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Structure–activity relationship study on polyglutamine binding peptide QBP1

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ABSTRACT

Aggregation and deposition of expanded polyglutamine proteins in the brain cause neurodegenerative diseases including Huntington disease. This pathogenic process is suppressed and delayed in the presence of polyglutamine binding peptide 1 (QBP1), which we previously identified as an undecapeptide binding to pathogenic polyglutamine proteins from phage display peptide libraries. In this paper, a structure–activity relationship study on QBP1 was conducted to determine the pharmacophores for inhibition of polyglutamine aggregation. Furthermore, a truncation study identified an octapeptide as the minimum structure for suppressing aggregation of polyglutamine proteins, which is equipotent to the parent undecapeptide QBP1.

1. Introduction

Conformational neurodegenerative diseases, such as Alzheimer and Parkinson disease, are caused by the abnormal accretion and deposition of misfolded proteins in the central nervous system.[1] Among them, the polyglutamine (polyQ) diseases, including Huntington disease, various types of spinocerebellar ataxia, spinobulbar muscular atrophy, and dentatorubral pallidoluysian atrophy, are caused by proteins containing an expanded polyQ repeat (more than 40 glutamines) encoded by a common mutation of a consecutive CAG trinucleotide repeat expansion.[2,3] In the pathogenesis of the polyQ diseases, a conformational change of the polyQ stretch caused by its pathogenic expansion is thought to be a key step leading to aggregation of the polyQ protein, which eventually results in its visible deposition as inclusion bodies inside affected neurons. Although the mechanism of polyQ aggregate formation has not been revealed, our recent study suggests that a β-sheet conformational transition of the expanded polyQ protein monomer precedes its assembly into β-sheet-rich amyloid-like fibrils.[4,5] To date, several inhibitors of polyQ protein aggregation have been identified. They include small peptides binding to the polyQ stretch,[6] intracellular antibodies (intrabodies) against the polyQ proteins,[7,8] molecular chaperones targeting misfolded proteins,[9–11] and small chemical compounds.[12–15] In spite of these findings, there are currently no effective therapies clinically available for polyQ disease patients.

We previously reported six novel undecapeptides binding to the expanded polyQ stretch, which were identified from combinatorial peptide phage display libraries (Table 1).[16] Among them, polyglutamine binding peptide 1 (QBP1) binds to pathologic-length polyQ more selectively compared to the other QBPs.[16] The inhibition of polyQ protein oligomerization by QBP1 is significantly observed not only against the purified polyQ protein but also that in cells.[17] A tandem repeat of QBP1, co-expressed with pathogenic polyQ proteins, rescues polyQ-induced compound eye generation and premature death in *Drosophila melanogaster*.[18] Furthermore, protein transduction domain (PTD)[19–21]-conjugates of QBP1 suppress polyQ-induced neurodegeneration in animal models.[22,23] Although the mechanisms of inhibition of polyQ protein aggregation by QBP1 has not been completely revealed, our recent research suggests that QBP1 prevents the toxic β-sheet conformational transition of the pathologic-length polyQ protein monomer as well as its subsequent oligomer formation.[4,5] These results indicate that QBP1 is a promising lead compound for preventing disease onset and for slowing progression of the polyQ diseases. Our previous work on several QBP1 derivatives demonstrated that truncation of two N-terminal (Ser1 and Asn2) and one C-terminal (Asp11) amino acid residue is tolerable,[24] and that a positively charged amino acid adjacent to the Trp residue is conserved among QBPs identified from phage display screening (Table 1). These data could be useful for development of novel inhibitors; however, a more comprehensive investigation is needed for elucidation of the structural requirements of QBP1. In this study, we conducted

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Table 1
Polyglutamine binding peptides (QBPs) identified from phage display peptide libraries

