mutant (80k/21k)has been used. After testing a wide range of crystallization conditions, we obtained crystals of this mutant with size of approximately 0.25x0.17x.03 mm, which were rather fragile and diffracted only to low resolution. Under somewhat different crystallization conditions, the mutant also crystallized in the presence of Ca^{2+}, although this was accompanied by heavy Ca^{2+} induced aggregation. Crystallization parameters were varied so that initial nucleation takes place before substantial precipitation, which is difficult to control. We are currently optimizing the conditions to improve crystal quality. (Supported by MRC of Canada and PENCE, Canada)

PS04.01.30 CRYSTAL STRUCTURE OF SUBTILISIN BPN' FOLDED WITHOUT THE PRODOMAIN. Orna Almog, D. Travis Gallagher, Maria Tordova, Joel Hoskins, Phillip Bryan, Gary L. Gilliland. Center for Advanced Research in Biotechnology of the University of Maryland Biotechnology Institute and the National Institute of Standards and Technology, 9600 Gudelsky Drive, Rockville, MD 20850

Secreted proteases such as subtilisin are usually synthesized as inactive proenzymes that are then processed into an active form. Once denatured, mature subtilisin BPN' refolds very slowly to the native state, but the refolding can be catalyzed by the addition of the prosegment [Bryan et al., (1995) *Biochemistry* **34**, 10310-10318]. Deletion of the high-affinity calcium-binding site (A-site; residues 75-83) from subtilisin BPN' can accelerate the refolding of the mature protein to its native form. To help substantiate that refolding without the prodomain produces a native-like fold, Sbt70, a subtilisin BPN' variant, was cloned and expressed in an *E. coli* system without the prodomain. Sbt70 has, besides the deletion of the calcium binding site (residues 75-83), six stabilizing mutations, K43N, M50F, A73L, Q206V, Y217K and N218S, were introduced to restore lost thermal stability. The active-site serine was also replaced by alanine (S221A) to prevent autolysis. The isolated variant was folded without the prodomain by dialysis against phosphate buffer and crystallized. Crystals of Sbt70 belong to the space group $P2_12_12_1$ with unit cell parameters a=60.67 Å, b=84.02 Å, c=54.17 Å. The structure was solved by molecular replacement and refined using 2.2 Å data. The final R factor is 0.17. The structure comparison with other high-resolution subtilisin BPN' structures shows that the conformation of Sbt70 is virtually identical to that of natural mature subtilisin BPN'. An analysis of three previously unreported stabilizing mutations, K43N, A73L, and Q206V, will also be described along with the interactions with the enzyme of the tetra-peptide ALAL that was unexpectedly found bound in the active site.

PS04.01.31 CRYSTAL STRUCTURE OF THE ASPARTIC PROTEINASE FROM *Rhizomucor miehei* at 2.15 Å. Jian Yang*, Alexei Teplyakov# and J. Wilson Quail*. *Department of Chemistry, University of Sasakatchewan, Saskatoon, Saskatchewan, Canada S7N 5C9, and #EMBL, Hamburg Outstation, Hamburg, Germany

The crystal structure of the aspartic proteinase from *Rhizomucor miehei* (RMP, EC. 3.4.23.23) has been refined to 2.15 Å resolution to a crystallographic *R*-factor of 21.5% and a *R*-free factor of 28.1%. The protein contains two domains, which consist predominantly of β-sheets. The C-terminal domain is less rigid than the N-terminal domain due to the crystal packing. A large substrate binding cleft is clearly visible between the two domains and the catalytic residues Asp38 and Asp237 are located in the middle of the cleft with a water molecule bridging these two carboxyl groups. We will report the results of the surface electrostatic potential calculations of RMP and other aspartic proteinases' active sites that show the pH optimum of each aspartic proteinase is determined by the electrostatic potentials of the two carboxyl groups of the two catalytic aspartates. The electrostatic potentials of the two aspartates' carboxyl groups are, in turn, determined by the active-site environment, especially residues 19 and 332. The protein is glycosylated at Asp79 and Asp188. The glycosylations are believed to stabilize the protein by sterically inhibiting the attack of other proteinases and contributing to the protein's high thermal stability. Three-dimensional structure alignment and sequence alignment of RMP with other aspartic proteinases have shown that RMP is structurally similar to *Mucor pusillus* aspartic proteinase (MPP). RMP and MPP are as distinct from other fungal enzymes as they are from the mammalian enzymes. This suggests that RMP and MPP diverged from the main stream of aspartic proteinases at an early stage of evolution.

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PS04.01.32 CRYSTAL STRUCTURES OF THE GLU^{18}, GLN^{18}, ASP^{18} AND ASN^{18} VARIANTS OF TURKEY OVOMUCOID INHIBITOR THIRD DOMAIN COMPLEXED WITH *STREPTOMYCES GRISEUS* PROTEINASE B AT VARIOUS pHs. K. Huang[1], M. Qasim[2], Wuyuan Lu[2], Michael Laskowski, Jr[2]. and Michael N.G. James[1]. [1]MRC Group in Protein Structure and Function, Department of Biochemistry, University of Alberta, Edmonton, AB T6G 2H7 Canada; [2]Department of Chemistry, Purdue University, West Lafayette, Indiana 47907-1393.

The ionizable P_1 residues Glu18I and Asp18I of turkey ovomucoid inhibitor third domain (OMTKY3) variants display unusually high pK_a values (8.7 and 9.4 respectively) when complexed with *Streptomyces griseus* Proteinase B. The complexes remain associated even after the carboxyl groups of the P_1 residues are ionized. The 1.8 Å crystal structures of the Glu^{18} and Asp^{18} variants of OMTKY3 have been determined in complex with SGPB at pH 6.5 (the neutral form) and at pH 10.7 (the ionized form). The crystal structures of the Gln^{18} and Asn^{18} variants of OMTKY3 have been determined at pH 6.5 (the Gln18 variant also at pH 10.7). Surprisingly, a potassium ion has been identified in the pH 10.7 structures of the Glu^{18} and Asp^{18} variants. Apparently it is recruited to the S_1 pocket to balance the negative charge of the P_1 residue at pHs above its pK_a. This K^+ ion forms a saltbridge with the carboxyl groups of the P_1 side-chains. Comparisons among the seven structures determined here show that they are remarkably similar except for the solvent structure in their S_1 pockets. Within the S_1 pocket of these complexes the solvent structure varies both with the size of the side-chain (Asp vs Glu) and with side-chain polarity (e.g. Glu^0, Glu^- and Gln).

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