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X-ray Laue Diffraction from Protein Crystals

Abstract. *In conventional x-ray diffraction experiments on single crystals, essentially monochromatic x-rays are used. If polychromatic x-rays derived from a synchrotron radiation spectrum are used, they generate a Laue diffraction pattern. Laue patterns from single crystals of macromolecules can be obtained in less than 1 second, and significant radiation damage does not occur over the course of an exposure. Integrated intensities are obtained without rotation of the crystal, and individual structure factors may be extracted for most reflections. The Laue technique thus offers advantages for the recording of diffraction patterns from short-lived structural intermediates; that is, for time-resolved crystallography.*

Structural intermediates in enzyme-catalyzed reactions or in the ligand binding reactions of myoglobin and hemoglobin typically have lifetimes at physiological temperatures of milliseconds or less. These transient intermediates cannot be crystallized and studied directly by x-ray crystallographic methods. Rather, their structures have been inferred from presumably similar, stable structures which can be crystallized, such as enzyme-product and enzyme-inhibitor complexes, complexes of hemoglobin with nonphysiological ligands, or hemoglobin locked in a single quaternary structure. In favorable cases, transient intermediates can be generated by photoactivation of a stable structure in the crystal, as in the photodissociation of carboxymyoglobin and carboxyhemoglobin by a brief light pulse and subsequent recombination with carbon monoxide in the dark (*1*). The crystal structures of photogenerated intermediates can be studied directly, provided that their lifetimes are long in relation to the minimum x-ray exposure time required to record a useful diffraction pattern. Even with an intense synchrotron x-ray source coupled to a conventional monochromator, a minimum exposure time of the order of minutes for an oscillation photograph is required (*2*). Although the lifetime of intermediates may be prolonged by cooling the crystal, the use of a monochromatic source requires that the crystal be rotated during the exposure in order to obtain integrated intensities from which quantitative Fourier structure amplitudes can be extracted. For exposures of less than about 1 second, unrealistically large angular velocities of the crystal would be required.

We now describe our experiments with an intense polychromatic x-ray source to generate a Laue diffraction pattern (*3*) and outline the underlying theory of these experiments. Instrumental and certain experimental aspects have been described (*4*). Although we focus on its applications to macromolecular crystallography, the Laue technique is equally applicable to chemical crystallography (*5*), solid-state physics, and surface diffraction.

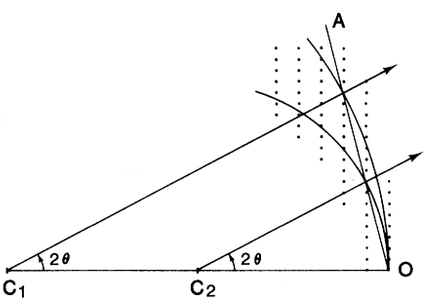

Fig. 1. The Ewald construction for Laue diffraction. A central section of the reciprocal lattice, origin O, is illuminated by x-rays of wavelength λ, where $\lambda_1 < \lambda < \lambda_2$. Diffraction from two reciprocal lattice points on the central lattice line OA occurs at the same scattering angle 2θ. Diffraction from other reciprocal lattice points not on this line occurs at different values of 2θ. $C_1O = 1/\lambda_1$ and $C_2O = 1/\lambda_2$.

Conventional diffraction experiments involving synchrotron radiation have used monochromatic radiation, in which a narrow bandpass, single-crystal monochromator with $\Delta\lambda/\lambda$ of the order of 10^{-4} selects a small portion of the continuous synchrotron x-ray spectrum. Monochromatization is generally believed (*2*) to be essential in preventing superposition of multiple orders of reflections, but our results show that this is not necessarily so.