Peptide	Sequence
QBP1	Ac-Ser-Asn-Trp-Lys-Trp-Trp-Pro-Gly-Ile-Phe-Asp-NH_2
QBP2	Ac-His-Trp-Trp-Arg-Ser-Trp-Tyr-Ser-Asp-Ser-Val-NH_2
QBP3	Ac-His-Glu-Trp-His-Trp-Trp-His-Gln-Glu-Ala-Ala-NH_2
QBP4	Ac-Trp-Gly-Leu-Glu-His-Phe-Ala-Gly-Asn-Lys-Arg-NH_2
QBP5	Ac-Trp-Trp-Arg-Trp-Asn-Trp-Ala-Thr-Pro-Val-Asp-NH_2
QBP6	Ac-Trp-His-Asn-Tyr-Phe-His-Trp-Trp-Gln-Asp-Thr-NH_2

alanine- and D-amino acid scanning of QBP1 to identify the pharmacophores for inhibitory activity against oligomerization of the polyQ protein and to investigate the importance of stereochemistry of each amino acid residue, respectively. Moreover, we examined a series of N- and/or C-terminal truncated QBP1 derivatives to determine the minimum sequence for inhibition of polyQ aggregation.

2. Results and discussion

2.1. Synthesis of QBP1 derivatives

All peptide chains were constructed by standard Fmoc-based solid phase peptide synthesis (Fmoc-SPPS) on Rink-amide resin. N-Terminal acetylation was operated by treatment with Ac_2O in the presence of *i*-Pr_2NEt. Final deprotection and cleavage from the resin with the cocktail, TFA/thioanisole/*m*-cresol/1,2-ethanedithiol/H_2O (80:5:5:5:5), followed by RP-HPLC purification afforded the peptides as TFA salts. All peptides were identified with MALDI-TOF-MS and the purity was more than 95% by analytical HPLC.

2.2. Biological evaluation of synthetic peptides

The bioactivity of synthesized peptides was evaluated based on the inhibitory potency against aggregation of a thioredoxin-conjugated polyQ protein (62-mer), which has advantages of high solubility, possibility of high concentration expression in *Escherichia coli* (*E. coli*), and easy purification.[25] Turbidity of this polyQ protein was traced by measuring at 405 nm. PolyQ protein aggregation increases in a time- and concentration-dependent manner and eventually reaches a plateau.[16] Relative inhibitory activity (% inhibition) induced by 20 μM of each QBP1 derivative was evaluated in duplicate by comparison to the plateau level (0% inhibition). Furthermore, IC_{50} values were calculated for potent inhibitors, exerting more than 50% inhibition at 20 μM.

2.3. Structure–activity relationships studies on QBP1

2.3.1. Alanine scanning of QBP1

In order to identify pharmacophores for the prevention of polyQ protein aggregation, each amino acid residue of QBP1 was substituted with alanine (Table 2). Replacement of Trp3, Trp5, Trp6, Ile9 and Phe10 resulted in a significant decrease of inhibitory activities (peptides **3**, **5**, **6**, **9** and **10**; less than 20% inhibition at 20 μM). This result indicates that functional groups of these five residues are involved in inhibition of polyQ protein aggregation through direct or indirect interaction. All QBPs identified from phage display libraries[16] possess more than two aromatic amino acid residues, in which at least one is a tryptophan residue (Table 1). Based on this result, aromatic groups, especially indole rings of tryptophan, might contribute to the biological activity.

On the other hand, alanine-substitutions of Ser1, Asn2, Lys4, Pro7, Gly8 and Asp11 are tolerable (peptides **1**, **2**, **4**, **7**, **8** and **11**; IC_{50} < 20 μM). Similar results were obtained for Lys4 and Pro7 substitutions in our previous study, which showed that each of them can be replaced with valine.[24] All hydrophilic functional groups of the side chain, hydroxy (Ser1), primary amide (Asn2), amine (Lys4) and carboxylic acid (Asp11), are not required for inhibition of polyQ aggregation. Taken together with the above result indicating that hydrophobic amino acid residues are pharmacophores, it is conceivable that hydrophobicity of QBP1 is important for its interaction with polyQ proteins. This is supported by the result that the C-terminal tetrapeptide (Gly-Ile-Phe-Asp) is replaceable with a tryptophan-rich tetrapeptide (Trp-Lys-Trp-Trp) without the loss of inhibitory activity.[24] Although proline and glycine are often ob-

