Poly-L-Glutamine Forms Cation Channels: Relevance to the Pathogenesis of the Polyglutamine Diseases

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INTRODUCTION

In the preceding reports (Monoi, 1995, 1997), we formulated, in terms of molecular mechanics, a new tubular, or pore-forming, single-stranded helix for all-L polypeptides. This helix, named the μ-helix, has a cylindrical pore along the longitudinal axis of the helix. The inner wall of the pore is composed of a hydrogen-bonded network of carbonyl and amino groups of the polypeptide backbone. The diameter of the pore is 3.7 Å when the closest-approach radii of C and N atoms are assumed to be 1.45 Å on average. A pore of this size is sufficient to accommodate small ions and molecules such as alkali cations and water molecules.

According to conformation-energy calculations (Monoi, 1995, and unpublished data), the μ-helix is usually unstable; it is not a preferred configuration for most polypeptide species. Interestingly, poly-L-glutamine forms a rare exception; the μ-helix of this polypeptide is expected to be very stable. Poly-L-glutamine may hence assume a μ-helical structure and behave as an ion channel if it is incorporated into artificial or biological lipid bilayer membranes.

In the present work, we experimentally inspected the channel-forming capability of poly-L-glutamine and found that long-chain poly-L-glutamine can actually produce ion channels when it is applied to artificial planar lipid bilayer membranes. The ion channel was cation selective and showed interesting characteristics. This finding suggests possible involvement of the cation channel formed by long-chain poly-L-glutamine in the pathogenesis of the polyglutamine diseases, which comprise Huntington's disease and related inherited neurodegenerative disorders that are caused by the expansion of the CAG trinucleotide repeat (encoding a polyglutamine stretch) present in each causative gene.

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MATERIALS AND METHODS

Synthesis of poly-L-glutamine

Poly-L-glutamine was synthesized by the continuous-flow FastMoc solid-phase method on an Applied Biosystems model 433A peptide synthesizer (Perkin-Elmer Corp., Applied Biosystems Division, Foster, CA). The reagents used were as follows: resin, Fmoc-L-Gln(Trt)-Alko resin (Watanabe Chemical Industries, Hiroshima, Japan); amino acid monomer, Fmoc-L-Gln(Trt)-OH; coupling reagent, 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU); deprotection reagent, piperidine. After 29 or 40 cycles of coupling, the peptide was uncoupled from the resin and from the trityl protection group by treatment with 95% aqueous trifluoroacetic acid (TFA). Before the cleavage from the resin, the terminal amino group of a portion of the 40-cycle peptide sample was acetylated by acetic anhydride in *N*-methylpyrrolidone.

In time-of-flight mass spectroscopy (on a Voyager Elite MALDI-TOF mass spectrometer; PerSeptive Biosystems, Framingham, MA), the 29-cycle sample showed two definite peaks corresponding to 29- and 28-residue chains; the 29-residue peak was predominantly higher. The *N*-unblocked 40-cycle sample exhibited a series of peaks ranging from 40 to 20 residues, with the highest peak at the 40-residue position. The *N*-acetylated 40-cycle sample was partitioned by adding CH_2Cl_2 to TFA in which the peptide had been dissolved. The peptide from the lower layer showed 10 definite peaks corresponding to 40 to 31 residues; the peaks were highest at the 40-residue position, decreasing rapidly with the decrease in the chain length. In what follows, the 40- and the 29-cycle sample are simply referred to as 40- and 29-residue poly-L-glutamine.

Single-channel measurement in black lipid membranes

Single-channel experiments were performed at 23 ± 0.5°C. Planar black lipid membranes were formed across a hole (100–200 μm in diameter) in

a polypropylene septum separating two aqueous electrolyte solutions in a Teflon chamber. Except for HCl solution, the pH of the chamber solutions was maintained at 7.0–7.3 with 0.2–5 mM HEPES–Tris buffer. All of the inorganic salts used had been roasted at 500°C for 24 h. The membrane-forming solution was asolectin (a mixture of soybean phospholipids) dissolved in *n*-decane, 2–3% w/v. Asolectin was purchased from Sigma Chemical Co. (St. Louis, MO); (asolectin type IV) and Avanti Polar-Lipids (Alabaster, AL); (soybean phosphatide extract, 20% phosphatidyl choline content, catalog no. 48-7416-01) and partially purified with acetone and diethyl ether.

Transmembrane electrical currents were recorded under voltage-clamp conditions through a patch-clamp amplifier (Nihon Kohden Co., Tokyo, Japan; model CEZ-2300). The electrodes were Ag–AgCl; no agar salt bridges were employed to minimize possible contamination. The difference in the equilibrium electrode potentials, which is prominent in the presence of asymmetrical Cl^- concentrations, was compensated for electronically. Poly-L-glutamine was applied to the chamber solution in the form of dilute aqueous solution (the final concentration was 5–50 pM), or it was added directly to the lipid–*n*-decane solution (usually 0.5–5 pmol/g lipid) in a glass test tube, sonicated on an ultrasonic cleaner. The two types of experiments gave the same results.

RESULTS

A brief preliminary consideration—minimum polypeptide length for transmembrane μ-helices

Before examining whether poly-L-glutamine can form ion channels, we estimated, by molecular modeling, the polypeptide chain length that is sufficient for the μ-helix to span the hydrophobic core of usual lipid bilayers. Atomic coordinate data for the energy-minimized μ-helix (Monoi, 1995) indicate that a polypeptide chain consisting of 37 residues produces, on the backbone basis, a μ-helix $\sim$30 Å in length (where the chain length is defined as the distance along the helical axis between the averaged van der Waals surface of one helix end and that of the other end). Accordingly, the minimum polypeptide chain length for transmembrane μ-helices will be $\sim$37 residues. When dimpling of the bilayer surface occurs at the ends of the helix, the minimum polypeptide length tends to decrease, possibly by a few residues at most.

