Interaction of Human \( \alpha \)-Synuclein and Parkinson’s Disease Variants with Phospholipids

**STRUCTURAL ANALYSIS USING SITE-DIRECTED MUTAGENESIS**

Received for publication, June 5, 2000
Published, JBC Papers in Press, August 21, 2000, DOI 10.1074/jbc.M004851200

Richard J. Perrin‡, Wendy S. Woods§, David F. Clayton§, and Julia M. George¶
From the ‡Department of Molecular and Integrative Physiology and §Department of Cell and Structural Biology, University of Illinois, Urbana, Illinois 61801

\( \alpha \)-Synuclein has been centrally implicated in neurodegenerative disease, and a normal function in developmental synaptic plasticity has been suggested by studies in songbirds. A variety of observations suggest the protein partitions between membrane and cytosol, a behavior apparently conferred by a conserved structural similarity to the exchangeable apolipoproteins. Here we show that the capacity to bind lipids is broadly distributed across exons 3, 4, and 5 (encoding residues 1–102). Binding to phosphatidylserine-containing vesicles requires the presence of all three exons, while binding to phosphatidic acid can be mediated by any one of the three. Consistent with a “class A2” helical binding mechanism, lipid association is disrupted by introduction of charged residues along the hydrophobic face of the predicted \( \alpha \)-helix and also by biotinylation of conserved lysines (which line the interfacial region). Circular dichroism spectroscopy reveals a general correlation between the amount of lipid-induced \( \alpha \)-helix content and the degree of binding to PS-containing vesicles. Two point mutations associated with Parkinson’s disease have little (A30P) or no (A53T) effect on lipid binding or \( \alpha \)-helicity. These results are consistent with the hypothesis that \( \alpha \)-synuclein’s normal functions depend on an ability to undergo a large conformational change in the presence of specific phospholipids.

\( \alpha \)-Synuclein (AS)\(^1\) is an abundant and highly conserved neuronal protein in vertebrates, implicated in both normal synaptic plasticity and neurodegenerative disease. In its molecular sequence, AS is very closely related to three other neuronal proteins, \( \beta \) and \( \gamma \)-synuclein (1) and synoretin (2), all of unknown function. In songbirds, expression of the protein coincides with a major synaptic reorganization in the circuit controlling learned song (3). In humans, AS is the primary fibrillar component of Lewy bodies and Lewy neurites (4, 5); these intraneuronal inclusions are typical of Parkinson’s disease and are sometimes found in Alzheimer’s disease (6, 7). Aggregated synuclein is also observed in Down’s syndrome (7) and multiple system atrophy (8–10). Additionally, two different alleles of AS are linked genetically to a rare form of autosomal dominant, early onset Parkinson’s disease (11, 12).

The normal function of AS is not yet understood. We have noted, however, a striking relationship to the family of apolipoproteins, apparent at the level of secondary structure (3, 13). Both protein families are organized around a repeating 11-residue periodicity. In the apolipoproteins, these repeats give rise to amphipathic \( \alpha \)-helical domains, which have been extensively studied and assigned to subclasses according to their unique structural and functional properties. AS shares the defining properties of the class A2 lipid-binding helix, distinguished by clustered basic residues at the apolar-apolar interface, positioned \( \pm 100^\circ \) from the center of the apolar face; a preponderance of lysine relative to arginine among these basic residues; and several glutamate residues localized to the polar face (14, 15). Consistent with these structural features, AS binds to synthetic vesicles containing acidic phospholipids, and this binding is accompanied by a shift to a largely \( \alpha \)-helical conformation (13).

In this report, we describe experiments to characterize the structural interaction of AS with acidic phospholipid vesicles. First, we used site-directed mutagenesis to map the domains that participate in lipid binding. Next, we tested several structural predictions of the class A2 helical model of lipid binding, by manipulating specific residues in the hydrophobic and interfacial domains of the predicted helix. Finally, we examined two Parkinson’s disease-associated point mutations, which (based on our structural model) are predicted to have different effects on lipid binding. The results reveal that lipid binding is a robust and broadly distributed property of the molecule that is nonetheless dependent upon specific conserved structural features shared with apolipoproteins.

