NMR spectroscopy reveals a preferred conformation with a defined hydrophobic cluster for polyglutamine binding peptide 1

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Several important human inherited neurodegenerative diseases are caused by “polyQ expansions”, which are aberrant long repeats of glutamine residues in proteins. PolyQ binding peptide 1 (QBP1), whose minimal active core sequence is Trp-Lys-Trp-Trp-Pro-Gly-Ile-Phe, binds to expanded polyQs and blocks their β-structure transition, aggregation and in vivo neurodegeneration. Whereas QBP1 is a widely used, commercially available product, its structure is unknown. Here, we have characterized the conformations of QBP1 and a scrambled peptide (Trp-Pro-Ile-Trp-Lys-Gly-Trp-Phe) in aqueous solution by CD, fluorescence and NMR spectroscopies. A CD maximum at 227 nm suggests the presence of rigid Trp side chains in QBP1. Based on 41 NOE-derived distance constraints, the 3D structure of QBP1 was determined. The side chains of Trp 4 and Ile 7, and to a lesser extent, those of Lys 2, Trp 3 and Phe 8, form a small hydrophobic cluster. Pro 5 and Gly 6 adopt a type II tight turn and Lys 2's ε-NH2 is positioned to form a favorable cation–π interaction with Trp 4's indole ring. In contrast, the scrambled QBP1 peptide, which lacks inhibitory activity, does not adopt a preferred structure. These results provide a basis for future structure-based design approaches to further optimize QBP1 for therapy.

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Introduction

Polyglutamine (PolyQ)1 sequences of unknown function are present in many proteins. Beyond a length threshold, polyQ stretches tend to adopt β-rich amyloidoic conformations which have been implicated in several fatal neurological pathologies, including Huntington's disease [1]. Identifying agents that block this critical transition at the beginning of the amyloidogenic cascade is a promising therapeutic approach that is being actively explored [2].

PolyQ Binding Peptide 1 (QBP1) is a duodecapeptide isolated from a phage display screen designed to find peptides capable of blocking polyQ aggregation [3]. QBP1 successfully blocks polyQ induced neurodegeneration at the whole organism level in Drosophila melanogaster [4] and in mice, where tandem constructs of QBP1 + HSC70-binding motifs help to clear mutant huntingtin in cellular and mouse models of Huntington's disease [5]. Further studies employing variant peptides with amino acid substitutions and chemical modifications lead to improved QBP1 variants [6,7]. Low micromolar concentrations of the minimal active core of QBP1 (generally known as QBP1-M8): Ac-Trp-Lys-Trp-Trp-Pro-Gly-Ile-Phe-NH2, which is the octapeptide studied here referred henceforth in this manuscript as QBP1 for simplicity, inhibits polyQ in vitro aggregation as well as the parent undecapeptide does [7].

Detailed kinetics and biophysical characterization of polyQ peptide models showed that a critical increase in beta structure accompanies aggregation; this key nucleation event occurs between four short polyQ peptides or within one peptide when the polyglutamine stretch is longer than 26 residues [8,9]. Further increases in the length of the polyQ segment lead to shorter nucleation times and higher fibril stabilities in vitro which parallel variation in the age of disease onset in humans [10]. Molecular dynamics simulations suggested that QBP1 adopts an unusual “α-hairpin” conformation that binds to β-hairpin conformation of polyQ, thus suppressing their further growth [11]. On the contrary, experimental circular dichroism (CD) measurements showed that QBP1 maintains polyQ in a non-cytotoxic, non-aggregating helical conformation [12]. Recently, we have shown that QBP1 has the remarkable capacity to decrease the conformational polymorphism associated with amyloid formation not in expanded...
polyQs but in an unexpectedly broad range of amyloidogenic peptides and proteins, including A53T α-synuclein and the Sup35NM prion peptide, but not Ap42. Another octapeptide with an identical amino acid residue composition, but a scrambled sequence: Ac-Trp-Pro-Ile-Trp-Lys-Gly-Trp-Phe-NH2, abbreviated as QBP1scr, showed no significant inhibition of polyQ aggregation and neurotoxicity. However, the conformation of these peptides has not been characterized in depth to date.

Here, our goals are to characterize the conformations of QBP1 and QBP1scr using CD and fluorescence spectroscopies, acquire and assign the NMR spectra of QBP1 and QBP1scr and then utilize the NMR-derived restraints to determine QBP1’s structure in solution. These results will aid the rational design of improved QBP1 peptides for therapy, with higher affinity or specificity or both, and provide a solid foundation for future NMR structural studies of QBP1-polyQ containing protein complexes.

