purified from buffalo colostrum. The purified lactotransferrin has been crystallized from a 10% ethanol solution. The crystals are orthorhombic and the space group is $P2_12_12_1$ with unit cell dimensions a = 111.40A, b = 150.80A, c = 158.10A. The asymmetric unit contains three molecules of the protein with a solvent content of about 59%. The crystals were stable in the X-ray beam and diffracted beyond 3.0A resolution. The intensity data upto 3.00A resolution on the native crystals have been collected. The molecular replacement method has been used to determine the structure of the protein using the models of human lactoferrin and rabbit serun transferrin protein. The protein possesses a bilobal structure. Further refinement of the structure is in progress.

PS–03.04.18 CRYSTAL STRUCTURE OF CYTOCHROME c_3 FROM *Desulfovibrio desulfuricans* ATCC 27774.

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The three-dimensional X-ray structure of cytochrome c_3 from sulphate-reducing bacteria *Desulfovibrio desulfuricans* ATCC 27774 (Dd 27774) (M.W. 13kDa, 107 residues, 4 heme groups) has been determined by the method of molecular replacement, using as model the refined structure of cytochrome c_3 from *Desulfovibrio vulgaris* Miyazaki F (DvM) (Higuchi *et al.*, J. Mol. Biol., 1984, 172, 109-139), since the two proteins share a 45% sequence homology. The c_3 DvM coordinates were retrieved from the Protein Data Bank.

Crystals of c_3 Dd 27774 were obtained in space group $P6_122$, with cell dimensions a=b=61.8Å and c=109.8Å, Z=12. An X-ray diffraction data set was measured with synchrotron radiation at SRS Daresbury, England, using an Enraf-Nonius FAST area detector diffractometer. The intensity data measurements were carried out with program MADNES. Using programs from CCP4 suite,19328 reflections were merged into 11179 unique (84.8% completeness) in the resolution range 1.75<d<20.0 Å, with $R_{merg}(I) = 5.5\%$.

Cross-rotation and translation functions were performed with ALMN and TFSGEN programs (CCP4 suite), respectively. The packing of the molecules in the unit cell was checked on a CYBER 910/470 graphics workstation with TOM/FRODO.

Rigid body refinement of the model and subsequent refinement using molecular dynamics were performed with XPLOR, achieving an R factor of 25.9%, for data up to 2.3 Å. RESTRAIN least-squares refinement is in progress, the current R factor being 22.5%, for data up to 1.9Å.

PS–03.04.19
CRYSTAL STRUCTURE STUDY OF BAR–HEADED GOOSE OXYHEMOGLOBIN. By Zhang Jian, Hua Ziqian, Lu Guangying and Gu Xiaocheng, Biology Department, Peking University, Beijing 100871, China.

Bar–headed goose (Anser indicus) is a migratory bird which inhabitates around west china's Qinhai lake. On its migratory flight over Mount Everest it is exposed to ambient pO_2 of about 50mmHg which accounts to 20% of pO_2 at sea level. The determination of its hemoglobin crystal structure may partly elucidate the high altitude respiration mechanism where the air is extremely sparse and also the effect of evolutionary pressure to the protein molecular structure.

Oxyhemoglobin crystals suitable for X– ray analysis were grown from polyethylene glycol(PEG) with average MW.6000, at 4° C, pH6.8. The crystals belong to space group $P4_22_12$. Its unit cell parameters are a=b=81.6A, c=107.3A. There is one ab dimer per asymmetric unit. The intensity data were collected from a single crystal on screenless Weissenberg camera using synchrotron X–ray(Sakabe, N.,J. Appl. Cryst. 1983, 20, 404–407). Total independent reflections were 24807 with upper resolution of 1.8A. Intensity R factor was 4.7.

The hemoglobin of Bar–headed goose has about 70% sequence homology to that of human. So the molecular replacement method was used in structure determination. 2.1A resolution human oxyhemoglobin coordinates was taken as model molecule(Shaanan, B. et al., J. Mol. Bio., 1983, 171, 31– 59). The orientation and position of the molecule in the unit cell were determined with AUTOMR program (Matsuura, Y., J. Appl. Cryst., 1991, 24, 1063–1066). The initial model was rotated about (30, 50, 40) degree (polar angle system). Cross rotation function was calculated using data between 10–4A resolution and pattern cutoff radius 30A. The highest peak appeared at (60.38, 175.73, 260.75) of polar angle system and its translation parameters were (40.8A, 0.0A, 32. 2A) calculated from 12–10A resolution data. Rigid body refinement was carried out with CORELS program, allowing only rotation and translation of molecular dimer. This brought the R–factor to 0.447 between 10–4A resolution. The model after CORELS program was then refined by the simulated annealing method with X–PLOR program(Brunger, A.T.,Science, 1987,235,458–460), the R–factor dropped to 28.9% against data between 8–2A resolution without intervention. The root–mean–square deviation of bond length and bond angle were 0.026A and 4.443 degree respectively for all atoms. The model was checked on the graphics with FRODO program. The hame group area fitted well with the 2Fo– Fc electron density map, while the N–terminal area of the peptide chain fitted relatively poor. Further refinement is under way.

