

21-Crystallography at Non-Ambient Temperatures and/or Pressures; Phase Transitions

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MS-21.02.04 LOW-TEMPERATURE COCRYSTALLIZATION

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Formation and crystal structure of low-melting adducts in certain small-molecule quasi-binary systems with hydrogen bonding as the pertinent interaction will be surveyed. Most are hydrates and hydrogen-halide adducts of various (other) weak and strong acids and bases of the Brønsted and Lewis type.

The respective melting diagrams are set up by DTA and X-ray powder diffraction. Single crystals of adducts established in this way are grown *in situ* in capillaries on a low-temperature X-ray diffractometer. Typically, a miniature zone-melting technique, based on focused heat radiation, is applied (Brodalla, Mootz, Boese & Osswald (1985). *J. Appl. Cryst.* **18**, 316-319).

Molecular adduct structures are determined as well as ionic ones. Some peculiar patterns and types of hydrogen bonding can clearly be attributed to the low temperature of their formation. Among them are dense layers of water molecules in certain hydrates and Cl-H... π interactions in adducts of hydrogen chloride with unsaturated hydrocarbons. Other findings include a strong H/D isotopic effect on phase formation and structure, the unusual hydrogen bonds F-H...N and F-H...O, and novel species in crystals, such as $(H_{n-1}Cl_n)^-$ and $(H_{n+1}F_n)^+$ with n up to 6.

Related work of others will also be discussed.

Support by the Deutsche Forschungsgemeinschaft is gratefully acknowledged.

MS-21.02.05

PROTEIN STRUCTURE AND DYNAMICS BY LOW TEMPERATURE CRYSTALLOGRAPHY.

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Dynamical motions in protein molecules are essential to their biological functioning. The use of low temperatures in the range of typically 80-250 K in combination with time-resolved Laue or monochromatic diffraction techniques provides a means of determining space and time averaged amplitudes of intramolecular motions, and of investigating the three-dimensional structure of the protein in "frozen" intermediate states of a biological reaction. Furthermore, cooling prolongs the lifetime of crystals in the synchrotron X-ray beam and causes a substantial reduction in the overall temperature factors. Thus, the diffraction limits, the contrast in electron density maps, and the localization of ordered solvent molecules may substantially improve.

Applications to enzymatic reactions are based on the Arrhenius type dependence of catalytic constants on the temperature, and on a possible accumulation of specific intermediates under cryo-conditions (P. Douzou and G.A. Petsko, 1984, *Adv. Protein Chem.* **36**, 245-361). Crystal structure analysis of serine proteases at very high resolution demonstrated that cooling in the presence of organic cryosolvents may leave the enzyme conformation essentially intact. On the other hand, the solvent structure in the vicinity of the active site and hence the reaction kinetics may be modified. The specificity, direction and speed of the reaction between enzyme and substrates depend on

transient changes in the conformation. Cooling to moderately low temperatures of 200-250 K extends the lifetimes of reaction intermediates from typically 1 ms - 1 s at room temperature to 1-100 minutes which is sufficient for synchrotron diffraction data collection to high resolution. The enzymatic reaction may be initiated in the crystal through jumps in temperature, pressure or pH, or by laser activation of caged metabolites. It has recently been possible to determine the structure of an acyl-enzyme intermediate of elastase at 2.0 Å resolution by combined use of low-temperature, Laue monitoring and monochromatic diffraction techniques (H.D. Bartunik et al., *Phil. Trans. R. Soc. London A*, 1992, **340**, 209-220).

Intra- and intermolecular motions in crystal-line proteins may be investigated on the basis of the temperature dependence in atomic Debye-Waller factors (e.g., F. Parak et al., 1987, *Euro. Biophys. J.* **15**, 237-249) and of diffuse scattering components (J. Doucet and J.P. Benoit, 1987, *Nature* **325**, 643-646; D.L.D. Caspar, 1988, *Nature* **332**, 659-662). In general, only isotropic temperature factors may be derived, except for very small proteins. The interpretation in terms of collective motions of molecular regions with an extent varying between a few residues and large domains of 50-100 residues is complicated by the time and space averaging of diffraction methods, and by a strong coupling between intra- and intermolecular modes. Theoretical molecular dynamics calculations and complementary studies with spectroscopic techniques broaden the basis for model-dependent interpretations.

MS-21.02.06

SYNCHROTRON-RADIATION X-RAY TOPOGRAPHY AND DIFFRACTOMETRY BELOW 1K BY MEANS OF HELIUM-3 DILUTION REFRIGERATOR. By Tetsuo Nakajima* and Haruhiko Suzuki†, Photon Factory, National Laboratory of High-Energy Physics, Oho, Ibaraki, Japan; *Dept of Physics, Faculty of Science, Kanazawa Univ., Kanazawa, Japan.

With development of synchrotron radiation, it became possible both to take X-ray diffraction patterns especially below 1K by the ^3He dilution refrigerator (hereafter abbreviated $^3\text{HeDR}$) and to understand changes of atomic arrangement within a unit cell and/or aggregated structure of unit cells. The former is the structure determination and the latter the X-ray topography. To do those, two sets of the $^3\text{HeDR}$ 200NS with top loading purchased from Oxford Instruments Inc. were installed at BL-3C₂ and 6C₁ in the experimental hall of PF. The difficult points are the positioning of specimens in the incident beam and the unavoidable temperature rise due to absorbed X-rays. The $^3\text{HeDR}$ at BL-3C₂ is used only for study of the quantum solid helium by X-ray topographs. The cylindrical sample cells made of Be in two sizes (10mm ϕ x 1mm x 30mm and 20mm ϕ x 2mm x 30mm) up to 100kg/cm² as a resisting pressure, were mounted on the two-circle goniometer of ω - χ ($\pm 20^\circ$ in each) controlled by two coaxial stainless steel tubes modified from top loading. The cell has a carbon-resistor thermometer (50 Ω 1/8 W made by Matsushita Co.) and a thin stainless capillary supplying cooled squeezed helium. The $^3\text{HeDR}$ at BL-6C₁, widely used for solid-state physics, was mounted on the three-circle Low Temperature Goniometer (LTG), with ω ($\pm 180^\circ$), 2θ ($\pm 150^\circ$) and χ (-20° , $\sim +35^\circ$) as reported in PF Act. Rept., 1982/3 p. V-25, ω of which is limited to $\pm 20^\circ$ by configuration interference of the peripheral instruments of $^3\text{HeDR}$, too. The sample holder in shape of ladder structure was made of OFHC Cu to hold single crystals and powdered specimens. The temperature rise by continuous irradiation of SR were controlled by use of Al-