

et al., *J. Biol. Chem.* 270: 7077-7087 (1995)). We have determined the structure of CTAP-III<sub>des10</sub> in a monoclinic space group ( $P2_1$ ), using the molecular replacement method, with a tetramer in the asymmetric unit, to a final R factor of 0.196 ( $R_{\text{free}}=0.251$ ) for 2 sigma data from 7.0 to 1.75 Å resolution. Clear, continuous density is seen for the extended amino terminus which does indeed fold back through a type-II turn and interact with the ELR region.

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**PS04.17.25 NEW CRYSTAL FORM AND CRYSTAL STRUCTURE OF S100B FROM BOVINE BRAIN AT 2.5 Å RESOLUTION.** Hiroyoshi Matsumura\*, Tomoo Shiba\*\*, Tsuyoshi Inoue\*, Shigeharu Harada\*\* and Yasushi Kai\*. \*Department of Applied Chemistry, Faculty of Engineering, Osaka University, Suita, Osaka 565, Japan; \*\*Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo 113.

S100b belongs to S100 proteins family and consists of a dimer of two S100β subunits (91 amino acid residues; MW 10,500) including two EF-hand (helix-loop-helix motif) calcium binding sites. S100b is sensitive to the concentration of  $\text{Ca}^{2+}$  and changes its conformation and activity in the form of dimer.

We have grown a new crystal of S100b from bovine brain and determined its three dimensional structure by X-ray diffraction method at 2.5 Å resolution. The crystal belongs to an orthorhombic system of space group  $C222_1$ . The unit-cell dimensions are determined as  $a=36.18$ ,  $b=89.75$  and  $c=58.36$  Å. The asymmetric unit of the crystal lattice includes one S100β subunit with a  $V_m$  value of  $2.22 \text{ \AA}^3 / \text{Da}$ . The crystal structure of S100b was determined by the molecular replacement method using calbindin  $D_{9k}$  in calcium binding state as starting structure model. The crystallographic R-factor of the structure refined by *X-PLOR* is 0.19.

#### REFERENCE

1. Isobe, T., Okuyama, T. (1978) *Eur. J. Biochem.* 89, 379-388
2. Doleth M.E. Szebenyi and Keith Moffat (1985) *J. Biological Chemistry* 261, 8761-8777

**PS04.17.26 CRYSTALLOGRAPHIC STUDIES OF CATALYTIC-SITE MUTANT  $\alpha$ -AMYLASE FROM *BACILLUS SUBTILIS*.** H. Mizuno, N. Doui, Z. Fujimoto, T. Matsumoto and K. Takase. National Institute of Agrobiological Resources, Tsukuba 305, Japan

$\alpha$ -Amylase catalyzes the hydrolysis of  $\alpha$ -D-(1,4)-glucosidic bond of starch or related carbohydrates. Site-directed mutagenesis of *Bacillus subtilis*  $\alpha$ -amylase has been performed to understand the role of active site residues in catalysis<sup>1</sup>). Further understanding of catalytic mechanism could be made by the X-ray analysis using a catalytic-site mutant EQ208.

EQ208 crystallizes in space group  $P2_12_12_1$ , cell constants  $a=72.6$ ,  $b=74.4$ ,  $c=116.7$  Å. All data sets were collected at PF, Tsukuba ( $\lambda=1.00$  Å). Heavy atom derivatives were obtained by soaking method. One platinum position was easily obtained from difference and anomalous Patterson maps. The Hg positions were located in a difference Fourier map made with single isomorphous replacement phases from the platinum derivative. Heavy atom positions were refined with MLPHARE using reflections from 50-3.0 Å resolution.

A multiple isomorphous replacement map was calculated and solvent-flattened with DM. The free R-factor was lowered from 0.55 to 0.365. The resulting maps allowed tracing of some helices. Interpretation of the main chain of EQ208 is in progress. Heavy atom derivative method is also in progress to get better phases.

