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Length-dependent conformational transitions of polyglutamine repeats as molecular origin of fibril initiation

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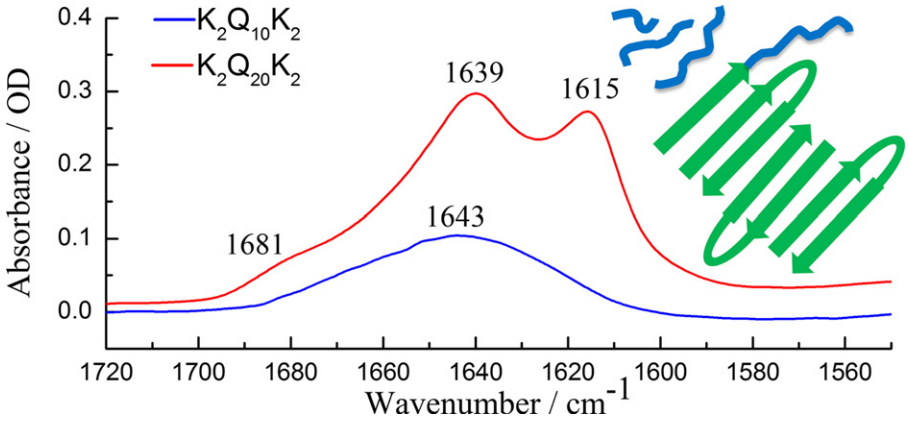
HIGHLIGHTS

- Molecular mechanisms of polyglutamine diseases have been studied spectroscopically.
- The glutamine repeat length is directly correlated to β-sheet formation.
- The concentration is another critical factor influencing the polyQ conformation.
- Different β-structures indicate different steps in polyQ fibrillogenesis.

GRAPHICAL ABSTRACT



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ABSTRACT

Polyglutamine (polyQ) sequences are found in a variety of proteins with normal function. However, their repeat expansion is associated with a number of neurodegenerative diseases, also called polyQ diseases. The length of the polyQ sequence, varying in the number of consecutive glutamines among different diseases, is critical for inducing fibril formation. We performed a systematic spectroscopic study to analyze the conformation of polyQ model peptides in dependence of the glutamine sequence lengths ($K_2Q_nK_2$ with $n = 10, 20, 30$). Complementary FTIR- and CD-spectra were measured in a wide concentration range and repeated heating and cooling cycles revealed the thermal stability of formed β-sheets. The shortest glutamine sequence $K_2Q_{10}K_2$ shows solely random structure for concentrations up to 10 mg/ml. By increasing the peptide length to $K_2Q_{20}K_2$, a significant fraction of β-sheet is observed even at low concentrations of 0.01 mg/ml. The higher the concentration, the more the structural composition is dominated by the intermolecular β-sheet. The formation of highly thermostable β-sheet is much more pronounced in $K_2Q_{30}K_2$. $K_2Q_{30}K_2$ precipitates at a concentration of 0.3 mg/ml. Our spectroscopic study shows that the aggregation tendency is enhanced with increased glutamine repeat expansion and that the concentration plays another critical factor in the β-sheet formation.

1. Introduction

The implication of polyglutamine (polyQ) repeats in proteins have become obvious by disease patterns. There are at least ten inherited polyQ diseases including the well-known Huntington's disease [1]. The proteins associated with polyQ diseases show no sequence

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homology except for an expanded polyQ region. There is a pathogenic threshold of repeat length that varies somewhat among diseases, but is generally in the range of 35–40 consecutive glutamines and the length of the polyQ sequence determines the age of the disease onset [2,3]. The processes of protein misfolding and aggregation seem to be a direct cause of neurodegeneration [4]. However, the molecular mechanisms that drive folding, misfolding or aggregation of a polyQ sequence are little understood. It is agreed that the length of the polyglutamine chain is a crucial factor for aggregation and disease. Structural properties of polyQ repeats in dependence of their sequence length are thus important for understanding the molecular mechanisms that are associated with a normal or abnormal biological function of polyQ proteins. Synthesized polyQ peptides have been used to analyze folding, misfolding and aggregation of polyQ repeats. However, detailed structure information has been difficult to obtain because polyQ peptides are quite insoluble. The observation that polyQ peptides tend to aggregate was already shown in an early study on synthetic polymers which are insoluble in water and readily aggregate to form viscous gels through the formation of interamide hydrogen bonds [5]. The first structure model of polyQ was proposed by Perutz et al. [6] who suggested that polyQ sequences could form polar zippers made of antiparallel β-strands held together by hydrogen bonds between main-chain and side-chain amides. These studies used synthetic polyQ peptides with 15 glutamines made soluble by the inclusion of flanking charged residues (Asp2-Gln15-Lys2). In subsequent experiments it was postulated that shorter polyQ tracts have a random coil conformation whereas longer repeats form β-sheet structures. A β-helix was suggested comprising about 20 Q residues per helical turn [7]. Other studies of polyQ peptides with repeat lengths from 5 to 44 Q residues indicated that, irrespective of their length, all peptides are in random structure [8]. This observation suggested that the existence of a disease threshold is not related to a conformational change in the monomeric state, but rather the threshold could be explained by the propensity of longer polyQ sequences to aggregate. A possible explanation for contradictions about structural states of polyQ peptides could be that random coil conformation is only observed when the solvent induces full solubility of the polyQ stretches, while β-sheet structures are observed when polyQ is partially aggregated. A method was described for dissolving and disaggregating chemically synthesized polyQ peptides up to a length of 44 glutamines by a mixture of trifluoroacetic acid (TFA) and hexafluoroisopropanol (FFIP) [9]. Other structure investigations of polyQ aggregates were carried out by use of polyQ peptides in which at different sequence intervals Gln–Gln pairs were replaced by Pro-Gly pairs, elements that are expected to favor β-turns and are incompatible with extended β-sheet chains [10]. The results indicate that polyQ aggregates consist of alternating elements of extended chains and turns, thus suggesting an antiparallel β-sheet folding motif as the fundamental aggregation repeat unit.

