s8a.m1.p7 **Systematic 3D-structure determination of enzymes involved in the plant phenylpropanoid pathway.** J.-L. Ferrer[1], J.M. Jez[2], C. Zubieta[2], M.E. Bowman[2], J.P. Noel[2]. *[1] IBS J.-P. Ebel CEA-CNRS / LCCP, 41 rue Jules Hororwitz, 38027 Grenoble cedex 1, France[2] The Salk Institute for Biol. Studies / SBL, 10010 N. Torrey Pines Rd., La Jolla, California 92037, USA.*
Keywords: enzyme catalysis, protein engineering.

Phenylpropanoid pathway is a part of the so-called secondary metabolism of plants. It produces, starting from the phenylalanine, a wide range of phenylated compounds. The utility of these compounds in plants varies widely and includes roles as structural polymers, defense barriers, defense chemicals synthesized in response to predation, signaling molecules for nitrogen-fixing rhizobia bacteria and UV-protective agents. In addition, phenylpropanoids are of considerable value in the food industry serving as important flavors, pigments, and nutritional components. Moreover, the regular dietary consumption of phenylpropanoid-derived compounds including lignans, stilbens, and isoflavonoids has considerable health benefits including lowered risk factors for both cancer and coronary artery disease. As medicinal natural products, the phenylpropanoids exhibit cancer chemopreventative, anti-mitotic, estrogenic, anti-malarial, anti-oxidant, anti-HIV, and anti-asthmatic activities. Recently, they have been associated to the positive effect of wine consumption (the French Paradox).

Compounds of the phenylpropanoids pathway are produced from phenylalanine by first the action of the phenylalanine-ammonia lyase (PAL). A set of small compounds is obtained like coumarins and lignans. A second branch starts from the condensation of one molecule of coumaroyl and three molecules of malonyl, and gives compounds like stilbenes and chalcones. This condensation reaction, and the specific cyclisation that leads to these two family of products, is performed by the stilbene synthase (STS) and the chalcone synthase (CHS) respectively. Other stereospecific enzymes and enzyme complexes perform hydroxylation, glycosylation, acylation, sulfation, and methylation reactions to produce the large variety of flavonoides family molecules.

In a first step, we determined the structure of CHS[1]. This key enzyme is actually part of a large family of single-domain polyketide synthases that perform condensation reactions similar to that involved in synthesis of fatty acids. In a second step, we are now starting the exploration of the others important enzymes of the secondary metabolism. Structural information on them should permit to manipulate the metabolism in order to obtain new products with medical and industrial interest. This project, which consists on the systematic determination of a large number of structures, is based on a collaboration between molecular biology and structural biology laboratories. Synchrotron beamlines take a large part in this project, as many of these structures will be determined by MAD experiments on Se-Met proteins.

s8a.m1.p8 **Crystallographic Studies of Thioredoxins from *Bacillus acidocaldarius*.** V. Menchise,[1] S. Galdiero,[1] G. De Simone,[1] E.Pedone,[2] S. Bartolucci,[2] M. Rossi,[2] C. Pedone[1] & M. Saviano[1], *[1]Dipartimento di Chimica & Centro di Studio di Biocristallografia- CNR, University of Naples "Federico II", via Mezzocannone 4; 80134 Napoli, Italy. [2]Dipartimento di Chimica Organica e Biologica, University of Napoli "Federico II", via Mezzocannone 16, I-80134 Napoli, Italy.*
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The utilization in biotechnology of many proteins is limited by their denaturation on exposure to operative conditions. Consequently, the knowledge of the relationship between the three dimensional structure of a protein and its stability is one of the most challenging problems in protein chemistry.

Thioredoxins are ubiquitous proteins known to function in a wide variety of cellular processes. This class of proteins is characterized by an active site containing two cysteine residues separated by two other residues. The reversible oxidation of these cysteine residues to the disulfide form serves as a redox couple for a number of biological reactions. Despite the large difference in amino acid sequence, thioredoxins from different species show a similar fold.

Recently, a thioredoxin from *Bacillus Acidocaldarius* (BacTrx), showing 49% sequence identity with *E. Coli* thioredoxin has been purified.[1] BacTrx is characterized by a greater resistance to temperature compared to *E. Coli* Trx. Molecular dynamic simulation studies *in vacuo* and in water solution have been undertaken in order to design new analogs with different thermal stability[2] (Mut1: Lys18Gly, Mut2: Arg82Glu, Mut3: Arg82Glu/Lys18Gly and Mut4: Asp102X).

In order to correlate the different thermostability with structural features for this class of protein we have carried out crystallographic studies on BacTrx and its mutants. We present the crystal data of three mutants and the comparative analysis of their structures.

[1] Pedone E., Bartolucci S., Rossi M. & Saviano M. "Computational Analysis of the Thermal Stability in Thioredoxins: a Molecular Dynamics Approach." Journal of Biomolecular Structure & Dynamics, 1998, 16, 2, 437-446.
[2] Pedone E., Cannio R., Saviano M., Rossi M. & Bartolucci S. "Prediction and experimental testing of Bacillus acidocaldarius thioredoxin stability." Biochem. J., 1999., 339, 309-317.