Preparation of poly-L-glutamines of two different chain lengths

On the basis of the above consideration, we synthesized 40- and 29-residue poly-L-glutamines to inspect them for the capability to form ion channels in artificial black lipid membranes. The method of synthesis employed and the qualities of the polyglutamines obtained are detailed in the Materials and Methods section and hence are described here only briefly.

The synthesis of poly-L-glutamine was performed by the continuous-flow FastMoc solid-phase method. Care was taken to maximize coupling yields and minimize side reactions. The terminal amino group of a portion of the 40-residue polyglutamine sample was acetylated. Time-of-flight mass spectroscopy showed that the *N*-unblocked 40-residue polyglutamine also contains shorter polyglutamine chains that range in length from 39 to 20 residues, with the highest mass peak at 40-residue polyglutamine. After being partitioned between dichloromethane and trifluoroacetic acid, the *N*-acetylated 40-residue polyglutamine exhibited 10 definite mass peaks corresponding to 40 to 31 residues, with the highest peak at 40-residue polyglutamine. This polyglutamine sample was usually employed for 40-residue experiments, but the *N*-unblocked 40-residue polyglutamine was also used for comparison. The 29-residue polyglutamine sample consisted predominantly of 29-residue chains.

Detection of single-channel currents; long-lived open states

When 40-residue poly-L-glutamine was applied to lipid bilayer membranes separating two appropriate electrolyte solutions, discrete steplike changes were detected in the transmembrane electrical current. The high, or open, conductance state often lasted for several minutes to tens of minutes with brief closings that were mostly less than a second in duration. Occasionally the open state persisted for more than 1 h. The unit conductance change, or the single-channel conductance, for 1 molal CsCl was 17 $\pm$ 2.4 pS (average of the upward and downward deflections for 40 different channels $\pm$ SD) at a membrane potential of 100 mV. This value is for *N*-acetyl poly-L-glutamine. The *N*-unblocked species, however, gave no significantly different conductance. A typical recording of single-channel current is shown in Fig. 1, which depicts an initial 18-min trace of three superposed open states; they lasted for more than 1 h, being interrupted by closings of varying duration.

Ion selectivity and current-voltage relationship

To examine the cation–anion selectivity of this ion channel, the zero-current membrane potential, or the reversal potential, was measured in the presence of concentration gradients of the same electrolytes across the membrane. It was -50.2 ± 0.2 mV (mixed average of six measurements for the *N*-acetyl and *N*-unblocked 40-residue chains; the value after the $\pm$ sign is SEM) at 23°C for 0.1 versus 1.0 molal solutions of CsCl (the former was placed on the reference electrode side). This value agrees with the theoretical cation-induced potential when cations alone are permeable to the channel. Therefore, the channel is cation selective.

The channel was also permeable to other alkali cations and H^+ ions. The conductance sequence was $H^+ \gg Cs^+ > K^+ > Na^+$. The single-channel conductances were 18, 17, 8, and 4 pS, respectively, for 10 mmolal H^+ and 1 molal alkali cations at a membrane potential of 100 mV (the anions were Cl^- throughout).

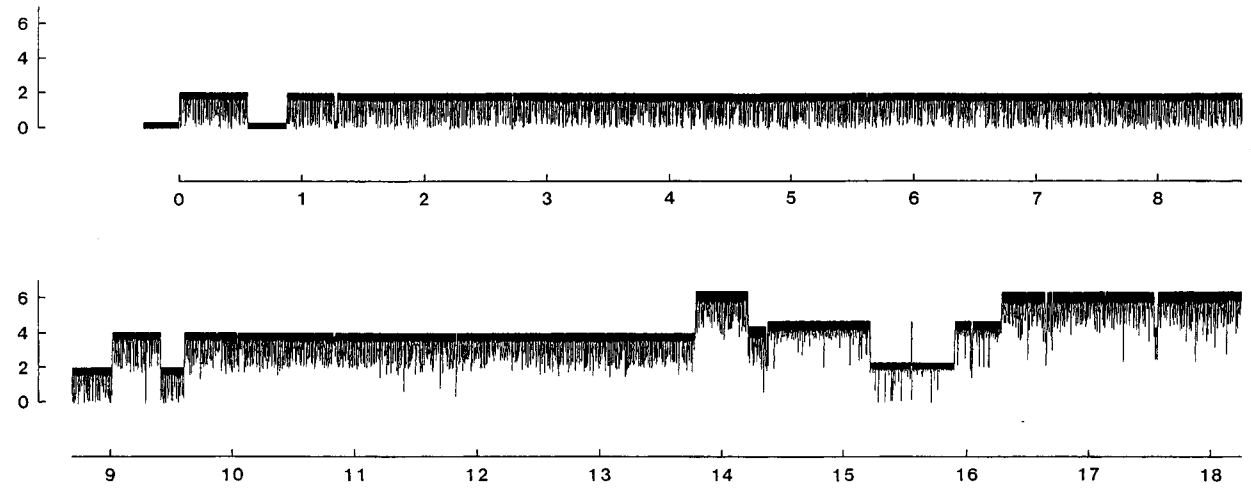

FIGURE 1 A trace of single-channel current in black lipid membrane, induced by poly-L-glutamine in the presence of 1 molal CsCl. The applied membrane potential is 120 mV. Three superimposed open states are seen; they lasted for more than 1 h, being interrupted by closings of various durations. The ordinate denotes transmembrane current expressed in pA, and the abscissa, time in minutes. A low-pass filtering at 80 Hz was taken.

Fig. 2 shows the dependence of the single-channel current I on the transmembrane potential V in the presence of 1 molal KCl in both sides of the membrane. The I–V curve is supralinear, i.e., concave upward in the positive potential domain and downward in the negative potential domain. The I–V plot is asymmetrical with respect to the zero-potential point. The degree of asymmetry is small but significant.

