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Pharmacological chaperones for curing enzymopathies: The case of lysosomal alpha-galactosidase

Valentina Citro
University of Naples Federico II, Italy

Pharmacological chaperones are useful for the treatment of enzymopathies arising from mutations that lower the free energy difference between an unfolded and a folded enzyme shifting the equilibrium towards the first form. The unfolded enzyme, although retaining the functional chemical groups is needed for the biological activity, does not maintain them in the appropriate spatial disposition which can be defined as native state. Improperly folded mutant enzymes are usually sensitive to proteolysis and are cleared by the protein quality control systems in the cytosol and endoplasmic reticulum. Activity can be rescued if the equilibrium is pushed back towards the native state. This can be obtained binding a pharmacological chaperone to the folded enzyme. In fact the binding energy of the ligand compensates for the loss in Delta G while unfolding. Lysosomal alpha-galactosidase represents a good model system for the therapy with pharmacological chaperones. Lysosomal alpha-galactosidase catalyzes the removal of α-galactosyl residues from a glycosphingolipid, globotriaosylceramide. Mutations of lysosomal alpha-galactosidase cause Fabry's disease. We used three methods to test the effect of pharmacological chaperones: 1) Thermal shift assay. This test takes advantage of an environmentally sensitive fluorescent dye which binds the enzyme when it reaches the melting temperature; 2) Urea induced unfolding coupled with limited proteolysis and Western blot detection. This test can be carried out on mutants in cell extracts; and 3) Administration of the pharmacological chaperone to cells expressing mutant enzymes. Open reading frames encoding mutated enzymes are introduced into vectors suitable for transient expression. Eukaryotic cells, COS7 or HEK293, are transfected and cultivated in the presence and in the absence of the drug. It can be interpreted that if the chaperones work and the mutants stabilize, a larger amount of protein can be detected by Western blot and consequently a higher enzymatic activity can be measured.

Biography

Valentina Citro is interested in developing Pharmacological Chaperones (PC) to cure rare diseases. She works on the identification of the mutations which can be responsive to chaperones and develop method for assays in vitro in two model systems: The Fabry disease, a lysosomal storage disorder and PMM2-CDG (CDG-Ia) disease, a disorder of glycosylation with no cure at present.

vale.ctr@gmail.com

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