**EXPERIMENTAL PROCEDURES**

Cloning and Purification of hAS and Mutants—The human \( \alpha \)-synuclein cDNA used in this study was the generous gift of M. Irizarry. For bacterial expression, the hAS cDNA was cloned into pET28(a) (Novagen), which directs inducible expression under a T7 lac promoter. For production of GST fusion proteins, the hAS cDNA was cloned into pGEX-2TK (Amersham Pharmacia Biotech). All mutant forms of hAS were constructed by long polymerase chain reaction-based techniques using \( \Phi 11 \) polymerase (Stratagene), 5’-phosphorylated primers, and subsequent recircularization of polymerase chain reaction products by T4 ligase. The correct DNA sequence of all constructs was confirmed by DNA sequencing (Life Technologies, Inc. double strand sequence system).

---

\(^1\) This work was supported by National Institutes of Health (NIH) Grant 1R01 AG13762 (to D. C.) and NIH Grant 5T32GM07143-20 (to R. P.). 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.

\(^\dagger\) To whom correspondence should be addressed: Dept. of Cell and Structural Biology, 617 Chemical and Life Science Laboratory, 601 S. Goodwin, Urbana, IL 61801. Tel.: 217-244-4525; Fax: 217-244-1648; E-mail: j-george@uiuc.edu.

\(^\ddagger\) The abbreviations used are: AS, \( \alpha \)-synuclein; hAS, human \( \alpha \)-synuclein; GST, glutathione S-transferase; A30P and A53T, Parkinson’s disease variants of hAS; POPC, 1-palmitoyl 2-oleyl phosphatidylcholine; POPA, 1-palmitoyl 2-oleyl phosphatidic acid; POPS, 1-palmitoyl 2-oleoyl phosphatidylserine; SUV, small unilamellar vesicles; PLD2, phospholipase D2; PA, phosphatidic acid; PS, phosphatidylserine.
Mapping the Lipid Binding Domain of α-Synuclein

Relative to unmodified wild-type human AS, the mutant constructs are described as follows.

**Single Exon Deletion Constructs**—In Δ3, residues 2–42 of hAS are replaced by a single residue, Ala; in Δ4, residues 43–56 are deleted; in Δ5, residues 56–102 are replaced by a single residue, Glu; and in Δ6, residues 103–130 are deleted.

**GST-hAS Fusion Constructs**—In GST-hAS, the 6 C-terminal residues of GST (as encoded by pGEX-2TK) are replaced by hAS residues 1–140; GST-3,7 is as above, but hAS residues 42–129 are deleted; in GST-4,7, hAS residues 2–42 are replaced by a single residue, Ala, and residues 56–130 are deleted; in GST-5,7, hAS residues 2–55 and 103–130 are deleted; and in GST-6, hAS residues 2–102 are replaced by a single residue, Glu.

pET28-hAS constructs were transfected into *Escherichia coli* BL-21, grown in LB at 37 °C with 30 μg/ml kanamycin, and induced at mid-log phase with 1 mM isopropyl-β-D-thiogalactopyranoside for 2 h. Purification of hAS was based upon a modification of Weirnb (16). Briefly, cell lysates were boiled, and the soluble fraction was brought to 30% ammonium sulfate, loaded onto a PorosPE column (Persptive Biosystems), and eluted with phosphate buffer. Synuclein-containing fractions, as assayed by Western blot with the C-terminal monoclonal antibody H3C (3), were diluted in 25 mM sodium acetate buffer, pH 4.0, loaded onto a PorosHS column (Persptive Biosystems), and eluted with phosphate buffer. Synuclein-containing fractions were pooled and exchanged into 25 mM Tris, pH 9.0, loaded onto a PorosHQ column (Persptive Biosystems), and eluted with 0–1 mM NaCl. Gel filtration (Superose 6; Amersham Pharmacia Biotech) was employed as a final polishing step and to exchange proteins into STB (10 mM Tris, pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.02% NaN₃). The molecular weights of purified hAS, A30P, and A53T and all single exon deletion mutants were determined by electrospray mass spectrometry and found to be as predicted from primary sequence + 2 Da.

