COMMENTARY

Pharmacological chaperoning: two 'hits' are better than one

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Protein folding disorders comprise a rapidly growing group of diseases that involve virtually every organ system and affect individuals of all ages. Their principal pathology is the inability of a protein to acquire or maintain its physiological three-dimensional structure. In cells, this generally results in one of three outcomes: accumulation of misfolded protein aggregates, cell death, or recognition by cellular quality control machinery and rapid degradation. Large-scale screening efforts to identify and design small molecules that either repair the folding defect or enable the protein to escape degradation have been encouraging. However, most compounds identified to date restore only a small fraction of molecules to the normal folding pathway, and hence are relatively poor therapeutic candidates. Results published by Wang et al. in this issue of the *Biochemical Journal* show that, for mutant forms of two ABC (ATP-Binding-Cassette) transporters, P-glycoprotein and CFTR (cystic fibrosis transmembrane conductance regulator), modest correction of trafficking by single agents can be additive when multiple compounds are used in combination. These findings raise the intriguing possibility that corrector molecules acting at different steps along the folding pathway might provide a multidrug approach to human protein folding disorders.

Key words: cellular chaperone, conformational disease, cystic fibrosis, cystic fibrosis transmembrane conductance regulator (CFTR), endoplasmic-reticulum-associated degradation (ERAD), protein folding, protein misfolding, pharmacological chaperone.

Biological organisms provide a unique environment for protein folding that is subject to a wide variety of physical, chemical and environmental stresses. It is therefore not surprising that cells have evolved elaborate and costly mechanisms to ensure that nascent proteins fold efficiently into stable conformations, and that, once folded, they are maintained in a soluble and well-behaved state. Molecular chaperone systems, which comprise a significant fraction of the total cellular protein, perform key roles in this process [1]. Collectively, the Hsp70 (heat-shock protein 70), Hsp90 and Hsp60 chaperone families bind nascent and partially unfolded polypeptides, shield hydrophobic aggregation-prone surfaces and peptide loops, stimulate protein maturation, provide a sequestered environment to prevent off-pathway interactions and promote productive folding outcomes [2]. Chaperones also continuously monitor the cellular folding environment and are directly linked, via cofactors, to degradation pathways that remove terminally damaged and misfolded proteins [1]. The importance of these homoeostatic mechanisms is evident from their high degree of evolutionary conservation, ubiquitous nature and functional redundancy in different intracellular protein folding compartments [2].

Even with these elaborate safeguards, many cellular proteins appear to reside very near to a critical thermodynamic threshold that renders them conformationally susceptible to minor perturbations in sequence and physical environment [3,4]. One example is the CFTR (cystic fibrosis transmembrane conductance regulator), in which mutations cause the lethal autosomal recessive disease cystic fibrosis. CFTR is a member of the ABC (ATP-Binding Cassette) transporter superfamily, and functions as an ATP-gated chloride channel in the apical epithelial cell membrane. In most cell types, CFTR trafficking is inefficient. Approx. 70 % of newly synthesized protein fails to attain a stable conformation and is rapidly degraded at its site of synthesis in the ER (endoplasmic reticulum). The most common CFTR mutation ΔF508 (with deletion of Phe^{508}), present in more than 90 % of CF patients, further tips this delicate balance to the side of instability such that less than 1 % of the mutant reaches the plasma membrane. However, ΔF508 CFTR, like numerous other disease-related misfolded proteins, retains some functional activity. Thus, despite major changes in stability and trafficking, inherited mutations often induce relatively minor perturbations in protein structure.

An important discovery in protein folding disorders is that manipulation of the cellular folding environment can stabilize and promote trafficking of mutant proteins. For example, plasma membrane chloride conductance can be partially restored when cells expressing ΔF508 CFTR are grown at reduced temperature [5]. Trafficking of many misfolded proteins can also be corrected pharmacologically by exogenous small molecules (glycerol, deuterated water and DMSO) and addition or induction of endogenous cellular osmolytes [6]. Finally, manipulating expression and/or function of cellular chaperones and/or their cofactor can also stabilize mutant proteins [7]. Such findings highlight a key relationship between chemical and thermodynamic aspects of protein biochemistry and the complex cellular environment that assists and monitors the folding process. These two interrelated themes, referred to as the chemical and the biological fold respectively, each offer potential therapeutic opportunities because the major checkpoints for conformational surveillance occur within a relatively brief interval during and/or following synthesis [4]. Transmembrane and secreted proteins that pass these checkpoint(s) in the ER can usually traverse the secretory pathway to their final cellular destination.

In a seminal study, Loo and Clarke previously demonstrated that small-molecule ligands can promote stability and trafficking of mutant misfolded substrates in a highly specific manner [8]. Using an unstable variant of the multidrug transporter P-gp (P-glycoprotein), they showed that addition of P-gp substrates and modulators to cells could partially reverse the trafficking defect.

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Similar results were soon reported for high-affinity antagonists of the V_2 vasopressin receptor, the voltage-dependent HERG (human *ether-a-go-go*-related gene) potassium channel and a variety of other proteins [9]. Moreover, native ligands (e.g. thyroxine) can also correct folding of their own endogenous mutant receptors [4]. It has therefore been proposed that the free energy imparted by high-affinity ligand binding can either stabilize native, or disfavour intermediate, folded states sufficiently for mutant proteins to escape degradation. These early findings stimulated major screening efforts to identify small molecules that could precisely target subtle folding defects in specific protein substrates. Results of these efforts are beginning to bear fruit. Several corrector compounds of different chemical classes were recently identified in two independent high-throughput screens ($\sim$150000 compounds each) of ΔF508 CFTR [10,11]. Their mechanism of action, although currently unknown, is the subject of intense study and will undoubtedly reveal new insights into cellular folding and quality control pathways.