Table 2
Alanine and D-amino acid scanning of QBP1

Peptide	Sequence	% Inhibition	IC_{50} (μM)
QBP1	Ac-Ser-Asn-Trp-Lys-Trp-Trp-Pro-Gly-Ile-Phe-Asp-NH_2	76.7	5.0
Alanine scanning			
1	Ac-*Ala*-Asn-Trp-Lys-Trp-Trp-Pro-Gly-Ile-Phe-Asp-NH_2	78.2	6.3
2	Ac-Ser-*Ala*-Trp-Lys-Trp-Trp-Pro-Gly-Ile-Phe-Asp-NH_2	82.2	4.3
3	Ac-Ser-Asn-*Ala*-Lys-Trp-Trp-Pro-Gly-Ile-Phe-Asp-NH_2	−4.0	—[a]
4	Ac-Ser-Asn-Trp-*Ala*-Trp-Trp-Pro-Gly-Ile-Phe-Asp-NH_2	78.2	8.5
5	Ac-Ser-Asn-Trp-Lys-*Ala*-Trp-Pro-Gly-Ile-Phe-Asp-NH_2	−5.4	—[a]
6	Ac-Ser-Asn-Trp-Lys-Trp-*Ala*-Pro-Gly-Ile-Phe-Asp-NH_2	−10.4	—[a]
7	Ac-Ser-Asn-Trp-Lys-Trp-Trp-*Ala*-Gly-Ile-Phe-Asp-NH_2	58.9	10.0
8	Ac-Ser-Asn-Trp-Lys-Trp-Trp-Pro-*Ala*-Ile-Phe-Asp-NH_2	64.9	11.5
9	Ac-Ser-Asn-Trp-Lys-Trp-Trp-Pro-Gly-*Ala*-Phe-Asp-NH_2	9.9	—[a]
10	Ac-Ser-Asn-Trp-Lys-Trp-Trp-Pro-Gly-Ile-*Ala*-Asp-NH_2	11.4	—[a]
11	Ac-Ser-Asn-Trp-Lys-Trp-Trp-Pro-Gly-Ile-Phe-*Ala*-NH_2	67.8	15.6
D-Amino acid scanning			
12	Ac-*D-Ser*-Asn-Trp-Lys-Trp-Trp-Pro-Gly-Ile-Phe-Asp-NH_2	83.2	4.4
13	Ac-Ser-*D-Asn*-Trp-Lys-Trp-Trp-Pro-Gly-Ile-Phe-Asp-NH_2	80.7	4.5
14	Ac-Ser-Asn-*D-Trp*-Lys-Trp-Trp-Pro-Gly-Ile-Phe-Asp-NH_2	41.1	—[a]
15	Ac-Ser-Asn-Trp-*D-Lys*-Trp-Trp-Pro-Gly-Ile-Phe-Asp-NH_2	9.9	—[a]
16	Ac-Ser-Asn-Trp-Lys-*D-Trp*-Trp-Pro-Gly-Ile-Phe-Asp-NH_2	6.4	—[a]
17	Ac-Ser-Asn-Trp-Lys-Trp-*D-Trp*-Pro-Gly-Ile-Phe-Asp-NH_2	8.4	—[a]
18	Ac-Ser-Asn-Trp-Lys-Trp-Trp-*D-Pro*-Gly-Ile-Phe-Asp-NH_2	25.7	—[a]
19	Ac-Ser-Asn-Trp-Lys-Trp-Trp-Pro-*D-Ala*-Ile-Phe-Asp-NH_2	16.3	—[a]
20	Ac-Ser-Asn-Trp-Lys-Trp-Trp-Pro-Gly-*D-Ile*-Phe-Asp-NH_2	33.2	—[a]
21	Ac-Ser-Asn-Trp-Lys-Trp-Trp-Pro-Gly-Ile-*D-Phe*-Asp-NH_2	65.3	>20.0
22	Ac-Ser-Asn-Trp-Lys-Trp-Trp-Pro-Gly-Ile-Phe-*D-Asp*-NH_2	77.2	5.0

[a] Not tested.