pGEX-hAS constructs were likewise transfected into BL-21 cells but were grown at 30 °C in 2× YTA media as per the manufacturer’s instructions. At mid-log phase, cultures were induced with 1 mM isopropyl-β-D-thiogalactopyranoside for 1–2 h and harvested by centrifugation. Cells were resuspended in GST-PBS (140 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, pH 7.3) plus 2.5 mM phenylmethylsulfonyl fluoride and frozen overnight at −20 °C. Suspensions were thawed in a 22 °C water bath, mixed with 1% Triton X-100 (v/v), and incubated for 30 min at 4 °C, followed by centrifugation at 12,000 × g. Supernatants were collected and incubated for 20 min at 22 °C with glutathione-agarose beads (Sigma). After incubation, beads were collected by centrifugation and washed four times with 20 bed volumes of GST-PBS. GST fusion proteins were eluted with 10 mM reduced glutathione in 50 mM Tris-HCl, pH 8.0, and exhaustively buffer-exchanged into STB using Centriprep 10 concentrators (Amicon).

**Biotinylation of Proteins**—Recombinant, purified hAS and A30P proteins were biotinylated after the method of Jensen et al. (17) using a commercially available kit (Pierce). 0.75 mM sulfosuccinimidobiotin (Pierce) was incubated with 0.2 μg/ml of protein for 1 h at 22 °C in 100 mM sodium carbonate buffer, pH 8.0. The reaction was terminated aseptically by boiling with 15 volumes of STB. Proteins were exhaustively buffer-exchanged into STB using Centriprep 3 concentrators (Amicon). By this technique, sulfosuccinimidobiotin levels were diluted below 0.4 μM. Biotin incorporation was determined by the method of Green (18).

**Circular Dichroism Measurements and Analysis**—CD spectra were collected for hAS and deletion mutants using a Jasco J-720 spectropolarimeter. Spectra were taken at 25 °C in a 0.1-cm path length quartz cuvette containing the sample at 0.1 mg/ml of protein in 20 mM phosphate buffer (pH 8.0), alone or in the presence of 2 mg/ml SUV. The spectral contributions of buffer and vesicles were subtracted as appropriate. The percentage of α-helix was determined from the mean residue ellipticities at 222 nm (22).

**RESULTS**

Mapping of Lipid Binding Domains—To identify the regions of hAS required for lipid binding, we first subcloned the hAS cDNA (generous gift of Michael Irizarry) into a bacterial expression vector and then prepared mutant versions, each lacking an individual exon of the coding region (Fig. 1).

The purified recombinant proteins were incubated with vesicles prepared from synthetic phospholipids in the following mass ratios: POPC/POPA (1:1), POPC/POPS (1:1), and POPC alone. These compositions were chosen because of our earlier studies indicating that canary synuclein (synelfin) binds preferentially to vesicles containing acidic phospholipids such as POPA and POPS but not to vesicles composed of the neutral phospholipid POPC alone (13). The protein/vesicle incubations were fractionated by gel filtration chromatography, and samples from consecutive 1-ml fractions were subjected to SDS-polyacrylamide gel electrophoresis and immunoblotting with monoclonal antibody H3C. Elution profiles of the recombinant proteins are presented in Fig. 2.

This factionation typically results in separation of three major peaks, corresponding to large multilamellar vesicles (a heterogeneous population that elutes in the column void volume), SUV (average diameter 25 nm), and free protein (13). Wild-type hAS eluted with the SUV fraction when incubated with either POPC/POPS or POPC/POPA phospholipids. In contrast, mutants lacking either exon 3, 4, or 5 eluted as free protein after incubation with POPC/POPS; only when incubated with POPC/POPA did these proteins elute in the SUV fractions. Thus, no one exon is essential for POPC/POPA binding, whereas all three together are required for binding to POPC/POPS under these experimental conditions.

