Polyglutamine expansion in huntingtin increases its insertion into lipid bilayers

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**Abstract**

An expanded polyglutamine (Q) tract (>37Q) in huntingtin (htt) causes Huntington disease. Htt associates with membranes and polyglutamine expansion in htt may alter membrane function in Huntington disease through a mechanism that is not known. Here we used differential scanning calorimetry to examine the effects of polyQ expansion in htt on its insertion into lipid bilayers. We prepared synthetic lipid vesicles composed of phosphatidylcholine and phosphatidylethanolamine and tested interactions of htt amino acids 1–89 with 20Q, 32Q or 53Q with the vesicles. GST-htt1–89 with 53Q inserted into synthetic lipid vesicles significantly more than GST-htt1–89 with 20Q or 32Q. We speculate that by inserting more into cell membranes, mutant huntingtin could increase disorder within the lipid bilayer and thereby disturb cellular membrane function.

**Introduction**

Huntington disease (HD) is caused by an expanded CAG repeat in the HD gene which encodes the protein huntingtin (htt). The CAG repeat is translated into an expanded glutamine (Q) repeat near the N-terminus of htt, a large protein comprised of 3144 amino acids. Htt is a soluble protein that is normally found in association with membranes in cells [1–3] and in brain [4,5]. We found that htt with a polyglutamine stretch in the normal range (<37Q) associates in vitro with large unilamellar vesicles (LUVs) containing specific phospholipids including: PI(3,4)P2, PI(3,5)P2, PI(3,4,5)P3 and cardiolipin [6]. A cationic membrane-binding domain of amino acids 171–372 is necessary for htt-membrane association in cells [7]. However, htt residues 1–18 and 1–89 (exon1) fused to GFP or GST respectively, can also target to membranes [8,9], and htt1–102 can interact with phospholipids, although much less than longer htt fragments containing htt amino acids 171–372 [6].

Htt interactions with membrane phospholipids are polyglutamine-dependent. For instance, compared to wild-type full-length htt with 7Q, mutant htt with 140Q had increased association with vesicles enriched in phosphatidylethanolamine (PE) as measured by binding to LUVs followed by sedimentation through sucrose gradients [6]. In vitro translated mutant htt1–102 with 100Q interacts more with lipid vesicles compared to its wild-type counterpart with 18Q. These data suggest that polyglutamine expansion in htt increases its membrane phospholipids association and that a region within amino acids 1–102 of htt is responsible for the interactions.

Pure polyglutamine tracts can form channels in planar lipid bilayers [10,11], indicating that polyglutamine tracts alone could damage membranes. A key question is whether polyglutamine expansion in the context of htt can alter htt membrane insertion. An increase in membrane insertion with expanded polyglutamine would provide support for a direct effect on membrane stability as a potential pathogenic mechanism in HD. Previous work using a dye release assay showed that htt1–89 could disrupt lipid bilayers but no change with polyglutamine length was found [9]. Differential scanning calorimetry (DSC) is a much more sensitive method for detecting membrane disturbances since it can detect small changes in the physical properties of lipid bilayers caused by interactions with the membrane, whereas dye release assays require outright loss of membrane integrity. In this study, we used DSC...
to study the effects of polyglutamine expansion near the N-termi-
nus of htt on membrane insertion into PE containing vesicles.
Results showed that polyglutamine expansion increased penetra-
tion of GST-htt1–89 into the lipid bilayer. These data support htt
interactions with membranes through direct phospholipid associ-
ation and membrane insertion. Through enhanced phospholipid
interaction and membrane insertion, mutant htt may change
normal membrane function.

Materials and methods

Western blots and silver staining. Proteins were separated by
SDS–PAGE using 4–12% bis–tris gels (Invitrogen); proteins were
transferred to nitrocellulose using iBlot semi-dry transfer apparatu-
us (Invitrogen) and developed using chemiluminescence or gels
were silver-stained using the Silver Stain Plus kit (BioRad). Blots
were probed with Ab1, an antibody to the N-terminus of htt
(htt1–17) [4].

Preparation of GST-htt fusion proteins. GST-htt exon1 with 18Q,
32Q, or 53Q were expressed in Escherichia coli BL21 Gold (Strata-
gene, La Jolla, CA) grown in 2YT broth and induced with isopropyl
1-thio-β-D-galactopyranoside. Proteins were purified and prepared
as previously described [7] and purity was verified by Coomassie
staining (BioSafe Coomassie G250 stain, BioRad) following SDS-
PAGE.

