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Peptide models for inherited neurodegenerative disorders: conformation and aggregation properties of long polyglutamine peptides with and without interruptions

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Abstract Several neurodegenerative diseases are caused by expansion of polyglutamine repeats in the affected proteins. In spino-cerebellar ataxia type 1 (SCA1), histidine interruptions have been reported to mitigate the pathological effects of long glutamine stretches. To understand this phenomenon, we investigated the conformational preferences of peptides containing both the uninterrupted polyglutamine stretches and those with histidine interruption(s) as seen in SCA1 normals. Our study suggests that substitution of histidines by glutamines induces a conformational change which results in decreased solubility and increased aggregation. Our findings also suggest that all the polyglutamine peptides with and without interruption(s) adopt a β-structure and not random coil.

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Key words: Polyglutamine; Insolubility; SCA1; β-Sheet; Aggregation; Neurodegenerative disease; Circular dichroism

1. Introduction

Polyglutamine expansions are known to be the cause of at least eight neurodegenerative disorders [1–3]. The expanded polyglutamines are thought to form potentially toxic intranuclear inclusions within the neurons, leading to cell death and subsequent neurodegeneration [4,5]. It has been proposed that these repeats might have a β-helical structure [6] related to that suggested for amyloid fibrils and prion particles [7,8]. However, a random coil conformation has also been suggested by Altschuler et al. for oligoglutamines having 9 and 17 glutamine repeats [9].

In all the diseases caused by polyglutamine expansions, the glutamine stretch is encoded by the CAG codon. In case of one of the disease loci, spino-cerebellar ataxia type 1 (SCA1), the CAG repeat stretch is interrupted approximately in the middle by CAT, which encodes histidine [10,11]. The range of repeats observed in normal individuals in SCA1 is 19–36, while mutant proteins carry 40–81 repeats [10,11]. Most of the normal proteins (93%) in SCA1 carry glutamine repeats interrupted by 1–2 histidine residues having a configuration of $(Gln)_n$-**His**-Gln-**His**-$(Gln)_n$. However, mutant proteins carry expanded repeats which have lost the His interruptions [11]. Thus, in the case of SCA1, the pathological allele differs both at the protein and DNA level, not only in repeat length but also in the configuration of the repeat stretch. It is known that at the DNA level, long stretches of CAGs are stabilised by non-CAG interruptions [11–13]. Do these interruptions also play a role at the protein level? Quan et al. [14] have reported an SCA1 allele with 44 repeats, which was transmitted stabily. The repeat length is well within the disease range for SCA1, however, the individual was normal. On sequencing, it was found that the glutamine repeat was interrupted twice by His-Gln-His configuration and was of the form $(CAG)_{12}$ **CAT** CAG **CAT** $(CAG)_{12}$ **CAT** CAG **CAT** $(CAG)_{14}$ and 21 CAG repeats encoding glutamine was the longest uninterrupted tract seen in normal alleles in SCA1 [14]. Thus, although the number of glutamines was within the disease range, their pathological effect seemed to be mitigated by the presence of non-glutamine interruptions. Since the pathological mechanism in SCA1 is thought to be mediated by aggregation, we have studied the effect of non-glutamine interruptions on the conformation and aggregation properties of polyglutamine stretches. Biophysical studies were carried out on peptides with a sequence similar to that of polyglutamine stretches observed in normal and uninterrupted SCA1 proteins. Results reported here show that His interruption within polyglutamine tracts, as observed in normal SCA1 proteins, reduces their potential to aggregate.

2. Materials and methods

2.1. Design and solid phase synthesis of peptides

Polyglutamine peptides were designed and synthesised with His interruptions (Q_8HQHQ_8 and $Q_{10}HQHQ_{10}$) as observed in the case of normal SCA1 protein. Although mutant SCA1 proteins have more than 40 glutamines, due to solubility reasons, Q_{22} was used as a model peptide for an uninterrupted polyglutamine stretch. Also, no normal SCA1 protein has been reported with more than 21 uninterrupted glutamine repeats [14]. Further, to study the effect, if any, of the number of glutamines present between the two histidine interruptions in the normal SCA1 protein, an analogue having four glutamines between the histidines ($Q_8HQ_4HQ_8$) was also synthesised.

