Pharmacological chaperones: a new twist on receptor folding

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Protein misfolding is at the root of several genetic human diseases. These diseases do not stem from mutations within the active domain of the proteins, but from mutations that disrupt their three-dimensional conformation, which leads to their intracellular retention by the quality control apparatus of the cell. Facilitating the escape of the mutant proteins from the quality control system by lowering the temperature of the cells or by adding chemicals that assist folding (chemical chaperones) can result in proteins that are fully functional despite their mutation. The discovery that ligands with pharmacological selectivity (pharmacological chaperones) can rescue the proper targeting and function of misfolded proteins, including receptors, might help to develop new treatments for 'conformational diseases'.

The endoplasmic reticulum (ER) is the first membrane compartment for the synthesis and processing of secretory and membrane proteins. Therefore, the function of the ER is to provide an environment in which the proper folding and assembly of polypeptides can occur. If polypeptides cannot fold correctly, mechanisms of quality control that reside in the ER must distinguish between correct and incorrect protein conformations to ensure that the aberrant proteins are not further processed along the secretory pathway. These quality control mechanisms are mediated, in part, by a family of proteins collectively referred to as molecular chaperones[1]. These chaperones interact with newly synthesized polypeptides to assist in their folding and eventually dissociate. At a mechanistic level, it has been hypothesized that misfolded proteins expose structural signals that are recognized by molecular chaperones.

Although the activities of the quality control system are generally advantageous to the cell, on occasion this stringent monitoring system leads to intracellular retention and ultimate destruction of salvageable proteins. In recent years, it has been observed that certain diseases stem from mutations that produce minor changes in a given protein structure. These changes are recognized by the quality control system and the mutant protein is retained intracellularly. The retention of an important protein in the ER results in the absence of this protein at its target site, which prevents it from performing its physiologically relevant function[2].

Recently, a class of compounds called chemical chaperones were shown to reverse the intracellular retention of several different misfolded proteins (Table 1). Among these, glycerol and other polyols stabilize protein conformation[3], increase the rate of *in vitro* protein refolding[4] and increase the kinetics of oligomeric assembly[5]. It has been proposed that such compounds favour the folding of mutant proteins into conformations that resemble the wild-type protein, allowing them to escape the quality control system.

Protein misfolding and chemical chaperones

Probably the best known example of protein misfolding that is responsible for a disease is the ΔF508 mutation in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), which causes cystic fibrosis. The ΔF508 allele of *CFTR* has been confirmed as a trafficking mutation that blocks the maturation of the protein in the ER and targets it for premature proteolysis[6]. However, if the ΔF508 protein is redirected to the cell surface, cAMP-mediated transport can be restored. The clinical importance of this mutation becomes evident when considering that the ΔF508 mutation accounts for nearly 70% of patients diagnosed with cystic fibrosis[7].

When overexpressed in heterologous systems, the ΔF508 mutation leads to the appearance of a small number of functional CFTR Cl^- channels in the plasma membrane[8]. As a result of this observation, it has been proposed that some

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Table 1. Mutations rescued by chemical and pharmacological chaperones[a]

Disease	Protein	Mutations rescued	Agents used	Refs
Cystic fibrosis	CFTR	ΔF508	Glycerol, DMSO, TMAO	11
Cystic fibrosis	CFTR	ΔF508	Glycerol	12
Emphysema and liver disease	α_1-Antitrypsin	D342K	Glycerol	15
Nephrogenic diabetes insipidus	Aquaporin-2	T126M, A147T, R187C	Glycerol, TMAO, DMSO	16
Nephrogenic diabetes insipidus	Vasopressin V_2 receptor	Δ62–64, L59P, L83Q, Y128S, S167L, A294P, P322H, R337X	SR121463A, VPA985	19
Fabry	α-Galactosidase A	R301Q, Q279E	1-Deoxy-galactonojirimycin	21
Cancer	p53	L173A, L175S, V249S, M273H	CP31398, CP257042	24
None	P-glycoprotein	G268V[b], DY490[b]	Capsaicin, cyclosporin, vinblastin, verapamil	17

[a]Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; DMSO, dimethylsulfoxide; TMAO, trimethylamine-*N*-oxide.
[b]Artificially induced mutations.

