example, one crystal of RNase-A was used to collect data from 1.5 to 1.2-Å resolution at 160K. The temperature was then lowered to 98K and data from ∞ to 1.5-Å resolution were collected on the same crystal. After a total of 28.3 days of X-ray exposure the decay in the intensity standards was about 3%. Data from two other RNase-A crystals have also been collected at 180K and 130K from ∞ to 1.5-Å resolution and, again, the decay in the standards after 13.1 days of X-ray exposure was only about 3% in both cases. Data were also collected from an RNase-A crystal at 220K and no radiation damage was observed over an 8.1 day period of X-ray exposure. The structures at each temperature have been refined to current R values of between 14 and 16%.

The reasons for the large reduction in radiation damage that we have observed for RNase-A crystals mounted on glass fibers are not at all obvious but may possibly be linked in some way to the removal of the glass of the capillary tube from proximity to the crystal, to the lack of excess mother liquor surrounding the crystal, or to the exclusion of oxygen from the crystal during data collection. The large reduction in radiation damage appears to be a combination of both the low temperature and the lack of the capillary tube.

01.1-2 USE OF POLYCHROMATIC SYNCHROTRON X-RADIATION IN PROTEIN CRYSTAL LAUE DIFFRACTION.

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The Daresbury SRS wiggler is used to provide an intense, smooth continuum of X-rays between 0.2Å - 2.5Å. Laue patterns can be recorded from protein crystals in approximately 100 milliseconds to 1 second using the SRS wiggler protein crystallography workstation (Helliwell et al (1986) Nucl.Instrum. and Methods A246, 617). On this station (9.6) the vertically diverging beam is collected by a cylindrical focussing mirror set to reflect λ's ≥ 0.5Å. A new workstation is currently under construction on the wiggler line. The design of the new station involves a toroid mirror to focus the vertically and horizontally diverging beam. Laue patterns are expected to be recorded in 1-10 milliseconds. The new station will also be used for point focussed, rapidly tunable monochromatic experiments.

The advantage of using a broad wavelength band is that a large continuous region of reciprocal space is sampled in a single shot. This "traditional" benefit of Laue geometry, taking advantage of the short exposures now possible, is useful for monitoring, in a qualitative way, crystal transitions. This has been used by Hajdu et al ((1986) Biochem Soc Trans) to study an order-disorder-reorder process in crystals of phosphorylase b.

The use of Laue diffraction data in a quantitative way, in difference Fourier maps, has necessitated new data processing software and new theory. The strategy for the processing software (Helliwell (1985) J Mol Struct 130, 63) involves the usual steps of sample refinement, prediction and integration. The single wavelength Laue spots and multi-component Laue spots are treated differently. Several reciprocal lattice points (rlps) stimulated in one spot can be unscrambled using differential film absorption (for the case of double and triple reflection spots). The integrated data are currently being used in three ways. Hajdu et al (pers comm) are using the fractional difference intensity in Fourier maps whereby wavelength dependent parameters cancel out, to study enzyme substrate interactions. Helliwell et al (this abstract) are comparing the data derived from Laue geometry with monochromatic data from the source crystals to check for systematic errors and improve the software; wavelength normalisation procedures have been developed (partly in collaboration with K Moffat). Harding et al (pers comm) is using wavelength normalized Laue data to solve small molecule crystal structures.

New theory has been necessary. Cruickshank, Helliwell and Moffat ((1987) submitted to Acta Cryst) have derived the observed multiplicity distribution of energy overlap spots. It has been established that the proportion of single wavelength spots is never less than 73% and depends on λmax/λmin and the number of Laue spots depends on (λmax-λmin).

01.2-1 FIBER DIFFRACTION AS AN ALTERNATIVE TO PROTEIN CRYSTALLOGRAPHY.

