

Random coil conformation for extended polyglutamine stretches in aqueous soluble monomeric peptides

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Several neurodegenerative diseases have been found to be strongly associated with proteins containing a polyglutamine stretch which is greatly expanded from approximately 20 glutamines in normal individuals to more than 40 in affected individuals. A conformational change in the expanded polyglutamine stretch has been suggested to form the molecular basis for disease onset. Model peptides containing polyglutamine tend to aggregate and become insoluble. We have synthesized readily water-soluble monomeric peptides by flanking polyglutamine stretches with sequences rich in alanine and lysine. Circular dichroism measurements show that polyglutamine stretches of length 9 or 17 adopt a random coil configuration in aqueous solution. We think that in the disease-associated peptides for normal individuals the stretches of ~20 glutamines are in a random coil conformation, whereas in affected individuals the polyglutamine stretch may be in some other conformation. Our method to design soluble monomeric peptides containing extended polyglutamine stretches may be generally useful in studying other highly aggregating peptides. © Munksgaard 1997.

Key words: polyglutamine; Huntington's disease; huntingtin; trinucleotide repeats

At least seven neurodegenerative diseases, Huntington's disease (HD), spinocerebellar ataxia type 1 (SCA1), spinocerebellar ataxia type 2 (SCA2), spinocerebellar ataxia type 7 (SCA7), dentatorubral-pallidoluysian atrophy (DRPLA), Macado-Joseph disease (MJD) and spinobulbar muscular atrophy (SBMA) (1, 2), have been found to be strongly associated with a protein containing a polyglutamine stretch which is greatly expanded from approximately 20 glutamines in normal individuals to more than 40 in affected individuals (1). Also, long polyglutamine stretches have been found in many transcription factors and other proteins (3). A conformational change in the expanded polyglutamine stretch has been suggested to form the molecular basis for disease onset (2, 4). Studying polyglutamine has been difficult because model peptides containing polyglutamine tend to aggregate and become insoluble (5–7). We have synthesized readily water-soluble monomeric peptides by flanking polyglutamine stretches with sequences rich in alanine and lysine. Circular dichroism (CD) measurements show that polyglutamine stretches of length 9 or 17 adopt a random coil configuration in aqueous solution. We think that in the disease-associated peptides for normal individuals the stretches of ~20 glutamines are in a random coil conformation, whereas in affected individuals the polyglutamine stretch may be in some other conformation. Our method to design soluble

monomeric peptides containing extended polyglutamine stretches may also be generally useful in studying other highly aggregating peptides.

EXPERIMENTAL PROCEDURES

The peptides were prepared in-house using a PS3 automated solid-phase peptide synthesizer (Protein Technologies Inc., Woburn, MA). On completion of the synthesis the free N terminus was acetylated with acetic anhydride. The peptide was cleaved from the Rink amide linker with trifluoroacetic acid/thioanazole/triethylsilane and purified to homogeneity by preparative HPLC (PRP-2 column [Hamilton Co., Reno, NV]). Presence of the peptides was confirmed by electrospray mass spectroscopy: expected for AKQ9 3061.5, found 3061.08 (0.98); expected for AKQ17 3751.9, found 3751.87 (0.14) g/mol. Molecular weights of the peptides dissolved in 50 mM phosphate, pH 7.0, were determined using a Beckman model XL-A analytical ultracentrifuge operating in sedimentation equilibrium mode at 50,000 rpm. The data were fit with a Levenberg-Marquart minimization to an ideal single-component model using a solvent density of 1.0 g/cm³ and a partial specific particle volume of 0.707 cm³/g. For AKQ9 at 20°C and recording at 245 nm, a molecular weight of 3140 (83) was found. For AKQ17 at 20°C (245

TABLE I
Ala/Lys and Gln, α -helix and coil content of peptides AKQ9 and AKQ17^a

Peptide	Amino acid content		0% TFE			40% TFE		
	Ala/Lys	Gln	α -helix	Coil	β -sheet	α -helix	Coil	β -sheet
AKQ9	71	29	41	59	0	71	29	0
AKQ17	44	50	26	67	7	64	36	0

^aValues stated are percentages.

nm) a molecular weight of 3804 (523) was found, but much of the sample aggregated. At 15°C there was much less aggregation of AKQ17. Recording at 235 and 276 nm, a molecular weight of 3639 (238) was found. CD spectra were recorded at room temperature using a computerized Cary model 60 spectropolarimeter (5 mm phosphate buffer, pH 7.0, 0.5 mm cells). Circular dichroism is reported as mean residue ellipticity in degree-cm²/dmol. The spectra were analyzed by a linear combination fit using the reference data of Greenfield and Fasman (8).