Table 3
Sequence and biological activity of QBP1 and its truncated analogs

Peptide	Sequence	% Inhibition	IC_{50} (μM)
QBP1	Ac-Ser-Asn-Trp-Lys-Trp-Trp-Pro-Gly-Ile-Phe-Asp-NH_2	76.7	5.0
N-Terminal truncation			
23	Ac-Asn-Trp-Lys-Trp-Trp-Pro-Gly-Ile-Phe-Asp-NH_2	78.2	5.0
24	Ac-Trp-Lys-Trp-Trp-Pro-Gly-Ile-Phe-Asp-NH_2	81.2	2.9
25	Ac-Lys-Trp-Trp-Pro-Gly-Ile-Phe-Asp-NH_2	5.4	—[a]
26	Ac-Trp-Trp-Pro-Gly-Ile-Phe-Asp-NH_2	5.0	—[a]
C-Terminal truncation			
27	Ac-Ser-Asn-Trp-Lys-Trp-Trp-Pro-Gly-Ile-Phe-NH_2	57.4	14.6
28	Ac-Ser-Asn-Trp-Lys-Trp-Trp-Pro-Gly-Ile-NH_2	7.4	—[a]
29	Ac-Ser-Asn-Trp-Lys-Trp-Trp-Pro-Gly-NH_2	5.9	—[a]
30	Ac-Ser-Asn-Trp-Lys-Trp-Trp-Pro-NH_2	11.4	—[a]
NC-Terminal truncation			
31	Ac-Trp-Lys-Trp-Trp-Pro-Gly-Ile-Phe-NH_2	85.6	3.6

[a] Not tested.

served in secondary structures of many bioactive peptides,[26] the substitutions of these amino acid residues in QBP1 with alanine are tolerable, which suggests that they are not involved in the stabilization of a turn or helix substructure. However, removal of Pro7 led to the significant loss of inhibitory activity,[24] indicating that Pro7 works as a spacer between pharmacophores in QBP1.

2.3.2. D-Amino acid scanning of QBP1

Next, D-amino acid scanning of QBP1 was performed in order to explore the importance of stereochemistry of each amino acid residue in QBP1 (Table 2). D-Amino acids can stabilize or destabilize some secondary structures such as β-turn and α-helix conformations, which are often observed as interactive surfaces in bioactive peptides and proteins.[26] The chirality inversion of each residue in the N- or C-terminal region (Ser1, Asn2, Phe10 and Asp11) retained the inhibitory activity (more than 60% inhibition at 20 μM). On the other hand, significantly less bioactivity was observed in the epimeric peptides with a substitution in the internal region (Trp3 to Ile9; less than 50% inhibition at 20 μM), especially from Lys4 to Trp6 (less than 10% inhibition at 20 μM). Although side chain functional groups of Lys4 and Pro7 were not required for inhibition of polyQ aggregation according to the above alanine-scanning study, their (*S*)-configurations are important for the potent inhibitory activity. The potency of peptide **19** (16.3% inhibition at 20 μM) having D-alanine at the original Gly8 position of QBP1 was significantly lower than the L-alanine-substituted congener **8** (64.9% inhibition at 20 μM). Taken together, substitution with D-amino acid residues in QBP1 is disfavored, and the effect of stereochemistry is dependent on the position of the amino acid residue. The internal region is more sensitive to chirality inversion compared to the terminal regions. These results imply that the substitution of D-amino acid disturbs the active conformation of QBP1, although circular dichroism (CD) analysis did not suggest a stabilized secondary structure of QBP1 and any conformational differences between QBP1 and peptides **12**–**22** (see Supplementary data).