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Many important biological macromolecules, for example actin, myosin, tubulin, flagellin, and the coat proteins of some viruses, form filamentous assemblies with functions specific to those assemblies. Even in cases where these molecules can be crystallized as monomers or small aggregates, it is important to know the molecular structure of the intact assembly in order to understand the function of the molecule. It is therefore necessary to use the methods of fiber diffraction.

The central problem of fiber diffraction is that the random azimuthal orientations of the diffracting particles cause the data to be cylindrically averaged. Considerable information is thus lost; for example, at 3Å resolution the effective number of observable diffraction data for tobacco mosaic virus (TMV) is reduced by a factor of 2.5, and for the bacteriophage Pf1 by 1.7. These factors are much higher for lower symmetry systems such as microtubules.

Multi-dimensional isomorphous replacement, analogous to isomorphous replacement in protein crystallography, can be used to compensate for the loss of information. Large numbers of heavy-atom derivatives are needed, but these numbers can be reduced by taking advantage of the fine splitting present in the diffracted layer lines when, as is usual, the helix in the diffracting system repeats approximately, but not exactly, in a small number of turns. Phases determined by MDIR and layer-line splitting can be refined by density modification (solvent flattening), which provides much more powerful constraints in fiber diffracting systems where the diffracted intensity is continuous along layer

lines than it does in crystals.

It has been necessary to develop for fiber diffraction many methods already established in crystallography. Restrained least-squares refinement has proved very useful, despite the limited number of data available. Information from partial structures will be increasingly important as crystal structures become available for the monomers of important biological assemblies. The difference Fourier method has been developed for fiber diffraction; the peak heights, noise levels and optimal Fourier coefficients are quite different from those found in crystallography, for example, in TMV at 3Å resolution, coefficients 6Fobs - 5Fcalc have been shown in both theory and practice to be most effective. These methods have been used in this laboratory to solve TMV at 2.9Å by MDIR and layer-line splitting, and to refine the structure by restrained least-squares in conjunction with difference Fourier-Bessel maps. Other helical viruses are under study; work on cucumber green mottle mosaic virus is at an advanced stage. The structure of microtubules has been solved at low-resolution (18Å) in collaboration with Drs. Cohen at Brandeis and Beese at Yale, using a model from electron microscopy and density modification refinement. The structure of Pf1 is being determined by Dr. Makowski's group at Columbia using model-building and restrained least-squares refinement.
Supported by NIH grant GM33265.

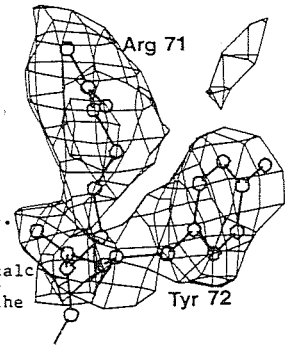

Figure: Part of a $6F_{obs} - 5F_{calc}$ map of TMV with Arg 71 omitted from the model clearly shows the position of the side-chain.

01.2-2 X-RAY CRYSTALLOGRAPHIC STUDIES OF HUMAN SERUM AMYLOID P-COMPONENT (SAP) .

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Serum Amyloid P-Component (SAP) is a normal plasma glycoprotein belonging to the pentraxin family of plasma proteins, of which the other member in man is the homologous protein C-Reactive Protein (CRP), the classical acute phase reactant (Mantzouranis et al., JBC, 280, 7752-7756,1985; Pepys & Baltz, Adv. Immunol. 34, 141-212, 1983). SAP is composed of ten glycosylated subunits, each of Mr 23,500, which are non-covalently associated in two pentameric discs interacting face-to-face, though under non-physiological conditions the pentamers can dissociate.
Pentameric SAP crystallizes in space group $P2_1$, cell constants a=69.0, b=99.3, c=96.8Å, β =96.1° , with one pentamer per asymmetric unit. The crystals diffract well to at least 2Å resolution. Data from native crystals and two isomorphous heavy atom derivatives have been collected on a FAST TV-detector diffractometer.
Analysis of the self-rotation function clearly indicates the orientation of the non-crystallographic 5-fold symmetry axis.
Progress on the analysis of the structure both by molecular replacement, using as search-model an unrelated pentameric molecule, and by isomorphous replacement will be presented.

