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Question & Answer

Q&A: What are pharmacological chaperones and why are they interesting?

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What is a chaperone in the context of pharmacology?

The term chaperone is borrowed from the name of a class of proteins that function in living cells [1]. Protein molecules are usually only marginally stable under physiological conditions, so some percent of them are often unfolded or misfolded. Such molecules can aggregate with one another, or with properly functioning proteins, with deleterious consequences to the cell. Protein chaperones prevent these unwanted associations by sequestering unfolded and misfolded proteins and providing them with an environment in which they have the opportunity to refold properly. In addition, the interior of a living cell is an extremely crowded environment, in which the concentration of macromolecules may exceed 100 μM [2,3]. As a protein is being synthesized on the ribosome, protein chaperones protect the nascent polypeptide chain from undesirable associations in that crowded environment until it can fold properly. In pharmacology, the role of a chaperone is similar, but instead of being proteins, pharmacological chaperones are small molecules, and instead of assisting in folding, they usually stabilize an already folded macromolecule (usually a protein) by binding to it and stabilizing it against thermal denaturation and proteolytic degradation [4-6].

So a pharmacological chaperone is a chemical chaperone?

No, a chemical chaperone is subtly different. Typical chemical chaperones are molecules such as glycerol and trehalose. Pharmacological chaperones are a special subset of chemical chaperones. Molecules like glycerol and trehalose are nonspecific: they bind to, and stabilize, practically any protein and usually do not have a specific binding site. Pharmacological chaperones, on the other hand, are designed specifically to bind to their target protein and, ideally, stabilize only that macromolecule. The difference, therefore, is one of specificity: a chemical chaperone used *in vivo* would stabilize virtually every macromolecule in the cell. A pharmacological chaperone acts on, at most, only a small number of protein targets. There is a great deal of excitement in the biomedical community these days about pharmacological chaperones because they may be the best approach to treating some serious human diseases, such as cystic fibrosis.

How do they work?

Small-molecule chaperones act like molecular glue, holding various parts of the protein structure together through the favorable interactions (electrostatic, van der Waals, and hydrogen bonding) they make with residues in the binding site. Since specific ligand binding sites are often located at the interfaces between protein domains or subdomains [7], such ligands can be particularly effective at stabilizing the whole protein structure.

Theoretically, a chemical chaperone could also act by binding to and stabilizing the transition state of protein folding (or a high-energy folding intermediate), which would increase the rate of folding. None of the pharmacological chaperones currently under development appear to act this way, but such a mechanism might be critical for secreted proteins, where slowly folding mutants could be degraded even if the folded state was stable.

Could ligand binding ever destabilize a protein?

It's rare, but it has happened. Usually this occurs either when the ligand induces a large conformational change in its target protein when it binds, and the bound conformation is inherently less stable, or when ligand binding disrupts a number of protein self-interactions in the binding site.

Why are small-molecule chaperones important?

There are only a few immortal proteins in the human body. Most proteins in all cell types turn over regularly. If such proteins have a mutation that makes them less stable than normal, they may be degraded more rapidly, thereby lowering their steady-state levels below what is required to maintain the health of the cell. Also, it is possible that the

unstable protein may aggregate when it unfolds, and such aggregates may themselves be toxic to the cell. There are many severe human diseases that arise from either mutations that destabilize an essential protein or the age-dependent build-up of toxic misfolded forms of normal proteins [8]. A chemical or pharmacological chaperone can stabilize the native fold of the protein, preventing aggregation and restoring proper steady-state levels.

Where are chaperones important?

Protein chaperones function in most cellular compartments, and small-molecule chaperones can do so as well, in theory. In practice, not all subcellular compartments may be permeable to a particular small-molecule chaperone. There is a particular need for such chaperones in compartments where proteins are subjected to unusual stress. Examples might include the mitochondrion, where large amounts of reactive oxygen species are present, the lysozome, which has a low pH and a high content of degradative enzymes, and the endoplasmic reticulum (ER), where many unstable mutant proteins may misfold during synthesis [9].

