PS16.01.09 CRYSTALLIZATION OF CYCLOMALTODEXTRIN GLUCANOTRANSFERASE FROM BACILLUS CIRCULANS VAR. ALKALOPHILUS. I. Kuranova, Institute of Crystallography, Moscow, Russia, P. Mattsson, T. Korpela, Biochemistry Department University of Turku, Turku, Finland

The crystallization of cyclomaltodextrin glucanotransferase from Bac. circulans var. alkalophilus (CGTase, EC 2.4.1.19) was studied in the presence of some low molecular weight compounds such as n-octyl beta-D-glucopyranoside (OG), 2-methyl-2,4-pentanediol (MPD), isopropanol, bivalent metal ions, alpha- and beta-cyclodextrin. Vapor diffusion technique was used for crystal growth. When ammonium sulfate (AS) and PEG 4000 were used as precipitants the protein was obtained in amorphous state only. When 3 % MPD was added into AS solution the microcrystalline tubes of CGTase were observed in electron microscope. The addition of 0.3 % of OG together with calcium chloride (0.5 mmol) to the PEG 4000 containing protein solution promoted the formation of thin needle-shaped crystals assembled into spherical druses. The substitution of calcium chloride for cobalt chloride in crystallization trials caused the formation of non-faceted spherical particles. When PEG 1500 or PEG 600 were used instead of PEG 4000 the bipyramidal crystals of size of 0.05 mm were obtained. The addition into protein solution of alpha- or beta-CD which are the products of enzymatic reaction reduced the amount of crystals in the drop and increased their size. The largest crystals up to 0.3 mm in each derection with limits of diffraction till 3 A were grown, when 10-15% of isopropanol was added into the reservoir solution.

PS16.01.10 PROGRESS WORK ON THE CRYSTALLISATION OF B800-820 LIGHT-HARVESTING COMPLEXES. K. McLuskey[1], N.W. Isaacs[1], R. J. Cogdell[2], S.M. Prince[1], A. A. Freer[1].Dept. of Chemistry[1] and Biochemistry[2], University of Glasgow, G12 8QQ.

In photosynthetic bacteria the cell membrane contains a reaction centre (RC) stoichiometrically associated with a light-harvesting (LH1) complex. In certain bacteria a second, so called LH2, complex is produced to increase the light-harvesting capacity. Depending on growth conditions, some bacteria are able to produce a second form of LH2 where the Bchl a absorption is observed at 800 and 820 nm, rather than at 800 and 850 nm.

The B800-820 complex is a more efficient light-harvesting complex than its B800-850 counterpart, being more effective in terms of energy transfer between the internal pigment molecules and more effectual in directing energy to LH1 and hence towards the RC. It has been suggested that the change in absorption is modulated by the apoprotein and it has been shown, using mutants, that changing the hydrogen-bonding patterns between protein residues and the Bchl a, causes a blue shift in the spectrum. A structure of a B800-820 complex would help us to understand the mechanisms of energy transfer in more detail and the role that proteins play in determining the characteristics of light harvesting.

LH2 B800-820 complexes from *Rps. acidophila* strain 7750 and *Rps. cryptolactis* have been crystallised (Guthrie, N. *et al., J. Mol Biol.* **224**, 527-528, (1992) and Halloreen, E. *et al., Photo. Res.* **44**, 149-155, (1995)). These crystals diffracted to resolutions of no higher than 5Å. The purification protocol employed was based mainly on charge, whereas experience, with the B800-850 complex, indicated that purification based on size exclusion gives better quality crystals. This work involves refining the protocol for both purification and crystallisation in order to attain crystals that diffract to the highest possible resolutions and results will be reported.

PS16.01.11 CROSSOVER OF LYSOZYME AGGREGATES BETWEEN SUPER- AND UNDER- SATURATED STATES IN AQUEOUS SOLUTION. N.Niimura, M.Ataka*, I.Tanaka, Y.Minezaki and Y.Karasawa, Advanced Science Research Center, Japan Atomic Energy Research Institute, Tokai-mura, Naka-gun, Ibaraki-ken 319-11, Japan, *National Institute of Bioscience and HumanTechnology, 1-1, Higashi, Tsukuba, 305, Japan

The structure of lysozyme aggregates at the wide concentration range of lysozyme and NaCl in the supersaturated state has been studied by small angle x-ray scattering (SAXS). Based on the interest in the nucleation stage of protein crystallization, we have already reported the aggregate formation in undersaturated aqueous lysozyme solutions (N.Niimura, Y.Minezaki, M.Ataka & T.Katsura: J. Crystal Growth 137(1994) 671, Y.Minezaki, N.Niimura, M.Ataka & T.Katsura, Biophys. Chem. 58(1996)355) and two types (Type I & II) of aggregates in supersaturated states by small angle neutron scattering measurement. (N.Niimura, Y.Minezaki, M.Ataka and T.Katsura: J. Cryst. Growth 154 (1995) 136). The SAXS measurements were performed by using a synchrotron-radiation x-ray scattering instrument. The radius of gyration Rg of Type II aggregates was obtained by carrying out Guinier plot in the Q range of $0.03<Q<0.08$. $(V-V_0)$ vs. natural logarithm of supersaturation ratio, where V and V_0 are the aggregates volume in the supersaturated and saturated states, respectively, are plotted. The volume increment from the one on the solubility curve at different NaCl concentration is described by the one unique straight line. On the other hand, the data in the undersaturated states are off the line. This means that the nature of the aggregates in the supersaturated states could be unified in reference to the supersaturation degree and the crossover between super and under saturated states was clearly demonstrated by the treatment.

Crystal Growth II
Fundamentals

MS16.02.01 DEFECT GENERATION DURING CRYSTAL GROWTH. J.P. van der Eerden, E.A. Huitema, M. Vlot and J. Huinink. Department of Interfaces & Thermodynamics, Debije Institute, Utrecht University, Padualaan 8, 3584CH Utrecht, The Netherlands

Although perfect crystals hardly exist, our fundamental knowledge of the origin of these defects is very limited. In this contribution we introduce general ideas about mechanisms of defect generation during (and due to) crystal growth. Both molecular scale phenomena and thermodynamic descriptions turn out to be relevant. These ideas will be supported by Monte Carlo and Molecular Dynamics simulation results.

In practice impurities often are the starting point for defect generation. From a thermodynamic point of view their incorporation in the crystal is expected to be given by the equilibrium distribution coefficient. In practice often much higher impurity levels are found. Moreover fluctuations in the impurity density couple to the local crystal growth rate and may lead to the well-known Mullins-Sekerka morphological instability.

The accumulation of impurities near the surface leads to macroscopic inclusions which generate planar and linear defects. Even in the absence of impurities such defects are to be expected at large growth rates.

The final defect structure is the result of a competition between *generation* processes at the growing surface (where sharp free energy gradients exist) and *healing* processes in a somewhat wider interface region (where relatively fast relaxation towards thermodynamic equilibrium still is possible). Some of the free energy parameters can be obtained from numerical simulations, others have to be estimated from macroscopic observations and descriptions.

The after-growth generation of defects due to temperature gradients, mechanical stresses and solid state phase transitions which occur during cooling of the crystal is not discussed here.