2.3.3. Truncated QBP1 analogs

Next, truncated analogs of QBP1 were evaluated to identify the minimum structure for inhibition of polyQ aggregation and to reduce its molecular size (Table 3), which could help towards development of novel low-molecular-weight inhibitors for polyQ aggregation. Initially, we removed N- and C-terminal amino acid residues, respectively. Among the N-terminally truncated analogs **23**–**26**, while N-terminal Ser1- **23** and Ser1-Asn2 dipeptide-truncated **24** analogs exerted anti-aggregation activity (78.2% and 81.2% inhibition at 20 μM, respectively), loss of activity was observed by removal of the N-terminal tripeptide and tetrapeptide containing Trp3 and Lys4 (peptides **25** and **26**, 5.4% and 5.0% inhibition at 20 μM, respectively). This observation is compatible with the result of alanine scanning (Table 1, peptide **3**), which showed that the indole ring of Trp3 is required for binding to the polyQ protein. In the C-terminal truncation, although removal of the C-terminal Asp11 residue was tolerable for maintenance of inhibitory activity (peptide **27**, 57.4% inhibition at 20 μM), the other C-terminally truncated analogs **28**–**30** showed significantly lower inhibitory activity (<20% inhibition at 20 μM). This may be caused by the loss of Ile9 and Phe10, which are also indispensable amino acid residues like Trp3 (Table 1, peptides **10**, **11**). Based on the above results, the octapeptide analog **31** equipotent (IC_{50} = 3.6 μM) to the parent peptide QBP1 (IC_{50} = 5.0 μM) was obtained. This octapeptide **31** seems to be the minimum peptide exerting inhibitory activity against aggregation of polyQ proteins.[24]

3. Conclusion

We conducted a structure–activity relationship study on QBP1 for inhibition of polyQ aggregation. Trp3, Trp5, Trp6, Ile9 and Phe10 were identified as pharmacophores for anti-aggregation of polyQ proteins through alanine-scanning. D-Amino acid scanning showed the importance of (*S*)-stereochemistry of each residue. Although this result may suggest the existence of a specific active conformation of QBP1, further investigation is required for its identification. Moreover, combination of the N- and C-terminal truncation studies identified the minimum octapeptide structure of the polyQ aggregation inhibitor, which includes five indispensable amino acids identified by alanine scanning. The information from the present study will be useful not only for the development of novel low-molecular-weight agents binding to polyQ proteins but also for elucidation of the mechanism of anti-polyQ aggregation activity.

4. Experimental

4.1. General procedure for preparation of peptides

The protected peptide-resin was manually constructed using Fmoc-based solid-phase synthesis on Novasyn® TGR resin (0.26 mmol/g, 190 mg, 0.05 mmol). *t*-Bu ester for Asp, *t*-Bu for Ser, Boc for Lys, and Trt for Asn were employed for side-chain protection. Fmoc-protected amino acid derivatives (0.15 mmol, 3.0 equiv) were successively condensed using *N*,*N*′-diisopropylcarbodiimide (DIC; 23 μL, 0.15 mmol, 3.0 equiv) in the presence of *N*-hydroxybenzotriazole monohydrate (HOBt·H_2O; 23 mg, 0.15 mmol, 3.0 equiv). Completion of each coupling reaction was ascer-