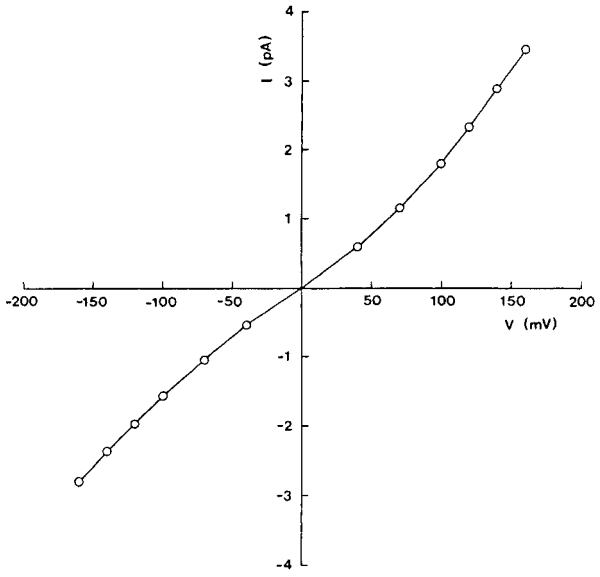

FIGURE 2 The I–V plot for the single-channel current I of the cation channel formed by 40-residue poly-L-glutamine in the presence of 1 molal KCl on both sides of the black lipid membrane. The plot is supralinear and asymmetrical with respect to the origin. The points represent averages of the same three channels. (The membrane-forming lipid solution was previously doped with poly-L-glutamine, and hence a channel in the membrane is expected to be randomly oriented with respect to the surface normal direction. Therefore, regardless of the real orientation of the applied potential V, the orientation of V for each channel is tentatively hypothesized to be such that I in the positive potential domain is greater than I in the negative potential domain.)

Channel-forming threshold length of poly-L-glutamine

The above results are for 40-residue chains. The case was quite different with 29-residue tracts. To examine the channel-forming nature of 29-residue poly-L-glutamine, 70 different black membranes were inspected over a total period of ~110 h. The observation time of one black membrane was usually more than 1 h and did not exceed 2 h. The membranes were previously doped with poly-L-glutamine, the concentration of which was 1–5 pmol/g lipid. The membrane potential was maintained at ± 100 mV in the presence of 1 molal CsCl. Under these conditions, no significant changes were detected in the transmembrane current. (Under corresponding conditions, the 40-residue poly-L-glutamine produced more than 100 ion channels.) Therefore, the channel-forming capability of the 29-residue chain, if it exists at all, is very low compared with that of the 40-residue chain. The channel-forming threshold length of poly-L-glutamine is hence between 29 and 40 residues.

DISCUSSION

The polyglutamine diseases

Seven dominantly inherited neurodegenerative disorders are now known to be caused by the expansion of the CAG

trinucleotide repeat (encoding a polyglutamine stretch) present in each causative gene: spinobulbar muscular atrophy (or Kennedy's disease) (La Spada et al., 1991), Huntington's disease (Huntington's Disease Collaborative Research Group, 1993), spinocerebellar ataxia type 1 (Orr et al., 1993), dentatorubral-pallidoluysian atrophy (Koide et al., 1994; Nagafuchi et al., 1994b), Machado-Joseph disease (or spinocerebellar ataxia type 3) (Kawaguchi et al., 1994), and spinocerebellar ataxia type 2 (Imbert et al., 1996; Sanpei et al., 1996) and type 7 (David et al., 1997). They are referred to as the CAG-repeat (or glutamine-repeat) expansion diseases or, simply, the CAG-repeat (or glutamine-repeat) diseases or the polyCAG (or polyglutamine) diseases.

In these illnesses, the expanded CAG repeats are located within the coding regions (La Spada et al., 1991; Huntington's Disease Collaborative Research Group, 1993; Orr et al., 1993; Koide et al., 1994; Nagafuchi et al., 1994b; Kawaguchi et al., 1994; Imbert et al., 1996; Sanpei et al., 1996; David et al., 1997) and are translated into the product proteins (Servadio et al., 1995; Sharp et al., 1995; Trottier et al., 1995; Yazawa et al., 1995; Ikeda et al., 1996). The disease genes share no homologous domains except the CAG repeats (La Spada et al., 1991; Huntington's Disease Collaborative Research Group, 1993; Orr et al., 1993; Koide et al., 1994; Nagafuchi et al., 1994b; Kawaguchi et al., 1994; Imbert et al., 1996; Sanpei et al., 1996; David et al., 1997). Accumulated evidence indicates that neuronal death can be caused by expanded glutamine repeats alone. How can long-chain polyglutamine be toxic?

One of the several common features shared by the seven diseases is the existence of a narrow and similar threshold in the CAG/glutamine repeat length for the onset of the diseases. In normal individuals the repeat length reported is 6–39 glutamines, and in affected individuals, 35–130 glutamines (La Spada et al., 1991; Huntington's Disease Collaborative Research Group, 1993; Orr et al., 1993; Koide et al., 1994; Nagafuchi et al., 1994b; Kawaguchi et al., 1994; Imbert et al., 1996; Sanpei et al., 1996; David et al., 1997; Andrew et al., 1993; Barron et al., 1993; Duyao et al., 1993; Snell et al., 1993; Stine et al., 1993; Novelletto et al., 1994; Rubinsztein et al., 1996; Ranum et al., 1994; Komure et al., 1995). The pathogenetic threshold is hence ~35–39 residues. How can there be such a narrow threshold for the toxicity of polyglutamine?

Little is known about the molecular mechanism of the pathogenesis of the polyglutamine diseases. Any pathological model for the disorders must explain their common pathological features, which are, besides the problem of the pathogenetic threshold, 1) the gain-of-function nature of the mutation, 2) delayed onset and relentless progression of the disorders, 3) correlation between the repeat length and the age of onset and between the repeat length and the severity of the phenotype, 4) cell death specific to neurons (despite the fact that the disease genes are widely expressed in nonneuronal cells), and 5) death of specific subsets of neurons characteristic of each disorder (even though each disease gene product is expressed in wider areas of the brain).

