FEBS 23518

Correspondence

Structure of polyglutamine

Eric Lewin Altschuler[a,*], Nicholas V. Hud[b], Joseph A. Mazrimas[c], Bernhard Rupp[c]

At least eight diseases (Huntington's disease (HD), spinobulbar muscular atrophy (SBMA or Kennedy's disease), dentatorubral-pallidoluysian atrophy (DRPLA), and spinocerebellar ataxias (SCAs) types 1, 2, 3, 6, and 7) are now known to be associated with a polyglutamine stretch which is greatly expanded in affected individuals [1,2]. While the function of only two of these genes is known (the gene for SBMA is the androgen receptor, and the gene for SCA6 is a sodium channel), it is clear from the DNA and protein sequences that the genes are diverse and unrelated. Thus, an expanded polyglutamine stretch itself may somehow play a role in disease etiology.

The study of the structure of polyglutamine has a long history. Apparently the first to study the structure of polyglutamine were Krull et al. in 1965 [3]. Using infrared, optical rotary dispersion and X-ray diffraction techniques they found polyglutamine to be in a β-sheet conformation. In the abstract of their paper they note, "These observations indicate that side-chain amides may associate with peptide amides in the same or other molecules through hydrogen bonding …". They pointed out that their own molecular models, as well as those they had received in a personal communication from Elkan Blout, founding editor of *Biopolymers*, indicated that polyglutamine was a highly aggregating molecule in a β-sheet conformation. That polyglutamine forms aggregates and is in a β-sheet conformation was independently rediscovered by Max Perutz and colleagues [4]. They found the peptide $Asp_2Gln_{15}Lys_2$ to be highly aggregating with a large circular dichroism (CD) peak at 197 nm.

The structure of polyglutamine as a monomer, however, was still not known. We synthesized a peptide $Lys_2Gln_{15}Lys_2$ [5]. This peptide was readily soluble in water at pH 7, and we got a similar CD spectra to that of Perutz. However, ultracentrifugation analysis showed that this peptide also formed large aggregates. We found that Robert Baldwin had made a monomeric short basic peptide Ac-$(Ala_4Lys)_3Ala$-NH_2 which is 80% helical in aqueous solution [6]. We thought that by flanking a polyglutamine stretch by such peptides we might be able to get a water-soluble monomeric peptide. We synthesized the peptide Ac-$(Ala_4Lys)_3$**Gln_9**$LysAla_4LysAla$-NH_2. Ultracentrifugation analysis showed that this peptide was monomeric. The CD spectra showed the peptide to be 59% random coil, and 41% α-helix. We synthesized another peptide Ac-$TyrGlyAla_2LysAla_4Lys$**Gln_{17}**$LysAla_4LysAla$-NH_2. This peptide was also found to be a monomer, with some nascent aggregate formation. The CD spectra showed 67% random coil, 26% α-helix, and 7% β-sheet. As the percentage of random coil in the peptides increased with a larger number of glutamines, and as the alanine/lysine containing segment of our peptides were nearly identical to Baldwin's helical peptide, we concluded that polyglutamine was in a random coil conformation [5]. Furthermore, in previous work with poly-[N^5-(hydroxyalkyl-glutamine)] compounds it was found that poly-[N^5-(hydroxybutyl-glutamine)] was strongly helical in aqueous solution, poly-[N^5-(hydroxypropyl-glutamine)] mixed helix and coil, and poly-[N^5-(hydroxyethyl-glutamine)] all coil [7,8]. Thus, we might expect polyglutamine itself to be in a random coil, rather than helical conformation.

From recent modeling of a wheat protein containing a number of short polyglutamine stretches, Masci et al. propose that these polyglutamine stretches will be "unordered and flexible" [9]. Also, they note that glutamine residues being in a flexible configuration is supported by chain flexibility predictions of Karplus and Schulz [10].