Materials and methods

Peptides preparation

QBP1 and QBP1scr were prepared by Merrifield solid phase peptide synthesis, as previously described [13]. Their identities and purities were corroborated by HPLC analysis, mass spectrometry and NMR spectroscopy. QBP1 and QBP1scr are sparingly soluble in aqueous solution. To achieve a sample sufficiently concentrated for NMR spectroscopy, the peptides were first dissolved in a small volume of deuterated DMSO (Sigma-Aldrich) and then diluted twenty fold with an aqueous buffer, prepared with MilliQ water or D2O (Cambridge Isotope Labs), containing 7 mM KH2PO4, 3 mM K2HPO4 (10 mM KPi) and 50 µM of DSS (sodium 4, 4-dimethyl-4-silapentane-1-sulfonate) as the internal chemical shift reference. These solutions showed visible cloudiness and, after some minutes, a white precipitation. After centrifugation, clear supernatants, whose pH was 6.9, were obtained. Utilizing a Cary 210 UV-Vis spectrometer and the extinction coefficient at 280 nm of 16,500 cm⁻¹/M⁻¹, concentrations of 0.745 and 0.404 mM were obtained for these QBP1 and QBP1scr solutions, respectively. The extinction coefficient was calculated based on 0.404 mM were obtained for these QBP1 and QBP1scr solutions, respectively.

Circular dichroism spectroscopy

For far-UV CD analysis, samples of QBP1 and QBP1scr were prepared by dissolving a small amount of peptide in 10 mM KPi buffer at pH = 6.9. Upon heating and vortexing, the peptide dissolved, yielding a transparent solution. Eight scans (wavelength range 190–260 nm, bandwidth = 1.2 nm, 8 scans, 0.5 nm steps, scan speed = 50 nm per min, temperature = 5 °C) were recorded for both sample and buffer reference spectra in a 0.1 cm quartz cuvette using a JASCO-J810 spectropolarimeter equipped with a Peltier temperature control unit. The buffer reference spectrum was subtracted and the peptides’ concentration, determined by UV absorbance, was used to express the signal as per-residue molar ellipticity. The CD spectra were analyzed with the CDSTR [15], CONTIN [16] and SELCON3 [17] algorithms as implemented in the on-line server Dichroweb (http://dichroweb.cryst.bbk.ac.uk).

Fluorescence spectroscopy

A Jobin Yvon Harobi Fluormax 4 instrument was used to record fluorescence spectra of QBP1 and QBP1scr using a 3 mm square quartz cuvette at 5.0°C in 10 mM KPi buffer, pH 6.8. These samples were prepared by dilution of the NMR sample. The following parameters were used for the peptides’ intrinsic fluorescence, which arises from the three Trp residues: excitation wavelength = 280 nm, emission range: 270–400 nm; Slit width = 2 nm for both the excitation and emission slits. The excitation and emission wavelengths were calibrated using a fine Xe emission line and the Raman water peak, respectively. ANS (8-anilino-1-naphthalenesulfonic acid) spectra were recorded using an excitation wavelength of 365 nm and emission ranges of 400–650 nm, with a 120 nm per minute scan speed. The ANS concentration was 120 µM and the peptide concentration ranged from 0 to 70 µM.

NMR spectroscopy

A series of NMR spectra: 1D ¹H {# scans = 32}, 2D ¹H TOCSY {# scans = 8 or 16, matrix size = 2xk × 512, mixing time = 60 ms}, 2D ¹H NOESY {# scans = 80, matrix size = 2xk × 512, mixing time = 80 or 150 ms}, 2D ¹H–¹³C HSQC {¹³C at natural abundance, # scans = 256, matrix size = 2xk × 512, centered at 37.5 ppm for aliphatic ¹H–¹³C, ¹³C sweep width = 65 ppm or centered at 123 ppm for aromatic ¹H–¹³C, ¹³C sweep width = 30 ppm} and 2D ¹H–¹⁵N HSQC {¹⁵N at natural abundance, # scans = 1024, matrix size = 2xk × 112} were recorded at 5°C using Bruker 600 and 800 MHz (¹H) spectrometers fitted with triple resonance cryoprobes and Z-axis gradients. To remove NOE peak ambiguities arising from overlapping HN and aromatic protons and to more clearly observe possible inter-residual ¹H–¹H NOE correlations, two additional NOESY spectra were recorded in 10 mM KPi buffer prepared with D2O and mixing times of 80 and 150 ms. Four additional 1D ¹H spectra were recorded at 5, 10, 15 and 20 °C in order to determine the temperature dependence of the ¹H resonance. Watergate and selective presaturation modules were used for ¹H₂O suppression in samples prepared in H₂O and D₂O, respectively. The same series of spectra were recorded for QBP1scr with the following two exceptions: (1) the 2D ¹H–¹³C HSQC, which is costly in terms of acquisition time for an unlabeled peptide, was not recorded and (2) two additional TOCSY spectra were recorded, at 15 and 25 °C, in order to measure the temperature dependence of the ¹H resonance. Due to the presence of two sets of peaks in the QBP1scr spectra, these two additional TOCSY spectra were recorded to overcome ambiguities in the crowded 1D ¹H spectra.