To determine whether individual exons by themselves are sufficient for binding to POPC/POPS vesicles, we engineered GST fusion proteins with hAS exons 3, 4, 5, or 6. In each construct, we also included the short hAS exon 7, which contains the H3C epitope for immunodetection. Without the GST sequence by itself was incubated with either POPC/POPA or POPC/POPS and fractionated by gel filtration, it eluted as free protein, indicating an inability to bind to acidic phospholipid vesicles (Fig. 3). The addition of hAS exons 6 and 7 did not significantly alter the lipid binding properties of GST. However, upon the addition of exon 3, 4, or 5, the resulting fusion protein eluted with POPC/POPA vesicles (although exon 4 imparted a somewhat reduced POPC/POPA binding capacity).
Mapping the Lipid Binding Domain of α-Synuclein

Consistent with the results of Fig. 2, all constructs except the full-length fusion protein eluted exclusively as free protein when incubated with POPC/POPS vesicles.

Tests of Class A2 Helical Model—The results in Figs. 2 and 3 reveal that any one of three exons, spanning from residue 1 to 102, is alone sufficient to mediate binding to SUV which contain POPA, whereas binding to POPS requires that all three exons be present. This result is consistent with our prediction of a series of lipid-binding amphipathic α-helices extending across this domain (13). To test this structural prediction, we analyzed protein/phospholipid mixtures of deletion mutants by CD spectroscopy. Wild-type hAS and single-exon deletion mutants, scanned in the presence of POPC/POPA or POPC/POPS vesicles, generated distinct minima at 208 and 222 nm that are characteristic of high α-helical content (Fig. 4, A and C). These minima were less pronounced for mutants lacking exons 3, 4, or 5, suggesting a loss of α-helical character. The mutant lacking exon 6, by contrast, gave evidence of a slightly increased proportion of α-helix relative to wild-type, consistent with the loss of a nonhelical domain. In the absence of lipid, all of these proteins displayed spectra with strong negative ellipticities around 200 nm, indicating a high percentage of random coil (Fig. 4A). The fractional contribution of α-helix to the secondary structure of hAS and mutants were estimated from the mean residue ellipticity according to the method of Chen (22), and these values are represented graphically in Fig. 4D.

These results show that lipid binding is associated with extensive α-helix formation, but they do not formally establish that the hydrophobic face of the amphipathic helix is the essential mediator of lipid binding. The extended helical domain is also distinctive in its abundance of lysine residues, which could mediate an electrostatic interaction with acidic phospholipid head groups. To discriminate between these two mechanisms of interaction, we substituted lysine residues for the six threonines (Thr22, Thr33, Thr44, Thr59, Thr81, and Thr92) that are structurally predicted to lie centrally along the helical face (13). This alteration should increase electrostatic attraction to acidic phospholipids but disrupt significant portions of the extended hydrophobic face of the helix. The recombinant protein (TsixK) was incubated with SUV of POPC/POPS (1:1) or POPC/POPA (1:1) and analyzed as in Fig. 2. As the elution profile of Fig. 5 demonstrates, the TsixK mutant completely failed to bind to POPC/POPS. This disruption would not be expected if electrostatic forces were primarily responsible for acidic lipid binding and supports the model of lipid binding based on formation of an amphipathic α-helix.

Despite the disruption of binding to POPC/POPS, TsixK still bound to POPC/POPA (Fig. 5). To probe the basis for this, we subjected the mutant protein to CD spectroscopy. By itself, TsixK produced a random coil signature indistinguishable from that of wild type (Fig. 4A). In the presence of POPC/POPA vesicles, induction of α-helicity was evident (Fig. 4D), consistent with the binding of the protein to the vesicles. However, the amount of helix in TsixK was only half of that induced in wild-type (35 versus 70%). Thus, the substitution of six lysines distributed along the predicted hydrophobic face is sufficient to eliminate the participation of approximately 35% of the sequence (49 residues) in helix formation, even under conditions of complete vesicle binding. The residual helix is apparently sufficient to mediate binding to POPA-containing vesicles. This interpretation is consistent with the results of Figs. 2 and 3, which indicate that binding to POPA can be mediated by even relatively short segments of the lipid-binding domain (i.e., exon 3, 4, or 5). In contrast, POPS binding by the wild-type protein requires all three exons (Figs. 2 and 3) and is completely abolished by the TsixK mutation. Consistent with its inability to bind POPS, TsixK exhibited only minimal induced helicity (9%, Fig. 4D) in the presence of POPC/POPS.