Table 4
Characterization data of the synthetic peptides

Peptide	Yield (%)	$[\alpha]_D$ (CH_3OH)	*c* (g/dL)	Temp. (°C)	Formula	MALDI-TOF	
						Found	Calculated
1	26	−31.5	0.15	28	$C_{74}H_{93}N_{17}O_{15}Na$	1482.6929	1482.9128
2	27	−31.8	0.16	28	$C_{73}H_{93}N_{16}O_{15}$	1433.7001	1433.5104
3	33	−30.9	0.12	29	$C_{66}H_{89}N_{16}O_{16}$	1361.6637	1361.5050
4	18	−24.0	0.11	29	$C_{71}H_{86}N_{16}O_{16}Na$	1441.6300	1441.5424
5	25	−26.5	0.11	29	$C_{66}H_{89}N_{16}O_{16}$	1361.6637	1361.6861
6	20	−25.7	0.11	29	$C_{66}H_{89}N_{16}O_{16}$	1361.6637	1361.6795
7	20	+8.7	0.10	29	$C_{72}H_{92}N_{17}O_{16}$	1450.6903	1450.6702
8	17	−25.5	0.12	29	$C_{75}H_{95}N_{17}O_{16}Na$	1512.7035	1512.7551
9	30	−35.7	0.10	29	$C_{71}H_{88}N_{17}O_{16}$	1434.6590	1434.6856
10	34	−39.2	0.11	28	$C_{68}H_{90}N_{17}O_{16}$	1400.6746	1400.6846
11	43	−29.5	0.11	28	$C_{73}H_{93}N_{17}O_{14}Na$	1454.6980	1454.5867
12	26	−21.3	0.16	28	$C_{74}H_{94}N_{17}O_{16}$	1476.7059	1476.7697
13	25	−39.9	0.11	28	$C_{74}H_{94}N_{17}O_{16}$	1476.7059	1476.7053
14	23	−37.3	0.10	27	$C_{74}H_{94}N_{17}O_{16}$	1476.7059	1476.6704
15	13	−33.7	0.13	27	$C_{74}H_{94}N_{17}O_{16}$	1476.7059	1476.4467
16	12	−35.3	0.15	27	$C_{74}H_{94}N_{17}O_{16}$	1476.7059	1476.4638
17	23	−47.2	0.13	27	$C_{74}H_{94}N_{17}O_{16}$	1476.7059	1476.5763
18	26	−52.4	0.12	27	$C_{74}H_{94}N_{17}O_{16}$	1476.7059	1476.5108
19	15	−40.2	0.13	28	$C_{75}H_{96}N_{17}O_{16}$	1490.7216	1490.4514
20	29	−29.2	0.12	28	$C_{74}H_{93}N_{17}O_{16}Na$	1498.6878	1498.3904
21	35	−41.4	0.15	28	$C_{74}H_{94}N_{17}O_{16}$	1476.7059	1476.3140
22	15	−40.8	0.12	28	$C_{74}H_{93}N_{17}O_{16}Na$	1498.6878	1498.3625
23	26	−47.0	0.14	28	$C_{71}H_{88}N_{16}O_{14}Na$	1411.6558	1411.6332
24	25	−35.7	0.14	29	$C_{67}H_{82}N_{14}O_{12}Na$	1297.6129	1297.6704
25	19	−50.0	0.13	28	$C_{56}H_{72}N_{12}O_{11}Na$	1111.5336	1111.6064
26	12	−33.5	0.11	28	$C_{50}H_{60}N_{10}O_{10}Na$	983.4386	983.2122
27	39	−42.2	0.20	27	$C_{70}H_{89}N_{16}O_{13}$	1361.6790	1361.7289
28	27	−25.6	0.15	27	$C_{61}H_{80}N_{15}O_{12}$	1214.6106	1214.5966
29	48	−42.0	0.13	28	$C_{55}H_{69}N_{14}O_{11}$	1101.5265	1101.5122
30	32	−44.4	0.14	28	$C_{53}H_{66}N_{13}O_{10}$	1044.5050	1044.4267
31	13	−34.6	0.12	28	$C_{63}H_{78}N_{13}O_9$	1160.6040	1160.5685

