MS–21.02.04 LOW-TEMPERATURE COCRYSTALLIZATION By D. Mootz* and W. Poll, Institut für Anorganische Chemie und Strukturchemie, Heinrich-Heine-Universität Düsseldorf, W-4000 Düsseldorf, Germany.

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---pi 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.

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 crysosolvents 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 A 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 inter- pretation 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.

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With development of synchrotron radiation, it became possible both to take X-ray diffraction patterns especially below 1K by the ^{3}He dilution refrigerator (hereafter abbreviated ^{3}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}HeDR 200NS with top loading purchased from Oxford Instruments Inc. were installed at BL-$3C_2$ and $6C_1$ 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}HeDR at BL-$3C_2$ 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 1mmt x 30mml & 20mm ϕ x 2mmt x 30mml) up to 100kg/cm^2 as a resisting pressure, were mounted on the two-circle goniometer of ω - χ ($\pm$ 20° in each) controlled by two coaxial stainless steel tubes modified from top loading. The cell has a carbon-resistor thermometer (50Ω 1/8 W made by Matsushita Co.) and a thin stainless capillary supplying cooled squeezed helium. The ^{3}HeDR at BL-$6C_1$, widely used for solid-state physics, was mounted on the three-circle Low Temperature Goniometer (LTG), with ω ($\pm$180°), 2θ ($\pm$150°) and χ (-20°, ~ +35°) as reported in PF Act. Rept., 1982/3 p. V-25, ω of which is limited to $\pm$20° by configuration interference of the peripheral instruments of ^{3}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-

foil attenuators as reported in PF Acta Rept. #7 (1989) 177. Fig 1 shows Laue spots of solid ^{3}He taken by use of white X-rays. The texture of solid ^{3}He changes with annealing time, which could not be observed in solid ^{4}He. Fig. 2 shows the Laue spots (422, 844) of Ho-elpasolite taken at 830mK, 65mK and 320mK by white X-rays and is more suitable for diffractometry than for topography from its characteristics. A structural phase transformation at 150mK predicted from other experiments was confirmed from splitting of a Laue spot as a change of atomic arrangement within unit cell as seen in Fig. 2.

Fig. 1 The topographs of solid ^{3}He in serial order (a)920705-22:25, (b)920705-23:15 and (c)920706-08:49.

Fig. 2 The temperature change of Laue spots of $Cs_2NaHoCl_6$ at (a)830mK, (b)65mK and (c)320mK.

Fig.1. Temperature dependence of hydrogen-bond lengths

MS-21.02.07   STRUCTURE OF LOW TEMPERATURE PHASE OF ISOLATED HYDROGEN BOND SYSTEM $K_3D(SO_4)_2$.

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The pair $K_3D(SO_4)_2$. and $K_3H(SO_4)_2$ is a textbook example of an extraordinary isotope effect for phase transition temperatures. The D-compound has a finite T_c at 84K while the H-compound has no phase transition. The structures feature two SO_4 groups connected by one hydrogen atom forming an isolated dimer. The high temperature phases of H and D compounds are isostructural with virtually identical atomic parameters. The structure analyses of the H-compound in the A2/a phase at various temperatures down to 27K were already performed. (Y. Noda, H. Kasatani, Y. Watanabe and H. Terauchi, J. Phys. Soc. Jpn., 1992, 61, 905-915): Several Bragg reflections violating the A-lattice centering were observed below T_c in the D-compound (Y. Noda, Y. Watanabe, H. Kasatani, H. Terauchi and K. Gesi, J. Phys. Soc. Jpn., 1991, 601, 1972-1977). The structure analysis of the low temperature phase of the D-compound described here was accordingly performed in space group P2₁/a. A Weissenberg photograph is presently being exposed at 12K, using a new technique with an image plate, to establish firmly the space group. Observed hydrogen bond length $R_{O-O}(D)$ is shown in Fig.1 as a function of temperature, above and below T_c. In the figure, the temperature dependence of $R_{O-O}(H)$ is also shown, which does not show any anomaly as a function of temperature. The low temperature phase seems to be characterized by an antiparallel ordering of hydrogen atoms.

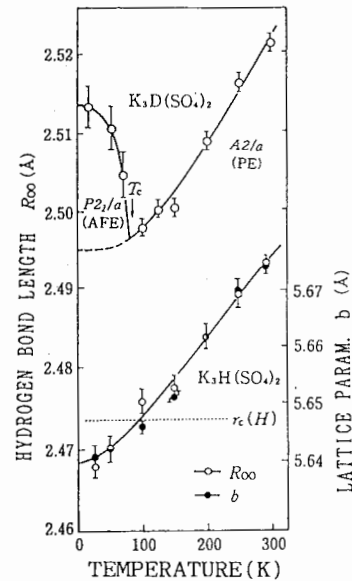The image plate study is supported by The Mitsubishi Foundation.

PS-21.02.08 CRYSTAL STRUCTURE OF K_2SO_4 AT 15 K.

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Crystal structure analyses at very low temperatures are fundamental for clarifying solid state properties such as superconductivity, ferro-electricity and magnetism. But there have not been so many reports because of instrumental difficulties. Recently we have set up a new diffractometer which can be easily used in a routine work to make crystal structure analyses at very low temperatures down to 10K; a four-circle automatic diffractometer with an offset χ-circle of inner diameter 400 mm (Huber Eulerian cradle model 512). The low-temperature device is a closed-cycle He-gas refrigerator (Cryomech GB15) which is mounted on the ϕ-circle of the four-circle diffractometer. This small and light refrigerator enables us to maintain the sample shift within 10μm from the center of the χ-circle during data collections. We can measure Bragg reflections in a wide range of χ-circle ($-90° \leq \chi \leq 90°$).
The crystal structure of K_2SO_4 at room temperature was reported by McGinnety(1972). It was suggested that K_2SO_4 crystal might undergo a phase transition at 56 K from measurements of specific heat and dielectric constant (Gesi, Tominaga & Urabe, 1982). The purpose of the present work is to examine the crystal structure of K_2SO_4 at 15 K and to examine whether such phase transition really occurs or not. The crystal structure was studied at both 296 and 15 K. Intensity data were collected up to $(\sin/\lambda)_{max}=0.904$ Å^{-1}. The structure was refined by a block-diagonal-matrix least-squares on F, using the program AXS-89 system which were rewritten from UNICS by Mashiyama(1991). The crystal at 296 K was confirmed to be orthorhombic, Pmcn, with Z=4. The calculation converged at R=0.045, wR=0.043 for 1281 independent reflections with $F \geq 3\sigma(F)$. The crystal at 15 K was found to be orthorhombic, Pmcn, with Z=4. The calculation converged at R=0.037, wR=0.034 for 1375 independent reflections with $F \geq 3\sigma(F)$. Isotropic thermal parameters U_{eq} of K and O atoms at 15 K reduce to