In a parallel set of experiments, we produced a mutant in which the six central threonines were replaced not with lysines but with glutamates (TsixE). In this case, we would expect lipid binding to be even more severely disrupted, since we have not only reduced hydrophobic interactions between the helix and the membrane interior but have also introduced negatively charged residues that should be electrostatically repulsed by acidic phospholipid head groups. As predicted, TsixE bound neither POPC/POPS nor POPC/POPA (Fig. 5).

Although substitution of lysines onto the predicted hydrophobic face (TsixK) reduces α-helix formation and lipid binding, numerous lysines lie along the predicted polar/apolar interface in the wild-type protein. In the class A2 helical model (14, 15), these interfacial lysines are believed to be important for lipid binding; their charged amino groups lie at the end of a long aliphatic side chain, which may facilitate “snorkeling” into the hydrophobic membrane interior. To test the contributions of hAS’s positively charged lysines to lipid binding, we chemically modified the recombinant wild-type protein with sulfosuccinimidobiotin. This reagent adds biotin groups to a random subset of primary amines. Molar biotin incorporation was determined to be ∼3:1 or ∼2:1 in our preparations. Biotinylated and wild-type proteins were incubated with POPC/POPA (1:1) and POPC/POPS (1:1) SUV, fractionated by gel filtration chromatography, and analyzed by immunoblotting (Fig. 6). Binding to POPC/POPS vesicles was virtually abolished by biotinylation.
and an increase in free protein in the presence of POPC/POPA vesicles was also detected. Effect of Parkinson’s Disease Variants—Jensen et al. (17) reported that the familial Parkinson’s disease mutation, A30P, completely abolished binding to vesicles derived from brain homogenates. However, their assays relied on biotinylation to detect the added recombinant proteins. In Fig. 6, we also tested recombinant biotinylated A30P protein (*A30P). No effect of the mutation could be observed on PS binding, since biotinylation alone was sufficient to eliminate binding (Fig. 6, *hAS).

The A30P mutation did decrease the proportion of protein bound to PA vesicles, however. To avoid the confounding effects of biotinylation on interpretation of the properties of A30P, we also compared the fractionation behavior of nonbiotinylated forms of A30P and hAS (Fig. 6). Here we observed complete binding to PA vesicles by the A30P protein. Most of the A30P protein also bound to PS-containing vesicles, although some of the protein remained unbound, in contrast to wild type. Lipid-induced helicity was also reduced for A30P, especially in the presence of PS (Fig. 7C). In related experiments, we also tested the effect of the A53T mutation on vesicle binding (data not shown) and lipid-induced helicity (Fig. 7) and observed no difference in its behavior compared with wild type.

**DISCUSSION**

This study provides a basis for understanding the observed tendency of AS to interact with a subset of cellular membranes. The evidence here supports a prediction made earlier on structural grounds that reversible membrane binding would arise intrinsically from an extended apolipoprotein-like domain apparent at the level of secondary structure (3, 13). This domain comprises two-thirds of the protein’s sequence and has been very specifically conserved among the related synuclein genes and across the range of vertebrate species for which synuclein sequences have been defined (1). Hence, it seems likely that this property is central to the cellular functions of the synuclein family. Our deletion analysis suggests that essentially all of this domain is required for binding to PS-containing vesicles, and even a single amino acid substitution (A30P) can cause a detectable reduction in PS binding.

We obtained further confirmation of the class A2 helical model for lipid binding by selectively manipulating the residues along the predicted hydrophobic face. We generated two mutants, one of which shows diminished lipid-induced helicity while retaining some capacity to bind lipids and another in which lipid binding is completely abolished. These mutants may be useful in future studies of the molecular and cellular functions of AS.

