FEBS Letters 585 (2011) 381–384

FEBS Letters

journal homepage: www.FEBSLetters.org

Polyglutamine shows a urea-like affinity for unfolded cytosolic protein

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ARTICLE INFO

Article history:
Received 2 November 2010
Revised 30 November 2010
Accepted 2 December 2010
Available online 19 December 2010

Edited by Miguel De la Rosa

Keywords:
Protein folding
Proteostasis
Denaturation
Polyglutamine
Huntington's disease
Urea

ABSTRACT

Noting that the glutamine (Q) amino acid side-chain bears a striking resemblance to urea, the chemical denaturant, we argue on biophysical grounds that polyQ chains should possess a potent denaturant activity. Using live-cell confocal microscopy, we demonstrate that the surface of a polyQ inclusion denatures cytosolic proteins by binding and trapping them in an immobilized ring. We also show the reverse effect: that elevated local concentrations of unfolded protein in the cytosol can drive the co-localization and accumulation of short polyQ tracts that normally do not aggregate. Such a urea-like mechanism explains many past observations about polyQ-driven disruption of proteostasis and neurodegeneration.

Polyglutamine disorders such as Huntington's disease are characterized by neurodegeneration brought on by the expression of mutant proteins containing expanded repeats of glutamine (Q) more than 40 residues in length [1]. Although the complete mechanism of polyQ-driven cytotoxicity is presently unclear, past work has established that polyglutamine chains cause a global disruption of protein folding homeostasis in the cell [2], that their toxicity correlates positively with their length [1], and that their protective partitioning into a large, intracellular inclusion known as the insoluble protein deposit (IPOD) promotes cell survival [3]. Here, we provide an explanation for all of these previous findings by using live-cell confocal microscopy to demonstrate that polyQ structurally destabilizes other proteins through direct association.

Our study was motivated by the observation that the glutamine side-chain bears a strong resemblance to the chemical denaturant urea (Fig. 1a). Simulations of polyglutamine peptides have suggested that a chain 37 residues in length forms a compact, globule that resembles a cube 20 Å on a side [4]. Simply by computing the volume of such a container, we may estimate that the concentration of the 37 glutamine residues in the globule is roughly 8 M ("[Q] in polyQ" in Fig. 1a), which stands well in excess of the activity of urea normally necessary to denature a wide range of proteins in vitro. We therefore reasoned that the surface of a polyglutamine molecule should share urea's high affinity for unfolded protein, and that a polyQ aggregate of sufficient size may possess the ability to bind and trap a protein in its unfolded state.

At a concentration of 300 mg/ml [5], the cytosol is highly crowded, and any given protein is likely to have several other polypeptide chains in near-direct contact with it. Thus, we may suppose that roughly one cytosolic protein should be denatured in the vicinity of each polyQ chain, much as it would be in a highly concentrated urea solution. In order to assess the impact of diffuse, cytosolic polyQ expression on overall proteostasis, we must estimate the amounts of natively folded and denatured protein in the cytosol, both in the presence and absence of the polyQ.

Approximating the mammalian cell as a sphere ~20 μm in diameter filled with proteins that are typically 60 kDa in mass, the total number of proteins N_p should be given by

$$N_p \sim \frac{(4/3)\pi(10\ \mu m)^3 \times (300\ mg/ml)}{60\ kDa/protein} = 1.3 \times 10^{10}\ \text{proteins}$$

Mammalian tissue culture cells overexpressing a toxic polyQ-expanded gene product frequently sequester the aggregating protein in an insoluble amyloid inclusion roughly 1 μm across at the time of its formation [3]. Assuming such an inclusion is mostly composed of amyloid of density ~1.3 g/cm^3 [6], the number of polyQ proteins N_{polyQ} that were dispersed in the cytosol before they accumulated in an inclusion should be given approximately by

$$N_{polyQ} \sim \frac{(4/3)\pi(1\ \mu m)^3 \times (1.3\ g/cm^3)}{12\ kDa/polyQ} = 2.7 \times 10^8\ \text{proteins}$$

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0014-5793/$36.00
doi:10.1016/j.febslet.2010.12.023