01.2-3 MULTIPLE-WAVELENGTH PHASE DETERMINATION IN PROTEIN CRYSTALLOGRAPHY. Janet L. Smith*, Arno Pähler, H. M. Krishna Murthy and Wayne A. Hendrickson, Dept. of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032, USA.

The tunability of synchrotron radiation provides an excellent means to exploit the dispersive anomalous-scattering properties of appropriate atoms in order to solve the phase problem. We report on methods developed to extract phase information from multiple-wavelength data.

Data were measured to 3.0Å spacings from crystals of selenobiotinyl streptavidin, lamprey hemoglobin (with W.E. Love) and Urechis hemoglobin (with R.L. Stanfield and M.L. Hackert) and to 2.5Å spacings from crystals of a bacterial ferredoxin (with W.H. Orme-Johnson) on the area-detector facility at the Stanford Synchrotron Radiation Laboratory with R.P. Phizackerly and E.A. Merritt. Absorption edges were measured from the data crystals in order to select wavelengths for data collection where anomalous-scattering effects are maximal. Bijvoet pairs were measured at each of four or five wavelengths.

Careful scaling of the integrated intensities from area-detector images was a crucial part of phase determination. Uncorrected systematic errors were minimized by local scaling first of Bijvoet pairs and then of data from multiple wavelengths. Dispersive differences in total scattering were considered only for ferredoxin, where iron anomalous scattering is a signficant part of the total. The data were placed on an approximately absolute scale by calculation of unit cell contents.

The phase equation for a single type of anomalous scatterer (adapted from J. Karle, Int. J. Quantum Chem. (1980) 7, 357-367) is

$$|F_\lambda^\pm|^2 = |Ft|^2 + (f_\lambda^A/f_0)^2|Fa|^2 + 2(f_\lambda'/f_0)|Ft||Fa|\cos(\phi_t-\phi_a) \pm 2(f_\lambda''/f_0)|Ft||Fa|\sin(\phi_t-\phi_a)$$

where $|F_\lambda^\pm|$ is the observed structure-factor amplitude for the + or - mate of a reflection at wavelength λ, $|Ft|$ and ϕ_t are the amplitude and phase for the total normal structure factor, and $|Fa|$ and ϕ_a are for the normal scattering of the anomalous scatterer. The scattering factors f_λ' and f_λ'' for the anomalous scatterer at wavelength λ were derived from absorption spectra measured from the data crystal; f_λ^A is the magnitude of anomalous scattering and f_0 is the normal scattering factor. A refinement procedure was developed to determine $|Ft|$, $|Fa|$ and $(\phi_t-\phi_a)$ from the multiple measurements for each reflection.

Patterson maps with coefficients $|Fa|^2$ yielded the anomalous-scatterer positions for lamprey hemoglobin (1 Fe) and streptavidin (2 Se). Atomic positions of anomalous scatterers were refined against the $|Fa|$'s. The ferredoxin and Urechis hemoglobin data are being analyzed. Values of ϕ_t were obtained by adding the calculated ϕ_a values to the refined phase differences. Electron-density maps were calculated from weighted $|Ft|$ coefficients and ϕ_t phases. Weights were based on lack-of-closure errors calculated for each reflection. Analysis of the streptavidin electron-density map is in progress.

The lamprey hemoglobin crystal structure is known and a model has been refined at 2.0Å resolution (Honzatko, Hendrickson & Love, J. Mol. Biol. (1985) 184, 147-164). Phases calculated from the refined model were used to judge the accuracy of the multiple-wavelength phases and to evaluate various error models. The multiple-wavelength analysis produced phases of equal quality to the isomorphous-replacement phases from which the structure was solved. Both sets of experimental phases have a mean phase error of 51° when compared with the model phases.

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