All the peptides were synthesised in house using Novasyn TGA resin (continuous flow method) [15] and Wang 1 resin (batch method) [16] on a Novabiochem and Advanced Chemtech ACT-90 peptide synthesisers, respectively, using standard protocols of Fmoc chemistry and purified by reversed phase high performance liquid chromatography. The integrity of peptide sequences was established by automated peptide sequencing (Procise 491, Applied Biosystem) and FABMS. The peptides synthetically obtained and studied were Q_8HQHQ_8, $Q_{10}HQHQ_{10}$, $Q_8HQ_4HQ_8$ and Q_{22}.

2.2. Physicochemical investigations

Circular dichroism (CD) measurements were made on a Jasco J-710 spectropolarimeter equipped with Pelteir Thermo Controller (PTC)-348. CD spectra reported are an average of 8–10 scans. All the peptides were previously dissolved in pure water and aliquots of the required amount were added to make samples of various concentrations of peptides in 3.3 mM sodium chloride sodium citrate buffer, pH = 7.2. All concentrations of peptides are calculated using per residue molecular weight for uniformity. Due to insolubility of Q_{22},

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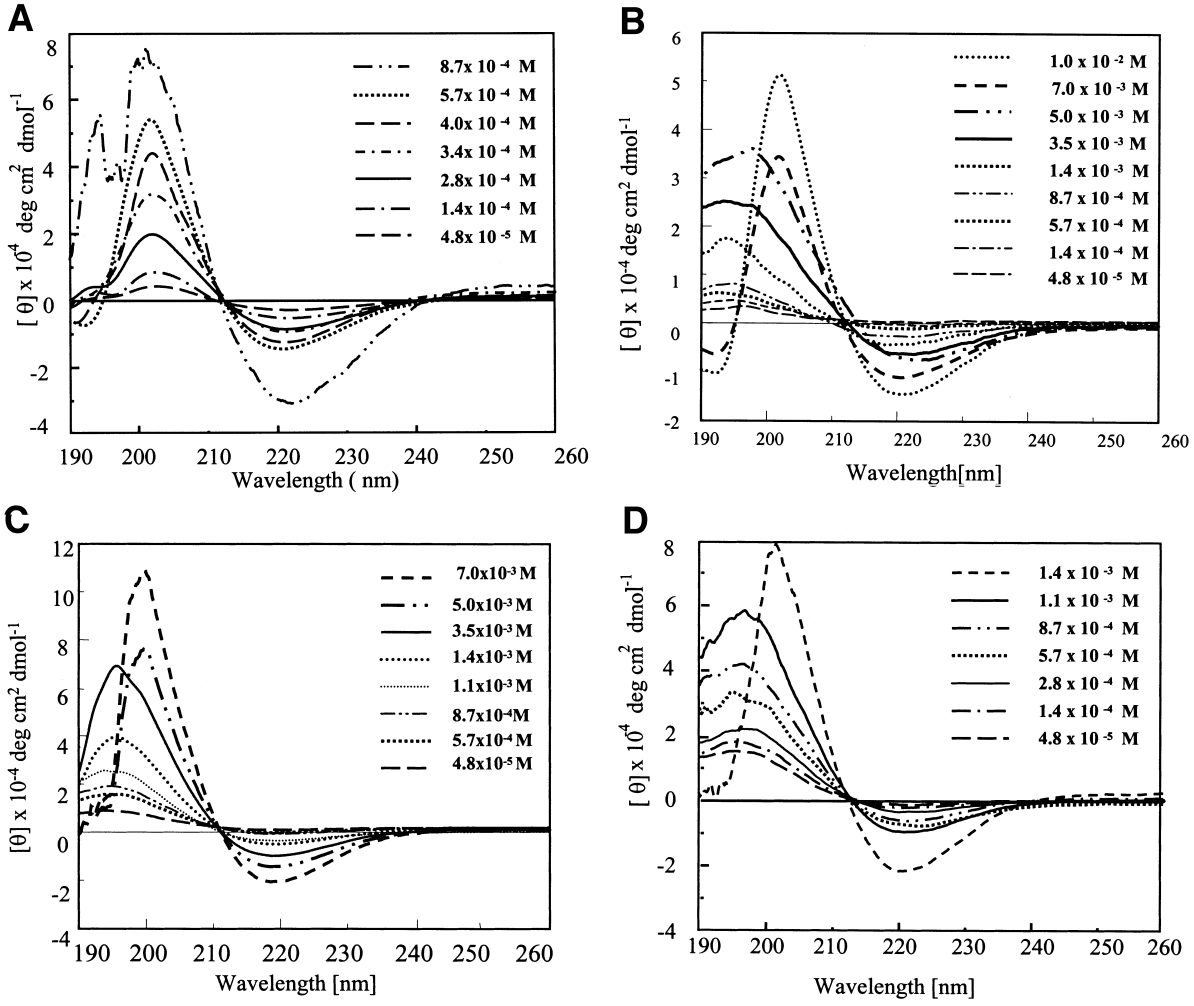