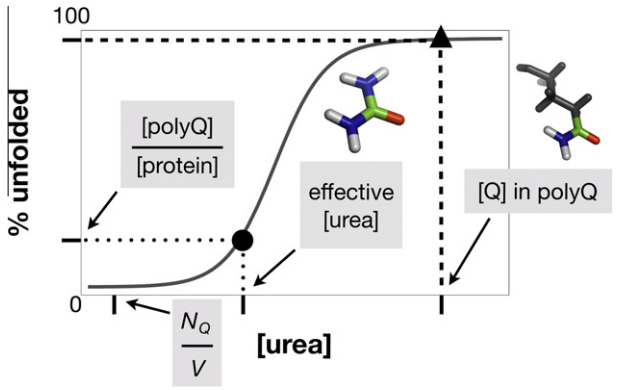

Fig. 1. Because of the cooperativity of protein folding, a denaturing agent's potency per molecule is dramatically enhanced when tethered to a protein backbone, as in a polyQ chain. The high local concentration in the polyQ molecule's vicinity fully denatures a nearby protein (triangle, dashed lines). This destabilization takes place for the fraction of all proteins found to be sitting adjacent to a polyQ chain, i.e., for [polyQ]:[protein]. This fraction in turn corresponds to some concentration of uniformly dispersed urea that would have achieved the same destabilization (circle, dotted lines), a concentration that vastly exceeds the ratio of the number of glutamine side-chains N_Q in the cell to the volume V of the cell. In the molecular models depicted, red atoms are oxygen, blue atoms are nitrogen, green atoms are carbon, and white atoms are hydrogen.

The ratio $N_{\mathrm{polyQ}}/N_{\mathrm{p}} \sim 0.02$ leads us to expect that roughly 2% of proteins should be destabilized by a urea-like polyQ globule in their local neighborhood. To weigh the potential significance of this effect, we note that globular proteins typically have an in vitro thermal stability of $\Delta G_{\mathrm{u}} \sim 5$ kcal/mol [7]. Making the admittedly simplistic assumption that this free energy difference determines the statistical equilibrium between populations of folded and unfolded proteins in the cytosol, we expect the unfolded fraction to be $e^{-\Delta G_{\mathrm{u}}/RT}/(1+e^{-\Delta G_{\mathrm{u}}/RT}) \sim 0.0002$ at a temperature $T \sim 300$ K. Put another way, if we define ΔG^* to be the free energy of unfolding for which $e^{-\Delta G^*/RT}/(1+e^{-\Delta G^*/RT}) = 0.02$, then $\Delta G_{\mathrm{u}} - \Delta G^* \sim 2.7$ kcal/mol, the rough equivalent of the destabilization brought about by treating the cytosol with a 1 M urea solution ("effective [urea]" in Fig. 1) [8]! Indeed, even if we have overestimated the number of polyQ molecules by 50-fold, we would predict a (still cytotoxic) effect comparable to that of 0.1 M urea. It should also be noted that while the presence of molecular chaperones in live cells would quantitatively alter this picture, the qualitative result remains the same: the stabilization of the unfolded states of proteins facilitated by polyQ should either increase the concentration of unfolded protein in the cytosol, or else should drive more proteins into complex with chaperones, titrating out components of the quality control machinery. In either event, the expected effect is a disruption of proteostasis.

This result may, at first glance, seem paradoxical. According to the assumptions made above, the total excess number of glutamine side-chains in one cell due to the overexpression of polyQ is $\sim 2 \times 10^{10}$ molecules. The same number of urea molecules in a cell 20 μm in diameter would have a concentration of ~0.01 M ("N_Q/V" in Fig. 1), yet we have just made the case that the effect of polyglutamine has the potential to be orders of magnitude stronger. The solution to this puzzle lies in the cooperativity of protein folding (Fig. 1). In the canonical case of a two-state folder, a protein exhibits a sharp transition between being natively folded and being denatured as the concentration of a chemical denaturant such as urea passes through a critical value. As a result, a given quantity of urea molecules (or glutamine side-chains) will have a dramatically more destabilizing effect on the same protein population to the extent that the denaturing agents are inhomogeneously spatially concentrated in microdomains that drive the proteins in their vicinity well past the unfolding transition point. Indeed, there is evidence that a similar principle is exploited by the cell itself, which modulates the process of protein folding in vivo by concentrating misfolded proteins in two distinct quality control compartments [9].

The claim that polyQ exerts a denaturing influence on the conformational equilibrium of proteins in the cytosol is equivalent, in statistical mechanical terms, to the assertion that polyQ must exhibit a high affinity for unfolded protein. To test for this property, we first co-expressed fluorophore-tagged fusions of various proteins and the fluorophore-tagged huntingtin fragment HttQ97 [9] in HEK293T cells (Fig. 2a). Ubiquitin accumulated in a bright ring around the HttQ97 inclusion, suggesting the presence of unfolded proteins tagged for proteasomal degradation [10]. The distinctive ring phenotype, which is only observed for certain proteins [11], also formed around HttQ97 when we instead expressed fluorescent fusions of luciferase [12], which is marginally stable, VHL, which is natively unstructured [13], and Ubc9ts, which is misfolded [9].