The experiments here used vesicles containing relatively high proportions of PA or PS (50%). Although these high concentrations are useful for experimental purposes, AS will bind to vesicles containing lower percentages of these lipids.2 In

**FIG. 4.** Targeted mutations in hAS sequence alter lipid-induced helicity. A–C, shown are circular dichroism spectra (mean residue ellipticity) of recombinant proteins (100 μg/ml) in 50 mM phosphate buffer, pH 7.6 (A), buffer plus POPC/POPA vesicles (2 mg/ml) (B); buffer plus POPC/POPS vesicles (2 mg/ml) (C). Spectra were measured at 25 °C in a 0.1-cm path length quartz cell. Each panel represents six different recombinant proteins, measured in triplicate (n = 3). hAS is shown in black; Δ3 in red, Δ4 in green, Δ5 in dark blue; Δ6 in pink, and TsixK in light blue. D, α-helix content estimated from [θ]222 by the method of Chen (22) for protein alone (black bars, all values <1%), or in the presence of POPC/POPA (red bars) or POPC/POPS (blue bars). S.D. of all calculated values was ±2%.

**FIG. 5.** Mutants TsixK and TsixE show altered lipid binding. Lipid binding assays were performed as described for Fig. 2. For the mutants, six threonines in the hAS sequence (Thr22, Thr33, Thr44, Thr59, Thr81, and Thr92) were replaced with positively charged lysines (TsixK) or negatively charged glutamates (TsixE).

**FIG. 6.** Effects of A30P mutation and biotinylation on lipid binding. Lipid binding assays were performed as described for Fig. 2, except immunoblots for biotinylated proteins were performed using horseradish peroxidase-conjugated avidin (Bio-Rad) (1:50,000). Biotinylation is indicated with an asterisk (*hAS, *A30P).
nervous tissue, PA comprises approximately 1–3% of total phospholipid, whereas PS is more abundant (12–22% of total phospholipids) (23). However, it is difficult to relate these values directly to the local composition of specific membranes inside the cell, since these lipids are not distributed evenly in the cell and are generated and metabolized rapidly. PA, for example, is generated by the highly localized action of phospholipase D on specific membrane domains (24).

Our results demonstrate that the strength of the interaction between AS and different phospholipids varies, even among different acidic phospholipids. As few as 14 residues (exon 4) are sufficient to confer partial binding to PA, whereas all three lipid binding exons (i.e. exons 3–5) are required for binding to PS. PA binding is also more resistant to the effects of the TsixK mutations. In other work, we have found that AS also binds to phosphatidylinositol and phosphatidylinositol 4,5-biphosphate. All four of these lipid binding partners (phosphatidylinositol, phosphatidylinositol 4,5-biphosphate, PS, and PA) are involved in cellular signaling pathways (for reviews, see Refs. 24–26).

In neurons, AS is a very abundant protein, with estimated concentrations in the micromolar range. In cultured cells, its distribution tends to be rather ubiquitous (27), but in the intact brain it is highly localized to presynaptic terminals (3, 28). The mechanism of localization is unknown, but a differential affinity for specific phospholipids could be a factor. PS, for example, is known to be enriched in synaptosomal fractions (29).

The consequences of AS-membrane interactions for cell function remain to be defined. Intercalation of AS into the lipid bilayer may functionally alter the biophysical properties of the membrane, specifically its packing density or surface pressure, to influence fusion or budding events. Alternatively, the pronounced conformational change of AS upon binding membranes may facilitate or inhibit its specific interactions with other proteins. AS molecules might thereby serve to recruit other cytosolic or membrane proteins to membrane regions of high acidic phospholipid content.

One specific functional hypothesis arises from AS’s potent inhibition of phospholipase D2 (PLD2) (30). This enzyme activity has been associated with movement of membranes from intracellular stores to the cell surface and with cytoskeletal reorganization (31), which may influence vesicle docking at the presynaptic terminal. For activity, PLD2 requires two lipids: phosphatidylinositol 4,5-bisphosphate for activation and phosphatidylcholine as a substrate. Upon activation, PLD2 cleaves phosphatidylcholine, producing free choline and PA, which may be processed further into additional signaling lipids (e.g. diacylglycerol and arachidonic acid). Consequently, AS may function in a local negative feedback loop wherein PA, the product of PLD2, selectively recruits AS to sites of PLD2 activity.