Fig. 1. CD spectra of polyglutamine peptides with and without histidine interruptions at various concentrations. (A) Q_{22}, (B) Q_8HQHQ_8, (C) $Q_{10}HQHQ_{10}$ and (D) $Q_8HQ_4HQ_8$. The spectra shown in (B–D) indicate a conformational transition from low to high concentration of the peptides. In contrast, the spectra in (A) do not show such a transition at different concentrations.

samples for a 8.7×10^{-4} M/residue concentration were prepared by adding 2% dimethyl sulfoxide (DMSO).

Solid phase FTIR spectra were recorded on a Perkin Elmer spectrum 1000 FTIR spectrometer, using KBr as medium. Deconvolution and second derivative analysis of the spectra were carried out using the software supplied by the manufacturer.

3. Results and discussion

Poly-L-glutamines are known to be insoluble in water and even short oligoglutamine chains tend to aggregate [6,17]. Therefore, the aqueous solubility of Q_8HQHQ_8, $Q_{10}HQHQ_{10}$, $Q_8HQ_4HQ_8$ and Q_{22} peptides at different concentrations were measured, by observing scattering, if any, at 320 nm in UV-vis absorption and CD spectroscopy [18,19]. The results are collated in Table 1. From the results (Table 1), it can clearly be observed that Q_8HQHQ_8, $Q_{10}HQHQ_{10}$ and $Q_8HQ_4HQ_8$, despite being of comparable length, are more soluble than Q_{22}. Moreover, Q_8HQHQ_8 and $Q_{10}HQHQ_{10}$, where histidine interruptions are separated by one glutamine, are 4–7 times more soluble than $Q_8HQ_4HQ_8$, where these interruptions are separated by four glutamines. It appears that the insolubility and aggregation phenomenon exhibited by these polyglutamine peptides are not just concentration or length de-

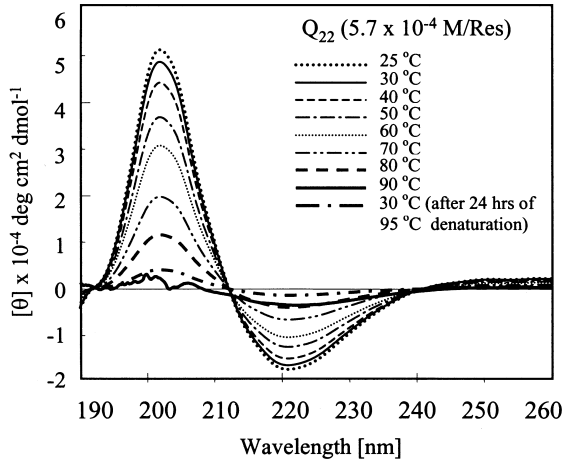

Fig. 2. CD spectra of 5.7×10^{-4} M/residue of Q_{22} at different temperatures suggest collapse of the β-structure at a high temperature and formation of this structure is irreversible.

