02.6-06 DTA AND X-RAY STUDY OF DRY PHOSPHATIDYL-DIPALMITOYL CHOLINE. By J. Doucet, N. Albon, A.M. Levelut, M. Lambert, Laboratoire de Physique des Solides, Université Paris-Sud, 91405 Orsay, France.

A study of extremely pure dipalmitoyl-phosphotidyl choline (DPPC) by thermal analysis and X-ray scattering has revealed several features not reported previously. The experimental observations were very sensitive to sample purity and experimental procedure. Nevertheless, reproducible results have been obtained. From both single domain and powder X-ray patterns we have shown that the dry region of the diagram [% water, T] exhibits some phases characterized by a three-dimensional order. The study of the diffuse scattering has permitted us to improve the knowledge of the Lδ phase and from the powder patterns we have deduced the existence of several novel structural features such as a three-dimensional "Lβ'" phase or phase transitions in the high temperature phase region Pδ + Qα.

02.6-07 CO-CRYSTALS OF GRAMICIDIN AND PHOSPHATIDYLCHOLINE: A SYSTEM FOR STUDYING ION-CHANNEL STRUCTURE AND LIPID-PROTEIN INTERACTIONS. By M.R. Kimball and B.A. Wallace, Dept. of Biochemistry, Columbia University, NY NY 10032.

In an effort to determine the membrane-bound structure of gramicidin A we have grown crystals for X-ray diffraction which contain gramicidin and phosphatidylcholine. Since CD studies suggest that the conformation of gramicidin in crystals prepared without lipids is unlikely to be the same as in membranes, several crystal forms containing both lipid and gramicidin have been prepared. The first, rolled up stacks of sheets with a high lipid/peptide ratio (19:1), exhibits primarily lipid reflections altered by the presence of peptide, as well as a number of reflections due to peptide itself. This form is useful for examining the effects of peptide on lipid molecules. The second form, which has a much lower lipid/peptide ratio (2:1), is a well-ordered single crystal which diffracts strongly to 2.4 Å, and differs from any of the crystals prepared in the absence of lipid. The symmetry of these crystals is orthorhombic with lattice dimensions 26 Å x 26 Å x 32 Å. A third crystal form which contains monovalent thallium in addition to lipid and protein is currently under investigation. Preliminary spectroscopic evidence suggests the gramicidin conformation in the mother liquor from which these crystals were formed is likely to be of the membrane-bound type. These crystal forms will be useful in defining the membrane-bound ion channel conformation at a resolution heretofore unseen for any membrane proteins. This work is funded by NSF PCM 8020063 and NIH GM27292.

02.7-01 INSULIN STRUCTURE AT 1.1Å RESOLUTION AND ITS DYNAMIC BEHAVIOR WITH ANISOTROPIC TEMPERATURE FACTORS. By N. Sakabe, K. Sasaki and K. Sakabe, Faculty of Science, Nagoya University, Chikusa, Nagoya, 464 Japan.

The compositions of rhombohedral 2 zinc insulin crystal prepared at pH 6.2 are insulin, zinc ion, water molecule and citrate. This crystal structure (positional parameters and isotropic temperature factors used have already been refined by FFT least-squares method ; Sakabe et al, proceeding of the symposium on proinsulin, insulin and C-peptide, p73, 1979, Excerpta Medica) was refined by using block-diagonal least-squares method with X-ray diffraction data to 1.1Å resolution collected at 4°C, and the structures of insulin and citrate were regularized by MODELFIT program. The ordered atoms whose isotropic temperature factors were less than 30Å^{2}were refined with anisotropic temperature factors in this procedure. R value reduced to 0.155 with 33038 data which correspond to 94.5% of the theoretical number of reflection to 1.1Å. 90% of hydrogen atoms bonded to insulin appeared at expected positions on D-Fourier maps. Thus the refinement with anisotropic temperature factors is effective to confirm hydrogen atoms. Hydrogen atom is bonded to Nε atom of B10His but is not bonded to the Nδ atom in this crystal, and those of terminal methyl groups of side chains keep a staggered conformation although they may move as a flipping mode. The geometrical analysis of hydrogen bonds was carried out about helical parts, β-sheet and β-bends and the geometry was compared between two molecules in an asymmetric unit. α-helix from B10 to B18 is a bit curved against inside of the molecule and the cooperation of hydrogen bonds exists in β-sheet.
The dynamic behavior was analysed with anisotropic temperature factors of individual atoms. We calculated translational vibration and libration of hexamer, two monomers and α-helix in B-chain by using TL or TLS method.

02.7-02 INVESTIGATIONS ON THE DISORDERED ACTIVATION DOMAIN IN TRYPSINOGEN BY CHEMICAL LABELLING AND LOW TEMPERATURE CRYSTALLOGRAPHY. By J. Walter and W. Steigemann, Max-Planck-Institut fuer Biochemie, 8033 Martinsried, FRG

The activation domain of the trypsinogen molecule is disordered. Upon complex formation with basic pancreatic trypsin inhibitor (PTI), trypsinogen undergoes a transition to a trypsin-like state, in which the activation domain is ordered (Huber & Bode, Acc. Chem. Res. (1978) 11, 114).

In the flexible activation domain of trypsinogen the disulphide Cys191-Cys220 can be selectively reduced and mercurated. The crystal structures of such modified trypsinogen have been analyzed in its native form and in its complex with PTI and the dipeptide ILE-VAL. The mercury atom, covalently bound between the two sulfur atoms, is crystallographically undetectable in the free form of trypsinogen, whereas well defined in the complexed form. After crystallographic refinement of the complex at 2.0Å resolution, the R-value was 0.20.

A trypsinogen crystal was crystallographically analyzed and refined at 173K. The coordinates as well as the thermal parameters were compared with the room temperature data. The overall B-factor is reduced by 4.4Å^2. A number of additional ordered water molecules are observed. The order of the activation domain is not increased detectably at 173K. Residues at the boundaries of the flexible segments, except at the N-terminus, indicate a considerable contribution of temperature independent static disorder. Within the significance level of our measurements it seems that most of the other residues behave like harmonic oscillators.