Unfortunately, most corrector compounds identified to date do not completely compensate for mutation-induced folding defects, and the small amount of protein salvaged may limit clinical benefit. In this issue of the *Biochemical Journal*, Wang et al. [12] identify a novel strategy to potentially overcome this problem. Again, the Clarke group took advantage of the well-developed transport pharmacology of P-gp. They first showed that rhodamine B and Hoechst 33342, which can bind simultaneously to different sites in P-gp transmembrane domains, elicit more efficient correction of mutant protein (P709G) processing when added together than either compound alone. This effect was also observed for two additional P-gp mutants in which either one or both of the cytosolic nucleotide-binding domains was removed, but not a third truncated mutant containing a single six-spanning transmembrane domain. Although drug binding was not directly examined in the study by Wang et al. [12], the implication of these results is that the combined binding energy of two substrates co-operatively augments folding of P-gp transmembrane domains into a trafficking-competent conformation. The authors then extended these findings to CFTR using three 'corrector' compounds identified by high-throughput screens either at the UCSF (University of California, San Francisco; corr-2b and corr-4a) or Vertex Pharmaceuticals (VRT325) [10,11]. Steady-state levels (determined by immunoblotting) of the CFTR mutant H1085R revealed that combinations of corr-2b/VRT325 or corr-4a/corr-2b rescued more CFTR than each compound independently. Similar additive effects were observed for combinations of corr-2b/VRT325 or corr-4a/corr-2b for the CFTR mutant H1085R, in which the second nucleotide-binding domain was removed. Finally, the combination of VRT325 together with corr-2b or corr-4a increased both the amount of Golgi-processed as well as plasma membrane ΔF508 CFTR when compared with individual compounds.

These results provide the first description of co-operativity between small molecules for correcting protein folding defects in cells. The simplest interpretation is that the unfavourable folding energetics imposed by mutations in P-gp and CFTR are overcome by the sum of binding energies imparted by corrector molecules. This may well be the case for P-gp because both drugs tested are direct substrates that bind to different sites on the molecule. Thus, whereas each individual ligand is insufficient to restore the native structure, both ligands together effect favourable conformational changes as viewed from the perspective of the cellular quality control machinery. In the case of CFTR, the mechanism of corrector action is not yet established, and it is not known whether these compounds bind to CFTR directly or whether they act indirectly through other cellular components. The non-selective activity of VRT325, as demonstrated by its ability to inhibit P-gp function and its pleiotropic correction of mutant HERG channel processing, suggests that the latter may be the case. In this respect, it would be interesting to test whether CFTR correctors exhibit co-operative effects with P-gp ligands, and vice versa. Regardless of the outcome, the results presented will undoubtedly stimulate further mechanistic studies to explain these intriguing observations.

REFERENCES

1 Brodsky, J. (2007) The protective and destructive roles played by molecular chaperones during ERAD (endoplasmic-reticulum-associated degradation). Biochem. J. **404**, 353–363

2 Hartl, F. and Hayer-Hartl, M. (2002) Molecular chaperones in the cytosol: from nascent chain to folded protein. Science **295**, 1852–1858

3 Sanders, C. and Myers, J. (2004) Disease-related misassembly of membrane proteins. Annu. Rev. Biophys. Biomol. Struct. **33**, 25–51

4 Kelly, J. and Balch, W. (2006) The integration of cell and chemical biology in protein folding. Nat. Chem. Biol. **2**, 224–227

5 Denning, G., Anderson, M., Amara, J., Marshall, J., Smith, A. and Welsh, M. (1992) Processing of mutant cystic fibrosis transmembrane conductance regulator is temperature-sensitive. Nature **358**, 761–763

6 Welch, W. and Brown, R. (1998) Influence of molecular and chemical chaperones on protein folding. Cell Stress Chaperones **1**, 109–115

7 Amaral, M. (2004) CFTR and chaperones: processing and degradation. J. Mol. Neurosci. **23**, 41–48

8 Loo, T. and Clarke, D. M. (1997) Correction of defective protein kinesis of human P-glycoprotein mutants by substrates and modulators. J. Biol. Chem. **272**, 709–712

9 Bernier, V., Lagace, M., Bichet, D. G. and Bouvier, M. (2004) Pharmacological chaperones: potential treatment for conformational diseases. Trends Endocrinol. Metab. **15**, 222–228

10 Pedemonte, N., Lukacs, G. L., Du, K., Caci, E., Zegarra-Moran, O., Galietta, L. J. and Verkman, A. S. (2005) Small-molecule correctors of defective DeltaF508-CFTR cellular processing identified by high-throughput screening. J. Clin. Invest. **115**, 2564–2571

11 Van Goor, F., Straley, K. S., Cao, D., Gonzalez, J., Hadida, S., Hazlewood, A., Joubran, J., Knapp, T., Makings, L. R., Miller, M. et al. (2006) Rescue of DeltaF508-CFTR trafficking and gating in human cystic fibrosis airway primary cultures by small molecules. Am. J. Physiol. Lung Cell. Mol. Physiol. **290**, L1117–L1130

12 Wang, Y., Loo, T. W., Bartlett, M. C. and Clarke, D. M. (2007) Additive effect of multiple pharmacological chaperones on maturation of CFTR processing mutants. Biochem. J. **406**, 257–263

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