PS04.18.09 ß-SHEET REARRANGEMENT BURIES ARGININE SIDE CHAINS IN THE HYDROPHOBIC CORE OF CLEAVED ANTICHYMOTRYPSIN HINGE REGION VARIANTS. Lukacs, C.M., University of Pennsylvania, Departments of Chemistry and Medicine, Philadelphia, PA 19104

The hallmark of serpin (*ser*ine *p*rotease *in*hibitor) function is a massive ß-sheet rearrangement involving the insertion of the P1-side of the cleaved reactive loop as strand s4A in ß-sheet A. This structural transition results in greatly enhanced stability and is required for inhibitory activity. Current dogma suggests that small hydrophobic residues at the P-even positions of the reactive loop (e.g., P14, P12, P10) facilitate this structural transition since these residues must pack in the hydrophobic core of the cleaved serpin. We have undertaken the X-ray crystal structures of cleaved P14, P12, and P10 arginine variants of antichymotrypsin (ACT), and our results challenge this dogma. All three variants show greatly enhanced thermostability upon cleavage, yet the P14 (T345R) and P12 (A347R) variants are substrates of chymotrypsin and elastase. Upon reactive loop cleavage as a substrate of chymotrypsin, A347R-ACT (P12) undergoes *full* strand s4A insertion despite the resultant burial of the bulky P12 arginine side chain in the hydrophobic core, and similar results are expected from the P14 variant. This feature has profound implications for structure-function and structure-stability relationships in ACT and as other members of the serpin superfamily.

PS04.18.10 CRYSTAL STRUCTURE OF NONSPECIFIC LIPID TRANSFER PROTEIN RICE SEEDS AT I.6 Å RESOLUTION. Kyeongsik Min, Jae Young Lee, Hoon Cha, Kwang Yeon Hwang, and Se Won Suh, Department of Chemistry, College of Natural Sciences, Seoul National University, Seoul 151-742,Korea

The crystal structure of non-specific lipid transfer protein (ns-LTP) from rice seeds, in the absence of a bound ligand, has been determined by molecular replacement using maize lipid transfer protein [Shin et al. (1995) Structure 3, 189]99, pdb ID code: lmzl] as a starting model and refined to 1.6 Å resolution. The final model for rice ns-LTP contains all of the 91 amino acid residues, 70 water molecules, a sulfate ion, and two CAPS (3-[cyclohexylamino]-1-propanesulfonic acid) molecules. The model for rice ns-LTP has rms deviations from ideal bond lengths and angles of 0.019 Å and 1.85°, respectively. The present crystallographic R-factor is 18.7 % for 9,420 unique reflections with $F_o > 2\ \sigma_f$ in the range 8.0-1.6 Å resolution. The structure of rice ns-LTP has a hydrophobic cavity formed by four α-helices similar to the previously determined structure of maize LTP. The root mean square deviation (rmsd) between them, for all common Cα atoms, is 1.4 Å. The largest difference is in the C-terminus which moves into the hydrophobic cavity. Especially, the side chains of Tyr79 and Ile81 block the hydrophobic cavity from solvent in the portal and bottom region, respectively. The high resolution structure of rice ns-LTP provides the structural basis for understanding its function.

PS04.18.11 STRUCTURES OF DIMORPHS OF RUBUREDOXIN FROM DESULFOVIBRIO VULGARIS MIYAZAKI F. S. Misaki, K. Muneo, S. Sugiyama, Y. Higuchi and N. Yasuoka, Faculty of Science, Himeji Institute of Technology, Japan

As a part of study to reveal relationship between physiological property and structure of proteins from sulfate-reducing bacteria, crystallographic structure determination of rubredoxin from Desulfovibrio vulgaris Miyazaki F (RdDvMF) has been carried out using molecular replacement method. RdDvMF has been crystalized in two forms, Form I and Form II. Form I belongs to $P3_221$ and Form II, P 21. Because of so small size of crystal, radiation power of ordinal conventional x-ray generators do not have enough radiation power for diffraction study, so diffraction study was carried out at Photon Factory, KEK in Japan. Diffraction data were collected using one crystal for Form I and using three crystals for Form II, respectively. Diffraction patterns were processed using Denzo and merged using Scalepack [Otwinowski, in Data Collection and Processing (eds Sawyer, L., Isaacs, N. & Bailey, S.) 56-62 (SERC Daresbury Laboratory, Warrington, 1993)]. Crystal data are as follows; One is space group $P3_221$, Z=6, cell parameters a=b=43.7, c=50.7Å, gamma=120, V=83849.7Å^3. The other is space group P 21, Z=6, cell parameters a=27.3, b=44.9, c=51.2Å. ß=90.6, V=62664.9Å^3. As the starting model for molecular replacement method, structure of rubredoxin from Desulfovibrio vulgaris Hildenborough (RdDvH) [Adman et al. (1991). J. Mol. Biol. 217, 337-352] was used. Final R value of Form I is 20.8% including 32 water molecules up to 2.0 Å resolution with 53.6% data completeness and that of Form II is 18.9% including 86 water molecules up to 1.9 Å resolution with 82.5% data completeness, respectively. Homology of amino acid sequences between RdDvMF and RdDvH is 90%. Molecular structure of Form I is compared with those of other rubredoxin from anaerobic bacteria. As the result essential similarity of core environment, including the structure of [Fe-4S(of Cys)] type active center, can be seen also in RdDvMF and it is considered that this similarity should be strongly related to the nature of rubredoxin as an electron carrier.

PS04.18.12 HIGH RESOLUTION STRUCTURES OF LIGANDED CANINE AND FELINE HEMOGLOBIN REVEAL AN ALTERNATIVE QUATERNARY CONFORMATION. Victoria L. Robinson, Timothy C. Mueser, Paul H. Rogers, Arthur Arnone, Dept. of Biochemistry, Univ. of Iowa, Iowa City, IA 52242

In addition to the well known T quaternary structure of deoxyhemoglobin and R quaternary structure of liganded hemoglobin, it was recently shown that liganded human hemoglobin can exist in yet another quaternary structure, the R2 structure (Silva *et al.* (1992) J. *Biol. Chem.* **267**, 17248). In higher vertebrate hemoglobins, the residues that have the greatest influence on the quaternary structure of the $\alpha_2\beta_2$ hemoglobin tetramer - the residues at the dynamic $\alpha_1\beta_2$ interface - are almost completely conserved. Thus a quaternary structure observed by crystallographic methods for one vertebrate hemoglobin should be accessible to all other vertebrate hemoglobins with the same $\alpha_1\beta_2$ interface residues. It may be possible, therefore, to crystallize each quaternary structure from more than one vertebrate hemoglobin, and in this way determine the full range of quaternary structures that are energetically accessible to the hemoglobin tetramer.

High quality (2.0Å resolution) crystals of canine and feline hemoglobin were grown under low-salt conditions at pH 8.5 and 5.8, respectively. The subunit tertiary structures of these two hemoglobins are essentially identical to those previously observed in all other liganded hemoglobin structures, and the quaternary structure of liganded dog hemoglobin exactly matches the R structure previously observed in human and equine hemoglobin. However, the quaternary structure of liganded feline hemoglobin is neither R-like nor R2-like in nature but is midway between the two. Moreover, this quaternary structure of liganded feline hemoglobin is identical to one found in liganded bovine hemoglobin (Mueser *et al.*, in preparation). Analysis of all the hemoglobin structures determined to date suggests that only one quaternary structure is energetically accessible to deoxygenated hemoglobin, whereas at least three structures are accessible to fully liganded hemoglobin.

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