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.

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SECONDARY AND TERTIARY STRUCTURE OF HUMAN $_1$-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 details by vibrational spectroscopy that it can represent a valuable contribution to understanding the role and behavior of AGP [4].

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MOLECULAR CLONING, E. COLI EXPRESSION AND PURIFICATION OF SCFV ANTIBODY FRAGMENTS OF DIAGNOSTIC/THERAPEUTIC INTEREST

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Fv fragments are the smallest antibody molecules that still retain the entire antigen-binding site. In single-chain Fv fragments (scFv), variable domains are joined by a flexible linker. Such scFv constructs, which generally retain full binding capacities towards the antigen, are the topic of very active research. First, they are of interest for structural studies because they usually yield crystals diffracting to higher resolution than the corresponding Fab fragments. Second, since they can be expressed in bacteria, they are also well suited for binding, mutagenesis and protein engineering studies [for review, see e.g.1].

In this work we describe molecular cloning, expression, purification and properties of two scFvs of potential diagnostic and immunotherapeutic use, scFv M75 and scFv TU?20.

Monoclonal antibody (mAb) M75 recognizes cell surface protein MN/CA IX strongly associated with several types of human carcinomas [2]. Radioactively labeled humanized scFv M75 could be used for tumor immunodetection and possibly for therapy. Monoclonal antibody TU-20 was raised against beta-III-tubulin, a specific neuronal marker in normal and neoplastic tissues. The antibody TU-20 fragments could thus be useful tools for probing beta-III-tubulin functions in neurons, as well as for immunohistochemical characterization of tumors of neuronal origin [3].

Coding sequences for light (V_L) and heavy (V_H) variable domains were obtained from total RNA, isolated from hybridoma cells, by RT-PCR using suitable pairs of primers. Single-chain Fv genes in the form V_L-linker-V_H-myc tag were then assembled and cloned into T7 promoter-driven expression plasmids. Bacterial strain *E. coli* BL21(DE3) was used for protein expression. The recombinant protein products accumulated in inclusion bodies as insoluble aggregates. To obtain refolded active proteins from inclusion bodies, several protocols were adapted to find optimal conditions for each scFv species.

Purification procedure comprising several conventional chromatography steps (ionex chromatography, gel filtration) yielded scFv proteins in amount and purity necessary for functional characterization. While scFv TU-20 in ELISA assay exhibits specificity and binding activity comparable to parental mAb TU-20, in case of scFv M75, ELISA assay was negative. The reasons for the lack of binding activity are under investigation.

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PROTEINS AND THEIR CRYSTALS

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Non-membrane proteins such as the pokeweed antiviral protein from Phytolacca acinosa (PAP-Saci) and the tryptophan (W)-repressor binding protein A (WrbA) and also membrane protein, the five-chlorophyll reaction center of photosystem II from Pisum sativum, have been crystallized in our laboratory.

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