1) Takase, K. et al. (1992) *Biochim. Biophys. Acta* 1120, 281-288

**PS04.17.27 STRUCTURE INVESTIGATION OF THE RIBOSOMAL PROTEIN L22 FROM *THERMUS THERMOPHILUS*.** A. Nikulin, N. Davidova, N. Nevskaya, N. Fomenkova, M. Garber, S. Nikonov; Institute of Protein Research, Russian Academy of Science, 142292, Pushchino, Moscow region, Russia. S. Al-Karadghi, A. Liljas; Molecular Biophysics, University of Lund, Chemical Center, POB 124, S-221 00 Lund, Sweden

L22 is a small protein of the 50S ribosomal subunit located in the erythromycin-binding center. It has been shown for *Escherichia coli* that erythromycin-resistant cells can have mutation in this protein.

The overexpressed protein L22 from *Thermus thermophilus* was purified and crystallized (1). The crystals belong to the space group  $P2_12_12_1$  with cell parameters of  $a=32.8$ ,  $b=65.8$ ,  $c=68.4$  Å and have one molecule in the asymmetric unit. Volume of asymmetric unit per molecular weight of a molecule ( $V_m$ ) is  $2.89 \text{ \AA}^3 / \text{Da}$ , molecular mass is 12.8 kDa.

Three good isomorphous derivatives were obtained and used for structure determination by MIR method. Phases have been calculated to 3.5 Å and improved and extended up to 2.8 Å. The structure of L22 protein at 2.8 Å resolution will be reported.

1. N.L. Davidova, O.I. Gryaznova, O.V. Maschenko, V.S. Vysotskaya, B.-H. Jonsson, S. Al-Karadghi, A. Liljas, M.B. Garber, *FEBS Letters* (1995), 369, 229-232.

**PS04.17.28 CRYSTAL STRUCTURE OF OXIDIZED AND REDUCED PSEUDOAZURIN FROM *ACHROMOBACTER CYCLOCLASTES* IAM1013.** Nobuya Nishio\*, Tsuyoshi Inoue\*, Shinnichiro Suzuki\*\*, Takamitsu Kohzuma\*\*\* and Yasushi Kai\*. \*Department Applied Chemistry, Faculty of Engineering, Osaka University, Suita, Osaka 565, \*\*Department Chemistry, Faculty of Science, Osaka University, Toyonaka, Osaka 560, \*\*\*Department of Chemistry, Faculty of Science, Ibaraki University, Mito, Ibaraki 310, Japan.

The crystal structure of the oxidized and reduced type pseudoazurin from the denitrifying bacteria *A. cycloclastes* IAM1013 have been solved at 1.6 Å and 2.0 Å resolution respectively. Pseudoazurin from *A. cycloclastes* (124 amino acid residues; MW 12900 Da) is believed to be electron donor to nitrite reductase which converts nitrite to nitric oxide in the denitrification.

Crystals of the oxidized pseudoazurin were obtained by the hanging-drop vapor diffusion method. The reduction of the crystal carried out by soaking to the solution including sodium ascorbate. The both crystals are isomorphous and belong to orthorhombic space group  $P2_12_12_1$ , cell constants  $a=56.69$ ,  $b=61.53$ ,  $c=30.20$  Å. Diffraction data of both crystals were collected on Rigaku R-AXIS IIC. Structure analysis and the following structure refinement have been carried out by molecular replacement method in *MERLOT* and by *X-PLOR* and *PROLSQ*. For the final refined models of the oxidized and reduced pseudoazurin, R-factor were 17.7% and 17.6% respectively.

#### REFERENCES

1. T. Inoue, N. Nishio, Y. Kai, S. Harada, Y. Ohshiro, S. Suzuki, T. Kohzuma, S. Shidara, H. Iwasaki *J. Biochem.* 114, 761-762 (1993)
2. T. Inoue, N. Nishio, T. Shimomura, S. Harada, S. Suzuki, T. Kohzuma, S. Shidara, H. Iwasaki, Y. Kai in preparation