RESULTS AND DISCUSSION

The peptide Ac-D₂Q₁₅K₂-NH₂ rapidly becomes insoluble in aqueous solution at neutral pH and is thought to form β -sheet aggregates (7). We synthesized a simi-

lar peptide, Ac-K₂Q₁₇YK₂-NH₂. This peptide was readily soluble in aqueous solution at neutral pH, but ultracentrifugation analysis showed that it formed large aggregates (data not shown) and the CD spectrum of this peptide strongly resembled the spectrum of the peptide Ac-D₂Q₁₅K₂-NH₂.

The basic peptide Ac-(A₄K)₃A-NH₂ is 80% α -helical in aqueous solution (9). We reasoned that by flanking a long polyglutamine stretch with sequences using the -(A₄K)- motif we might be able to obtain a soluble, monomeric peptide containing an extended polyglutamine stretch. We synthesized two peptides, AKQ9 (Ac-(A₄K)₃Q₉KA₄KA-NH₂) and AKQ17 (Ac-YGA₂KA₄KQ₁₇KA₄KA-NH₂), containing 9 and 17 glutamines, respectively (Table 1). These two peptides are readily soluble in aqueous solution at neutral pH, and

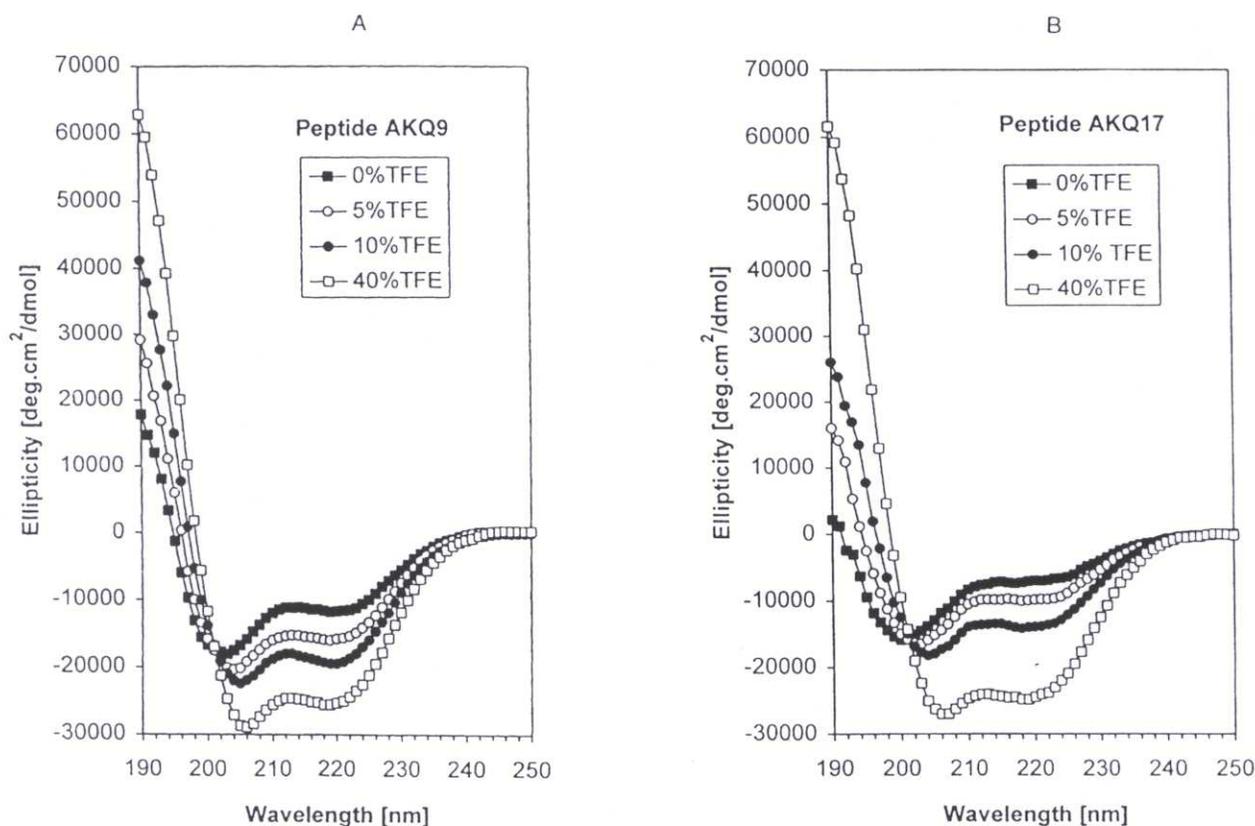


FIGURE 1
CD spectra for (A) AKQ9 and (B) AKQ17.

results from ultracentrifugation show that the peptides are in fact monomeric (see above).