Recently Sharma et al. [11] have studied peptides containing polyglutamine stretches. In their abstract, they suggest "that all the polyglutamine peptides with and without interruptions(s) adopt a β-structure and not random coil". Sharma et al. have studied four peptides Gln_{22}, $Gln_8HisGlnHisGln_8$, $Gln_{10}HisGlnHisGln_{10}$, and $Gln_8HisGln_4HisGln_8$. For Gln_{22} they found a spectrum qualitatively similar to the one found by Perutz. Their peak, however, is at 202 nm, rather than at 197 nm as in Perutz' spectrum. They note that the histidine containing compounds are more soluble in aqueous solution than pure polyglutamine. They suggest that histidine plays a 'major role' in this solubility. However, we had already shown that a peptide, $Lys_2Gln_{15}Lys_2$, with more consecutive glutamines than any of the peptides of Sharma et al., is soluble in aqueous solution at pH 7. Based on a blue-shifted positive CD band in the 193–195 nm range, Sharma et al. conclude that their Gln/His containing peptides are in intramolecular β-sheet conformation. However, details of CD spectra can be notoriously difficult to interpret. The suggestion that a peptide adopts an intramolecular β-sheet conformation requires demonstration that the peptide is a monomer.

Sharma et al. suggest that histidine interruptions of polyglutamine stretches in the SCA1 protein promote protein solubility, and that loss of histidines in mutant proteins leads to protein aggregation and disease. However, all SCA1 patients have both a loss of histidines and an extended polyglutamine stretch [12], which does not prove the suggestion of Sharma et al.

As all evidence required to show that the peptides studied by Sharma et al. are intramolecular β-sheets has not been given, we stand by our demonstration that polyglutamine stretches of moderate length, e.g. nine glutamines, are in a random coil conformation. Even if peptides containing polyglutamine and histidine are in intramolecular β-sheets, this does not imply that moderate length stretches of polyglutamine itself are in a β-sheet conformation.

In summary, to support their claim that their peptides form intramolecular β-sheets, Sharma et al. would have to demonstrate that their peptides are monomers. Even if they did so, this does not imply that moderate length polyglutamine stretches are in a β-sheet conformation. Future work using NMR or other techniques may be able to study the structure of polyglutamine in more detail using our monomeric polyglutamine containing peptides [5].

References

[1] Klockgether, T. and Evert, B. (1998) Trends Neurosci. 21, 413–418.
[2] Zhuchenko, O., Bailey, J., Bonnen, P., Ashizawa, T., Stockton, D.W., Amos, C., Dobyns, W.B., Subramony, S.H., Zoghbi, H.Y. and Lee, C.C. (1997) Nature Genet. 15, 62–69.

PII: S0014-5793(00)01381-8

[3] Krull, L.H., Wall, J.S., Zobel, H. and Dimler, R.J. (1965) Biochemistry 4, 626–632.
[4] Perutz, M.F., Johnson, T., Suzuki, M. and Finch, J.T. (1994) Proc. Natl. Acad. Sci. USA 91, 5355–5358.
[5] Altschuler, E.L., Hud, N.V., Mazrimas, J.A. and Rupp, B. (1997) J. Peptide Res. 50, 73–75.
[6] Marqusee, S., Robbins, V.H. and Baldwin, R.L. (1989) Proc. Natl. Acad. Sci. USA 86, 5286–5290.
[7] Lupu-Lotan, N., Yaron, A. and Berger, A. (1966) Biopolymers 4, 365–368.
[8] Adler, A.J., Hoving, R., Potter, J., Wells, M. and Fasman, G.D. (1968) J. Am. Chem. Soc. 90, 4736–4738.
[9] Masci, S., D'Ovidio, R., Lafiandra, D. and Kasarda, D.D. (1998) Plant Physiol. 118, 1147–1158.
[10] Karplus, P.A. and Schulz, G.E. (1985) Naturwissenschaften 72, 212–213.
[11] Sharma, D., Sharma, S., Pasha, S. and Brahmachari, S.K. (1999) FEBS Lett. 456, 181–185.
[12] Zoghbi, H.Y. and Orr, H.T. (1995) Semin. Cell Biol. 6, 29–35.

