New and Notable

Polyglutamine Toxicity: A New Idea for a Tough Problem

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INTRODUCTION

Many progressive neurodegenerative diseases including Huntington's disease, spinobulbar muscular atrophy, dentatorubral pallidoluysian atrophy, and spinocerebellar ataxia types 1, 2, 3, 6, and 7 are caused by an expansion of CAG (polyglutamine) codons at the ends of genes coding for various proteins (reviewed in, e.g., Reddy and Housman, 1997). In every case except the type 6 ataxia, which involves a defective calcium channel (Lunkes and Mandel, 1997), expansions of up to about 35 residues are benign, whereas expansions of >40 residues are pathogenic. Perutz (1999) has hypothesized that sufficiently long glutamine repeats can form hairpin structures that destabilize proteins and produce insoluble aggregates. The presence of protein aggregates inside afflicted neurons is, in fact, a post-mortem diagnostic marker of these diseases (Lunkes et al., 1998). However, it is not known whether the aggregates themselves are pathogenic or are, instead, by-products of other cellular processes. Recent work with yeast, for example, has shown that aggregated fragments of polyglutamine-expanded huntingtin protein, the causative factor in Huntington's disease, are not particularly toxic to cells expressing them (Krobitsch and Lindquist, 2000). Also, Saudou et al. (1998) found that inhibiting ubiquination and consequent formation of aggregates in the cell nucleus increased rather than decreased the toxic effect of polyglutamine-expanded huntingtin expressed in transfected striatal neurons. Although the evidence linking glutamine expansion length with both disease and protein aggregates is clear and abundant, a mechanistic relationship between protein aggregation and cellular toxicity has yet to be established. In this issue of the *Biophysical Journal*, Monoi et al. (2000) provide evidence in support of a strikingly simple and direct alternative to the aggregation hypothesis to explain the length dependence of polyglutamine toxicity: only repeats greater than 40 residues are long enough to form membrane-depolarizing ion channels in cells.

At the heart of this hypothesis is a novel protein structure, the μ-helix, predicted to be stable only for polyglutamine sequences (Monoi, 1995). This structure is remarkable in that a single, all L-amino acid polypeptide chain forms a helix with a 3.7 Å diameter cylindrical pore along its axis, physically large enough to accommodate passage of small ions and water. In the helix, each residue adds only about 0.81 Å to the length, so spanning 30 Å of bilayer hydrocarbon requires at least 37 residues instead of the 20 required for an α-helix. The interior of the pore is lined with backbone amide and carbonyl groups, much like gramicidin. The exterior surface of the pore is made up of glutamine side chains whose amide carbonyls form hydrogen bonds to side chain amide NH_2 groups 6 residues away toward the peptide's amino terminus. These hydrogen bonds, together with those of the oppositely directed backbone amide hydrogen bonds, presumably provide sufficient energy to stabilize an otherwise energetically improbable structure. Ordinarily, one might dismiss the suggestion that an ion channel structure has polar glutamine residues exposed to bilayer hydrocarbon. However, Monoi et al. (2000) point out that the hydrogen bonding of glutamine's side chain amides significantly reduces their unfavorable free energy of transfer to the hydrocarbon, so bilayer insertion is not implausible.

Given that such a structure, albeit never observed in protein solution or crystal structures, is possible, Monoi and coworkers then set about to determine experimentally if polyglutamine could make ion channels. Because polyglutamine peptides are virtually impossible to purify to homogeneity, the authors compared two samples that had been prepared and partially purified in the same way, but which contained peptides of 29 versus 40 residues maximum length. Only the sample containing the longer peptides made ion channels in multiple, well-controlled planar bilayer experiments. Moreover, the observed channels had the well-defined unit conductances characteristic of stable ion channel structures, rather than the erratic conductance fluctuations often observed with membrane-perturbing protein fragments and peptide toxins such as melittin. Strikingly, the channels were small, cation-selective, and only very weakly rectifying, all properties one might predict from the μ-helix structure.

Where might this work lead? Certainly, it should inspire efforts to make and characterize μ-helix structures in smaller, soluble polyglutamine peptides (see, e.g., Perutz et al., 1994), as well as to confirm and extend the present ion channel study to a wider range of glutamine repeat lengths. In addition, there are many questions to be answered about how polyglutamine repeats might produce cell-specific

Received for publication 10 April 2000 and in final form 12 April 2000.

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0006-3495/00/06/2733/02 $2.00

toxic effects in vivo. Does insolubility precede, parallel, or follow ion channel formation? Could it be that certain membrane lipid compositions are more susceptible than others, similar to the tropism observed with some natural peptide toxins? Are other proteins involved in the process? Might these small, probably sparse channels cause slow neuronal death by chronically overtaxing already highly active ATP-dependent ion pumps?

The challenge of biophysics has always been to find, in the complex processes observed in living systems, relatively simple underlying physical mechanisms. This class of devastating, incurable illnesses presents this challenge in the very urgent context of human medicine, and Monoi and coworkers are to be commended for a creative and inventive effort in addressing it.

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