Selective membrane binding could also be relevant to the phenotypic effects reported for AS knockout mice (32). These mice are viable and superficially normal, but significant alterations are detected in the dynamic properties of dopamine release from electrically stimulated terminals in slices of striatum. Specifically, dopamine release recovers more quickly in the AS-deficient mice, in a stimulation paradigm referred to as “poststimulus depression.” From this and other evidence, the authors infer that AS may function to regulate the rate at which the “readily releasable pool” of vesicles (33–36) is regenerated following particular patterns of synaptic excitation. Consistent with such a role, AS is especially enriched in a vesicle-containing presynaptic compartment displaced from the active zone (37, 38), and antisense disruption of AS synthesis in cultured hippocampal neurons causes an apparent reduction in this “distal pool” of synaptic vesicles (38).

Apart from its undefined role in normal brain function, AS has a direct involvement in neurodegenerative disease, since it is the major protein component of Lewy bodies and Lewy neurites. In the great majority of cases, Lewy body formation is not linked to genetic mutations in AS, so pathological aggregation of AS must depend most commonly on its interactions with other entities in the cell. Growing evidence suggests that Lewy body formation may involve oxidative cross-linking of AS (39, 40). Membranes are major sites of lipid peroxidation (41), hence recruitment of AS to membrane environments could be an early step in the generation of pathological AS aggregates. The ability of AS to interact selectively and reversibly with specific phospholipids may thus be of central relevance not only to its normal function but also to neurodegenerative disease.

REFERENCES

1. Clayton, D. F., and George, J. M. (1998) Trends Neurosci. 21, 249–254
2. Surguchov, A., Surgucheva, I., Solessio, E., and Baehr, W. (1999) Mol. Cell. Neurosci. 13, 95–103
3. George, J. M., Jin, H., Woods, W. S., and Clayton, D. F. (1995) Neuron 13, 361–372
4. Irizarry, M. C., Growdon, W., Gomez-Isla, T., Newell, K., George, J. M., Clayton, D. F., and Hyman, B. T. (1998) J. Neuropathol. Exp. Neurol. 57, 334–337
5. Spillantini, M. G., Crowther, R. A., Jakes, R., Hasegawa, M., and Goedert, M. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 6469–6473
6. Lippa, C. F., Fujisawa, H., Mann, D. M. A., Giasson, B., Baba, M., Schmidt, M. L., Nee, L. E., O’Connell, B., Pollen, D. A., George-Hyslop, P. S., Ghetti, B., Nochlin, D., Bird, T. D., Cairns, N. J., Lee, V. M. Y., Iwatsubo, T., and Trojanowski, J. Q. (1999) J. Neuropathol. Exp. Neurol. 58, 952–962
7. Lippa, C. F., Schmidt, M. L., Lee, V. M. Y., and Trojanowski, J. Q. (1999) Ann. Neurol. 45, 353–357
8. Wakabayashi, K., Yoshimoto, M., Tsuji, S., and Takahashi, H. (1998) Neurosci. Lett. 249, 180–182
Mapping the Lipid Binding Domain of α-Synuclein