In all cases, the ring width remained small compared to the radius of the inclusion, which suggests that a protein must interact directly with the HttQ97 inclusion in order to add itself to the ring. With the expected exception of Hsp104 (which disrupts polyQ aggregation [14]) fluorescence recovery after photobleaching (FRAP) revealed that proteins in the ring were immobile, indicating they had bound irreversibly to the polyglutamine surface. The ring phenotype was not observed, however, for a non-ligatable C-terminal fusion of ubiquitin and YFP, indicating that ubiquitin must be attached to another protein to join the ring (Fig. S1). Since the ubiquitin ring is displaced and eliminated in the presence of the Hsp104 disaggregase (Fig. S2), it is also clear that the ubiquitin does not become covalently attached to the polyQ inclusion itself. We concluded from these observations that the surface of the polyQ inclusion binds and traps various ubiquitylated cytosolic proteins in unfolded conformations.

Having established that polyQ in the cell can drive the accumulation of unfolded protein, we next sought to test whether the reverse is also the case, that is, whether unfolded protein can act as a lure for polyglutamine. When VHL is expressed by itself in mammalian cells, it is ubiquitinated and targeted to the juxtanuclear quality control (JUNQ) compartment [9]. In contrast, the short polyQ protein HttQ25 remains diffusely distributed throughout the cytosol when expressed alone [15]. When the two proteins are co-expressed, however, HttQ25 is found to co-localize with the VHL-rich JUNQ structure, suggesting that polyQ is attracted to the high local concentration of unfolded protein (Fig. 2b). The same co-localization was also observed for Q25 and Ubc9ts (Fig. S3). This finding supports the hypothesis that polyglutamine stretches have high affinity for unfolded protein.

The urea-like mechanism for polyQ action is consistent with a large number of past empirical observations. Perhaps the most basic of these is that, much as one would expect from a concentrated urea cloud, polyQ expansions are known to denature many other proteins to which they are fused [16–18] (with GFP, oft-cited for its extreme stability, being the exception that proves the rule [19]). The resemblance runs in the other direction as well, since urea, like polyQ, is thought to have the tendency to aggregate on the nanometer scale [20], despite its high aqueous solubility.

More importantly, the urea-like mechanism also provides a natural explanation for the observation that polyglutamine has a global destabilizing impact on proteostasis: our calculations suggest that, as a combined consequence of the crowdedness of the cytosol and the cooperativity of protein folding (Fig. 1), diffuse overexpression of polyQ in tissue culture could have the equivalent effect on proteostasis to treatment of cells with toxic levels of urea [21]. In light of this, the origin of the protective effect of sequestering polyQ in an inclusion body becomes obvious: the exposed surface area per molecule of polyQ may drop by as much as several