Using CD spectroscopy we have found that the polyglutamine stretches in these peptides adopt a random coil configuration (Table 1, Fig. 1): The CD spectra for AKQ9 and AKQ17 with increasing amounts of trifluoroethanol (TFE), a known α -helix inducer, are similar in their qualitative appearance and both show well-defined isometric points indicative of a two-state system. AKQ9 in aqueous solution consists of 41% helix and 59% random coil. Adding TFE increases the helix content to 71% helix, 29% coil (saturation at about 40% TFE). For AKQ17 in aqueous solution, the fit yields a larger amount of random coil (67%) than for AKQ9. The remainder consists of 26% helix and a small amount of β -sheet (7%). In 40% TFE AKQ17 is 64% helix and 36% coil. We take the fact that these peptides adopt increasingly α -helical conformations with increasing concentrations of TFE as further evidence that the peptides are monomers. In contrast, for example, the highly aggregating peptides Ac-D₂Q₁₅K₂-NH₂ (7) and Ac-K₂Q₁₇YK₂-NH₂ (data not shown) do not adopt helical conformations in solutions containing high concentrations of TFE.

For several reasons we infer that the alanine/lysine-rich regions are helical, whereas the polyglutamine stretches adopt random coil configurations. First, the N-terminal 15 amino acids of AKQ9 are the first 15 of 16 amino acids of the peptide Ac-(A₄K)₃A-NH₂, which was shown to be strongly helical in aqueous solution (9), and the other N-terminal of AKQ9 and the C-terminal regions of AKQ9 and AKQ17 use the -(A₄K)- motif. Second, the percentage of helix decrease and of coil increase from AKQ9 to AKQ17 parallels the decrease of alanine/lysine content and increase in glutamine content. Finally, working with poly-[N⁵-(hydroxyalkyl-glutamine)] compounds, Lupu-Lotan and colleagues (10) found that poly-[N⁵-(hydroxybutyl-glutamine)] was strongly helical in aqueous solution, poly-[N⁵-(hydroxypropyl-glutamine)] was mixed helix and coil, and poly-[N⁵-(hydroxyethyl-glutamine)] was all coil. A coil conformation for poly-[N⁵-(hydroxyethyl-glutamine)] was confirmed by Alder *et al.* (11). Following this trend of increasing coil content with shorter alkyl chains, one would expect polyglutamine itself to be a coil rather than a helix. (The small amount of β -sheet (7%) in AKQ17 represents only about 3 residues and is of the order of uncertainty for a CD spectrum fit, although it may indicate some nascent hairpin-forming ability, or aggregation of the polyglutamine stretch in AKQ17.)

We have shown that the polyglutamine stretches in the water-soluble peptides AKQ9 and AKQ17 adopt a random coil conformation. With a readily water-soluble monomeric peptide in hand, we can now use NMR to confirm the random coil conformation for the main chain atoms of polyglutamine, and hopefully be able

to study the conformation of the side chains. We predict that when fragments of the disease-associated proteins containing polyglutamine stretches are crystallized, the polyglutamine stretches will be in a random coil conformation. It has recently been suggested (2, 4) that polyglutamine stretches in the disease-associated proteins makes a conformational transition, so that ~20 glutamines adopt one conformation and >40 glutamines another, and that glutamine stretches with more than 40 glutamines adopt a hairpin conformation. Perhaps this hypothesis of a hairpin conformation for >40 glutamines can be tested using peptides such as Ac-(A₄K)₃Q_NK(A₄K)₃A-NH₂ with N > 40, however, the solid-state synthesis of such long peptides may be difficult. Eventually, there might be drugs which stabilize a random coil conformation for polyglutamine stretches in the disease (>40) range, or which break up the putative hairpin. The method used here to produce readily soluble monomeric peptides containing long polyglutamine stretches might be applicable to studying other strongly aggregating peptides.

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