*Corresponding author.
E-mail: ealtschuler@ucsd.edu

[a] *Institute for Neural Computation, University of California, San Diego, 9500 Gilman Drive, 0109, La Jolla, CA 92093-0109, USA*
[b] *School of Chemistry and Biochemistry, Georgia Tech, Atlanta, GA 30332-0400, USA*
[c] *Lawrence Livermore National Laboratory, Livermore, CA 94551, USA*

FEBS 23519

Probing the polyglutamine puzzle in neurological disorders

Samir K. Brahmachari*, Deepak Sharma, Sunitha Sharma, Santosh Pasha, Somdatta Sen, Quasar Saleem

We have recently studied the structure and aggregation properties of a series of peptides containing polyglutamine stretches in order to understand the mechanism of diseases caused by polyglutamine expansion [1]. In particular, we have used the SCA1 polyglutamine stretch as a model. Peptides Q_{22}, Q_8HQHQ_8, $Q_{10}HQHQ_{10}$, and $Q_8HQ_4HQ_8$ were synthesized to mimic the natural sequences present in the SCA1 protein. Most of the normal SCA1 proteins (93%) carry glutamine repeats interrupted by 1–2 histidine residues having a configuration of Q_mHQHQ_m [2]. Mutant proteins, however, carry expanded repeats that have lost the histidine residue interruptions. Q_{21} was the longest uninterrupted tract seen in normal individuals in SCA1. Our interest in the SCA1 protein was kindled by a report by Quan et al. [3], in which they described an individual with 44 repeats in the SCA1 protein. Although the repeat length was well within the disease range for SCA1, the individual was normal. On sequencing, it was found that the glutamine repeat was interrupted twice by an HQH configuration. Thus, although the number of glutamine residues was within the disease range, their pathological effect seemed to be mitigated by the presence of histidine interruptions.

Altschuler et al. [4] had studied synthetic peptides containing nine and 17 polyglutamine stretches flanked by other amino acids (to make them water soluble) and concluded that the glutamine stretches of moderate size adopt a random coil conformation in the monomer form in solution. Earlier, Perutz et al. [5] had demonstrated the formation of aggregates of β-sheet structure by the $E_2Q_{15}K_2$ peptide both in solution and in solid state.

Altschuler et al. [6] have asked for evidence of the monomeric state of peptides in solution studied by us. We feel that they have overlooked the primary objective of our study. We would like to reassert and strengthen our original observation based on the solubility, concentration and temperature dependent circular dichroism (CD) spectra and Fourier transform infrared data shown in Sharma et al. [1]. We showed that in 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 histidine residue interruptions undergo such aggregation at much higher concentrations compared to uninterrupted polyglutamine tracts. Interrupted polyglutamine peptides with an 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. We showed that at low concentrations, the histidine residue 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 [1]. In addition, the effect of TFE on the conformation was also investigated at different concentrations of the peptides (unpublished results). At low concentrations of Q_8HQHQ_8, $Q_{10}HQHQ_{10}$, $Q_8HQ_4HQ_8$, an increase in molar ellipticity at 220 nm was observed whereas at high concentrations this increase was negligible. This suggests that at low concentrations, the structure is primarily intramolecularly hydrogen bonded and at high concentrations there is formation of intermolecular β-sheet structure. This further substantiates our earlier conclusion based on the observation that these peptides at low concentration undergo reversible thermal denaturation–renaturation unlike the Q_{22} peptide, which forms irreversible disordered aggregates on denaturation [1].