 0165-6147/00/$ – see front matter PII: S0165-6147(00)01575-3

nascent ΔF508 molecules can fold correctly, thereby escaping degradation. Interestingly, *Xenopus* oocytes[9] and mammalian cells[10] incubated at reduced (20–30°C) temperatures, express more ΔF508 molecules at the cell surface than those incubated at standard temperatures. At these lower temperatures, a fivefold increase in cAMP-stimulated whole-cell currents was detected[10]. These data indicate that many of the mutant proteins adopt a conformation compatible with cell-surface transport when the folding and/or the degradation processes are slowed down by reducing the temperature (kinetic effect). This temperature-dependent recovery of Cl^- channels at the cell surface was mimicked by treating cells with the chemical chaperones glycerol, dimethylsulfoxide (DMSO), trimethylamine-*N*-oxide (TMAO) or deuterated water[11]. For example, glycerol treatment of cells that express ΔF508 *CFTR* caused an eightfold increase in cAMP-dependent Cl^- currents[12]. Such chemical chaperones are believed to function by stabilizing misfolded mutant proteins into conformations that are not targeted for degradation and can escape the ER (conformational effect).

In addition, certain forms of liver disease and emphysema have been linked to mutations that lead to ER retention[13]. One such example is D342K, which is the most common mutation found in the gene encoding α1-antitrypsin (α1-AT), referred to as the Z deficiency variant (α1-ATZ)[14]. Normally, α1-AT is secreted into the bloodstream and body fluids, where it accumulates in the lung to inhibit primarily neutrophil elastase, cathepsin G and proteinase 3. In patients harbouring this mutation, the absence of circulating α1-AT leads to lung emphysema and its marked accumulation in the liver is responsible for liver injury[13]. In cells that synthesize α1-ATZ, treatment with glycerol produces a fivefold increase in the secreted level of the mutant enzyme[15].

Another disease in which intracellular retention of proteins has been invoked is nephrogenic diabetes insipidus (NDI). Patients afflicted with this disease cannot concentrate urine in response to arginine vasopressin (AVP). Mutations responsible for this disorder have been identified in the gene encoding vasopressin V_2 receptors (*AVPR2*) (90% of cases) and in the gene encoding the AVP-responsive aquaporin-2 water channel (10% of cases) (see NDI Mutation Database at http://www.medcor.mcgill.ca/~nephros/). To date, over 150 different *AVPR2* mutations occurring in unrelated families have been reported. Of these, over 40 have been expressed in heterologous expression systems and ~70% result in misfolded, trafficking-deficient V_2 receptors. Similar to many mutant V_2 receptors, mutations of the aquaporin-2 water channel can prevent maturation of this protein to the plasma membrane. Although fewer NDI-causing mutations have been reported in the gene encoding aquaporin-2, a recent report describes three trafficking-deficient aquaporin-2 water channels (T126M, A147T and R187C) that were corrected with glycerol, DMSO or TMAO, as assessed by protein maturation, cellular targeting and water permeability[16]. When cells that express the T126M mutant aquaporin-2 were incubated with these chemical chaperones, the water-transport rate increased twofold.

Pharmacological chaperones

Taking the concept of chemical chaperones a step further, Loo and Clarke have functionally characterized artificial mutations of the multidrug resistance 1 gene (*ABCB1*), which codes for P-glycoprotein 1, an energy-dependent transporter at the plasma membrane that interacts with a wide variety of cytotoxic agents[17]. The P-glycoprotein 1 mutants generated were retained in the ER as core-glycosylated biosynthetic intermediates. Given that chemical agents such as glycerol can correct protein folding nonspecifically, most probably by stabilizing adequate folding intermediates, the effects of specific substrates or modulators that bind P-glycoprotein 1, such as capsaicin, cyclosporin, vinblastine and verapamil, were assessed. Pulse-chase metabolic labelling studies showed that each of these compounds increased the proportion of correctly folded mutant P-glycoprotein 1 and promoted a 7–12-fold increase in activity[17].