PS–03.04.20 CRYSTAL STRUCTURE ANALYSIS OF HEMOGLOBINS WITH MG-SUBSTITUTED α-HEMES AND EITHER UNLIGANDED OR CO β-HEMES. By S-Y. Park*[1] A. Nakagawa[2], H. Morimoto[1], [1]Department of Biophysical Engineering, Faculty of Engineering Science, Osaka University, Osaka 560, Japan., [2]Photon Factory, KEK, Oho, Tsukuba, Ibaraki 305, Japan.

We have been studying metal substituted hybrid hemoglobins represented as $\alpha_2(M)\beta_2(Fe)$ and $\alpha_2(Fe)\beta_2(M)$, where M denotes metal ions substituted for Fe^{2+}. Depending on the kind of metal ion substituted Fe^{2+}, the subunits change their O_2 affinity from the lowest to the highest of the human hemoglobins (Morimoto et al., *Tanpaku Kakusan Koso*, 1987, 32, 557-565). Thus we can study the interaction between the state of the central ion of heme and the state of the globin moiety by comparing their properties.

The $\alpha_2(Mg^{2+})\beta_2(Fe^{2+})$ shows a very low O_2 affinity. It is constrained more strongly to the deoxy quaternary structure than native deoxy hemoglobin (Minagawa, 1990, M.thesis, Osaka Univ.). Crystalization of deoxy $\alpha_2(Mg^{2+})\beta_2(Fe^{2+})$ was carried out in ammonium phosphate/sulphate buffer (pH=6.5) according to the method described by Perutz (*J.Crystal Growth*, 1968, **2**, 54-56). Crystals were grown from solution whose final ammonium phosphate/sulphate buffer concentration was 2.4-2.5M. Crystals of $\alpha_2(Mg^{2+})\beta_2$(Fe-CO) were grown at room temperature using the batch method previously described by Ward (*J.Mol.Biol.*, 1975, **98**, 237-256). The best crystals were grown from 20-25% PEG.1000(w/v). X-ray diffraction data of $\alpha_2(Mg^{2+})\beta_2(Fe^{2+})$ and $\alpha_2(Mg^{2+})\beta_2$(Fe-CO) crystals were collected to about 1.7Å and 2.0Å resolution, respectively, using synchrotron radiation at the Photon Factory (wavelength=1.04Å, I=341-327mA, E=2.5GeV). The refinement of the $\alpha_2(Mg^{2+})\beta_2(Fe^{2+})$ structure was carried out with the restrained least-squares program PROLSQ of Hendrickson and Konnert (*Methods Enzymol*, 1985, **62**, 241-246). An *R*-factor of 17.1% was obtained for the data between 10.0 and 1.7Å with magnitudes greater than 3.0σ. We are refining the structure of $\alpha_2(Mg^{2+})\beta_2$(Fe-CO) crystal, and refined structures will be presented in the conference.

Crystal	Total No. of reflections	Unique reflections	*R*-merge (%)	Space Group
$\alpha_2(Mg^{2+})\beta_2(Fe^{2+})$	209163	90366	6.87	$P2_1$
$\alpha_2(Mg^{2+})\beta_2$(Fe-CO)	150689	44289	7.58	$P2_12_12$

PS-03.04.21 THE CRYSTALLOGRAPHIC STRUCTURE OF BAR-HEADED GOOSE METHEMGLOBIN. By Hua Jing, Guangying Lu,* Ziqian Hua and Xiaocheng Gu Department of Biology, Peking University, Beijing 100871, P.R.China

The protein under investigation is the methemoglobin of Bar-headed Goose, a bird which can fly over the summit of Mt.Everest(8848m) during its normal migration between Tibet and India. In order to elucidate the molecular mechanism of high altitude respiration, the structure at high resolution of Bar-headed Goose hemoglobin is necessary. We present here the method to prepare the crystal of methemoglobin, the way to solve phase problem using molecular replacement method and the procedure of structure refinement.

