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 prodomain [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, residues 75-83) from subtilisin BPN’ of the tetra-peptide ALAL that was unexpectedly found bound in the calcium binding site (residues 75-83), six stabilizing mutations were introduced that improved the 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

The crystal structure of the aspartic proteinase from Rhizomucor miehei at 2.15 Å. Jian Yang1, Alexei Teplyakov2, and J. Wilson Quail1. 1Department of Chemistry, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5C9, and #EMBL, Hamburg Outstation, Hamburg, Germany

The crystal structure of the aspartic proteinase from Rhizomucor miehei (RMP, EC. 3.4.23.25) 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 GLU18, GLN18, ASP18 AND ASN18 VARIANTS OF TURKEY OVOMUCOID INHIBITOR THIRD DOMAIN COMPLEXED WITH STREPTOMYCES GRISEUS PROTEINASE B AT VARIOUS pHs. K. Huang1, M. Qasim2, Wuyuan Lu2, Michael Laskowski, Jr.1, and Michael N.G. James1. 1MRC Group in Protein Structure and Function. Department of Biochemistry, University of Alberta, Edmonton, AB T6G 2H7 Canada; 2Department of Chemistry, Purdue University, West Lafayette, Indiana 47907-1393.

The ionizable P1 residues Glu18I and Asp18I of turkey ovomucoid inhibitor third domain (OMTKY3) variants display unusually high pKa 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 P1 residues are ionized. The 1.8 Å crystal structures of the Glu18 and Asp18 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 Glu18 and Asn18 variants of OMTKY3 have been determined at pH 6.5 (the Glu18 variant also at pH 10.7). Surprisingly, a potassium ion has been identified in the pH 10.7 structures of the Glu18 and Asp18 variants. Apparently it is recruited to the S1 pocket to balance the negative charge of the P1 residue at pHs above its pKa. This K+ ion forms a saltbridge with the carboxyl groups of the P1 side-chains. Comparisons among the seven structures determined here show that they are remarkably similar except for the solvent structure in their S1 pockets. Within the S1 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. Glu6, Glu and Gin).

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