Suggestion of a novel pathogenetic hypothesis for the diseases: the toxic-channel hypothesis

In this work, we have revealed that long-chain poly-L-glutamine can form characteristic cation channels in vitro. This finding has led us to suggest a novel pathogenetic hypothesis for the polyglutamine diseases, as follows. In cells affected by a polyglutamine disease, causative protein molecules, which have expanded glutamine repeats, will be proteolyzed to yield fragments that contain long polyglutamine stretches. If the same channel species as found in vitro is also produced in vivo from the polyglutamine domains of such fragments, then the cation channel, which has long-lived open states, will dissipate the potential energies of permeant cations across subcellular membranes. Especially in mitochondria, the channel would dissipate the electrochemical proton gradient and the voltage gradient across the inner membrane and would reduce ATP production. In the course of time, the toxic channel will gradually accumulate to a critical level, finally to trigger lethal cascade processes leading to cell death. In this toxic-channel hypothesis, the cation channel formed by poly-L-glutamine assumes a crucial role.

Recent hypotheses proposed so far about the pathogenesis of the polyglutamine diseases focus on the formation of aggregates (or complexes) of polyglutamine with itself and/or other intracellular macromolecules. Two major mechanisms have been offered for the aggregation: hydrogen-bond formation involving the amide groups of glutamine side chains (Perutz et al., 1994; Stott et al., 1995) and covalent bonding catalyzed by transglutaminases (Green, 1993; Kahlem et al., 1996). Although such aggregate formation generally increases with the expansion of the glutamine repeats (Cooper et al., 1997; Gentile et al., 1998; Kahlem et al., 1998), it shows no sharp dependence on the repeat length. Therefore, the aggregation hypotheses cannot successfully explain the existence of the narrow pathogenetic threshold in the CAG/glutamine repeat length. Within the framework of the new hypothesis, the aggregation is secondary or side responses; it might play a part in the fatal mechanism and/or constitute a cellular protective device to sequester the toxic polypeptide.

More recently, intranuclear aggregates or inclusions have often been found in neurons of affected brain regions of polyglutamine disease patients; those inclusions are immunoreactive for antibodies to portions of disease proteins and to ubiquitin (e.g., DiFiglia et al., 1997; Paulson et al., 1997). However, evidence now available suggests that the inclusions are not pathogenetic and, instead, may play a role in

the sequestration of the causative protein (see, e.g., Zoghbi and Orr, 1999, for a review).

Explanation of pathological features of the diseases

Within the framework of the toxic-channel hypothesis presented above, the pathogenetic threshold must be approximately equal to the channel-forming threshold length of poly-L-glutamine; or, more properly stated, the former threshold is expected to be longer, more or less, than the latter because intracellular glutamine repeats will be subject to enzymic cleavage. The channel-forming threshold length found above (which was between 29 and 40 residues) agrees approximately with a range expected from the reported range (35–39 residues) of the pathogenetic threshold. Therefore, the hypothesis can explain the existence and approximate magnitude of the pathogenetic threshold.

Evidently, the toxic-channel hypothesis can also account for the gain-of-function nature of the mutation and the delayed onset of the diseases. Taking into account the intracellular proteolysis of glutamine repeats, it can explain the correlation between the repeat length and the age of disease onset and between the repeat length and the severity of disease symptoms.

In the hypothesis, the toxicity of expanded polyglutamine is obviously not restricted to nerve cells alone. This is consistent with the observation (Ikeda et al., 1996) that apoptosis is induced in nonneuronal cultured COS-7 cells transfected with cDNA containing expanded CAG repeats. In the polyglutamine diseases, however, cell death is specific to neurons despite the fact that the disease genes are widely expressed in nonneuronal cells (Li et al., 1993; Strong et al., 1993; Banfi et al., 1994; Nagafuchi et al., 1994a). This selective vulnerability of nerve cells may be attributable, at least in part, to their long-lived postmitotic nature, which will make them susceptible to the accumulation of the toxic channels. Cellular processes specific to neurons, such as excitotoxicity, may also be responsible for their vulnerability. The problem of region-specific neuronal death, however, cannot be explained straightforwardly and requires further investigation.

Comparison with other ion channels formed by protein fragments presumed to be neuropathogenic

So far two different polypeptides that are presumed to be involved in neurodegenerative diseases have been reported to form ion channels in lipid bilayer membranes: Alzheimer amyloid β protein (1–40) (Arispe et al., 1993a,b) and a peptide fragment (residues 106–126) of the prion protein (Lin et al., 1997). The former peptide is a proteolytic product of amyloid precursor protein and is presumed to be involved in the pathogenesis of Alzheimer's disease. The latter was reported to be toxic to cultured neurons (Forloni et al., 1993; but not confirmed by Kunz et al., 1999).

These two species of ion channels share several permeability characteristics: 1) both are permeable to common physiological cations; 2) they are also permeable to Cl^- ion, with permeability coefficients of 0.1 (amyloid β protein) and 0.4 (prion fragment) of those for K^+ and Na^+ ion, respectively; 3) each shows a linear current–voltage relationship in the presence of symmetrical solutions; and 4) they have multiple subconductance states (amyloid β protein) or widely distributed single-channel conductances (prion fragment), and their single-channel conductances are large, going up to a few nanosiemens and hundreds of picosiemens, respectively, at the most.

These characteristics are quite different from those of the ion channel produced by long-chain poly-L-glutamine in all of the points raised above, except for the first point.

The μ-helix as a candidate molecular structure for the toxic channel

What is the molecular structure of the cation channel formed by poly-L-glutamine? The observed value for the channel-forming threshold length of this polypeptide (between 29 and 40 residues) is difficult to explain in terms of bundled α-helices and β-barrel structures. As reported above (see the section titled A brief preliminary consideration), the minimum polypeptide length for transmembrane μ-helices is calculated to be $\sim$37 residues or a few residues less. Therefore, the observed channel-forming threshold can be accounted for if the channel has a μ-helical conformation. We suggest the μ-helix as a candidate molecular structure for the novel channel.

