Predicting the responsiveness of lysosomal human alpha-galactosidase to pharmacological chaperones

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Motivation

Fabry disease is an X-linked lysosomal storage disorder caused by inherited genetic mutations of alphagalactosidase (GLA). The disease has several genotypes and different phenotypes: patients with early-onset (classic) Fabry disease generally have undetectable or very low GLA activity, later-onset patients usually have low, but measurable, GLA activity. A novel pharmacological therapy for Fabry disease has been recently proposed. It relies on substrate competitors of GLA used at sub-inhibitorory concentration. These drugs have been defined "pharmacological chaperones" (PC). Clinical trials are currently carried out with 1-Deoxy-galactonojirimycin (DGJ), but, regrettably not all genotypes in GLA associated with Fabry disease respond to this drug.It is therefore desirable to address three points: how PC work, why they work only on some genotypes of GLA, can we develop new PC with broader or different specificity? Prediction of the effects of missense mutations depends critically on exploiting all information available on the three-dimensional structure of proteins. Luckily the structure of GLA has been solved by X-ray crystallography both in the presence (1r47 in pdb) and in the absence (1r46 in pdb) of a substrate analog.

Methods

The structure of GLA is a homodimer and each monomer contains a(beta/alpha)8 domain with the active site and an antiparallel beta domain. We localised 72 mutations whose responsiveness to DGJ is known on each chain in 1r46. As a general rule, responsive mutations do not occur in spine elements of the structure or in buried residues, but we could not find a single property, that clearly correlates with susceptibility to chaperones. In order to classify mutations more effectively, we used state of the art multivariate methods from the statistical learning theory. In particular, we built a training set consisting of mutations whose class is known. For each mutation, we have assigned a binary label depending on the DGJ responsiveness. Then, we trained a Fisher linear discriminant algorithm to produce a model that can predict the class label for mutations for which it is unknown. To train the algorithm we need numerical input data, that is structural, functional, evolutionary attributes for each mutation in the dimer protein. Models of 72 mutants were constructed using 1r46 as a template and the program Andante. To determine the influence of each replacement on the structure, the mutant model was superimposed on the wild type structure based on the Ca atoms by the least square-mean fitting method and the "all atom" rmsd was calculated. Residue accessibilities, both for mainchain and sidechain atoms, were calculated. The effect of mutations on stability was assessed with SDM, a program which incorporates a statistical potential energy function. Shannon Entropy was calculated on suitable multiple alignment of orthologous proteins obtained from Pfam.

Results

Accuracy results have been evaluated using Matlab 2007a, and MatlabArsenal implementation of Fisher linear discriminant algorithm. First, we normalized the dataset to have zero mean and standard deviation equal to one. Then, we choose a subspace spanned by two features. In this space we evaluated, with a tenfold cross validation, the accuracy of the prediction of decision rules. Tenfold cross validation experiments were repeated 10 times for each choice of the subspace. Results show an accuracy above 80% in predicting the activity of DGJ using features describing stability assessed by SDM and Shannon entropy.

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