Table 1
Aqueous solubility of Q_8HQHQ_8, $Q_{10}HQHQ_{10}$, $Q_8HQ_4HQ_8$ and Q_{22}

Peptide concentration in M/residue	Q_8HQHQ_8	$Q_{10}HQHQ_{10}$	$Q_8HQ_4HQ_8$	Q_{22}
1.4×10^{-4}	Soluble No scattering	Soluble No scattering	Soluble No scattering	Soluble No scattering
5.7×10^{-4}	Soluble No scattering	Soluble No scattering	Soluble No scattering	Soluble No scattering
8.7×10^{-4}	Soluble No scattering	Soluble No scattering	Soluble No scattering	Soluble (2% DMSO) Mild scattering
1.4×10^{-3}	Soluble No scattering	Soluble No scattering	Soluble No scattering	Partly soluble Scattering
7.0×10^{-3}	Soluble No scattering	Soluble No scattering	Partly soluble Mild scattering	Insoluble
1.0×10^{-2}	Soluble No scattering	Partly soluble Mild scattering	Partly soluble Scattering	Insoluble
1.2×10^{-2}	Partly soluble Mild scattering	Insoluble	Insoluble	Insoluble
5.0×10^{-2}	Partly soluble Scattering	Insoluble	Insoluble	Insoluble

pendent, but the histidine interruptions have a major role to play in this regard. The reason for this differential solubility behaviour cannot be just the presence of histidine residues, because as such, histidine residues are slightly more hydrophobic than glutamines at neutral pH [20]. Hence, it was thought that these histidine-interrupted polyglutamine peptides might be adopting different conformation(s) from Q_{22}, which in turn play a key role in determining their solubility and aggregation properties.

CD spectra of Q_{22}, Q_8HQHQ_8, $Q_{10}HQHQ_{10}$ and $Q_8HQ_4HQ_8$ peptides were measured at various concentrations (Fig. 1A–D). The CD spectra of Q_{22}, at all measured concentrations, exhibited a positive band at 202 nm and a negative band around 222 nm. The molar ellipticity of both the bands increased with an increase in peptide concentration up to 5.7×10^{-4} M. These spectra resembled that of strongly twisted and tightly linked wide intermolecular β-sheets which are characterised by the large magnitude of the π-π* positive band near 200 nm compared to the weak n-π* negative band centred around 220 nm, i.e. $|\Delta\varepsilon_{\pi-\pi^*}|/|\Delta\varepsilon_{n-\pi^*}| \sim 5$–7 [21]. At higher concentrations, scattering was observed due to aggregation of β-sheet structures (Fig. 1A), eventually leading to precipitation. At low concentrations, His-interrupted peptides, Q_8HQHQ_8, $Q_{10}HQHQ_{10}$ and $Q_8HQ_4HQ_8$, exhibited a blue-shifted positive CD band in the range 193–195 nm, suggesting that β-sheets formed by these peptides do not involve more than two to three strands and these structures are mostly intramolecular in nature. Since the exciton component for the π-π* positive CD band is directed along the H-bond direction, the position of this band depends upon the width of the sheet [22]. This band shifts to a longer wavelength (red-shifted to 202 nm) at higher concentrations of Q_8HQHQ_8 (7 mM and above), $Q_{10}HQHQ_{10}$ (5 mM) and $Q_8HQ_4HQ_8$ (1.4 mM) peptides. At these concentrations, Q_{22} is completely insoluble. This suggests that with an increasing concentration, there is either widening of the existing intramolecular β-sheet structure and/or formation of intermolecular species. From Fig. 1, it can be observed that at similar concentrations, the molar ellipticity of the positive band is more in case of Q_{22} compared to $Q_8HQ_4HQ_8 > Q_{10}HQHQ_{10} > Q_8HQHQ_8$ peptides. This once again suggests that Q_{22} forms highly twisted and tightly linked wide intermolecular β-sheets even at low concentrations. Formation of β-sheets by polyglutamines via