In order to carry out biophysical studies on the monomeric state of polyglutamine containing peptides, we feel that Altschuler et al. [4] have compromised on the biological relevance of the peptide sequences they selected as models to understand the etiology of polyglutamine diseases. They ques-

Table 1
Amino acid residues flanking the polyglutamine stretches in the proteins associated with polyglutamine expansion diseases

	N-terminal residues		C-terminal residues
DRPLA	H H	Q_n	H H
HD	S F	Q_n	P P
SBMA	L L	Q_n	E T
SCA1	A E	Q_n [a]	H L
SCA2	K P	Q_n	P P
SCA3	F R	Q_n	R D
SCA6	P P	Q_n	A V
SCA7	A R	Q_n	P P

[a] SCA1 is the only disease protein where the polyglutamine stretch is interrupted by non-glutamine residues. The most common configuration observed is Q_mHQHQ_m.

tioned our suggestion that histidine residues play a major role in the solubility of polyglutamine tracts by citing their work on $K_2Q_{15}K_2$ and claimed that this peptide is soluble and contains longer polyglutamine stretches than the peptides studied by us. Our analysis indicates that this configuration of flanking residues is never seen in any of the eight disease proteins (DRPLA, SBMA, SCA1, SCA2, SCA3, SCA6, SCA7, HD) involved in the various polyglutamine repeat disorders (Table 1). Altschuler et al. have therefore used peptides which, unlike ours, are not known to occur in any of the disease proteins.

On the basis of our experiments, we have suggested that histidine interruptions within the glutamine stretch of the SCA1 protein promotes solubility and prevents aggregation [1]. Altschuler et al. [6] note that for our model to be correct, there should be both a loss of histidine residues and an expansion of the glutamine stretch. They are mistaken in that they consider these two events to be independent of each other. In the case of SCA1, the polyglutamine tract (encoded by the CAG repeat) is interrupted by two histidine residues (encoded by CAT) in normal individuals, whereas in the case of SCA2, CAG repeats are interrupted by CAA triplets, also encoding glutamine [7]. In both cases, loss of interruption of the CAG repeat by point mutation predisposes to the expansion of CAG repeats, leading to a long stretch of uninterrupted glutamine repeats and subsequent disease [2,7,8]. Our model is also validated by the findings of Quan et al. [3] that long glutamine stretches fail to evoke disease pathology when interrupted by histidine residues even when the length of the repeat is in the 'disease range'. To further test this we have also synthesized the Q44 peptide with two HQH interruptions and found it to be soluble (unpublished result). We therefore feel that our model of modulation of aggregation by histidine interruptions still holds true. Further studies should be carried out on polyglutamine peptides flanked by sequences that are similar to the natural context, rather than peptides used by Altschuler et al. that are not found in the context of the disease proteins.

References

[1] Sharma, D., Sharma, S., Pasha, S. and Brahmachari, S.K. (1999) FEBS Lett. 456, 181–185.
[2] Chung, M.Y., Ranum, L.P.W., Duvick, L.A., McCall, A.E., Servadio, A., Zoghbi, H.Y. and Orr, H.T. (1993) Nature Genet. 5, 254.
[3] Quan, F., Janas, J. and Popovich, B.D. (1995) Hum. Mol. Genet. 4, 2411.
[4] Altschuler, E.L., Hud, N.V., Mazrimas, J.A. and Rupp, B. (1997) J. Peptide Res. 50, 73.
[5] Perutz, M.F., Johnson, T., Suzuki, M. and Finch, J.T. (1994) Proc. Natl. Acad. Sci. USA 91, 5355–5358.
[6] Altschuler, E.L., Hud, N.V., Mazrimas, J.A. and Rupp, B. (2000) FEBS Lett. 472, 166–168.
[7] Saleem, Q., Choudhry, S., Mukerji, M., Padma M.V., Maheshwari, M.C., Jain, S. and Brahmachari, S.K. (2000) Human Genet. (in press).
[8] Bhandari, R. and Brahmachari, S.K. (1995) J. Biosci. 20, 613.

*Corresponding author.
E-mail: skb@cbt.res.in

Functional Genomics Unit, Centre for Biochemical Technology (CSIR), Delhi University Campus, Delhi 110 007, India