As is also stated above (see the Introduction), a molecular mechanics calculation implies that poly-L-glutamine, as a rare exception, can form a stable μ-helix. What is the origin of this exceptional stability? From the results of a detailed energy analysis presented previously (Monoi, 1995), this stability can be ascribed to the presence of a unique conformational motif (here named the "amide string"), which is a string of side-chain amide groups that are serially hydrogen-bonded to one another. As illustrated in Fig. 3, each amide group ($CONH_2$) of the glutaminyl side chains is hydrogen-bonded to the side-chain amide groups of the sixth residues before and after it along the primary structure; thus six strings of side-chain amide groups are formed. The hydrogen-bond length in the strings is only slightly longer (by 0.08 Å) than that in the main chain and falls within the range of standard hydrogen-bond length. The μ-helical main chain itself is not stable (Monoi, 1995). In the polyglutamine μ-helix, however, the main chain is stabilized by six pieces of the amide string motif.

We here add some comments on the degree of hydrophobicity of the outer surface of the polyglutamine μ-helix. In

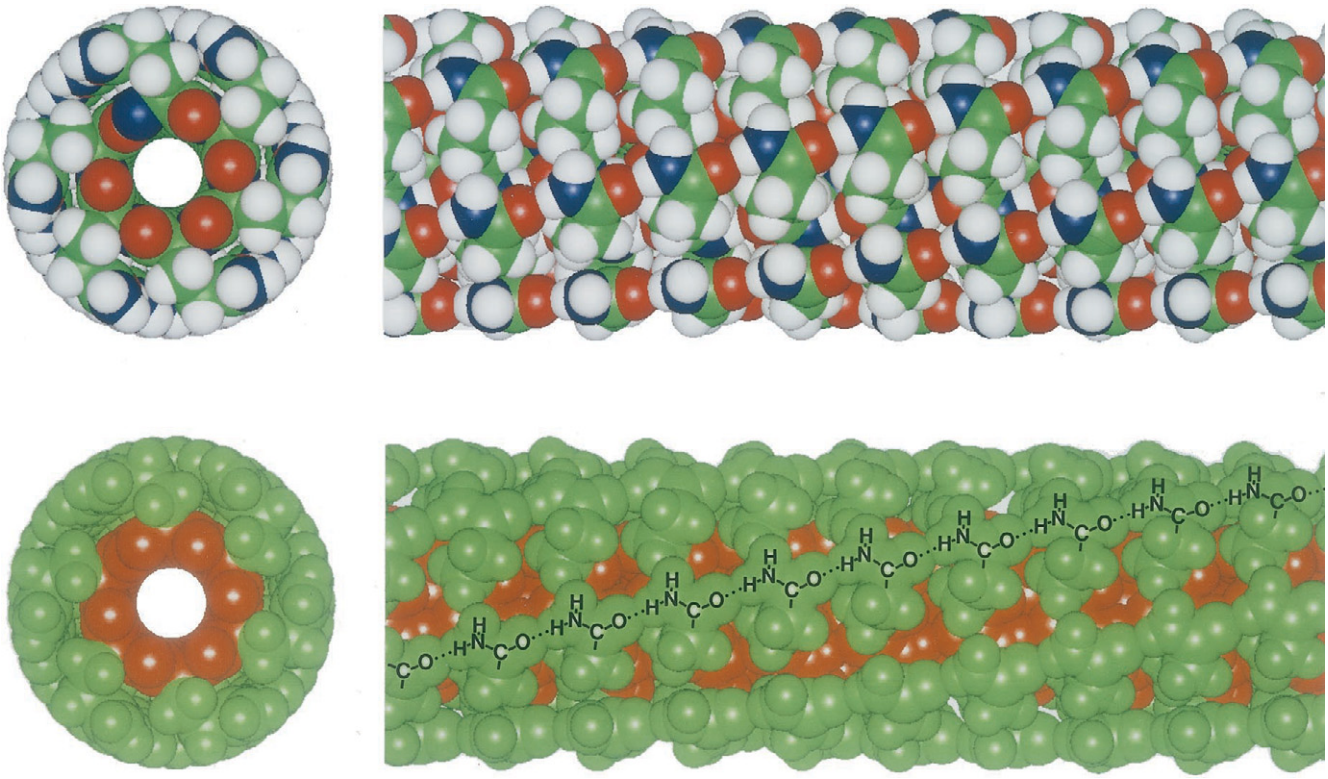

FIGURE 3 The μ-helix of poly-L-glutamine. (*Top*) Atom species are denoted by a standard coloring: C, green; N, blue; O, red; H, white. (*Bottom*) Main-chain atoms are colored red, and side chains green. (*Left*) Longitudinal view from the N-terminal end of a 73-residue chain; the terminal H atom is removed. (*Right*) Lateral view; the N-terminal end is to the left. Atomic radii follow the standard of the CPK molecular models. The μ-helix has a cylindrical pore, the diameter of which is 3.7 Å when the closest-approach radii of C and N atoms are assumed to be 1.45 Å on average. In the polyglutamine μ-helix, each amide group ($-CONH_2$) of the side chains is hydrogen-bonded to the side-chain amide groups of the sixth residues before and after it along the primary structure; thus strings of side-chain amide groups are formed. The helical conformation is stabilized by six pieces of this conformational motif or the amide string motif. One of the amide strings is indicated by superimposed chemical symbols in the bottom right figure. A side-chain interamide hydrogen bond is approximately parallel to the nearest-neighbor main-chain hydrogen bond, and their dipole moments point in opposite directions. This figure represents the lowest-energy conformation of an infinitely long μ-helix calculated in terms of molecular mechanics with a modified ECEPP83 force field (Monoi, 1995).

the μ-helix, the inner wall of the central pore is composed of hydrogen-bonded carbonyl and amino groups of the peptide backbone, and the side chains are situated outside. As seen in Fig. 3, the outer surface of the polyglutamine μ-helix is chiefly occupied by hydrogen-bonded amide groups and γ-methylene groups. The amide group is considerably hydrophilic by nature, and hence one might be doubtful about whether the polyglutamine μ-helix can be incorporated effectively into lipid bilayers. However, the hydrophobicity of the amide group increases when it is hydrogen-bonded. According to Roseman (1988), the free energy change of transferring the peptide C═O···H—N hydrogen-bonded group from water to CCl_4 is +0.6 kcal/mol, which is much less than the corresponding free energy change for a non-hydrogen-bonded peptide CONH group (+6.1 kcal/mol). Therefore, it is expected that the outer surface of the polyglutamine μ-helix is relatively insensitive to solvent polarity.