Crystallizatoin of methemoglobin was carried out at 18° C by the hanging drop method.The protein was precipitated by polyethylene glycol(PEG) at optimum pH 7.8. After one week, crystals could be found in some drops. The crystal belongs to space group $P4_22_12$, with 4 molecules in the unit cell of dimension:a=b=81.6A,c=107.2A. Crystallographic data were collected on Area Detector to resolution 2.3A with Rmerge 7.21. The ratio of the observed reflections to the number of theoretical reflections is 94.6%, to resolution 2.3A.

The initial phases of measured reflections were solved by molecular replacement method. In order to find out the possible orientation of search model in the unit cell and to locate it correctly in the cell, an automatic processing program system AUTOMR(Yoshiki Matsuura, Appl.Cryst., 1991, 24, 1063-1066) was used to carry out these calculations. The 2.0A structure of horse hemoglobin from Protein Data Bank(PDB) was used as the search model. It has 70% homology with Bar-headed Goose hemoglobin. Firstly, the search model molecule was put in a cubic P1 cell with cell dimension as large as 100A and the structure factor was calculated by conventioanl algorithm to the 4A resolution. Secondly, the rotation function was calculated and a number of higest independent peaks were listed and put into next calculation. Thirdly , translation function was calculated to find the correct position of rotated search model. In this step, only 100 to 300 reflections at low resolution were used to define correct solutions. Finally, rigid-body refinement was carried out to refine the rotation and translation parameters. The correct solution is usually obtained as a clear minumum of R value in the listing. In our calculation, the minimum R value was 0.428, which corresponds to the angular position (49.48, 270.56, 125.47) in spherical polar coodinates and translation position(0.00, 40.25, 21.83). By plotting the main chain models in the unit cell, a reasonable molecular packing was observed.

From the correctly rotated and translated model of horse methemoglobin, we calculated the initial phases of measured reflections and the initial electron density map. The residues of horse hemoglobin were substituted by that of Bar-headed Goose hemoglobin with FRODO program. Phases of the substituted structure was then calculated and input into XPLOR program together with the measured intensity of reflections. In XPLOR program, simulating annealing method was applied to refine the structure. The final crystallographic R value is 0.245 to 2.5A resolution. Further refinement of the structure with FRODO program is still in progress. We hope that the mechanism of high altitude respiration could be partly explained after the structure at high resolution is solved.

PS-03.04.22 STRUCTURE OF THE RECOMBINANT N-TERMINAL LOBE OF HUMAN LACTOFERRIN AT 2.0Å RESOLUTION. By Catherine L. Day, Bryan F. Anderson, John W. Tweedie and Edward N. Baker,* Department of Chemistry and Biochemistry, Massey University, Palmerston North, New Zealand.

The three-dimensional structure of the N-terminal half-molecule of human lactoferrin, Lf_N, prepared by recombinant DNA methods, has been determined at 2.0Å resolution. The protein is in its iron-bound form and is deglycosylated. X-ray diffraction data were obtained by diffractometry to 3.2Å resolution and synchrotron data collection, using Weissenberg photography with imaging plates, to 1.8Å resolution. The structure was solved by molecular replacement using as search model the N-lobe (residues 1-333) of native diferric human lactoferrin (Lf) (Anderson, Baker, Norris, Rice & Baker, J. Mol. Biol., 1989, 209, 711-734). Restrained least squares refinement (program TNT) has resulted in a model structure with R = 0.184 for all data (34180 reflections) in the resolution range 8.0 to 2.0Å. The model comprises 2490 protein atoms (residues 4-327), 1 Fe^{3+} ion, 1 CO_3^{2-} ion and 180 water molecules.

The structure of Lf_N is essentially the same as that of the N-lobe of intact Lf, being folded into two similar α/β domains, with the Fe^{3+} and CO_3^{2-} ions bound in a specific site in the interdomain cleft. These details are not affect by either deglycosylation or expression from cloned DNA. The C-terminus has unfolded, however; whereas in Lf residues 321-333 form a helix crossing between the domains at the back of the iron site, in Lf_N 321-326 are extended, forming a third interdomain β-strand, and 327-333 are disordered. The conformational change is attributed to the loss of stabilising interactions from the C-lobe, and is mediated by Gly residues at 321 and 323. The conformational change also causes Lf_N to release iron more readily by increased solvent exposure of the hinge region at the back of the iron site. The metal and anion binding geometry, hydrogen bonding in the molecule, and solvent structure have been analysed. Two cis-proline residues and a clear example of amino-aromatic hydrogen bonding have been found.