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AMINOALDEHYDE DEHYDROGENASE 1 FROM TOMATO – ENZYME STRUCTURE AND POSSIBLE USING AS A TOOL TO ANALYZE ALDEHYDES IN BEVERAGES

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Aminoaldehyde dehydrogenases (AMADH, EC 1.2.1.19) belong to the aldehyde dehydrogenase (ALDH) superfamily and oxidize -aminoaldehydes to the respective -amino acids. First reported X-ray structures of plant aminoaldehyde dehydrogenase were two isoenzymes from pea (*Pisum sativum*, PsAMADH1 and 2). In this work we focused on characterization of the isoenzyme 1 from tomato (*Lycopersicon esulentum*, LeAMADH1), which has unusually wide substrate specificity. The enzyme crystals belong to $P2_12_12_1$ space group and contain one dimer per asymmetric unit in line with gel-filtration measurements indicating the enzyme exists as dimer in solution. Each monomer is composed from oligomerization, catalytic and coenzyme binding domains.

Our study on substrate specificity shows, LeAMADH1 oxidizes not only linear aminoaldehydes but a wide range of N-containing heterocyclic aldehydes such as pyridinecarbaldehydes, 3-pyridinylpropanals and aromatic aldehydes too. The K_m values for the best substrates is 10^{-4} – 10^{-5} M and the V_{max}/K_m values compared with that for 3-aminopropanal usually was lower than 10%. There were only three substrates (4-pyridinecarbaldehyde, 2-brom-4-pyridinecarbaldehyde and 3-methylthiopropanal) with this value above 10%. By testing several analogues of NAD^+ coenzyme we observed that good coenzymes are deamino-NAD^+ and 3-acetylpyridine-NAD^+ - the activity of LeAMADH1 reached 110% and 75% of those by using NAD^+ respectively. By using others coenzymes the activity was drastically reduced.

Almost every alcoholic beverage contains several aldehydes which are products of sugar degradation by higher temperatures or as fermentation products. In higher concentrations these aldehydes are not welcomed in spirits because of their toxicity. Many of these aldehydes are substrates of our enzyme, so we tested several samples of spirits – mostly slivovitz – as substrates of LeAMADH1. Its activity usually was around 1% of the activity reached with APAL. In order to create a biosensor for detection of aldehydes in alcoholic beverages we immobilized LeAMADH1 on magnetic nanoparticles and performed first electrochemical measurement by linear sweep voltametry to detect NADH produced by the enzyme reaction.

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INTERACTION OF THE MYRISTOYLATED M-PMV MATRIX PROTEIN AND HIS MUTANTS WITH PHOSPHOLIPIDS

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Mason-Pfizer monkey virus (M-PMV) is widely used as a model organism for studies of the late phase of the retroviral life cycle. Matrix protein forms the N-terminus of a polyprotein Gag and plays key role in the transport of the immature viral particle (IVP) to the cell membrane and in its interaction with membranes. Previous studies suggest that matrix protein interacts with the phospholipids of the cell membrane and thus allows budding of the IVP. This work focuses on the interaction of the matrix protein with the main components of the cell membrane: phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine and, the marker of the cytoplasmic membrane, phosphatidylinositol-4,5-bisphosphate. The wild type matrix protein and his two mutants (T41I/T78I and Y28F/ Y67F) were used. Both mutants have decreased budding capability, which could be explained by their impaired interaction with the cell membrane. We studied the interaction by a combination of surface plasmon resonance (SPR) and nuclear magnetic resonance (NMR).

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MOLECULAR MOTIONS MONITORED BY NMR RELAXATION – A CONSERVATIVE APPROACH

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The aim of the lecture is to explain how NMR relaxation can describe motions of molecules such as nucleic acids, proteins, or saccharides with an atomic resolution. The basic principles of NMR relaxation studies will be reviewed . Rather than trying to provide rigorous description, general ideas will be described. Attention will be paid to the limits of applicability of various manners of interpretation of the NMR relaxation rates. In this context, a special attention will be paid to the methods of spectral density mapping, applicable to studies of molecules whose motions are difficult to describe by particular physical models (e.g. disordered proteins and other highly flexible molecules).