9. Gai, W. P., Power, J. H., Blumbergs, P. C., and Blessing, W. W. (1998) Lancet 352, 547–548
10. Tu, P. H., Galvin, J. E., Baba, M., Giasson, B., Tomita, T., Leight, S., Nakajo, S., Iwatsubo, T., Trojanowski, J. Q., and Lee, V. M. Y. (1998) Ann. Neurol. 44, 415–422
11. Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dutra, A., Pike, B., Roel, H., Rubenstein, J., Boyer, R., Stenroos, S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, W. G., Lazzarini, A. M., Duvoisin, R. C., Di Iorio, G., Golbe, L. I., and Nussbaum, R. L. (1997) Science 276, 2045–2047
12. Kruger, R., Kuhn, W., Muller, T., Wei alta, D., Graeber, M., Kosel, S., Przuntek, H., Epplen, J. T., Schols, L., and Riess, O. (1998) Nat. Genet. 18, 106–108
13. Davidson, W. S., Jonas, A., Clayton, D. F., and George, J. M. (1998) J. Biol. Chem. 273, 9440–9449
14. Segrest, J., De Loof, H., Dohlman, J., Brouillette, C., and Anantharamaiah, G. (1990) Proteins Struct. Funct. Genet. 8, 103–117
15. Segrest, J., Jones, M., De Loof, H., Brouillette, C., Venkatachalapathi, Y., and Anantharamaiah, G. (1992) J. Lipid Res. 33, 141–166
16. Weinreb, P. H., Zhen, W., Poon, A. W., Conway, K. A., and Lansbury, P. T. J. (1996) Biochemistry 35, 2045–2047
17. Jensen, P. H., Nielsen, M. S., Jakes, R., Dotti, G., and Goedert, M. (1998) J. Biol. Chem. 273, 26292–26294
18. Green, N. M. (1970) Methods Enzymol. 18A, 418–424
19. Barenholz, Y., Gibbes, D., Littman, B. J., Gell, J., Thompson, T. E., and Carlson, R. D. (1977) Biochemistry 16, 2806–2810
20. Davidson, W. S., Rodriguez, W. V., Lund-Katz, S., Johnson, W. J., Rothblat, G. H., and Phillips, M. C. (1995) J. Biol. Chem. 270, 17106–17113
21. Sokoloff, L., and Rothblat, G. H. (1974) Proc. Soc. Exp. Biol. Med. 146, 1160–1172
22. Chen, Y. H., Yang, J. T., and Martinez, H. M. (1972) Biochemistry 11, 4120–4131
23. Sastry, P. S. (1985) Prog. Lipid Res. 24, 69–176
24. Lisovitch, M., Czarny, M., Fiucci, G., and Tang, X. (2000) Biochem. J. 345, 401–415
25. Martin, T. P. (1998) Annu. Rev. Cell Dev. Biol. 14, 231–264
26. Huang, K. P., and Huang, F. L. (1993) Neurochem. Int. 22, 417–33
27. Withers, G., George, J., Banker, G., and Clayton, D. F. (1997) Dev. Brain Res. 99, 87–94
28. Iwai, A., Maslia, E., Yoshimoto, M., Ge, N., Flanagan, L., Rohan de Silva, H., Kittel, A., and Saitoh, T. (1995) Neuron 14, 467–475
29. Salem, N., Jr., and Niebylski, C. D. (1995) Mol. Membr. Biol. 12, 131–4
30. Jenco, J. M., Rawlingson, A., Daniels, B., and Morris, A. J. (1998) Biochemistry 37, 4901–4909
31. Colley, W. C., Sung, T. C., Roll, R., Jenco, J., Hammond, S. M., Altschuller, Y., Barb-Sagi, D., Morris, A. J., and Frohman, M. A. (1997) Curr. Biol. 7, 191–201
32. Abeliovich, A., Schmitz, Y., Farinas, I., Choi-Lundberg, D., Ho, W. H., Castilla, P. E., Shiinsky, N., Verdugo, J. M., Armanini, M., Ryan, A., Hynes, M., Phillips, H., Sulzer, D., and Rosenthal, A. (2000) Neuron 25, 239–252
33. Stevens, C. F., and Wesseling, J. F. (1999) Neuron 24, 1017–1028
34. Dittman, J. S., and Regehr, W. G. (1998) J. Neurosci. 18, 6147–6162
35. Gomis, A., Hurvone, J., and Lagnado, L. (1999) J. Neurosci. 19, 6309–6317
36. Wang, L. Y., and Kazmierczak, L. K. (1998) Nature 394, 384–388
37. Clayton, D. F., and George, J. M. (1999) J. Neurosci. Res. 58, 120–129
38. Murphy, D. D., Rueter, S. M., Trojanowski, J. Q., and Lee, V. M. (2000) J. Neurosci. 20, 3214–3229
39. Souza, J. M., Giasson, B. I., Chen, Q., Lee, V. M., and Ischiropoulos, H. (2000) J. Biol. Chem. 275, 18344–18349
40. Hashimoto, M., Hsu, L. J., Xia, Y., Takeda, A., Sisk, A., Sundsmo, M., and Masliah, E. (1999) Neureport 10, 717–721
41. Refsgaard, H. H., Tsai, L., and Stadtman, E. R. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 611–616
42. Ueda, K., Fukushima, H., Masliah, E., Xia, Y., Iwai, A., Yoshimoto, M., Otero, D., Kondo, J., Ibara, Y., and Saitoh, T. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 11262–11266