PROTEIN CHEMISTRY AND MASS SPECTROMETRY IN STRUCTURAL ANALYSIS OF LARGE PROTEINS

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According to the textbook knowledge the use of automated Edman degradation and protein mass spectrometry for the determination of complete primary structure is limited to small proteins with molecular size less than 10 kDa, larger proteins being analyzed by DNA sequencing of the corresponding genes or cDNA clones. Here we present two examples of our recent work in which the sequence of rather large proteins has been determined completely or nearly completely by protein sequencing and mass spectrometry. The first example includes the pokeweed antiviral protein from *Phytolacca acinosa* (PAP-S) that belongs to the family of type-1 ribosome-inactivating proteins. The purified PAP-S proteins resolve on SDS electrophoresis into two closely related bands with Mr of about 29 kDa. Interestingly, the upper protein band, PAP-S_{up} has been shown to crystallize through carbohydrate-protein interactions based on a rare type of N-glycosylation, namely N-linked GlcNAc monosaccharide substitutions at the canonical Asn-Xxx-Ser/The sequons [1]. The sequence of PAP-S_{up} is not known from the genetic data, but is essential for unambiguous solving of the crystal structure in positions that cannot be called directly from the electron density. We have thus determined the complete structure of PAP-S_{up} by Edman degradation of N-terminal and internal peptide sequences in combination with MALDI peptide mapping and tandem mass spectrometry using an ion trap. The complete sequence has 261 amino acids and includes three sites of the above N-glycosylation. The sequence coverage was 92 % by Edman degradation data, 93 % by peptide mapping and 90 % by tandem MS data. The second example is -N-acetylhexosaminidase from *Aspergillus oryzae* CCF1066, a robust extracellular secreted enzyme used in enzymatic syntheses of oligosaccharides and biotechnology [2]. This enzyme has 600 amino acids (including 6 cysteins and 6 sites of N-glycosylation), of which 466 has been verified by direct analysis of the protein (sequence coverage 77 %). Identification of large N-terminal segment in the protein proved difficult pointing to the fast cleavage of this protein segment. Enzyme is composed of cleaved signal peptide, the propeptide sequence involved in regulated secretion, the inactive zincin domain, and the catalytical domain belonging to family 20 of glycohydrolases.

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DYNAMICS OF 1,2-DICHLOROETHANE IN THE ACTIVE SITE OF HALOALKANE DEHALOGENASE LINB: EFFECT OF SOLVENT AND HALIDE ION ON PRODUCTIVE BINDING

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1,2-dichloroethane (DCE) is a toxic and carcinogenic chlorinated compound that is not known to be formed naturally. As many other synthetic halogenated aliphatic compounds, DCE is rather resistant to biodegradation and persists in the environment. Nevertheless, several bacterial cultures that are able to use DCE as the only carbon and halogen source have been isolated. The most efficient catalysis of DCE has been observed with haloalkane dehalogenase DhlA from *Xanthobacter autotrophicus* GJ10. Even lower activity with DCE was observed for haloalkane dehalogenase LinB from *Sphingomonas paucimobilis* UT26. Crystallographic analysis of LinB-DCE complex showed non-productive binding of DCE to the enzyme active site, while molecular docking suggested that DCE molecule can possibly bind to the active site but is prevented by chloride ion and/or water molecules [1].

Two nanoseconds-long trajectories of LinB with different number of ligands bound to the active site were carried out and compared. The results show that productive binding of DCE (i.e., binding to the Michaelis-Menten complex) is blocked by the presence of chloride ion or water molecule in the halide-stabilization pocket of the active site. In case of empty halide-stabilization pocket, the productive binding of DCE occurs very rapidly (in less then 20 ps). On the other hand, DCE locks the chloride ion in the halide-stabilization pocket as was confirmed by steered molecular dynamics simulations and by fact that chloride ion can easy leave LinB active site in the system without DCE.

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MOLECULAR MODELING AS A TOOL IN MOLECULAR BIOLOGY OF MEMBRANE-BOUND RECEPTORS

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The importance of computer modeling of membrane proteins in molecular biology is worked out. We give three examples that models gained by a combined approach of homology and energetic modeling with vibrational spectroscopy are a useful help in site-directed mutagenesis, truncation, binding-studies and even in crystallography. The study of the vanilloid receptor is a successful application of a computer model in the construction of truncations that served for the identification of functionally important protein parts. In the case of CD69 computer docking helped to identify the Ca^{2+}–binding site that was not observed in the crystal structure of this protein due to the non-physiological conditions of crystallisation.

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PROTON TRANSFER IN SHORT OLIGOPEPTIDES

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Peptides belong to key biomolecules. Their activity can be influenced by several impacts and the interaction with protons is one of them. Such a type of interaction can also influence behaviour of peptides in gas phase, which can lead to different way of fragmentation during mass spectroscopy analysis [1].

Our quantum-chemical study has been focused on detailed analysis of proton interaction with short oligopeptides. Density functional theory employing hybrid functional B3LYP and 6-31G(d',p') basis set was used. The study was performed on terminally blocked diglycine and triglycine models. It implies that the proton can only interact with the oxygen and nitrogen atoms of the amidic groups. Because of appropriate geometry, the proton transfer can occur between these positions [2, 3].

In general, the proton transfer process consists of two repeating steps. In the first step, the proton is moved around the double bond of the carbonyl group by isomerization from *E* to *Z* configuration. Then the process continues by proton jump between adjacent carbonyl oxygens. The isomerization processes have significantly higher activation barriers than the jump steps [3]. Also changes in proton transfer were examined when a single water molecule was presented in the system. Strong influence to all steps, and also active participation of the water molecule due to proton exchange processes was found.

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APPLICATION OF POWDER DIFFRACTION IN BIOLOGY? THE EGG-SHELL MICROSTRUCTURE

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In last years, renaissance of rather old and traditional technique - X-ray powder diffraction can be observed. This was initiated by both the interest in design of new materials (in materials science, physics and chemistry, where it plays the role of a basic method), and also by fast development in instrumental techniques - X-ray optics and detection which enhanced its possibilities.

Powder diffraction pattern contains different kind of information. Peak positions and intensities are related to crystal (atomic) structure, i.e. the type and size of lattice cell and atomic positions and consequently it can be used for structure refinement and even structure determination in some cases. As a finger print of each individual phase, the diffraction pattern can be an ideal tool for phase analysis.

However, there is much more hidden in the pattern. Variations of lattice parameters and intensitites can detect lattice defects. This is related to the so-called real structure of material, the term which is also used for structural features in the scale of nanometers, i.e. grains or subgrains. The topics which is now of great interest because of intense