A model of polyQ aggregate initiation and elongation was proposed where an unstructured polyQ monomer serves as nucleus and undergoes a structural transition to a four-stranded antiparallel β-sheet before elongation [11]. Other mechanisms have been proposed how glutamines are involved in aggregation, one involving intrasheet hydrogen bonding of their side-chain amide groups (polar zipper model) [6], and a second involving reorientation of intrasheet glutamine hydrogen bonding resulting in close van der Waals contacts between the glutamine methylene groups (steric zipper model) [12]. Model β-hairpin systems have been designed to analyze which interactions are involved in early assembly processes [13].

Fret studies analyzed the role of polyQ length on the aggregation [14]. It was shown that the peptides become increasingly collapsed as the number of glutamines increased with an estimated effective persistence length decreasing from ~11 Å to ~7 Å as the numbers of glutamines increased from 8 to 24. Molecular dynamics simulations made also predictions about the length dependence of the polyQ-mediated aggregation states. It was concluded that the longer the glutamine tract length, the higher is the propensity to form β-helices [15–18]. A mechanism for protein aggregation has been proposed by simulations where expanded polyQ tracts destabilize the affected proteins by the formation of partially intermediate states [19].

From the above summarized studies, it becomes obvious that different models about aggregation mechanisms of polyQ proteins exist. A reason therefore is given by the fact that the conformational transitions are influenced by the used residue sequence and/or the specific measurement conditions. Since a common property of all polyQ diseases is an abnormal extended glutamine sequence in the polyQ protein, polyQ peptides consisting primarily out of glutamine residues are ideal model systems to study their conformational properties [20]. Here we report a systematic spectroscopic study to get insight into the molecular origin for fibril initiation. The studied peptides differ only in the glutamine sequence length varying between 10, 20 and 30 consecutive glutamines. The impact of the concentration on the conformation, the thermal stability of formed β-sheets and the conformational influence of the flanking charged lysine residues are analyzed in detail by complementary FTIR- and CD-measurements. Both approaches facilitate marker-free conformational studies of polyQ repeats under physiological conditions and contribute to understand the molecular mechanism of fibrillogenesis in polyQ proteins.

2. Experimental section

2.1. Peptides

PolyQ peptides were synthesized by Peptide Specialty Laboratories GmbH (Heidelberg, Germany) using FMOC-based solid-state synthesis methods. The studied peptides vary in the glutamine sequence lengths and are denominated as $K_2Q_nK_2$ with n = 10, 20, 30. Two lysines were introduced at each terminus to enhance the solubility. The peptides were lyophilized and dissolved in D_2O, resulting in an acidic pD of ~2.9. $K_2Q_{30}K_2$ doesn't dissolve even at concentrations of 0.1 mg/ml and was treated with ultrasound for 30 min directly before the measurement. For the pD study, the pD was adjusted by adding DCl, D_3PO_4 or NaOD and measured with a pH electrode using the conversion pD = pH + 0.4 [21].

2.2. CD sample preparations and CD measurements

$K_2Q_nK_2$ peptides were dissolved at the desired concentration in either H_2O or D_2O. CD measurements were carried out with a J815 spectrometer (JASCO, USA). Data were recorded between 300 nm and 180 nm with a scanning speed of 200 nm/min and a digital integration time of 0.25 s. The bandwidth was 1 nm. Final spectra were recorded as an average of seven scans, smoothed and a smoothed water background spectrum was subtracted. Variable-temperature experiments were performed with a 1 °C/min ramp speed and the temperature was controlled by a regulated flow from a water bath (FL300, Julabo, Germany) through a cell holder. 1 mm quartz cells (Starna) were used except for peptide concentrations of 0.01 mg/ml and 0.03 mg/ml that were analyzed with 1 cm cell path length. A heating run was carried out from 5 °C to 90 °C in steps of 5 °C.

2.3. IR sample preparation and FTIR measurements

For IR measurements, the peptides were dissolved in 0.1 M DCl and lyophilized three times to remove the trifluoroacetic acid (TFA) counterions remaining from the peptide synthesis. TFA absorbs at 1672 cm^{-1} [22] and thus interferes the analyzed amide I′ region. The samples were redissolved in D_2O after the lyophilization procedure. D_2O is used as solvent for all IR measurements since H_2O has a strong absorption of the HO bending vibration at ~1650 cm^{-1} overlaying the amide I region (1600–1700 cm^{-1}). Due to the mass effect on the vibrational frequency, the DO bending vibration is shifted to ~1200 cm^{-1} and thus out of the amide I region. The amide I band, mainly the C═O stretching vibration