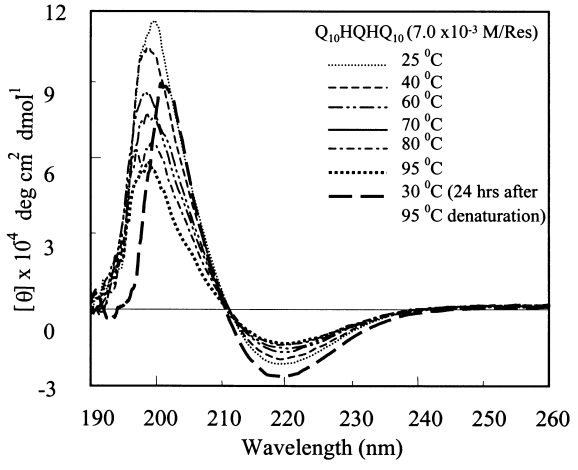

Fig. 3. CD spectra of 7.0×10^{-3} M/residue of $Q_{10}HQHQ_{10}$ at different temperatures suggest melting of the β-structure at a high temperature but the peptide regains approximately 60% of its native structure on cooling at 30°C for 24 h.

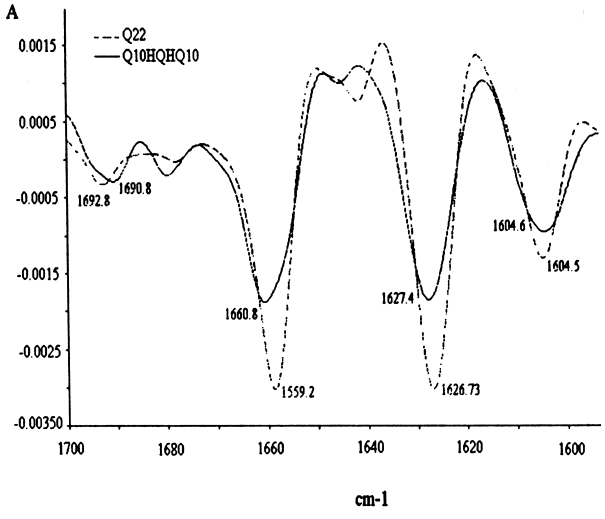

Fig. 4. Second derivative FTIR spectra (in KBr) of Q_{22} and $Q_{10}HQHQ_{10}$. The similarity of these spectra suggests that in solid phase, both uninterrupted and histidine-interrupted polyglutamine peptides form aggregates of an intermolecular β-structure.