CONCLUDING REMARKS

In this work we have revealed that long-chain poly-L-glutamine forms a characteristic cation channel. The channel is permeable to monovalent cations, including K^+, Na^+, and H^+ ions, and has a long-lived open state. These characteristics give the channel a cytotoxic nature. In living cells, the cation channel would behave as toxic channel by dissipating the potential energies of the permeant cations across subcellular membranes. Especially in mitochondrial inner membranes, the channel would dissipate the electrochemical proton gradient and the voltage gradient and would reduce ATP production.

For the polyglutamine diseases, there is now a consensus that neuronal death can be produced by expanded polyglutamine alone. Little is known, however, about the molecular mechanism of the pathogenesis of the disorders. From the above considerations, we suggested a novel hypothesis for

the pathogenetic mechanism of the disorders. In this hypothesis, the toxic channel formed by glutamine repeats assumes a crucial role.

Another characteristic of the cation channel is that the channel-forming threshold length of poly-L-glutamine is between 29 and 40 residues. The toxic-channel hypothesis thus provides a straightforward explanation for the existence and magnitude of the pathogenetic threshold of the CAG/glutamine repeat length.

Although this study was motivated by the theoretical prediction that poly-L-glutamine may exceptionally produce a stable μ-helix with a tubular shape, the available evidence for the μ-helical structure of the novel channel is circumstantial at present. Further study is now proceeding on this point as well as the question of whether the characteristic cation channel found in vitro is also produced in cells affected by a polyglutamine disease.

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REFERENCES

Andrew, S. E., Y. P. Goldberg, B. Kremer, H. Telenius, J. Theilmann, S. Adam, E. Starr, F. Squitieri, B. Lin, M. A. Kalchman, R. K. Graham, and M. R. Hayden. 1993. The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington's disease. *Nature Genet.* 4:398–403.

Arispe, N., H. B. Pollard, and E. Rojas. 1993a. Giant multilevel cation channels formed by Alzheimer disease amyloid β-protein [AβP-(1–40)] in bilayer membranes. *Proc. Natl. Acad. Sci. USA.* 90:10573–10577.

Arispe, N., E. Rojas, and H. B. Pollard. 1993b. Alzheimer disease amyloid β protein forms calcium channels in bilayer membranes: blockade by tromethamine and aluminum. *Proc. Natl. Acad. Sci. USA.* 90:567–571.

Banfi, S., A. Servadio, M.-Y. Chung, T. J. Kwiatkowski Jr, A. E. McCall, L. A. Duvick, Y. Shen, E. J. Roth, H. T. Orr, and H. Y. Zoghbi. 1994. Identification and characterization of the gene causing type 1 spinocerebellar ataxia. *Nature Genet.* 7:513–520.

Barron, L. H., J. P. Warner, M. Porteous, S. Holloway, S. Simpson, R. Davidson, and D. J. H. Brock. 1993. A study of the Huntington's disease associated trinucleotide repeat in the Scottish population. *J. Med. Genet.* 30:1003–1007.

Cooper, A. J. L., K.-F. R. Sheu, J. R. Burke, O. Onodera, W. J. Strittmatter, A. D. Roses, and J. P. Blass. 1997. Polyglutamine domains are substrates of tissue transglutaminase. Does transglutaminase play a role in expanded CAG/poly-Q neurodegenerative diseases? *J. Neurochem.* 69: 431–434.

David, G., N. Abbas, G. Stevanin, A. Dürr, G. Yvert, G. Cancel, C. Weber, G. Imbert, F. Saudou, E. Antoniou, H. Drabkin, R. Gemmill, P. Giunti, A. Benomar, N. Wood, M. Ruberg, Y. Agid, J.-L. Mandel, and A. Brice. 1997. Cloning of the SCA7 gene reveals a highly unstable CAG repeat expansion. *Nature Genet.* 17:65–70.

DiFiglia, M., E. Sapp, K. O. Chase, S. W. Davies, G. P. Bates, J. P. Vonsattel, and N. Aronin. 1997. Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science.* 277: 1990–1993.

Duyao, M., C. Ambrose, R. Myers, A. Novelletto, F. Persichetti, M. Frontali, S. Folstein, C. Ross, M. Franz, M. Abbott, J. Gray, P. Conneally, A. Young, J. Penney, Z. Hollingsworth, I. Shoulson, A. Lazzarini, A. Falek, W. Koroshetz, D. Sax, E. Bird, J. Vonsattel, E. Bonilla, J. Alvir, J. B. Conde, J.-H. Cha, L. Dure, F. Gomez, M. Ramos, J. Sanchez-Ramos, S. Snodgrass, M. de Young, N. Wexler, C. Moscowitz, G. Penchaszadeh, H. MacFarlane, M. Anderson, B. Jenkins, J. Srinidhi, G. Barnes, J. Gusella, and M. MacDonald. 1993. Trinucleotide repeat length instability and age of onset in Huntington's disease. *Nature Genet.* 4:387–392.

Forloni, G., N. Angeretti, R. Chiesa, E. Monzani, M. Salmona, O. Bugiani, and F. Tagliavini. 1993. Neurotoxicity of a prion protein fragment. *Nature.* 362:543–546.