Differential scanning calorimetry. The phospholipids, 1,2-dimyri-
stoyl-sn-glycero-3-phosphocholine (DMPC) and 1-palmitoyl-2-
oleyl-sn-glycero-3-phosphoethanolamine (POPE) were purchased
from Avanti Polar Lipids and used without further purification. Li-
ipid stock solutions were prepared by dissolving the lipid powders
in chloroform/methanol 2/1 (v/v). From aliquots of these solutions,
a dry lipid film was formed on the walls of an extensively rinsed
glass beaker by evaporating the solvent with a stream of nitrogen
followed by vacuum desiccation for at least 3 h. The lipid film was
dissolved in 15 mM NaCl, 1 mM EDTA, 10 mM HEPES, pH 7.5 for
the preparation of multilamellar vesicles (MLVs). The lipid disper-
sion was then pressed 10 times through 200-nm filters to make
LUVs and used in experiments at these buffer conditions [12]. A
differential scanning calorimeter Q100 from TA Instruments was
used. Briefly, the reservoirs for the sample and reference solution
are made of stainless steel to hold a volume of around 100
l each. Lipid vesicles solutions are placed in the reference cell and
the lipid vesicle solutions plus the huntingtin constructs in the sample
cell. Under sealed conditions, both solutions are heated/cooled at a rate
of 0.5 °C/min between +7 °C and +35 °C in six to eight cycles until
the equilibrium of the phase transition enthalpy was reached,
using a mixture of DMPC:POPE (50:50 M ratio) at 5 mg/ml. A phase
transition was observed at around 20 °C. Traces from various cycles
(at different protein/lipid molar ratios) were averaged and data
analysis was performed using the software from Universal Analysis
2000 (TA Instruments) and Origin 7G [13].

Results

Protein–phospholipid interactions can modify membrane fluid-
ity [14]. Altered fluidity of lipid bilayers can be measured by vari-
ations in the thermodynamic behavior of the membrane, which is
determined by the amount of heat required to bring about a phase
transition of the lipid bilayer. Protein–lipid interactions also
change the melting temperature (Tm), which is otherwise a fixed
value intrinsic to the lipid composition of the membrane. Having
observed wild-type and mutant htt associated with synthetic lipids
vesicles [6], we asked if an N-terminal fragment bearing the poly-
glutamine region can change the membrane fluidity. We used DSC
to examine membrane insertion of bacterially expressed and
purified GST-htt1–89 with 20Q, 32Q or 53Q. GST-htt1–89 could
be purified in sufficient quantities needed for DSC measurements.
Purified proteins of the expected sizes of (~26 kDa for GST) and
35–45 kDa for GST-htt1–89 with 20Q, 32Q and 53Q were eluted
from the glutathione beads (Fig. 1). Note, that the polyglutamine
tract reduces mobility in SDS–PAGE beyond what is predicted.
Additional low molecular weight bands were observed on the Coo-
massie gel for each of the GST-htt products (Fig. 1A). We verified by
Western blot that the full-length protein was GST-htt and addi-
tional bands were proteolytic breakdown products of the ex-
gressed GST-htt fusion proteins (Fig. 1B) and that the band
around 26 kDa was GST (not shown). We used LUVs with the lipid
combination of DMPC:POPE (1:1 M ratio) that is suitable for DSC
analysis. Thermal heating profiles (heat flow in mW) are shown
in Fig. 2. The phase transition peak or melting temperature (Tm)
for DMPC:POPE (1:1) was ~19.2 °C. The relative flattening of the
curves together with a shift of Tm to lower temperatures at increas-
ing protein concentration are indicative for protein insertion into
lipid bilayers [15,16]. Results showed that, for LUVs composed of
DMPC:POPE, all three GST-htt1–89 proteins reduced the enthalpy
required to bring about a phase change compared to GST alone
(Fig. 3). The reduced enthalpy requirement reflects insertion of
GST-htt1–89 in the lipid bilayer, causing more disorder in the bi-
layer and a reduced energy requirement for melting. At 5 μM
protein concentration, LUVs required significantly less heat for phase
transition in the presence of GST-htt1–89Q32 compared to GST-
htt1–89Q20, and GST-htt1–89Q53 compared to GST-htt1–89Q20
(p < 0.0001, n = 4–6, unpaired Student’s t-test). At 10 μM protein
concentration, GST-htt1–89Q53 was significantly different from
both GST-htt1–89Q20 and GST-htt1–89Q32 (p < 0.0001, n = 6,
unpaired Student’s t-test). Protein concentrations higher than
10 μM (data not shown) gave variable results in the assay, possibly
due to increased aggregation of htt during the reaction [9]. Due to
the presence of proteolytic breakdown products in our preparation,
we cannot say whether the insertion was due to the GST-htt1–89
fusion protein or due to free htt1–89 or smaller htt fragments gen-
erated through proteolysis. GST alone did not insert into LUVs.
These results show that a region in htt1–89 can insert into a lipid
bilayer in a polyglutamine-dependent manner.