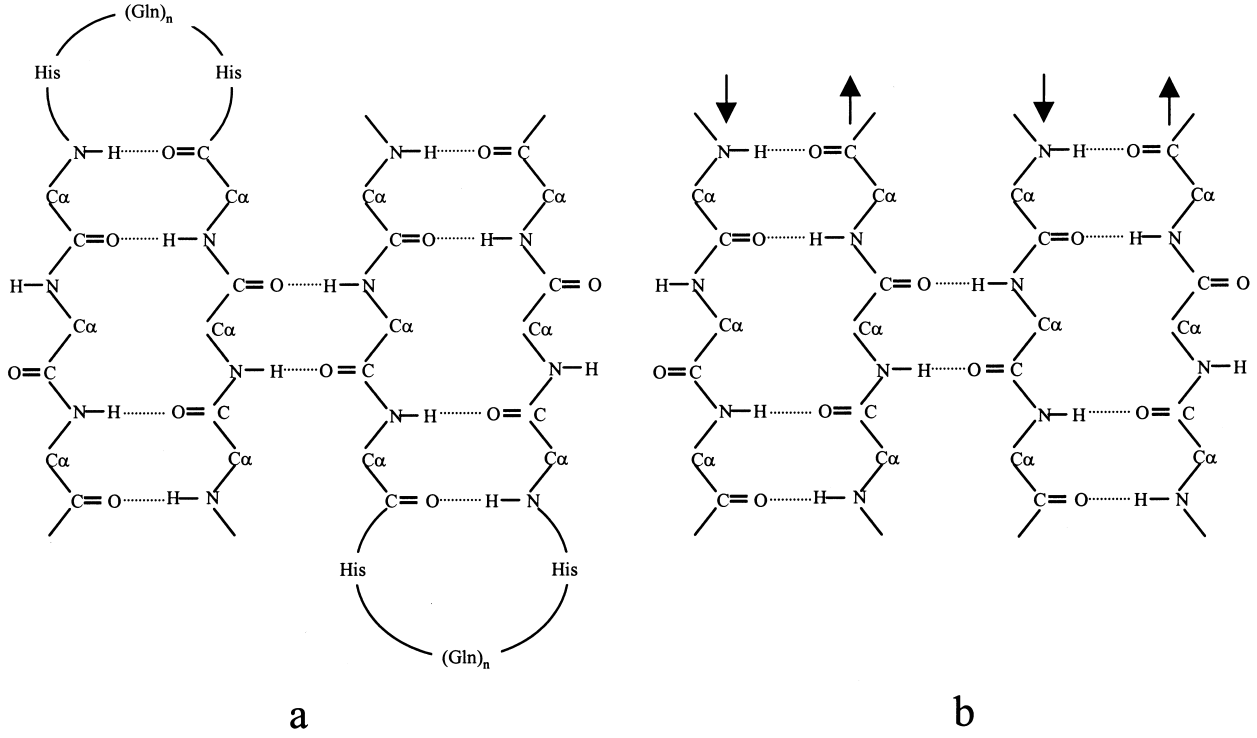

Fig. 5. Schematic representation of (a) intramolecular β-sheet structure that is proposed to be formed by polyglutamine peptides interrupted with the HQH motif. (b) Intermolecular β-sheet structure which is adopted by an uninterrupted polyglutamine tract.

the polar zipper mechanism has been proposed by Perutz et al. [6]. Moreover, the difference between the λ_{max} of CD and absorption spectra of Q_{22} was about 12 nm, compared to Q_8HQHQ_8, $Q_{10}HQHQ_{10}$ and $Q_8HQ_4HQ_8$ peptides where it was only 5 nm, which also suggest that the Q_{22} adopts an anti-parallel β-sheet conformation whereas others form parallel and/or anti-parallel β-sheets. Our results are in accordance with Woody's predictions and theoretical calculations on anti-parallel and parallel β-sheets [23].

From the solubility data (Table 1) and concentration dependent CD profiles (Fig. 1), it can be concluded that in all the polyglutamine peptides with or without histidine interruption(s), the insolubility and aggregation phenomena occur only after the formation of large (wide) intermolecular β-sheet structures. However, polyglutamine peptides with His interruptions undergo such an aggregation at much higher concentrations compared to uninterrupted polyglutamine tracts. Interrupted polyglutamine peptides with a HQH motif in the middle as observed in normal SCA1 proteins showed the least propensity to form large β-sheet structures and hence a lower aggregation potential.

