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SUBSTRATE PROMISCUITY OF AN AMINOGLYCOSIDE ANTIBIOTIC RESISTANCE ENZYME VIA TARGET MIMICRY

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Due to the prolific use and abuse of antibiotics, pathogenic bacteria have evolved means to render these drugs ineffective. Resistant bacteria can counter the effects of antibiotics in several ways. For aminoglycosides, a group of bactericidal antibiotics that target the A-site of the 16S ribosomal RNA, the most common mode of resistance is enzyme-catalyzed chemical modification and detoxification of the drug. Aminoglycoside kinase (3') type IIIa (APH(3')-IIIa), an enzyme produced by pathogenic Gram-positive bacteria such as *Enterococci* and *Staphylococci*, is capable of conferring resistance to at least ten clinically important aminoglycoside antibiotics. In an effort to understand the promiscuity in substrate recognition by APH(3')-IIIa, we have determined the crystal structures, by molecular replacement, of APH(3')-IIIa in complex with ADP and kanamycin A, ADP and neomycin B, and AMPPNP (an ATP analog) and butirosin A. Refinement for the kanamycin and neomycin enzyme complexes have been completed to 2.4 and 2.7 angstroms with R-factors 0.234 and 0.219, respectively. Refinement for enzyme complexed with butirosin is in progress. These structures reveal that the basis for this enzyme's broad substrate spectrum is the presence of a flexible antibiotic-binding loop and a versatile antibiotic-binding pocket composed of three sub-sites. Comparison between the A site of the bacterial ribosome and APH(3')-IIIa shows a high degree of similarity in the pattern of hydrogen bonds to the aminoglycoside. However, they differ in their van der Waals interactions with the substrate, suggesting a potential strategy for the design and development of novel antibiotics and adjuvants.

Keywords: ANTIBIOTIC RESISTANCE KINASE MULTI-DRUG BINDING

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CRYSTAL STRUCTURE OF PLANT PECTIN METHYLESTERASE

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Pectin is a principal component in the primary cell wall of plants. During cell development, pectin is modified by pectin methylesterases to give different properties to the cell wall. This report describes the first crystal structure of a plant pectin methylesterase. The α-helical structure embodies a central cleft, lined by several aromatic residues, that has been deduced to be suitable for pectin binding. The active site is found at the center of this cleft where Asp157 is suggested to act as the nucleophile, Asp136 as an acid/base and Gln113/Gln135 to form an anion hole to stabilize the transition state.

Keywords: **β-HELIX PLANT ENZYME CATALYTIC MECHANISM**

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CRYSTAL STRUCTURE OF THERMOSTABLE ASPARTASE AND EXPLORATION OF FUNCTIONAL SITES IN ASPARTASE FAMILY

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Aspartase plays an important role in the bacterial nitrogen metabolism by catalyzing the reversible conversion of l-aspartate to fumarate and ammonium ion. The crystal structure of the thermostable enzyme from *bacillus sp*. Ym55-1 was solved by the molecular replacement method using the *e. Coli* enzyme as a search model. The structural model was refined up to 2.5 Å resolution for data collected at 100k with the R-factor of 22.2%. The present *bacillus* enzyme is a homotetramer of four subunits, each of which is composed of 406 amino-acid residues distributed into three domains. It exhibits no allosteric effects, in contrast to the *e. Coli* enzyme which is activated by l-aspartate and divalent metal cations, and has four-times higher activity than e. Coli enzyme. The overall folding of the present subunit is similar to those of e. Coli aspartase and *e. Coli* fumarase both of which belong to the same family with the present enzyme. The local structural comparisons among these three enzymes revealed seven structurally different regions. Four of the regions were located around putative functional sites, suggesting the involvement of these regions into functions characteristic of the individual enzymes. Based on these results, we have proposed possible mechanisms of substrate recognition, catalytic reaction, and enzyme activation in the aspartase family. Moreover, increase in the number of intersubunit hydrogen-bonds and salt-bridges was observed in the bacillus aspartase compared with those of the others, which explains the thermostability of the present aspartase.

Keywords: **ASPARTASE THERMOSTABILITY CRYSTAL STRUCTURE**

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CRYSTAL STRUCTURE OF IRPEX LACTEUS ASPARTIC PROTEINASE IN COMPLEX WITH THE INHIBITOR PEPSTATIN

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Irpex lacteus aspartic proteinase (ilap) has almost the same value of the ratio of milk-clotting activity to proteolytic activity (mca/pa) as commercial microbial milk-clotting enzymes from *mucor pusillus* or *mucor miehei*. In actual cheese making trials, irap could produce some kinds of cheeses of good quality including fiber-structured cheese, gouda cheese and cheddar cheese. Ilap is most active at ph = 3 toward protein substrates and is inhibited by aspartic proteinase inhibitors such as pepstatin. However, compared to other milk clotting enzymes, ilap is apparently unstable at pH = 6.0. This is important as calf rennet substitute because excess proteolytic activity remaining in the curd affects physical properties of cheese and makes it bitter during ripening. To understand the stability and the catalytic mechanism of this enzyme, we conducted structure analysis of ilap.

Crystals of ilap have been obtained by the hanging drop method using ammonium sulfate as a precipitant. The crystals diffracted beyond 1.2 Å resolution at photon factory. Structure analysis was conducted by molecular replace method using the program amore and model building was done by the program arp/warp. The model was refined by the programs cns and refmac, and the present model contains one protein molecule with the inhibitor pepstatin and 423 water molecules with an r-factor of 0.172 and Rfree-factor of 0.208.

Keywords: **ASPARTIC PROTEINASE, PEPSTATIN**