Discussion

We used DCS to show that a small N-terminal fragment of htt
inserts into vesicles composed of PC and PE, which are major

![Fig. 1. Purity of GST-htt fusion proteins. (A) Coomassie stained gel of GST and GST-htt fusion proteins. GST runs at ~26 kDa. GST-ht11–89Q20, GST-ht11–89Q32 and GST-ht11–89Q53 run between 35 and 45 kDa (arrows). Due to proteolysis, GST alone was present in all preparations (arrowhead). Molecular mass is indicated in kDa on the left. (B) Western blot analysis was performed using anti-htt antibody Ab1. The lower molecular mass products (easily visible by Coomassie staining) were confirmed to be proteolytic products of htt by Western blot analysis.](image-url)
constituents of biological membranes. We found a decreased enthalpy (reduced $\Delta H_H$) for phase transition observed with increasing polyglutamine length, indicating that polyglutamine expansion in htt increases its insertion into lipid bilayers. Previous studies using a dye release assay showed an association of htt1–89 with lipid vesicles that was not polyglutamine-dependent. Using a more sensitive method (DSC), we were able to demonstrate that polyglutamine expansion increases htt1–89 insertion into lipid bilayers.

Whether the increased insertion into model membranes by a purified mutant htt fragment is sufficient to explain membrane dysfunction in vivo is unknown. Early studies of membranes from control or HD brains suggested that the HD mutation could alter the physical properties of membranes. Membranes from HD brain samples showed increased binding affinities of tritium-labeled $\gamma$-aminobutyric acid (GABA) that were normalized by treatment with Triton X-100, phospholipase C, and glycerophospho-ethanolamine (a metabolite of PE) [17]. It is noteworthy that the addition of the polyunsaturated fatty acid ethyl-eicosapentaenoic acid (ethyl-EPA) to the diet reduces motor symptoms in a HD mouse model [18] and reduces motor symptoms and brain atrophy in HD patients [19,20]. The mechanism through which ethyl-EPA exerts its effect is unknown, but ethyl-EPA changes the physical properties of model membranes, including the melting temperature [21]. Thus, EPA may work to counteract adverse effects of mutant htt on the physical properties of biological membranes. A more dramatic effect on neuronal membranes would occur if mutant htt formed ionic conductance channels, which could dissipate membrane potentials across the plasma membrane and in mitochondria [22]. Pure polyglutamine tracts can form channels that accommodate physiological ions such as $K^+$, $Na^+$ and $H^+$ in planar lipid bilayers [22]. However, these studies did not provide direct evidence for an alteration in the lipid bilayer by mutant htt.

There is ample evidence for membrane dysfunction in a HD transgenic mouse expressing mutant htt1–89 (R62) with large polyglutamine repeats (>120Q). This mouse model develops a severe motor dysfunction and early death [23]. Levels of receptors important for normal synaptic transmission and membrane
conductance, including NMDARs, GluR1 and GluR2 were changed in post-synaptic membranes isolated from R6/2 mice [9]. Amphip- physin and synaptojanin are membrane-associated proteins important for vesicle trafficking of such receptors [24] and their levels were also changed in the same membrane preparations [9].

In summary, our findings using DSC suggest that mutant htt causes direct disturbance in the stability of the lipid bilayer and thereby could be a basis for the membrane dysfunction previously observed in brain of HD patients and R62 mice. Mutant htt could also indirectly affect membrane function by changing the physical properties of lipid bilayers and by altering binding of other soluble proteins. We propose that mutant htt interaction at membranes may represent a target for therapeutic intervention in HD.

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References