Gentile, V., C. Sepe, M. Calvani, M. A. B. Melone, R. Cotrufo, A. J. L. Cooper, J. P. Blass, and G. Peluso. 1998. Tissue transglutaminase-catalyzed formation of high-molecular-weight aggregates in vitro is favored with long polyglutamine domains: a possible mechanism contributing to CAG-triplet diseases. *Arch. Biochem. Biophys.* 352: 314–321.

Green, H. 1993. Human genetic diseases due to codon reiteration: relationship to an evolutionary mechanism. *Cell.* 74:955–956.

Huntington's Disease Collaborative Research Group. 1993. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell.* 72:971–983.

Ikeda, H., M. Yamaguchi, S. Sugai, Y. Aze, S. Narumiya, and A. Kakizuka. 1996. Expanded polyglutamine in the Machado-Joseph disease protein induces cell death in vitro and in vivo. *Nature Genet.* 13: 196–202.

Imbert, G., F. Saudou, G. Yvert, D. Devys, Y. Trottier, J.-M. Garnier, C. Weber, J.-L. Mandel, G. Cancel, N. Abbas, A. Dürr, O. Didierjean, G. Stevanin, Y. Agid, and A. Brice. 1996. Cloning of the gene for spinocerebellar ataxia 2 reveals a locus with high sensitivity to expanded CAG/glutamine repeats. *Nature Genet.* 14:285–291.

Kahlem, P., H. Green, and P. Djian. 1998. Transglutaminase action imitates Huntington's disease: selective polymerization of huntingtin containing polyglutamine. *Mol. Cell.* 1:595–601.

Kahlem, P., C. Terré, H. Green, and P. Djian. 1996. Peptides containing glutamine repeats as substrates for transglutaminase-catalyzed cross-linking: relevance to diseases of the nervous system. *Proc. Natl. Acad. Sci. USA.* 93:14580–14585.

Kawaguchi, Y., T. Okamoto, M. Taniwaki, M. Aizawa, M. Inoue, S. Katayama, H. Kawakami, S. Nakamura, M. Nishimura, I. Akiguchi, J. Kimura, S. Narumiya, and A. Kakizuka. 1994. CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. *Nature Genet.* 8:221–228.

Koide, R., T. Ikeuchi, O. Onodera, H. Tanaka, S. Igarashi, K. Endo, H. Takahashi, R. Kondo, A. Ishikawa, T. Hayashi, M. Saito, A. Tomoda, T. Miike, H. Naito, F. Ikuta, and S. Tsuji. 1994. Unstable expansion of CAG repeat in hereditary dentatorubral-pallidoluysian atrophy (DRPLA). *Nature Genet.* 6:9–13.

Komure, O., A. Sano, N. Nishino, N. Yamauchi, S. Ueno, K. Kondoh, N. Sano, M. Takahashi, N. Murayama, I. Kondo, S. Nagafuchi, M. Yamada, and I. Kanazawa. 1995. DNA analysis in hereditary dentatorubral-pallidoluysian atrophy: correlation between CAG repeat length and phenotypic variation and the molecular basis of anticipation. *Neurology.* 45:143–149.

Kunz, B., E. Sandmeier, and P. Christen. 1999. Neurotoxicity of prion peptide 106–126 not confirmed. *FEBS Lett.* 458:65–68.

La Spada, A. R., E. M. Wilson, D. B. Lubahn, A. E. Harding, and K. H. Fischbeck. 1991. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature.* 352:77–79.

Li, S.-H., G. Schilling, W. S. Young, III., X.-J. Li, R. L. Margolis, O. C. Stine, M. V. Wagster, M. H. Abbott, M. L. Franz, N. G. Ranen, S. E. Folstein, J. C. Hedreen, and C. A. Ross. 1993. Huntington's disease gene (IT15) is widely expressed in human and rat tissues. *Neuron.* 11: 985–993.

Lin, M.-C., T. Mirzabekov, and B. L. Kagan. 1997. Channel formation by a neurotoxic prion protein fragment. *J. Biol. Chem.* 272:44–47.

Monoi, H. 1995. New tubular single-stranded helix of poly-L-amino acids suggested by molecular mechanics calculations. I. Homopolypeptides in isolated environments. *Biophys. J.* 69:1130–1141.

Monoi, H. 1997. The μ helix, a tubular single-stranded helix of poly-L-amino acids. *In* Progress in Cell Research, Vol. 6. M. Sokabe, A.

Auerbach, and F. J. Sigworth, editors. Elsevier Science, Amsterdam. 233–249.

Nagafuchi, S., H. Yanagisawa, E. Ohsaki, T. Shirayama, K. Tadokoro, T. Inoue, and M. Yamada. 1994a. Structure and expression of the gene responsible for the triplet repeat disorder, dentatorubral and pallidoluysian atrophy (DRPLA). *Nature Genet.* 8:177–182.

Nagafuchi, S., H. Yanagisawa, K. Sato, T. Shirayama, E. Ohsaki, M. Bundo, T. Takeda, K. Tadokoro, I. Kondo, N. Murayama, Y. Tanaka, H. Kikushima, K. Umino, H. Kurosawa, T. Furukawa, K. Nihei, T. Inoue, A. Sano, O. Komure, M. Takahashi, T. Yoshizawa, I. Kanazawa, and M. Yamada. 1994b. Dentatorubral and pallidoluysian atrophy expansion of an unstable CAG trinucleotide on chromosome 12p. *Nature Genet.* 6:14–18.

Novelletto, A., F. Persichetti, G. Sabbadini, P. Mandich, E. Bellone, F. Ajmar, M. Pergola, L. Del Senno, M. E. MacDonald, J. F. Gusella, and M. Frontali. 1994. Analysis of the trinucleotide repeat expansion in Italian families affected with Huntington's disease. *Hum. Mol. Genet.* 3:93–98.