tained using the Kaiser ninhydrin test.[27] The Fmoc-protecting group was removed by treating the resin with a DMF/piperidine solution (80/20, v/v). After removal of Fmoc protection of the N-terminal amino acid residue, the amino group was reacted with Ac_2O (40 μL, 0.50 mmol, 10.0 equiv) in the presence of *i*-Pr_2NEt (44 μL, 0.25 mmol, 5.0 equiv). The resulting protected resin was treated with TFA/thioanisole/*m*-cresol/1,2-ethandithiol/H_2O (5 mL; 80:5:5:5:5) at room temperature for 2 h. After removal of the resin by filtration, the filtrate was poured into ice-cold dry diethyl ether (40 mL). The resulting powder was collected by centrifugation and then washed three times with ice-cold dry diethyl ether (3 × 40 mL). The crude product was purified by preparative RP-HPLC on a Cosmosil 5C18-ARII preparative column (Nacalai Tesque, 20 × 250 mm) and a Shimadzu LC-6AD (Shimadzu corporation, Ltd.) in an isocratic mode of CH_3CN solution containing 0.1% (v/v) TFA at a flow rate of 10 mL/min to afford the desired peptide as a colorless powder. All peptides were characterized by MALDI-TOF-MS (AXIMA-CFR plus, Shimadzu, Kyoto, Japan, Table 4) and the purity was calculated as >95% by HPLC on a Cosmosil 5C18-ARII analytical column (Nacalai Tesque, 4.6 × 250 mm, flow rate 1 mL/min) at 220 nm absorbance on a Shimadzu LC-10ADvp (Shimadzu corporation, Ltd., Kyoto, Japan). Optical rotations were measured with a JASCO P-1020 polarimeter.

4.2. Thioredoxin–polyglutamine constructs and protein purification

Thioredoxin fused to 62 glutamines (thio-Q62) were expressed in *E. coli* DH5α and purified using B-PER Bacterial Protein Extraction Reagent (Pierce Chemical, Rockford, IL) and nickel-chelating ProBond Resin columns (Invitrogen, Carlsbad, CA) as described previously.[16] The final concentration of thio-Q62 was determined by the Lowry method using a DC protein assay kit (Bio-Rad Laboratories, Hercules, CA), and its purity was examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by Coomassie brilliant blue staining.

4.3. Protein aggregation turbidity assay

Aggregation of thio-Q62 protein was assessed by the turbidity assay as described previously.[16] Briefly, the thio-Q62 protein (7.5 μM) was incubated with compound in phosphate-buffered saline (PBS, pH 7.5) at 37 °C in a low-protein-binding 384-well plate, and turbidity at 405 nm was measured every 12 h using a Spectramax Plus plate reader (Molecular Devices).

4.4. CD measurement

QBP1 and peptides **12**–**22** were dissolved in PBS (pH 7.4) at a concentration of 10 μM. The wavelength-dependent of molar ellipticity [*Q*] was monitored at 20 °C as the average of eight scans in a spectropolarimeter (Model J-710; Jasco, Tokyo, Japan).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.12.018.

