PS04.11.27 MAPPING THE STRUCTURAL FEATURES OF METAL- AND CARBOHYDRATE BINDING TO CONCANAVALIN A. Julie Bouckaert(1), Remy Loris(1), Dominique Maes(1), Freddy Poortmans(2) and Lode Wyns(1); (1)Laboratorium voor Ultrastructuur, VUB, Paardenstraat 65, B-1640 Sint-GenesiusRode, Belgium and (2)VITO, Boeretang 200, B- 2400 Mol, Belgium

The lectin concanavalin A (Con A) sequentially binds a transition metal ion in the metal-binding site S1 and a calcium ion in the metal-binding site S2 in order to form its saccharide-binding site. Zn^{2+} or Co^{2+} soaked into metal-free Con A crystals (apoZn-Con A or apoCo-Con A) bind only partially in the proto-transition metal-binding site, not followed by any conformational changes. These structures can represent the very first step in going from metal-free Con A towards the holoprotein. In the co-crystals of metal-free Con A with Zn^{2+} (Zn-Con A), the Zn-ion fully occupies the S1 site. It orientates Asp10 optimally for calcium binding in the S2 site and stabilizes Asp19 by hydrogen bonding to one of its water ligands. Zn^{2+} binding in S1 apparently is necessary to allow for subsequent Ca^{2+} binding in the S2 site. Ca^{2+} binding is critical to induce the large conformational changes, that comprise the trans to cis isomerization of the Ala207-Asp208 peptide bond accompanied by the formation of the saccharide-binding site: the co-crystals of metal-free Con A with both Zn^{2+} and Ca^{2+} contain the active holoprotein (Con A ZnCa). Con A interacts with all three saccharide units in the complex with its most specific epitope, the trisaccharide methyl-3,6-di-O-(alpha-D- mannopyranosyl)-alpha-D-mannopyranoside. The mannose on the alpha(1-6) arm is bound in the primary or monosaccharide-binding site. The reducing core mannose interacts with the side chains of Tyr12 and Asp16 via the hydroxyl groups on C_4 and C_2 respectively. The alpha(1-3) linked mannose shows two distinct conformations, but in each of them the hydroxyl group on the C_3 position makes a hydrogen bond to the main chain carbonyl of Pro13. These results are discussed in view of recent thermodynamic data on the ConA-trimannosyl system and in view of differences in oligosaccharide specificity between Con A and the mannose/glucose specific lectins from the Viciae tribe.

PS04.11.28 STRUCTURE OF RECOMBINANT SALIVARY α-AMYLASE: POSSIBLE ROLE OF THE INDIVIDUAL DOMAINS IN DENTAL PLAQUE FORMATION. Kalaiyarasi Ramalingam, Ching-Chung Tseng, Narayanan Ramasubbu, Michael J. Levine, Department of Oral Biology and Dental Research Institute, School of Dental Medicine, State University of New York at Buffalo, Buffalo, NY 14214, USA

Salivary α-amylase (sAmy) is a multifunctional enzyme involved in the initial hydrolysis of starch and in the colonization of bacteria which leads to dental plaque formation. Amylases are monomeric, calcium binding proteins with a single polypeptide chain folded into three domains and contain the $(ß/\alpha)_8$-barrel topology. Domains A and B appear to be involved in enzymatic activity. The role of the C domain in either of the functions is unknown. To better understand the functional roles of the various domains of this enzyme we have optimized a baculovirus expression system for the production of recombinant amylase (rAmy) with biochemical and biological properties similar to native sAmy. Crystallization of rAmy was carried out by vapor diffusion method using 2-methyl-2,4-pentanediol as the precipitant in the presence of $CaCl_2$ at pH 9.0. The crystals are of the space group $P2_12_12_1$ with unit cell dimensions of a=52.63, b=75.20 and c=137.11 Å with one molecule per asymmetric unit. A total of 21,505 unique reflections were collected on a R-AXIS II imaging plate system to a resolution of 2.0 Å using two crystals (Rmerge = 9.38%). The structure of rAmy was solved by molecular replacement techniques using the sAmy structure as a starting model. The structure has been refined at 2.5 Å resolution to an R factor of 20% with excellent stereochemistry. The overall topological fold of the molecule is identical to the native enzyme. The comparison of the recombinant amylase structure to other functionally related enzymes has revealed potential sites for bacterial binding. Consequently, we have initiated the generation of mutants targeted against the selective control of bacterial binding and enamel binding.

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Drug Design II-Macromolecule Based

MS04.12.01 EARLY DAYS OF STRUCTURE-BASED DRUG DESIGN, THE TRUSOPT STORY Brian M. McKeever, Department of Biophysical Chemistry, Merck Research Laboratories, RY80M-136, 126 East Lincoln Ave., Rahway, N.J., 07065

Carbonic anhydrase (CA) has long been recognized as a potential therapeutic target for conditions involving the abnormal movement of fluid. As such, it was "targeted" for treatment of high intraocular pressure found in patients suffering from glaucoma. What was needed was not a powerful, specific enzyme inhibitor (that part was done), but a superior drug that could be safely bottled, delivered directly to the afflicted organ and lead to effortless patient compliance. CA was chosen as a model system for trying out a new approach to developing a compound to meet the clinical specifications, structure-based drug design.

MS04.12.02 HIV-PROTEASE AS A TARGET MOLECULE IN THE TREATMENT OF AIDS. J. P. Priestle, A. Fässler, G. Bold, H.-G. Capraro, J. Rösel, M. Tintelnot-Blomley, M. G. Grütter, Marc Lang, Pharmaceuticals Division, Ciba-Geigy, Ltd., CH-4002 Basel, Switzerland

The maturation of the Human Immunodeficiency Virus (HIV) is dependent on a protease that cleaves the *gag*- and the *gag-pol* polyprotein into individual, active proteins. The HIV-protease (HIV-Pr) is an aspartic protease composed of two identical subunits of 99 residues each. Each subunit donates one aspartyl residue to the active site, which possesses two-fold molecular symmetry. The HIV-protease cleaves substrates with preferentially large hydrophobic residues in P1/P1' (e.g. Phe, Tyr, Leu) and small either hydrophobic (e.g. Val, Ile) or hydrophilic (Asn) residues in P2/P2' . Substrate is bound in an extended conformation.

We found initial HIV-Pr inhibitors by screening compounds originally designed to inhibit renin, a human aspartic protease involved in blood pressure regulation. Thereby we identified CGP 43026, a peptide mimic with high affinity to both enzymes (IC_{50} of 27nM and 17nM for HIV-Pr and renin, respectively). Optimization of this lead compound with respect to specificity vs. renin and pharmacokinetic properties was achieved based on structural investigations and modeling studies and afforded first CGP 53437 and ultimately CGP 61755, the actual development compound. Independently, a second class of compounds was evaluated which took advantage of the two-fold molecular symmetry of HIV-Pr. These pseudosymmetric inhibitors are less peptidic and synthetically less demanding. The typical representative, CGP 53820, displays an azapeptide derived moiety instead of the central hydroxyethyl dipeptide isostere. Compounds of this class are currently in preclinical investigation. Thus, examination of the crystallographic structures of inhibitors in complex with both HIV-1 and HIV-2 protease have provided the basis for modeling studies which have led to the discovery of potent and pharmacologically attractive anti-HIV agents suitable for clinical evaluation.