Application of computational methods for structural and functional characterization of mutants of GALT enzyme

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Motivations

Galactose-1-phosphate uridylyltransferase (GALT) catalyses the conversion of galactose-1-phosphate to UDP-galactose, a key step in the galactose metabolism. Deficiency of GALT activity in humans caused by mutations in the GALT gene are associated to a rare genetic disease called Classic Galactosemia. To date, more than 180 mutations are known on GALT gene, most of which are missense mutations. A previous study applied computational methods to predict the effect of known mutations [1] and results were stored in a publicly available database: http://bioinformatica.isa.cnr.it/GALT [2]. In this work, we have characterized the effects on structure and function of GALT enzyme of 14 novel missense mutations, applying not only static modelling, but also molecular dynamics simulations. Results were compared to the biochemical characterization of these mutants, expressed and purified from bacteria.

Methods

Starting from the 3D model of human GALT enzyme [3] (the crystallographic structure of the human enzyme is not yet available), homodimeric mutans were created using Modeller 9v8 [4]. Each resulting mutant was analysed for variations in structural feature such as intersubunit interactions, secondary structures, solvent accessibility, H-bond and salt bridge patterns, and for predicted stability of the protein. Molecular dynamics simulations were applied to those selected mutants, for which the static modelling of mutations did not allow to highlight any variation in these properties, using GROMACS program [5]. Results were compared with those obtained on wild type molecule and on the most characterized mutant, Q188R. The human homodimeric mutant proteins were expressed in E. coli, purified and assayed for their activity and kinetic properties (Vmax and KM).

Results

Our static modelling simulations predicted for most of the mutant GALT enzyme alterations at structural level: in two cases the alteration of intersubunit interactions, in other two cases the involvement of residues indirectly affecting the active site, and in most cases the alteration of the network of H-bonds and salt bridges, with either local or global effects. In addition, in most cases these mutants have predicted stability problems In two cases, however, static modelling was not able to predict any structural effect. In these cases, the results of molecular dynamics simulations were able to suggest possible effects that impair the correct activity and the stability of the enzyme, too. The comparison with simulations made on mutant Q188R allowed also to confirm the deleterious effects of this mutation at structural level. The biochemical characterization of these mutants showed results that are well explained by the simulations. In particular, those mutations that were predicted to have a large impact on protein structure are also those with minimal or no activity, whereas those mutants showing a residual activity, also show localized effects on protein structure.

Acknowledgments

This work was supported by a Parents of Galactosemic Children Research Grant (to A.M.), and by project FIRB MIUR ITALBIONET (RBPR05ZK2Z and RBIN064YAT _ 003) (to A.M., L.M). This work has been made in the frame of the Flagship Project InterOmics and of the project CNR-Bioinformatics. Research grant support to K.L. includes U.S. NIH grants 5R01 HD054744-04 and 3R01 HD054744-04S1.

References

1. Facchiano A, Marabotti A. (2010) Protein Eng Des Sel 23, 103-113.
2. d'Acierno A, Facchiano AM, Marabotti A. (2009) Genomics Proteomics Bioinformatics 7, 71-76.

3. Marabotti A., Facchiano AM (2005) J Med Chem 48, 773-339.
4. Sali A, Blundell T L (1993) J Mol Biol 234, 779-815.
5. Hess B, Kutzner C, Van der Spoel D, Lindahl E (2008) J Chem Theory Comput 4, 435-447.