The CD profiles of Q_{22} (5.7×10^{-4} M/residue) at different temperatures are shown in Fig. 2. With an increasing temperature, the molar ellipticity of both the positive band at 202 nm and negative band at 220 nm decreased, indicating collapse of the ordered β-structure. It was interesting to note that even after cooling for 24 h at 30°C, Q_{22} did not regain its native conformation, suggesting formation of a disordered aggregated structure, most likely involving main chain-side chain hydrogen bonding. Such disordered aggregated structures have been proposed to be responsible for neurodegenerative diseases [6]. In contrast, all the histidine-interrupted peptides at a high concentration regain about 60% of the native structure after complete thermal denaturation (CD profile shown only for $Q_{10}HQHQ_{10}$ at 7×10^{-3} M/residue) (Fig. 3). The histidine-interrupted peptides at low concentrations ($Q_{10}HQHQ_{10}$ at 5.7×10^{-4} M/residue) (data not shown) regain almost the complete native structure on cooling at 30°C after 95°C thermal denaturation. These results suggest that peptides with histidine interruptions at a low concentration form intramolecular β-hairpins which are reversibly folded on renaturation (Fig. 5a). Denaturation at a higher concentration causes partial non-specific aggregation for these peptides. Whereas Q_{22}, even at a low concentration, does not regain the native structure after denaturation as the native structure is stabilised through ordered intramolecular hydrogen binding (Fig. 5b). On denaturation, random main chain-side chain hydrogen bonding, as proposed by Pertuz et al. [6], could be responsible for the formation of irreversible aggregates. Since the conformational analysis of these peptides in a solution phase at higher concentrations was difficult because of solubility and scattering problems, we carried out conformational analysis of these in solid phase by recording second derivative FTIR spectra in KBr. In all the peptides, the major amide I peaks were in the 1604 ± 1 cm^{-1}, 1626 ± 2 cm^{-1}, 1659 ± 2 cm^{-1} and 1691 ± 2 cm^{-1} wavelength range (Fig. 4). The band at 1658 ± 2 cm^{-1} indicates presence of an anti-parallel orientation of β-strands [24], whereas the peaks at 1605 ± 1 cm^{-1}, 1626 ± 2 cm^{-1} and 1691 ± 2 cm^{-1} are mainly due to aggregates of intermolecular β-structures [25–27]. Hence, in solid state, all the peptides seem to form aggregated intermolecular β-structures.

Our results strongly suggest that the peptides containing a polyglutamine stretch (with or without interruption(s)) at low concentrations are soluble in water and exhibit β-structure and not a random coil as suggested by Altschuler et al. [9].

Our study also supports that the molecular basis of the disease may be formation of large aggregates as proposed by Perutz et al. [6,28] and Subirana et al. [29]. Moreover, we propose that the aggregation phenomenon is preceded by a conformational change, i.e. aggregation takes place via the formation of large intermolecular β-sheet structures (Fig. 5). Therefore, the aggregation phenomenon is not only length dependent but also depends upon the propensity to form large intermolecular β-sheets. Histidine interruptions of the polyglutamine stretch seems to favour intramolecular β-hairpin formation and reduces the potential to form large intermolecular β-sheet structures and aggregation.

This study provides experimental evidence that polyglutamine tracts with His interruption(s) as observed in the normal SCA1 sequence are highly soluble and possess less aggregation propensity, whereas uninterrupted polyglutamine tracts exhibit a high aggregation potential even at low concentrations. These peptide models also provide evidence about how a point mutation with a change in amino acid(s) could bring change in conformational properties and lead to aggregation which may be responsible for the disease processes.

