MODELLING OF ENZYME-SUBSTRATE COMPLEXES FOR COMBINE ANALYSIS OF HALOALKANE DEHALOGENASE BY MEANS OF MOLECULAR DOCKING AND QUANTUM MECHANICAL CALCULATIONS

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The applicability of automated molecular docking techniques and quantum mechanical calculations for the construction of enzyme-substrate complexes for use in Comparative binding energy (COMBINE) analysis [1-6] was evaluated. The data set studied consists of the complexes of eighteen substrates with the haloalkane dehalogenase (DhlA) isolated from bacterium *Xanthobacter autotrophicus* strain GJ10. An automated molecular docking procedure provided the structures for a set of DhlA-substrate complexes that was used to derive a robust COMBINE model. Quantum-mechanical calculations were successfully used as an additional and complementary computational tool for selection of correct binding modes obtained from the docking. The resulting COMBINE model is compared with a previously reported COMBINE model [7] derived for the same data set using structures of complexes built according to experimentally determined structure of the DhlA-dichloroethane complex. Both models were similar in terms of overall fit and internal predictive power even though the conformations and orientations of the substrates in the complexes were significantly different. The new COMBINE model derived from the automatically docked structures performed notably better in external prediction. Small differences in the relative contributions of important residues to explaining binding affinities can be directly linked to structural differences in the modelled enzyme-substrate complexes.

1. A. R. Ortiz, M. T. Pisabarro, F. Gago & R. C. Wade, *J. Med. Chem.*, **38** (1995) 2681-2691.
2. R. C. Wade, A. R. Ortiz, and F. Gago: Comparative Binding Energy Analysis, in 3D QSAR in Drug Design. H. Kubinyi, G. Folkers, and Y.C. Martin, Editors. Dordrecht 1998. Kluwer Academic Publishers. p. 19-34.
3. C. Perez, M. Pastor, A. R. Ortiz & F. Gago, *J. Med. Chem.*, **41** (1998) 836-852.
4. T. Wang & R. C. Wade, *J. Med. Chem.*, **44** (2001) 961-971.
5. C. Cuevas, M. Pastor, C. Perez & F. Gago, *Comb. Chem. High Throughput Screen.*, **4** (2001) 627-642.
6. R. C. Wade: Derivation of QSARs using 3D structural models of protein-ligand complexes by COMBINE analysis, in Rational approaches to drug design: 13th European symposium on Quantitative Structure-Activity Relationships. H.-D. Holtje and W. Sippl, Editors. Barcelona 2001. Prous Science. p. 23-28.
7. J. Kmuníček, S. Luengo, F. Gago, A. R. Ortiz, R. C. Wade & J. Damborský, *Biochemistry*, **40** (2001) 8905-8917.

SECONDARY AND TERTIARY STRUCTURE OF HUMAN A1-ACID GLYCOPROTEIN BY HOMOLOGY MODELING AND VIBRATIONAL SPECTROSCOPY

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Human $_1$-acid glycoprotein (AGP), also known as orosomucoid, is a 41-kDa single polypeptide formed of 183 amino acids. It contains 42% carbohydrate in weight and has up to 16 sialic acids residues. AGP, a human blood plasma protein, belongs to the lipocalin family of proteins, a heterogeneous group of proteins that bind a variety of small hydrophobic ligands. It is known that AGP plays a role under inflammatory or other pathophysiological conditions and is able to bind basic drugs and certain steroid hormones such as progesterone, however its biological function and 3D structure remains unknown [1].

The aim of our work was to predict and verify the three-dimensional structure of AGP. A structural model, using available lipocalin structures as templates, was constructed by means of the Modeller program [2]. The model shows that AGP folds as a highly symmetrical all- protein dominated by a single eight-stranded antiparallel -sheet. For the first time secondary and tertiary structures of AGP have been studied by infrared and Raman spectroscopy. Vibrational spectroscopy confirmed details of the secondary structure predicted by modeling, i.e. 15% -helices, 41% -sheets, 12% -turns, 8% -bands and 24% unordered structure at pH 7.4. Thermal dynamics in the range 20-70 °C monitored by Raman spectroscopy and analyzed by principle component analysis revealed full reversibility of the protein motion upon heating dominated by decreasing of -sheets, probably thermal "breathing" of the -barrel.

Docking of progesterone into the binding pocket of our model was explored with the AutoDock program [3]. Then Raman difference spectroscopy confirmed the predicted proximity of Trp122 to the progesterone binding pocket. We can conclude that our model was verified in so many