Based on the fact that pharmacological ligands act by binding to and stabilizing specific conformations of receptors, selective V_2 receptor antagonists were assessed to reveal whether they facilitate the folding of mutant V_2 receptors that are retained in the ER and are responsible for NDI. The biosynthesis of mutant V_2 receptors was monitored in the presence of the selective V_2 receptor non-peptide antagonist SR121463A (Ref. 18). This cell-permeable antagonist converted precursor forms of a mutant V_2 receptor (Δ62–64) into fully glycosylated mature receptor proteins that were targeted to the cell surface, as determined by pulse-chase metabolic labelling analysis and cell-surface immunofluorescence microscopy. Once at the correct cellular location, this mutant receptor bound to AVP and produced an intracellular cAMP response 15 times greater than that produced in cells not exposed to the SR121463A antagonist[19]. V_2 receptor antagonists that are membrane-impermeable were unable to mimic this effect or compete with SR121463A, which indicates that SR121463A was mediating its effect intracellularly. In addition, seven other NDI mutant V_2 receptors that are retained intracellularly were rescued by SR121463A treatment. Such an effect is not unique to SR121463A because another V_2 receptor non-peptide antagonist, VPA985 (Ref. 20), rescued cell-surface expression and function of the same subset of mutant receptors.

On the basis of these data, we proposed a model in which small non-peptide V_2 receptor antagonists permeate the cell and bind to unstable folding intermediates of the mutant receptors. This would stabilize a conformation of the receptor that allows its release from the ER quality control apparatus. The stabilized receptor would then be targeted to the cell surface where it could bind AVP and promote signal transduction upon dissociation from the antagonist (Fig. 1). Given that these antagonists are specific to the V_2 receptor and that they perform a chaperone-like function, we termed these compounds 'pharmacological chaperones'[19].

The stabilizing effects of pharmacological compounds are not restricted to the secreted and plasma membrane proteins discussed thus far. Further support for the hypothesis that small-molecule ligands can rescue ER-trapped mutant proteins is provided by a recent report on the functional rescue of