NMR spectral processing and analysis

NMR spectra were processed and Fourier transformed using Bruker Toppspin software (version 2.1). This program’s integration module was used to quantify the populations of cis and trans isomers of the Trp-Pro peptide bond. The ¹H signals were assigned using a standard approach based on TOCSY intra-residual and NOE inter-residual connectivities [18] with the aid of the program Sparky [19]. The assignments were corroborated and, in the case of some side chains, completed by studying the ¹H–¹⁵N and ¹H–¹³C HSQC spectra. The program TALOS+ was utilized to detect possible tendencies to adopt secondary structure and the backbone order parameters using the ¹Hzz, ¹⁵N, ¹³Cα and ¹³Cβ chemical shift data as input [20].

The NOESY spectra recorded in buffer prepared using 90% H₂O, 10% D₂O was examined to identify correlations arising from medium to long range contacts to provide distance constraints for structure determination calculations. Additional NOESY spectra with 80 ms and 150 ms mixing times were recorded on samples
prepared with deuterated water. The analysis of these spectra allowed us to resolve the ambiguities arising from QBP1’s W4 HN, which has the same δ value as W3 Hc1 and W1 Hc3. Most signals seen in the NOESY spectrum recorded in 150 ms also appear in the spectrum recorded in 80 ms, and by comparing their relative intensities, a semi-quantitative assessment of internuclear distances was obtained.

Structure calculation

We used a starting set of unambiguous NOEs to calculate a first group of twenty 3D structures of QBP1 with CYANA (version 2.1) [21]. These structures were examined and used to tentatively assign additional NOEs and to repeat the structure calculations. Three rounds of NOE assignment and structure calculations were carried out until a set of twenty structures with no consistent NOE violations and low RMSD values were obtained. The geometry of the resulting structures was validated using tools provided by the RCSB PDB server. Additional analysis to detect H-bonds (with <3.0 Å and <45° angle cutoffs), assess van der Waals contacts and solvent exposure (with a 1.4 Å water probe radius) were carried out using MOLMOL [22].

Results and discussion

The far-UV CD spectrum of QBP1 shows a maximum at 227 nm (Fig. 1). This spectral feature has been observed previously in folded peptides containing well ordered Trp side chains [23–25]. In the spectrum of QBP1scr, this maximum is blue shifted and less intense. Although the CD spectra are dominated by contributions from the aromatic moieties, for the sake of completeness, we analyzed the spectra for secondary structure content. In the case of QBP1, the algorithm CDSSTR gave the best least squares fit to the data and yielded an average secondary structure content of 7% helix, 39% strand, 28% turn and 22% coil. None of the algorithms utilized successfully fit QBP1scr’s CD spectrum.

QBP1’s and QBP1scr’s intrinsic fluorescence spectra show emission maxima of 360 nm, which is characteristic of solvent exposed Trp indole moieties (Sup. Fig. 1A). Both peptides enhance and blue shift the fluorescence emission spectrum of ANS, indicating the presence of solvent exposed hydrophobic clusters (Sup. Fig. 1B) [26].

To study the conformation of QBP1 and QBP1scr in more detail, we turned to NMR spectroscopy. The 1D 1H NMR spectra of QBP1 show modest chemical shift dispersion, although some aliphatic moieties including the γ and δ methyls of Ile 7 are shifted upfield significantly, which suggests a proximity to aromatic groups. The backbone and most side chain resonances of QBP1 (Fig. 2) and QBP1scr (Fig. 3) could be assigned by analysis of the 2D TOCSY & NOESY spectra. By studying the HSQC spectra, it was possible to corroborate the backbone assignments and complete the side chain assignment. The 1H–13C HSQC spectra focused on the aromatic region were particularly useful for assigning the indole and phenyl ring protons, which are strongly overlapped in the 1D and 2D 1H spectra. QBP1’s and QBP1scr’s chemical shift values are listed in Sup. Table 1 and Sup. Table 2, respectively. In addition, the chemical shifts of QBP1 have been deposited at the BMRB under accession number 25022.