Can chemical chaperones be useful as drugs?

In theory, yes, and there are some efforts to use molecules such as trehalose therapeutically, but in general it is probably not desirable to stabilize most of the proteins in a cell, so specific chaperones are preferable therapeutically. In addition, the concentration of a chemical chaperone required to achieve stability may be millimolar or even molar, which is impractical for use in treatment.

For what sorts of diseases might pharmacological chaperones be useful?

Obvious examples are the protein-misfolding diseases such as cystic fibrosis, the amyloidoses, Parkinson's disease, Alzheimer's disease and Lou Gehrig's disease. Less obviously, most metabolic disorders involve mutations that destabilize proteins rather than simply inactivating them. For example, Gaucher disease, an autosomal-recessive lysosomal storage disorder, arises from mutations in the gene coding for the lysosomal enzyme acid-β-glucosidase (GCase). More than a hundred such mutations are known, and only a handful are nonsense mutations or involve the replacement of a residue in the active site of GCase. The vast majority of the disease-causing mutations occur randomly throughout the protein and lead to an unstable form that is either degraded in the lysosome (where it normally functions), or never manages to exit the ER in the first place. Enzyme-replacement therapy using injections of the normal enzyme can alleviate many of the symptoms of Gaucher disease, but the injected enzyme does not reach every affected organ system, and the treatment is onerous and extremely expensive. In principle, a pharmacological chaperone could be orally available, relatively inexpensive and might be able to stabilize GCase in every tissue of the body [10]. There are literally hundreds of diseases where the ability to stabilize a specific protein could have similar therapeutic benefits.

What kinds of molecules can act as specific chaperones?

It has been known for decades that the binding of an inhibitor to an enzyme stabilizes the enzyme against thermal denaturation, sometimes by 10°C or more [11]. Structural biologists have taken advantage of this property from the early days of protein crystallography: liganded proteins tend to crystallize more readily than their unliganded counterparts because their structures are more stable. Therefore, the obvious candidates for specific pharmacological chaperones for enzyme-related diseases are enzyme inhibitors.

Wait a minute! How can an inhibitor, which by definition interferes with a protein's activity, give you more active protein?

We should have said, reversible inhibitor. That is the key to the use of active-site-directed ligands as pharmacological chaperones. An inhibitor that binds irreversibly to a target protein takes that protein out of circulation, activity-wise. But a reversible inhibitor always allows the presence of some equilibrium amount of free enzyme [12], which is then available for substrate binding (which will also stabilize the protein). The affinity of the inhibitor is also important. If it binds too tightly, it may be effectively irreversible. But if it binds too weakly, it may impossible to supply a high enough concentration of the chaperone to be effective. In practice, inhibitors with a K_i (equilibrium disassociation constant) close to the K_m (Michaelis-Menten constant) of the substrate or a bit tighter seem to be useful.

Isn't it possible that if you are trying to stabilize a protein with a reversible inhibitor, you could give so high a dose that it would still attenuate the activity below necessary levels?

Yes, that is sometimes observed. Too high a dose of any pharmacological chaperone that inhibits its target protein is counterproductive, because it starts to reduce the activity. The dosing regimen that can be used is limited by this. It may be necessary to 'spike' the dose of the chaperone - that is, for example, giving it three days on, four days off, or every other day - rather than dosing continuously. Controlled release may also be needed in some cases. It is also possible that some mutated proteins may be so unstable that they cannot be effectively chaperoned by inhibitors because the concentration required to achieve beneficial stabilization would lead to loss of activity.

Is it possible to stabilize an enzyme without binding to the active site?