Orr, H. T., M.-Y. Chung, S. Banfi, T. J. Kwiatkowski, Jr., A. Servadio, A. L. Beaudet, A. E. McCall, L. A. Duvick, L. P. W. Ranum, and H. Y. Zoghbi. 1993. Expansion of an unstable trinucleotide CAG repeat in spinocerebellar ataxia type 1. *Nature Genet.* 4:221–226.

Paulson, H. L., M. K. Perez, Y. Trottier, J. Q. Trojanowski, S. H. Subramony, S. S. Das, P. Vig, J.-L. Mandel, K. H. Fischbeck, and R. N. Pittman. 1997. Intranuclear inclusions of expanded polyglutamine protein in spinocerebellar ataxia type 3. *Neuron.* 19:333–344.

Perutz, M. F., T. Johnson, M. Suzuki, and J. T. Finch. 1994. Glutamine repeats as polar zippers: their possible role in inherited neurodegenerative diseases. *Proc. Natl. Acad. Sci. USA.* 91:5355–5358.

Ranum, L. P. W., M.-Y. Chung, S. Banfi, A. Bryer, L. J. Schut, R. Ramesar, L. A. Duvick, A. McCall, S. H. Subramony, L. Goldfarb, C. Gomez, L. A. Sandkuijl, H. T. Orr, and H. Y. Zoghbi. 1994. Molecular and clinical correlations in spinocerebellar ataxia type 1: evidence for familial effects on the age of onset. *Am. J. Hum. Genet.* 55:244–252.

Roseman, M. A. 1988. Hydrophobicity of the peptide C═O···H—N hydrogen-bonded group. *J. Mol. Biol.* 201:621–623.

Rubinsztein, D. C., J. Leggo, R. Coles, E. Almqvist, V. Biancalana, J.-J. Cassiman, K. Chotai, M. Connarty, D. Craufurd, A. Curtis, D. Curtis, M. J. Davidson, A.-M. Differ, C. Dode, A. Dodge, M. Frontali, N. G. Ranen, O. C. Stine, M. Sherr, M. H. Abbott, M. L. Franz, C. A. Graham, P. S. Harper, J. C. Hedreen, A. Jackson, J.-C. Kaplan, M. Losekoot, J. C. MacMillan, P. Morrison, Y. Trottier, A. Novelletto, S. A. Simpson, J. Theilmann, J. L. Whittaker, S. E. Folstein, C. A. Ross, and M. R. Hayden. 1996. Phenotypic characterization of individuals with 30–40 CAG repeats in the Huntington disease (HD) gene reveals HD cases with 36 repeats and apparently normal elderly individuals with 36–39 repeats. *Am. J. Hum. Genet.* 59:16–22.

Sanpei, K., H. Takano, S. Igarashi, T. Sato, M. Oyake, H. Sasaki, A. Wakisaka, K. Tashiro, Y. Ishida, T. Ikeuchi, R. Koide, M. Saito, A. Sato, T. Tanaka, S. Hanyu, Y. Takiyama, M. Nishizawa, N. Shimizu, Y. Nomura, M. Segawa, K. Iwabuchi, I. Eguchi, H. Tanaka, H. Takahashi, and S. Tsuji. 1996. Identification of the spinocerebellar ataxia type 2 gene using a direct identification of repeat expansion and cloning technique, DIRECT. *Nature Genet.* 14:277–284.

Servadio, A., B. Koshy, D. Armstrong, B. Antalffy, H. T. Orr, and H. Y. Zoghbi. 1995. Expression analysis of the ataxin-1 protein in tissues from normal and spinocerebellar ataxia type 1 individuals. *Nature Genet.* 10:94–98.

Sharp, A. H., S. J. Loev, G. Schilling, S.-H. Li, X.-J. Li, J. Bao, M. V. Wagster, J. A. Kotzuk, J. P. Steiner, A. Lo, J. Hedreen, S. Sisodia, S. H. Snyder, T. M. Dawson, D. K. Ryugo, and C. A. Ross. 1995. Widespread expression of Huntington's disease gene (IT15) protein product. *Neuron.* 14:1065–1074.

Snell, R. G., J. C. MacMillan, J. P. Cheadle, I. Fenton, L. P. Lazarou, P. Davies, M. E. MacDonald, J. F. Gusella, P. S. Harper, and D. J. Shaw. 1993. Relationship between trinucleotide repeat expansion and phenotypic variation in Huntington's disease. *Nature Genet.* 4:393–397.

Stine, O. C., N. Pleasant, M. L. Franz, M. H. Abbott, S. E. Folstein, and C. A. Ross. 1993. Correlation between the onset age of Huntington's disease and length of the trinucleotide repeat in IT-15. *Hum. Mol. Genet.* 2:1547–1549.

Stott, K., J. M. Blackburn, P. J. G. Butler, and M. Perutz. 1995. Incorporation of glutamine repeats makes protein oligomerize: implications for neurodegenerative diseases. *Proc. Natl. Acad. Sci. USA.* 92:6509–6513.

Strong, T. V., D. A. Tagle, J. M. Valdes, L. W. Elmer, K. Boehm, M. Swaroop, K. W. Kaatz, F. S. Collins, and R. L. Albin. 1993. Widespread expression of the human and rat Huntington's disease gene in brain and nonneural tissues. *Nature Genet.* 5:259–265.

Trottier, Y., D. Devys, G. Imbert, F. Saudou, I. An, Y. Lutz, C. Weber, Y. Agid, E. C. Hirsch, and J.-L. Mandel. 1995. Cellular localization of the Huntington's disease protein and discrimination of the normal and mutated form. *Nature Genet.* 10:104–110.

Yazawa, I., N. Nukina, H. Hashida, J. Goto, M. Yamada, and I. Kanazawa. 1995. Abnormal gene product identified in hereditary dentatorubral-pallidoluysian atrophy (DRPLA) brain. *Nature Genet.* 10:99–103.

Zoghbi, H. Y., and H. T. Orr. 1999. Polyglutamine diseases: protein cleavage and aggregation. *Curr. Opin. Neurobiol.* 9:566–570.