The observation of QBP1’s W4 1Hα . . . 1Hδ P5 NOE correlations and the chemical shift values of P5’s 13Cβ and 13Cγ clearly indicate that the W4–P5 peptide bond is mainly in the trans conformation. In contrast, two sets of signals appear in the spectra of QBP1scr. Based on the P2’s 13Cβ, 13Cγ chemical shift values and the observation of strong W1 1Hα . . . 1Hδ P2 NOEs signals in one set of resonances, and the presence of a strong W1 1Hα . . . 1Hδ P2 NOE in the other set of resonances, we conclude that first set of signals is due to the W1–P2 amide bond being in the trans isomeric state, with the second set corresponding this amide bond adopting the cis isomeric state. Based on peak integration, the relative isomeric populations are 66% trans and 34 cis (±3%, n = 9) for QBP1scr. This proportion is very similar to the 62% trans/38% cis ratio previously reported for a Trp-Pro amide bond in a short unstructured peptide [27]. The proportion of cis isomer, as estimated by peak integration, is less than 7% for QBP1. This is significantly less than the proportion expected for a W–P peptide bond [27], which leads us to advance that QBP1 adopts a preferred structure that holds the W–P peptide bond in the trans conformation.

The temperature coefficients of the QBP1 backbone 1HN signals’ chemical shifts (δ/δT) were found to be −9.4, −4.2, −7.7, −5.9, −6.9 and −9.4 ppb/°C for W1, K2, W3, G6, I7 and F8. The δ/δT values for QBP1scr are −8.4, −8.5, −12.3, −7.0, +6.4, −5.1 and −7.7 for W1, I3, W4, K5, G6, W7 and F8 when the Trp1-Pro2 peptide bond is cis and −9.1, −10.7, −11.3, −6.9, +4.7, −6.0, −7.6 when that bond is in trans. Except for K2 in QBP1 and G6 in QBP1scr, whose values are highly anomalous, these values are smaller than −6 ppb/°C, which is consistent with a lack of stable backbone hydrogen bonds. In agreement with these results, when we utilized the TALOS+ program and the peptides’ chemical shift values as input, the backbones of both QBP1 and QBP1scr were predicted to be chiefly flexible random coils.

In the NOESY spectra of QBP1scr, there are few aromatic–aliphatic 1H crosspeaks and the signals that are observed chiefly arise from 1H belonging to sequential residues (Fig. 4). These signals likely result from chance contacts in the unfolded state ensemble. In contrast, the NOESY spectra of QBP1 show dozens of correlations between aromatic and aliphatic 1H, indicating the QBP1’s side chains form a hydrophobic cluster (Fig. 4). Employing 41 distance restraints derived from these backbone and side chain NOE signals, a set of twenty 3D solution structures was determined for QBP1 (Fig. 5). These structures are well determined and show good geometry, low root mean square structural differences (RMSD) and no distance restraint violations (Table 1). These structures represent the major conformation of QBP1 and are available at the BMRB as a small molecule structure deposition under accession number 21055. Alternative minor conformations will also be present and may contain a Trp 4–Pro 5 cis peptide bond, which
would account for some weak $^1$H$^1$C Pro 5, Ile 7 γ2 and Ile 7 δ signals (Fig. 2). Pro 5 and Gly 6 adopt a type II tight turn, which is closed by a Trp 4 C=O ||| H–N–Ile 7 H-bond in 17 of the 20 structures. The side chains of Trp 4 and Ile 7 are packed against
each other and are largely shielded from solvent. Trp 3, Phe 8 and the aliphatic methylene groups of Lys 2 make additional hydrophobic contacts with Trp 4 and Ile 7. The ε-NH$_2$ of Lys 2 is positioned to make a cation–π interaction with Trp 4’s indole ring in some structures.

We advance that these structural features could be important for QBP1’s function since amino acid substitutions or chemical modifications that alter the high turn propensity of Pro 5 & Gly 6 or the hydrophobicity of Trp 4 and Ile 7 are detrimental to QBP1’s inhibition of polyQ aggregation [6,7]. Moreover, QBP1scr, which we have shown here does not adopt a preferred conformation, lacks the QBP1’s ability to decrease neurotoxicity, ameliorate neurological symptoms and prolong life span in *Drosophila* [4] and mouse models [5] of polyQ pathologies.

In conclusion, we have determined the structure QBP1 by NMR methods and shown that Pro 5–Gly 6 form a type II turn while Trp 4 and Ile 7, together with Lys 2, Trp 3 and Phe 8, form a hydrophobic cluster. These findings will be useful to develop improved QBP1 peptoids and for future NMR structural studies of QBP1 complexed with polyQ-containing proteins and other neurotoxic proteins aimed to shed light on the biophysical basis of their interaction.
oligomerization and to efficiently prevent and treat neurodegenerative diseases.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.abb.2014.06.025.

References