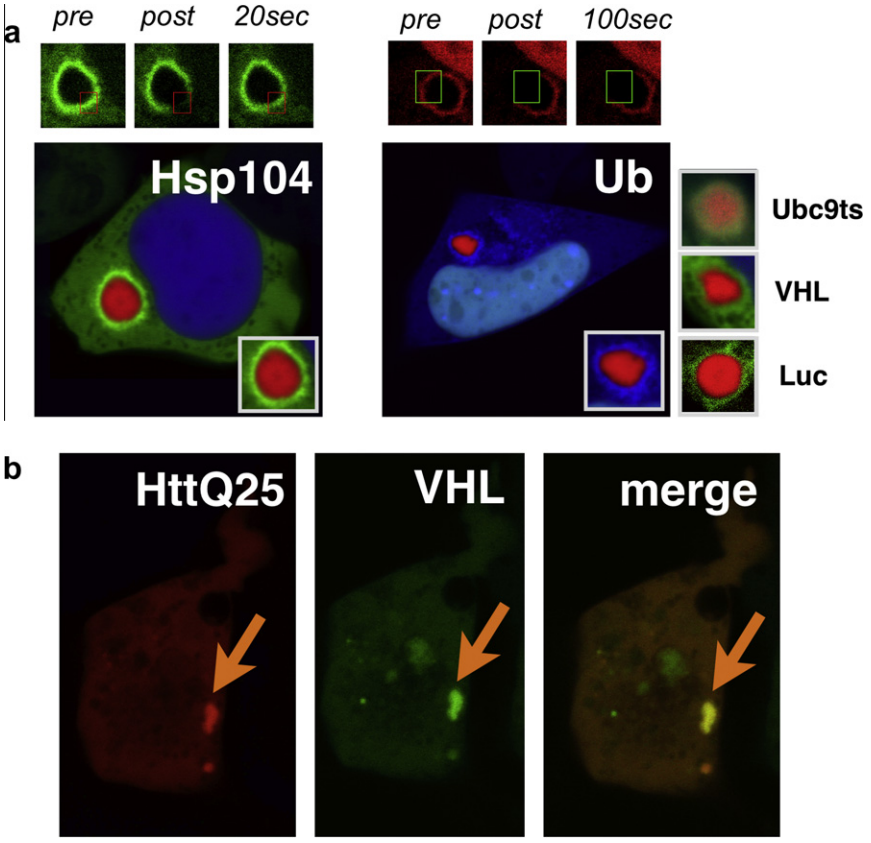

Fig. 2. (a) Various fluorophore-tagged proteins (ubiquitin in blue (right panel), Hsp104, VHL, and luciferase in green) accumulate in rings around polyglutamine inclusions (bright red). Though the Hsp104 ring remains mobile (top row, green), the ubiquitin ring (top row, red) and all others are immobile and do not recover fluorescence after photobleaching. (b) Upon co-expression, fluorescently tagged HttQ25 (red) and VHL (green) were found to co-localize (yellow) in a JUNQ compartment (orange arrows) that recovered rapidly from photobleaching (data not shown).

thousandfold upon formation of an inclusion. It should also be possible to reduce toxicity by counteracting the urea-like activity of the polyQ. Thus, it is quite notable that treating cells with an osmolyte known to reverse the denaturing effects of urea has been shown to bring about a partial rescue from the toxic impact of polyQ expression [22].

Perhaps most striking of all, the analogy between polyglutamine and concentrated urea provides us with a means to understand the dependence of polyQ toxicity on chain length. At least two scenarios must be considered. In the first, which provided the basis for the initial calculation performed in this work of the effective urea concentration in a polyQ 37mer, we assume that a single polyQ chain can, in principle, act by itself to denature another protein its vicinity. In this case, it may be argued that in order to drive the unfolding of a protein, a localized cloud of urea must not only be concentrated, it must also be big enough to be a substantial determinant of that protein's local environment. Thus, we would expect that a polyQ globule should only be able to affect the stability of another protein if it is large enough to surround or adsorb a sizeable portion of that protein. This criterion could explain why short polyQ stretches such as Q9 or Q25 are unable to exert a significant influence on proteostasis: they are simply too small.

A second possibility, however, is that the smallest polyQ-rich structures that provide a large enough scaffold for denaturation are larger than a single polyQ chain. In this case, the key determinant of polyQ toxicity would be the competency of the chain to aggregate into a larger, oligomeric species whose surface would be expansive enough to adsorb a whole unfolded protein. In this scenario, the fate of a cell expressing polyQ is substantially more complicated than for the simple process sketched in Fig. 1a; the non-equilibrium dynamics of the aggregation of polyQ and the formation of various oligomeric species of different sizes and surfaces areas is likely to be actively modulated by molecular chaperones and other components of the cellular quality control system. Whether polyQ toxicity be a property of a single polyQ chain or that of the surface of a larger aggregate, however, one would in either case predict that polyQ stretches must be at least ~40 residues in length to be toxic (either so that they are as large as a single protein domain, or so that they are competent to form aggregates [1]), and that their toxicity should increase with their length (either because they provide more surface area per chain and can therefore disrupt a larger number and broader range of substrates, or because they have a more pronounced tendency to aggregate [1]).

The denaturant picture of polyQ toxicity suggests a simple therapeutic approach to polyglutamine diseases. Presumably, by coating the urea-like surface of a polyQ aggregate with the right ligand, one might totally abrogate the aberrant protein's toxicity. Future small molecule screens should look for a compound that binds to glutamine-studded surfaces and occludes their denaturing activity from proteins in the surrounding environment. The potential role for polyglutamine chain in destabilization of proteins with which they directly interact also merits further attention. Because of polyQ's notorious tendency to aggregate in vitro [6], it will likely be necessary to investigate this issue using single-molecule fluorescence techniques that probe the conformations of proteins in the proximity of polyglutamine tracts. Future studies along such lines would undoubtedly help to define the requirements for polyQ-driven denaturation more precisely.

Acknowledgments

The authors thank E. Perlstein and M. Schuldiner for fruitful discussions, M. Brandeis, H. Kampinga, and R. Morimoto for reagents, and the Lewis-Sigler Institute for support. This work was also supported in part by NSF Grant PHY-0650617 and by NIH Grant P50 GM071598.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2010.12.023.

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