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PEPTIDES WITH DISTORTED TRANS-PROLINE IN THE COUNTER DIRECTION BUT NOT THE INTERMEDIATE FROM TRANS TO CIS ISOMERASIZATION BIND CYCLOPHILIN B

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It was reported that the isomerization reaction from trans- to cis-proline for proteins is enhanced in the present of cyclophilin (CyP) A. CyPB with membrane binding signal sequence in N-terminal proceeds cis to trans isomerization reaction for model substrate suc-AAPF-pNA as well as CyPA in the cytoplasm. Suc-APA-pNA, Ac-AAPA-AMC and Suc(Ome)-AAPV-AMC have inhibitory role as substrate analogy for this reaction. In order to ascertain that CyPB forms complexes with intermediates of trans to cis isomerization, we tried crystal analysis of the complexes of CyPB with these three peptides. Crystallization of *E. coli* CyPB did not succeeded until K187T mutant was used. K187T mutant has peptidyl-prolyl cis to trans isomerase activity. There is the cleft with the hydrophobic pocket in the upper sheet of β-barrel structure consisting of two β-sheets. Three peptides, which are located in the cleft, are distorted over -35° around peptide bond from trans-form of Ala-Pro. This distotion is in not direction of the rotation from trans to cis but the counter direction. This fact indicates that this enzyme not only distorts peptides into intermediate in trans to cis process but also interacts with and fixes trans-proline containing molecules distorted in the counter direction to play some important physiological role.

Keywords: CYCLOPHILIN PPIASE PEPTIDE-BINDING

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STRUCTURAL STUDIES OF CDP-TYVELOSE 2-EPIMERASE

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Deoxysugars play important roles in numerous physiological reactions including antibiotic synthesis, cellular adhesion, and cell-cell interaction (1). Tyvelose is a 3,6-dideoxyhexose found in the O-antigen of lipopolysaccharide in the pathogenic bacteria *Yersinia pseudotuberculosis* IVA and *Salmonella typhi*. One enzyme involved in its synthesis is CDP-D-tyvelose 2-epimerase, which catalyzes the C-2 epimerization reaction on 3,6-dideoxyhexose CDP-D-paratose to produce CDP-D-tyvelose (2). This enzyme is a homotetramer with a monomeric molecular weight of 38 kDa and contains 1 molecule of bound NAD+ per monomer. CDP-D-tyvelose 2-epimerase belongs to a group of enzymes, including UDP-D-galactose 4-epimerase, that perform epimerization at unactivated stereocenters. We have cloned, over-expressed and purified CDP-D-tyvelose 2-epimerase from *S. typhi*. Crystals of the enzyme have been obtained and results from our crystallographic investigation will be presented.

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CRYSTAL STRUCTURES OF DIMERIC HEXOKINASE HKB FROM YEAST

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The first crystal structure, form 1, of a homodimer of yeast hexokinase B, hkB (subunit, ca. 50 kDa), was reported at 6 Å resolution [Steitz et al., 1973]. A structure of an hkB 'monomer', form 2, was later reported to 2.1 Å, [Anderson et al., 1978]. Recently, a 2.2 Å structure of a new crystalline form (3) was characterized [Kuser et al., 2000], and we have obtained a 2.2 Å structure of another form (4).
We have obtained the high resolution structures of forms 1, 2, and 4 hkB. We will show that all known structures of hkB contain the dimeric motif. This is most interesting in the light of the developing insight at the regulatory role of the hkB monomer-dimer equilibrium in yeast metabolism, [Golbik et al., 2001]. Further, the hkB dimer has a similar S-shape to the human hkI monomer [Rosano et al., 1999, Aleshin et al., 2000] indicating that this shape has been selected in the course of evolution for optimal functioning of hk.
Finally, in comparing the yeast hkB homodimer with human hkI we will discuss the possibility of a regulatory ATP binding site in hkB in a cavity between the two subunits.

Keywords: **HEXOKINASE, DIMERIC YEAST HEXOKINASE B, X-RAY STRUCTURE**

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STRUCTURAL STUDIES OF POLAR PEPTIDES DERIVED FROM THE YEAST PRION SUP35

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Amyloid is an ordered, extremely stable, fibrillar protein aggregate involved in the pathogenesis of multiple diseases. Proteins capable of forming amyloid vary widely in sequence and native secondary structure. Though amyloid formation is accompanied by an increase in β-structure, the quaternary interactions that give amyloid fibers such unusual stability are not well understood. We have characterized three peptides from the prion-determining domain of the yeast prion Sup35 via x-ray powder diffraction. These polar peptides, GNNQQNY, NNQQNY, and NNQQ, form bundles of needle-shaped microcrystals having related amyloid-like structures. The peptides form extended β-strands. Parallel sheets are formed by a 4.8-4.9 Å crystallographic translation of the strands. The dense packing of peptides in the microcrystals largely excludes water, admitting only 2-4 water molecules per peptide. This lack of water suggests a highly interconnected H-bonding network between peptide strands and may explain the extreme stability of amyloid.

Keywords: **AMYLOID, POWDER DIFFRACTION, PEPTIDE**