It should be, in theory. In practice, nearly all of the pharmacological chaperones now under development are

active-site ligands. However, tight specific binding anywhere on the surface of a protein ought to confer stabilization as a result of the increased number of interactions. The problem is how to find suitable sites. For many years it was thought that essentially the whole surface of a protein should be available for small-molecule binding - that is, that proteins are sticky everywhere. But in the early 1990s, one of us established that only a small number of sites were actually available to bind organic molecules, because tightly bound water prevents access to much of the protein surface [13]. These sites can be mapped crystallographically [14] and computationally [15]. Recently, these methods have been applied to a few enzymes, such as GCase, for the purpose of identifying sites other than the active site, known as exosites, that are suitable for pharmacological chaperone binding. For GCase a single exosite was identified and a library of small organic compounds has been docked to that position on the protein surface. The top 20 predicted binders were screened for thermal stabilization of the enzyme in a fluorescence-based assay. One compound was found that increased thermal stabilization by several degrees (the corresponding stabilization with a tight-binding inhibitor is about 10°C). Whether such non-inhibitory chaperones will work *in vivo* is still under investigation, but at least it has been shown that they can work *in vitro*.

Has there been any clinical success with pharmacological chaperones?

There has. It turns out that an established therapy for a metabolic disorder probably works this way, but no one recognized this until recently. Phenylketonuria (PKU) is caused by mutations in the gene coding for the enzyme phenylalanine hydroxylase. For years, one treatment for a subset of patients with this disease was high doses of tetrahydrobiopterin, the cofactor involved in the reaction catalyzed by PKU. It has since been shown that most of the PKU mutations destabilize the enzyme and, in some cases, the increased levels of cofactor enable it to act as a chaperone to stabilize the mutated enzyme [16]. Efforts are under way to apply this technology to cystic fibrosis, the prototypic genetic protein-folding disease, in which many of the mutations, including the most common, lead to a failure to produce enough properly folded protein, called CFTR, in the right place in the cell [17].

In mouse models of obesity and type 2 diabetes, the development of insulin resistance correlates with elevated levels of ER stress and induction of the 'unfolded protein response'. Ozcan *et al.* [18] have shown that the chemical chaperones phenylbutyric acid and taurine-conjugated ursodeoxycholic acid, both of which are known to attenuate ER stress [19], improve glucose tolerance and insulin action in a mouse model of type 2 diabetes. These findings offer a potential new approach to improve insulin action and glucose tolerance in diabetic individuals.

A number of other pharmacological chaperones are in late-stage clinical trials, including drugs for transthyretin-based amyloidosis [20-22], the imino sugar isofagomine for Gaucher disease [23-25], and 1-deoxygalactonojirimycin for Anderson-Fabry disease [26]. All these compounds are analogs of normal physiological substrates or reaction products that interact with these proteins, and all have shown efficacy in animal models of the disease. They have been shown to be safe, and in some cases to have a therapeutic effect in humans.

Do pharmacological chaperones have wider potential?

The answer appears to be yes. Although most of the work up to now has concerned the chaperoning of unstable mutant proteins, there is no reason, in principle, why the normal form of any protein cannot be stabilized in this way, thereby increasing its steady-state level. Imagine using a small-molecule chaperone to boost, perhaps temporarily, the activity of a tumor suppressor such as p53 or a transcriptional regulator that controls the immune response. Pharmacological chaperoning may be as effective as gene therapy, and much easier to implement, whenever elevated levels of a beneficial protein are desirable. But one of the most exciting applications of small-molecule chaperoning may actually be its use *in vitro*. Biopharmaceuticals are relatively unstable, both during manufacture and in storage. This is one reason why even injected human proteins often cause an immune response: misfolded proteins are known to break tolerance [27]. By including a pharmacological chaperone in the manufacture and storage of biopharmaceuticals it may be possible to reduce or eliminate many of these problems. Combining the chaperone with the biopharmaceutical during treatment may also improve *in vivo* stability, reducing the need for frequent dosing. Joint use of chemical and biological therapeutics is, we think, an exciting new approach to the treatment of disease.

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