PS04.17.21 CRYSTALLIZATION AND PRELIMINARY X-RAY STUDIES OF PHOSPHOENOLPYRUVATE CARBOXYLASE FROM *Escherichia coli* Y. Kai*, T. Inoue*, Y. Nagara*, H. Matsumura*, K. Izui**, *Department of Applied Chemistry, Faculty of Engineering, Osaka University, Suita, Osaka 565, Japan, **Department of Applied Botany, Faculty of Agriculture, Kyoto University, Sakyou-ku, Kyoto 606, Japan

Phosphoenolpyruvate carboxylase (PEPC†; EC4.1.1.31) from *Escherichia coli* catalyzes the fixation of carbonate ion to form oxaloacetate and inorganic phosphate. It is composed of four identical subunits with molecular weight of *ca.* 100,000 dalton. Although the primary structure of PEPC has been determined in 1984,[1] the molecular structure of PEPC has not been determined. In order to study its biological function based on the three dimensional structure, we have crystallized PEPC by using PEG4000 as precipitant,[2] however, the quality of the diffraction pattern was rather low. To get crystals suitable for X-ray crystallographic studies, hanging drop vapor diffusion method was examined in various conditions with variety of pH, molecular weight of PEG, and additives as control parameters. The crystals under the best conditions appeared in tetragonal bipyramidal shape with orthorhombic crystal lattice, space group *I*222. The unit cell parameters were determined to be *a*=117.9, *b*=250.0, and *c*=81.8Å (1Å= 0.1 nm). Two sets of intensity data for native PEPC were obtained by using Rigaku RAXIS-IIc system and the Sakabe's Weissenberg camera in the Photon Factory, up to 3.4Å and 2.5Å resolution, respectively. These data sets were merged with *PROTEIN* . Among 143,033 accepted observations up to 2.5Å resolution, 34,452 were independent reflections, the completeness of which was 66.4% with an *R*-merge of 10.4%. More than 20 sets of heavy-atom derivatives have been measured for the structure determination by multiple isomorphous replacement method. Among these derivatives the Hg-derivative gave an excellent difference-Patterson map. The two sites of Hg atom were refined to have a mean figure of merit of 0.40 and the phasing power of 1.75 with the Cullis's R factor of 0.67 in the 20.0 - 3.5 Å resolution range by using the program *MLPHARE* in the *CCP4* program package. The search for other good heavy-atom derivatives and the phase improvement with the solvent-flattening method are in progress.

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PS04.17.22 CRYSTAL STRUCTURE OF THE AMINO TERMINAL DOMAIN OF ENZYME I OF THE E. COLI PHOSPHOENOLPYRUVATE:SUGAR PHOSPHOTRANSFERASE SYSTEM. Der-Ing Liao[1], Enid Silverton[1], Yeong-Jae Seok[2], Byeong Ryong Lee[2,3], Alan Peterkofsky[2], David R. Davies[1]. [1]Laboratory of Molecular Biology, NIDDK, National Institutes of Health, Bethesda, MD 20892; [2]Laboratory of Biochemical Genetics, NHLBI, National Institutes of Health, Bethesda, MD 20892; [3]Present address: Department of Biological Education, Seo-Won University, Mochung-Dong, Chong-Ju City, Chung-Buk, South Korea.

The crystal structure of the amino terminal domain of enzyme I (EIN) from E. coli has been determined and refined at 2.5Å resolution with the crystallographic R factor of 22.5%. Enzyme I catalyzes the first reaction in the multi-protein phosphoenolpyruvate: sugar phosphotransferase system (PTS), which couples the sugar translocation across bacterial cell membranes and phosphorylation. Enzyme I transfers the phosphoryl group from phosphoenolpyruvate (PEP) to a histidine containing phosphocarrier protein HPr through phosphorylation and dephosphorylation of a histidine residue HIS 189. EIN contains this phosphorylation site histidine and can transfer phosphoryl group back and forth between HPr and itself. Unlike the intact enzyme I, EIN can not be autophosphorylated by PEP. It has an elongated two-domain structure consisting of a four- helix α-domain and an α/β domain. In addition, there is a helical linker to the missing C-terminal domain of the intact enzyme I. The α/β domain of EIN is topologically similar to the phospho- histidine domain of the enzyme pyruvate-phosphate dikinase (PPDK), which can be phosphorylated by PEP on a histidyl residue. The crystal structure of EIN as well as its comparisons with PPDK and EIIA, another PTS protein which also interacts with HPr, will be presented. A model of the interaction between EIN and HPr will also be purposed.

