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The fusogenic tilted peptide (67–78) of α-synuclein is a cholesterol binding domain

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ABSTRACT

Parkinson's disease-associated α-synuclein is an amyloidogenic protein not only expressed in the cytoplasm of neurons, but also secreted in the extracellular space and internalized into glial cells through a lipid raft-dependent process. We previously showed that α-synuclein interacts with raft glycosphingolipids through a structural motif common to various viral and amyloidogenic proteins. Here we report that α-synuclein also interacts with cholesterol, as assessed by surface pressure measurements of cholesterol-containing monolayers. Using a panel of recombinant fragments and synthetic peptides, we identified two distinct cholesterol-binding domains in α-synuclein. One of these domains, which corresponds to the tilted peptide of α-synuclein (67–78), bound cholesterol with high affinity and was toxic for cultured astrocytes. Molecular modeling suggested that cholesterol binds to this peptide with a tilt angle of 46°. α-synuclein also contains a cholesterol recognition consensus motif, which had a lower affinity for cholesterol and was devoid of toxicity. This motif is encased in the glycosphingolipid-binding domain (34–45) of α-synuclein. In raft-like model membranes containing both cholesterol and glycosphingolipids, the head groups of glycosphingolipids prevented the accessibility of cholesterol to exogenous ligands. Nevertheless, cholesterol appeared to 'signal' its presence by tuning glycosphingolipid conformation, thereby facilitating α-synuclein binding to raft-like membranes. We propose that the association of α-synuclein with lipid rafts involves both the binding of α-synuclein (34–45) to glycosphingolipids, and the interaction of the fusogenic tilted peptide (67–78) with cholesterol. Coincidentally, a similar mechanism is used by viruses (HIV-1, HTLV-I, Ebola) which display a tilted peptide and fuse with host cell membranes through a sphingolipid/cholesterol-dependent process.

1. Introduction

α-synuclein is 140 kDa protein expressed in the brain, involved in the traffic of synaptic vesicles, and associated with Parkinson's disease (PD) [1]. It belongs to the category of intrinsically disordered proteins (IDPs) that lack a stable 3D structure in water but can adopt various conformations depending on environmental factors [2]. Although generally considered as a cytoplasmic protein, recent data have shown that α-synuclein is also secreted from neuronal cells [3]. In line with these data, α-synuclein has been detected in extracellular biological fluids, including human cerebrospinal fluid and blood plasma, in both PD and normal human subjects [4]. Several studies have shown that α-synuclein can penetrate into various cell types, including neurons, microglia, platelets, and fibroblasts [5–8]. In addition, α-synuclein oligomers can form annular structures with pore-like properties that might affect membrane permeability in the brain cells of PD patients [9].

Several studies have shown that α-synuclein has an affinity for lipid rafts, suggesting a role for these membrane microdomains enriched in sphingolipids and cholesterol in the normal and/or the pathological functions of α-synuclein [6,10–12]. In vitro, α-synuclein binds to model membranes, especially those containing typical raft components such as phosphatidylserine [11] and glycosphingolipids (GSLs) [13]. We recently reported that α-synuclein interacts with GSLs through a specific domain spanning amino acid residues 34–45 of the protein [14]. This GSL-binding motif (GBM), which is structurally conserved among several virus, bacterial, and amyloidogenic proteins [15], mediates the insertion of α-synuclein in raft-like membranes containing various GSLs such as gangliosides GM1 and GM3. A growing body of evidence suggests that these gangliosides have a strong impact on the biological activity of α-synuclein. On one hand, GM1 has been shown to inhibit α-synuclein fibrillation [13] and to promote its internalization into microglial cells [6]. On the other hand, GM3 markedly affected the function of α-synuclein channels in membrane bilayers [16]. Interestingly, the GSL that displays the highest affinity for α-synuclein [14], i.e. GM3, is the most abundant

Abbreviations: βMCD, β-methyl-cyclodextrin; CRAC, cholesterol recognition/interaction amino acid consensus sequence; GBM, glycosphingolipid-binding motif; GSL, glycosphingolipid; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; $\Delta\pi_{max}$, maximal surface pressure increase; π_c, critical pressure of insertion; π_i, initial surface pressure; NAC, sequence 61–95 of α-synuclein, originally termed non β-amyloid component; PD, Parkinson disease; SBD, sphingolipid-binding domain; v_i, initial velocity

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ganglioside expressed by astrocytes [17]. This finding supports the view that the interaction of extracellular α-synuclein with glial cells might play an important role in PD [18].

Apart from GSLs, the exofacial leaflet of lipid raft domains contains two other major lipids, sphingomyelin and cholesterol [19]. Recent data suggested that α-synuclein has no particular affinity for sphingomyelin [14]. In contrast, cholesterol has been reported to promote α-synuclein aggregation in transfected neuronal cells [20]. Moreover, statin treatment of α-synuclein transgenic mice was associated with a marked reduction of α-synuclein aggregation and abrogation of neuronal pathology [21]. A possible interpretation of these data could be that α-synuclein physically interacts with cholesterol and that this lipid controls α-synuclein aggregation. Yet little is known about the cholesterol-binding properties of α-synuclein at the molecular level [22].