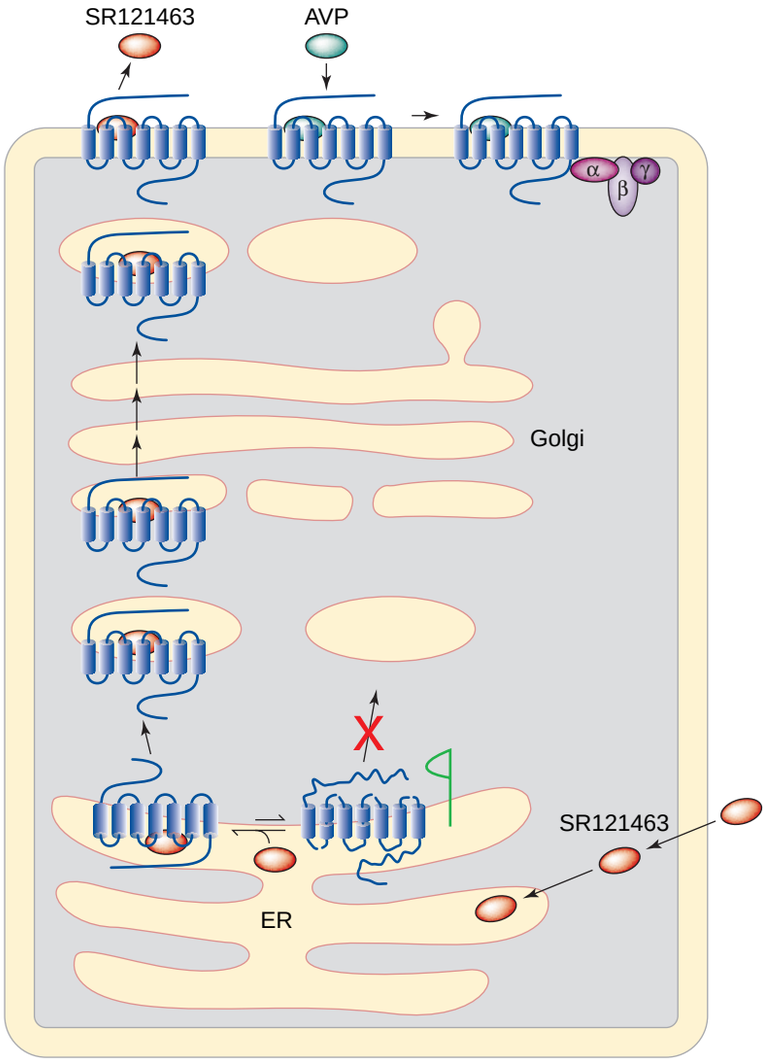

Fig. 1. Misfolded, ER-retained mutant vasopressin V_2 receptors can be rescued by pharmacological chaperones. Misfolded or incompletely folded proteins are retained in the ER through their interactions with molecular chaperones (green) until an 'acceptable' protein conformation is reached. In this model, the pharmacological chaperone is the non-peptide, selective V_2 receptor antagonist SR121463A. This compound is sufficiently hydrophobic to cross both the plasma and ER membranes, where it can bind misfolded V_2 receptors and stabilize a conformation that is released from the molecular chaperone and is compatible with cell-surface transport. At the cell surface, the receptor can bind its natural ligand vasopressin and stimulate effector activation through G-protein coupling. Abbreviation: ER, endoplasmic reticulum.

lysosomal α-galactosidase A (α-Gal A) mutants responsible for Fabry disease[21]. The mutant enzyme causes aberrant glycosphingolipid metabolism, which leads to renal failure, premature myocardial infarctions and strokes[22]. Two naturally occurring mutations (R301Q and Q279E) in the gene encoding α-Gal A result in the intracellular aggregation of this protein in the ER (Ref. 23). Lymphocytes derived from Fabry patients were treated for 4 days with 1-deoxy-galactonojirimycin (20 μM), a potent inhibitor of α-Gal A, which resulted in a sevenfold increase in enzymatic activity that was still present up to 5 days after the withdrawal of the drug. When administered to transgenic mice that express the R301Q mutation, 1-deoxy-galactonojirimycin caused an 18-fold increase of enzyme activity in the heart, with more modest increases in kidney, spleen and liver. This provides yet another example of a compound that can bind misfolded proteins with pharmacological selectivity and promote release from ER retention, concomitant with proper subcellular protein localization.

Furthermore, the concept of pharmacological chaperones extends to cytosolic proteins. Mutant forms of the tumour suppressor protein p53 that have reduced thermodynamic stability of their DNA-binding domain were stabilized by two newly identified, small-molecule compounds[24].

Concluding remarks

As the molecular mechanisms of a growing number of genetically inherited diseases are uncovered, it is increasingly appreciated that errors in folding and cellular trafficking are more frequent than anticipated[25]. Thus, the development of strategies aimed at promoting proper folding and maturation of mutant proteins could provide new therapies for a wide spectrum of diseases. The observation that small cell-permeable molecules can act as either chemical chaperones or pharmacological (ligand-mediated) chaperones to rescue mutant protein function has taken us one step further towards this goal. The selectivity of action offered by ligand-mediated chaperones might represent a significant advantage over chemical chaperones by allowing tailor-made interventions that are less likely to result in toxic effects. Clinical trials testing the effects of pharmacological chaperones are currently underway; thus, we should know soon whether a new class of drug will be added to our therapeutic arsenal.

