SNAP-25 Glu-170 shows just such an interaction buttressing SNAP-25 Gln-174 at 2.79 Å through the Gln-174 ε-nitrogen (Fig. 5b). This interaction stabilizes the SNAP-25 Gln-174 ε-oxygen, which in turn interacts with synaptobrevin Arg-56 Nε2 at a distance of 2.65 Å (Fig. 5b). The close interaction of synaptobrevin Arg-56 Nε2 with the SNAP-25 Gln-174 ε-oxygen as compared with the Arg-56 Nε1 and syntaxin Gln-226 interaction probably reflects the proximity of the negatively charged carboxylic group from SNAP-25 Gln-170. We note further that the three glutamines exhibit very similar conformations (Fig. 5b). The close interaction of synaptobrevin Arg-56 Nε2 with the SNAP-25 Gln-174 ε-oxygen may be involved in oligomerization. This Arg-56 rotamer is also visible in the structure of the squid neuronal SNARE complex with complexin (38). Thus, Arg-56 exhibits significant conformational variability among the different structures, whereas the three glutamines exhibit very similar conformations (Fig. 5a). The observed conformational variability of the central layer may suggest a possible functional role in the disassembly process (39).

**REFERENCES**

Structure of a Truncated Neuronal SNARE Complex