References and notes

1. Ross, C. A.; Poirier, M. A. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 891.

2. Gusella, J. F.; MacDonald, M. E. *Nat. Rev. Neurosci.* **2000**, *1*, 109.
3. Orr, H. T.; Zoghbi, H. Y. *Annu. Rev. Neurosci.* **2007**, *30*, 575.
4. Nagai, Y.; Inui, T.; Popiel, H. A.; Fujikake, N.; Hasegawa, K.; Urade, Y.; Goto, Y.; Naiki, H.; Toda, T. *Nat. Struct. Mol. Biol.* **2007**, *14*, 332.
5. Nagai, Y.; Popiel, H. A. *Curr. Pharm. Des.* **2008**, *14*, 3267.
6. Kazantsev, A.; Walker, H. A.; Slepko, N.; Bear, J. E.; Preisinger, E.; Steffan, J. S.; Zhu, Y.-Z.; Gertler, F. B.; Housman, D. E.; Marsh, J. L.; Thompson, L. M. *Nat. Genet.* **2002**, *30*, 367.
7. Lecerf, J.-M.; Shirley, T. L.; Zhu, Q.; Kazantsev, A.; Amersdorfer, P.; Housman, D. E.; Messer, A.; Huston, J. S. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 4764.
8. Colby, D. W.; Chu, Y.; Cassady, J. P.; Duennwald, M.; Zazulak, H.; Webster, J. M.; Messer, A.; Lindquist, S.; Ingram, V. I.; Wittrup, K. D. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 17616.
9. Chai, Y.; Koppenhafer, S. L.; Bonini, N. M.; Paulson, H. L. *J. Neurosci.* **1999**, *19*, 10338.
10. Cummings, C. J.; Mancini, M. A.; Antalffy, B.; DeFranco, D. B.; Orr, H. T.; Zoghbi, H. Y. *Nat. Genet.* **1998**, *19*, 148.
11. Warrick, J. M.; Chan, H. Y. E.; Gray-Board, G. L.; Chai, Y.; Paulson, H. L.; Bonini, N. M. *Nat. Genet.* **1999**, *23*, 425.
12. Heiser, V.; Scherzinger, E.; Boeddrich, A.; Nordhoff, E.; Lurz, R.; Schugardt, N.; Lehrach, H.; Wanker, E. E. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6739.
13. Heiser, V.; Engemann, S.; Bröcker, W.; Dunkel, I.; Boeddrich, A.; Waelter, S.; Nordhoff, E.; Lurz, R.; Schugardt, N.; Rautenberg, S.; Herhaus, C.; Barnickel, G.; Böttcher, H.; Lehrach, H.; Wanker, E. E. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 16400.
14. Sánchez, I.; Mahlke, C.; Yuan, J. *Nature* **2003**, *421*, 373.
15. Tanaka, M.; Machida, Y.; Niu, S.; Ikeda, T.; Jana, N. R.; Doi, H.; Kurosawa, M.; Nekooki, M.; Nukina, N. *Nat. Med.* **2004**, *2*, 148.
16. Nagai, Y.; Tucker, T.; Ren, H.; Kenan, D. J.; Henderson, B. S.; Keene, J. D.; Strittmatter, W. J.; Burke, J. R. *J. Biol. Chem.* **2000**, *275*, 10437.
17. Takahashi, Y.; Okamono, Y.; Popiel, H. A.; Fujikake, N.; Toda, T.; Kinjo, M.; Nagai, Y. *J. Biol. Chem.* **2007**, *282*, 24039.
18. Nagai, Y.; Fujikake, N.; Ohno, K.; Higashiyama, H.; Popiel, H. A.; Rahadian, J.; Yamaguchi, M.; Strittmatter, W. J.; Burke, J. R.; Toda, T. *Hum. Mol. Genet.* **2003**, *12*, 1253.
19. Joliot, A.; Prochiantz, A. *Nat. Cell Biol.* **2004**, *6*, 189.
20. Wadia, J. S.; Dowdy, S. F. *Curr. Opin. Biotechnol.* **2002**, *13*, 52.
21. Futaki, S.; Suzuki, T.; Ohashi, W.; Yagami, T.; Tanaka, S.; Ueda, K.; Sugiura, Y. *J. Biol. Chem.* **2001**, *276*, 5836.
22. Popiel, H. A.; Nagai, Y.; Fujikake, N.; Toda, T. *Mol. Ther.* **2007**, *15*, 303.
23. Popiel, H. A.; Nagai, Y.; Fujikake, N.; Toda, T. *Neurosci. Lett.* **2009**, *449*, 87.
24. Ren, H.; Nagai, Y.; Tucker, T.; Strittmatter, W. J.; Burke, J. R. *Biochem. Biophys. Res. Commun.* **2001**, *288*, 703.
25. Yasukawa, T.; Kanei-Ishii, C.; Maekawa, T.; Fujimoto, J.; Yamamoto, T.; Ishii, S. *J. Biol. Chem.* **1995**, *270*, 25328.
26. Tyndall, J. D. A.; Pfeiffer, B.; Abbenante, G.; Fairlie, D. P. *Chem. Rev.* **2005**, *105*, 793.
27. Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595.