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Chemical names

CP31398: *N*′-{2-[2-(4-methoxy-phenyl)-ethyl]-quinazolin-4-yl}-*N*,*N*-dimethyl-propane-1,3-diamine

CP257042: 5-(benzo[*g*]quinolin-5-ylamino)-2-diethylaminomethyl-phenol

SR121463A: 1-[4-(*N*-tert-butylcarbamoyl)-2-methoxybenzene sulfonyl]-5-ethoxy-3-spiro-[4-(2-morpholinoethoxy)cyclohexane]indol-2-one, fumerate

VPA985: 5-fluoro-2-methyl-*N*-[4-(5*H*-pyrrolo[2,1-*c*]-[1,4] benzodiazepin-10(11*H*)-ylcarbonyl)-3-chlorophenyl] benzamide

PPAR-γ agonists: therapeutic role in diabetes, inflammation and cancer

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The recent development of a novel class of insulin-sensitizing drugs, the thiazolidinediones (TZDs), represents a significant advance in antidiabetic therapy. One key mechanism by which these drugs exert their effects is by activation of the peroxisome proliferator activated receptor γ (PPAR-γ), a member of the nuclear receptor family. Evidence supporting this mechanism of action of the TZDs will be reviewed in this article. Recent data suggests that PPAR-γ agonists might also have therapeutic potential in the treatment of inflammatory diseases and certain cancers.

Type 2 diabetes, also known as non-insulin dependent diabetes mellitus (NIDDM), is a chronic disease that affects 5–10% of adults over the age of 30 in most populations[1]. Type 2 diabetes is characterized by resistance to the effects of insulin in peripheral tissues, which is manifested as reduced insulin-stimulated glucose uptake into skeletal muscle (which normally disposes of 90% of post-prandial plasma glucose) and adipose tissue, defective insulin-dependent suppression of hepatic glucose output and reduced insulin secretion from pancreatic B-cells[2]. A new class of drugs, the thiazolidinediones (TZDs), has been developed recently that directly targets insulin resistance, a primary defect of type 2 diabetes, and potentially represents a major therapeutic advance in the treatment of this disease[3]. Compelling data now indicate that the peroxisome proliferator activated receptor γ (PPAR-γ), an orphan nuclear receptor, has a central role in the insulin-sensitizing actions of TZDs.

PPARs and their expression

PPARs are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors that are related to retinoid, steroid and thyroid hormone receptors[4]. All members of this superfamily have a similar structural organization. An N-terminal region that allows ligand-independent activation[5] can confer constitutive activity on the receptor and is negatively regulated by phosphorylation[6]. This region is followed by a DNA-binding domain, comprising two zinc fingers separated by a linker region, and the C-terminal ligand-binding domain[7] (Fig. 1). The PPAR subfamily consists of three members, PPAR-α, -δ (also known as β) and -γ (Fig. 2), so called because activation by xenobiotics of the first receptors to be characterized, PPAR-α, results in peroxisome proliferation in rodent hepatocytes[8]. Activation of the PPAR-δ or PPAR-γ isoforms, however, does not elicit this response. Most tissues in *Xenopus*, rodents and humans express all three receptor subtypes, although there is considerable variability in relative expression levels. In rats and humans, mRNA encoding PPAR-α is most abundant in tissues with a high capacity for lipid oxidation, such as the liver, kidney, skeletal and cardiac muscle, and adrenal glands. PPAR-δ is expressed ubiquitously in all tissues of adult mammals, whereas two isoforms of PPAR-γ, γ_1 and γ_2, which arise from alternative promoter usage and differential splicing, are preferentially expressed in adipose tissue[8]. PPAR-γ_1 is also expressed in vascular smooth muscle cells[9] and macrophages[10,11].

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