In the present study, we have analyzed the interaction of α-synuclein with cholesterol with a combination of physicochemical, computational, and cellular approaches. The Langmuir film balance technique [23] was used to study the insertion of recombinant α-synuclein (full-length and fragments) and synthetic α-synuclein peptides into cholesterol-containing monolayers at the air–water interface. We showed that although α-synuclein displays a cholesterol recognition/interaction amino acid consensus sequence (CRAC) spanning amino acid residues 37–43, this domain behaves as a low affinity cholesterol-binding site. This CRAC domain is encased in the GBM (34–45) of α-synuclein. In addition, we have discovered a second, high-affinity cholesterol-binding motif in the 67–78 region. This domain corresponds to the previously characterized fusogenic tilted peptide of α-synuclein [24]. We discussed the respective roles of the GSL and cholesterol-binding domains of α-synuclein and proposed that GSLs and cholesterol could act in synergy to promote the insertion of α-synuclein in lipid rafts through a virus-like fusion mechanism.

2. Materials and methods

2.1. Materials

Recombinant human α-synuclein was obtained from rPeptide (Bogart, GA). Recombinant α-synuclein fragments [8] were purchased from ATGen Co., Ltd. (Seongnam-si, South Korea). Synthetic peptides with a purity>95% were obtained from Schafer-N (Copenhagen, Denmark). Ultrapure apyrogenic water was from Biorad (Marnes La Coquette, France). All lipids were purchased from Matreya (Pleasant Gap, PA).

2.2. Detection of a CRAC domain in α-synuclein

We used the consensus of the cholesterol recognition amino acid (CRAC) consensus sequence determined by Jamin et al. [25] (L/V)–X_{1-5}–(Y)–X_{1-5}–(K,R) to identify potential cholesterol binding domains in α-synuclein.

2.3. In silico studies of α-synuclein-cholesterol interactions

Molecular modeling studies were performed with the Hyperchem 8 program (ChemCAD, Obernay, France). To generate peptide–cholesterol complexes, one cholesterol molecule was positioned in the vicinity of the peptide (manual search for geometric fit). Several initial conditions were tested (with distinct orientations of cholesterol with respect to the tilted peptide) to select the complex having the highest energy of interaction. In particular, initial conditions in which the methyl groups of cholesterol were not directed towards the α-helical peptide did not allow the formation of a stable complex. Geometry optimization of the selected complex was first achieved using the unconstrained optimization rendered by the Polak–Ribière conjugate gradient algorithm [14]. Molecular dynamics simulations were then performed for 10 ns in vacuo with the Bio+ (CHARMM) force field [26] of the Hyperchem software suite. The energy of interaction was determined with the Molegro Molecular Viewer [27].

2.4. Lipid monolayer assay

α-Synuclein-lipid interactions were studied with the Langmuir film balance technique [23]. The surface pressure of lipid monolayers [14] was measured with a fully automated microtensiometer (μTROUGH SX, Kibron Inc. Helsinki, Finland). All experiments were carried out in a controlled atmosphere at 20 ± 1 °C. The lipids were dissolved in hexane: chloroform:ethanol (11:5:4, vol:vol:vol) at a concentration of 1 mg.mL^{-1} and stored at −20 °C under saturated nitrogen atmosphere. Monomolecular films of the indicated lipid were spread on ultrapure water subphases totally devoid of any surfactant contaminant as described previously [28]. After spreading of the film, 5 min was allowed for solvent evaporation. Stock solutions of recombinant proteins and synthetic peptides were prepared in ultrapure water, stored at −20 °C and thawed immediately before use. 8 μL of the protein (or the synthetic peptide) solution were injected in the subphase (800 μL, pH 7) with a 10-μl Hamilton syringe, and pressure increases produced were continuously recorded as a function of time. The surface pressure at the time of injection is indicated in the figure legends. Control experiments were run in parallel to ensure that the injection of 8 μL of the ultrapure water in which proteins and peptides were dissolved did not induce by itself any surface pressure change. The data were analyzed with the FilmWareX 3.57 program (Kibron Inc.). The accuracy of the system under our experimental conditions was ±0.25 mN.m^{-1} for surface pressure.

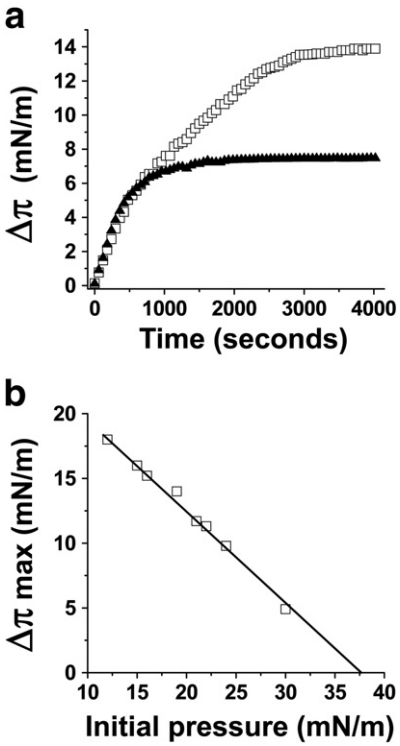

Fig. 1. Interaction of recombinant α-synuclein with cholesterol. a. Kinetics studies of recombinant α-synuclein (100 nM) interaction with pure cholesterol (□) or mixed cholesterol:POPC (1:1, mol:mol) (▲) monolayers. The data show the real-time changes of surface pressure following injection of 100 nM recombinant α-synuclein beneath each lipid monolayer prepared at an initial surface pressure of 17 mN.m^{-1}. Each curve is representative of three separate experiments (SD<10%). b. Interaction of recombinant α-synuclein (100 nM) with pure cholesterol monolayers prepared at various values of the initial surface pressure. The maximal surface pressure ($\Delta\pi_{max}$) was determined after reaching the equilibrium. The critical pressure of insertion (π_c) is the value of the pressure extrapolated at the intercept of the $\Delta\pi_{max} = f(\pi_i)$ slopes.