PS04.17.23 THE NOVEL ANTITUMOR NUCLEASE RC-PUP FROM RANA CATESBEIANA OOCYTES. Yen-Chywan Liaw[1], Faik N. Musayev[1], You-Di Liao[2], [1]Institute of Molecular Biology and [2]Institute of Biomedical Science, Academia Sinica, Taipei, Taiwan, ROC

The crystal structure of RC-PUP, a pyrimidine-guanine sequence-specific ribonuclease which is isolated from *Rana Catesbeiana* (bullfrog) oocytes, has been determined. A number of anticancer proteins, which shown sequence highly homologous to RNase A, were recently found from frog eggs. These proteins are abundant in the frog oocytes and reveal cytotoxic ribonuclease activity, which they appear to bind to cells, enter the cytosol where they degrade the RNA and kill the target cells. It is very likely that exert this kind of cytotoxic ribonuclease as antiparasitic and anticancer agent in human.

The RC-PUP is crystallized in the space group $P4_1$, with cell dimensions a = 68.7 Å, b = 68.7 Å, and c = 52.3 Å and diffract up to 2.4 Å resolution. The structure was solved by molecular replacement method using P-30 protein (Onconase) as initial model. The overall folding is similar to other RNase. Three loop regions show different folding.

Many specific features of RC-PUP and this class of proteins are under investigation, such as, the protein cell binding and their cytotoxic mechanism. The detail refinement and comparism with other RNases is in progress.

PS04.17.24 CTAP-IIIdes10: IMPLICATIONS OF AMINO TERMINAL RESIDUES ON CHEMOTACTIC ACTIVITY AND RECEPTOR ACTIVATION. Michael G. Malkowski[1], Jerome B. Lazar[2], Paul H. Johnson[2], and Brian F.P. Edwards[1]. Department of Biochemistry, Wayne State University, Detroit, MI 48201[1] and Department of Molecular Biology, SRI International, Menlo Park, CA 94025[2].

CTAP-IIIdes10, which was generated by recombinant methods, with Met-11 replaced by leucine, has 10 fewer amino terminal residues than Connective Tissue Activating Protein-III (CTAP-III) but 5 more than Neutrophil Activating Peptide-2 (NAP-2). CTAP-III (85 residues) and NAP-2 (70 residues), members of the CXC chemokine family, are carboxy terminal fragments of Platelet Basic Protein (PBP, 94 residues) which arise from cleavage by monocyte-derived proteases. NAP-2 has powerful neutrophil stimulating effects involved in inflammation, whereas the larger precursors, PBP and CTAP-III, are inactive. NAP-2 behaves like a typical chemotactic receptor agonist inducing a rise in cytosolic calcium, chemotaxis, and exocytosis at concentrations between 0.3 and 10nM. PBP, CTAP-I1I, and NAP-2 all contain the highly conserved Glu-Leu-Arg (ELR) region that is critical for receptor binding. The longer isoforms, PBP and CTAP-III, are inactive toward receptors at physiological concentrations. We have proposed that the extended amino terminus folds back to interact with the ELR region and block access to the receptor (Malkowski

et al., J. Biol. Chem. 270: 7077 7087 (1995)). We have determined the structure of CTAP-IIIdes10 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_{free}=0.251) for 2 sigma data from 7.0 to 1.75Å 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,Bunkyou-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 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 *Vm* value of 2.22 $Å^3$ / 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.

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

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

EQ208 crystalizes in space group $P2_12_12_1$, cell constants a=72.6, b=74.4, c=116.7Å. All data sets were collected at PF, Tsukuba (λ=1.00Å). 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.

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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 erythromicin-bindind center. It has been shown for Escherichia coli that erithromicin-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 (Vm) is 2.89 Å 3/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.

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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 oxidised 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.

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