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References

[1] Timchenko, L.T. and Caskey, T. (1996) FASEB J. 10, 1589–1597.
[2] Zhuchenko, O., Bailey, J., Bonnen, P., Ahsizawa, T., Stockton, D.W., Amos, C., Dobyns, W.B., Subbramony, S.H., Zoghbi, H.Y. and Lee, C.C. (1997) Nat. Genet. 15, 62–69.
[3] David, G., Abbas, N., Stevanin, G., Durr, A., Yvert, G., Cancel, G., Weber, C., Imbert, G., Saudou, F., Antoniou, E., Drabkin, H., Gemmill, R., Giunti, P., Benomar, A., Wood, N., Ruberg, M., Agid, Y., Mandel, J.L. and Brice, A. (1997) Nat. Genet. 17, 65–70.
[4] Davies, S.W., Turmaine, M., Cozens, B.A., DiFiglia, M., Sharp, A.H., Ross, C.A., Scherzinger, E., Wanker, E.E., Mangiarini, L. and Bates, G.P. (1997) Cell 90, 537–548.
[5] Martindale, D., Hackam, A., Wieczorek, A., Ellerby, L., Wellington, C., McCutcheon, K., Singaraja, R., Kazemi-Esfarjani, P., Devon, R., Kim, S.U., Bredesen, D.E., Tufaro, F. and Hayden, M.R. (1998) Nat. Genet. 18, 150–154.
[6] Perutz, M.F., Johnson, T., Suzuki, M. and Finch, J.T. (1994) Proc. Natl. Acad. Sci. USA 91, 5355–5358.
[7] Lazo, N.D. and Downing, D.T. (1998) Biochemistry 37, 1731–1735.
[8] Kelly, J.W. (1998) Proc. Natl. Acad. Sci. USA 95, 930–932.
[9] Altschuler, E.L., Hud, N.V., Mazrimas, J.A. and Rupp, B. (1997) J. Pept. Res. 50, 73.
[10] Ranum, L.P., Chung, M.Y., Banfi, S., Bryer, A., Schut, I.J., Ramesar, R., Duvick, L.A., McCall, A., Subramony, S.H., Goldfarb, L., Gomez, C., Sandkuijl, L.A., Orr, H.T. and Zoghbi, H.Y. (1994) Am. J. Hum. Genet. 55, 244–252.
[11] Chung, M.Y., Ranum, L.P., Duvick, L.A., Servadio, A., Zoghbi, H.Y. and Orr, H.T. (1993) Nat. Genet. 5, 254–258.
[12] Brahmachari, S.K., Meera, G., Sarkar, P.S., Balagurumoorthy, P., Tripathi, J., Raghavan, S., Shaligram, U.S. and Pataskar, S.S. (1995) Electrophoresis 16, 1705–1714.
[13] Bhandari, R. and Brahmachari, S.K. (1995) J. Biosci. 20, 613–627.
[14] Quan, F., Janas, J. and Popovich, B.W. (1995) Hum. Mol. Genet. 4, 2411–2413.
[15] Sieber, P. (1987) Tetrahedron Lett. 28, 6147–6153.
[16] Wang, S.W. (1973) J. Am. Chem. Soc. 95, 1328–1334.
[17] Nozaki, Y. and Tanford, C. (1971) J. Biol. Chem. 246, 2211–2217.
[18] Levine, R.L. and Fedrici, M.M. (1982) Biochemistry 21, 2600–2606.
[19] Timasheff, S.N. (1966) J. Colloid Interface Sci. 21, 489–497.
[20] Kyte, J. and Doolittle, R.F. (1982) J. Mol. Biol. 157, 105–132.
[21] Tonilo, C., Bonora, G.M. and Fontana, A. (1974) Int. J. Pept. Protein Res. 6, 371–378.
[22] Chothia, C. (1973) J. Mol. Biol. 75, 295–302.
[23] Woody, R.W. (1996) Circular dichroism and the conformational analysis of biomolecules (Fasman, G.D., Ed.), in: Theory of Circular Dichroism of Proteins, pp. 25–68, Plenum Press, New York.
[24] Byler, D.M. and Susi, H. (1986) Biopolymers 25, 469–487.
[25] Surewicz, W.K. and Mantsch, H.H. (1990) Biochemistry 29, 8106–8111.
[26] Jackson, M., Haris, P.I. and Chapman, D. (1991) Biochemistry 30, 9681–9686.
[27] Jackson, M. and Mantsch, H.H. (1992) Biochim. Biophys. Acta 1118, 139–143.
[28] Perutz, M.F. (1999) Trends Biochem. Sci. 24, 58–63.
[29] Subirana, J.A. and Paula, J. (1999) FEBS Lett. 448, 1–3.