The principle of Laue diffraction is illustrated in the Ewald construction (*6*) of Fig. 1. If polychromatic x-rays of wavelength λ, where λ lies in the range from λ_1 to λ_2 and $\Delta\lambda = \lambda_2 - \lambda_1$, fall on a crystal at point O, then all reciprocal lattice points that lie between the limiting Ewald spheres of radii $1/\lambda_1$ and $1/\lambda_2$ will be in diffracting position for some incident wavelength λ and will contribute to a Laue reflection. For all points on a radial reciprocal lattice line such as OA, diffraction will occur at a single value of 2θ; that is, the Laue reflection is multiple, with contributions from several structure factors.

Consider, for example, a reciprocal lattice point $(h'k'l')$ where $h' = nh$, $k' = nk$, and $l' = nl$; that is, $(h'k'l')$ is the nth order of (hkl). Then it may be shown that the condition for a Laue reflection to arise from a single structure factor $\mathbf{F}(h'k'l')$ is $n\lambda \leqq \lambda_1\lambda_2/\Delta\lambda$, where λ is the wavelength at which $(h'k'l')$ is in diffracting position. For small $\Delta\lambda$, this condition is approximated by $n \leqq \lambda/\Delta\lambda$. The fraction of lattice points that is of the nth order varies only slightly with the volume of reciprocal space included (determined by unit cell size, the resolution of crystal diffraction, and λ_1 and λ_2). Approximately 80 percent of all points are first order, and 98 percent are fifth order or less. With a bandpass $\Delta\lambda/\lambda$ of about 0.2, $\lambda/\Delta\lambda = 5$, and up to fifth order reflections will arise from only a single structure factor. Superposition of multiple orders of a reflection is therefore not a problem.

Laue diffraction from a stationary crystal results in an intensity whose integration is carried out over wavelength (*6*) rather than angle. Thus, rotation of the crystal is not required to record integrated intensities as it is in conventional oscillation and precession photography or single crystal diffractometry. By

adapting the derivation of Zachariasen (*6*) to the synchrotron case and introducing an absorption factor, $T(\lambda,\mathbf{r})$, a polarization term, $P(\lambda,\mathbf{r})$, and a detector sensitivity and obliquity factor, $S(\lambda,\mathbf{r})$, the integrated intensity I_L of a Laue reflection $\mathbf{k}$ can be expressed as

$$I_L \sim I_0(\lambda)V^{*2}V_cT(\lambda,\mathbf{r})P(\lambda,\mathbf{r})S(\lambda,\mathbf{r}) \cdot |\mathbf{F}(\mathbf{k},\lambda)|^2\lambda^4 = M(\lambda,\mathbf{r})T(\lambda,\mathbf{r})|\mathbf{F}(\mathbf{k},\lambda)|^2$$

where $I_0(\lambda)$ is the incident intensity between λ and $\lambda + d\lambda$, V^* is the reciprocal unit cell volume, V_c is the crystal volume, and $\mathbf{r}$ is the direction of the diffracted beam. The factor $M(\lambda,\mathbf{r})$ is a function of the source and detector only and can be determined in a separate experiment via a rotation (*5*) or low-angle precession photograph. Extraction of the desired structure factor, $|\mathbf{F}(\mathbf{k},\lambda)|$, requires accurate location and measurement of the optical density of reflections and an estimate of $T(\lambda,\mathbf{r})$ (*7*).

Preliminary experiments with a stationary crystal yielded Laue patterns such as those shown in Fig. 2, A and B. Significant radiation damage occurred only after several patterns had been obtained. Exposure times as short as 450 msec were obtained for a hemoglobin crystal in subsequent experiments (*4*). Increases in x-ray intensity could reduce exposures to about 10 msec for proposed single-shot exposures (*4*).

With a stationary crystal, each Laue reflection is recorded with a different wavelength. If the crystal is now oscillated during the exposure through a small angle (for example, about the normal to the plane of the paper through O in Fig. 1), each reciprocal lattice point is exposed to a range of wavelengths. An example of the resulting Laue oscillation pattern is shown in Fig. 2C. Each reflection is drawn out into a small streak that extends in a nearly radial direction. There is a one-to-one correspondence between position in the streak and wavelength; that is, each streak is a diffraction spectrum that displays directly the variation with wavelength of the integrated

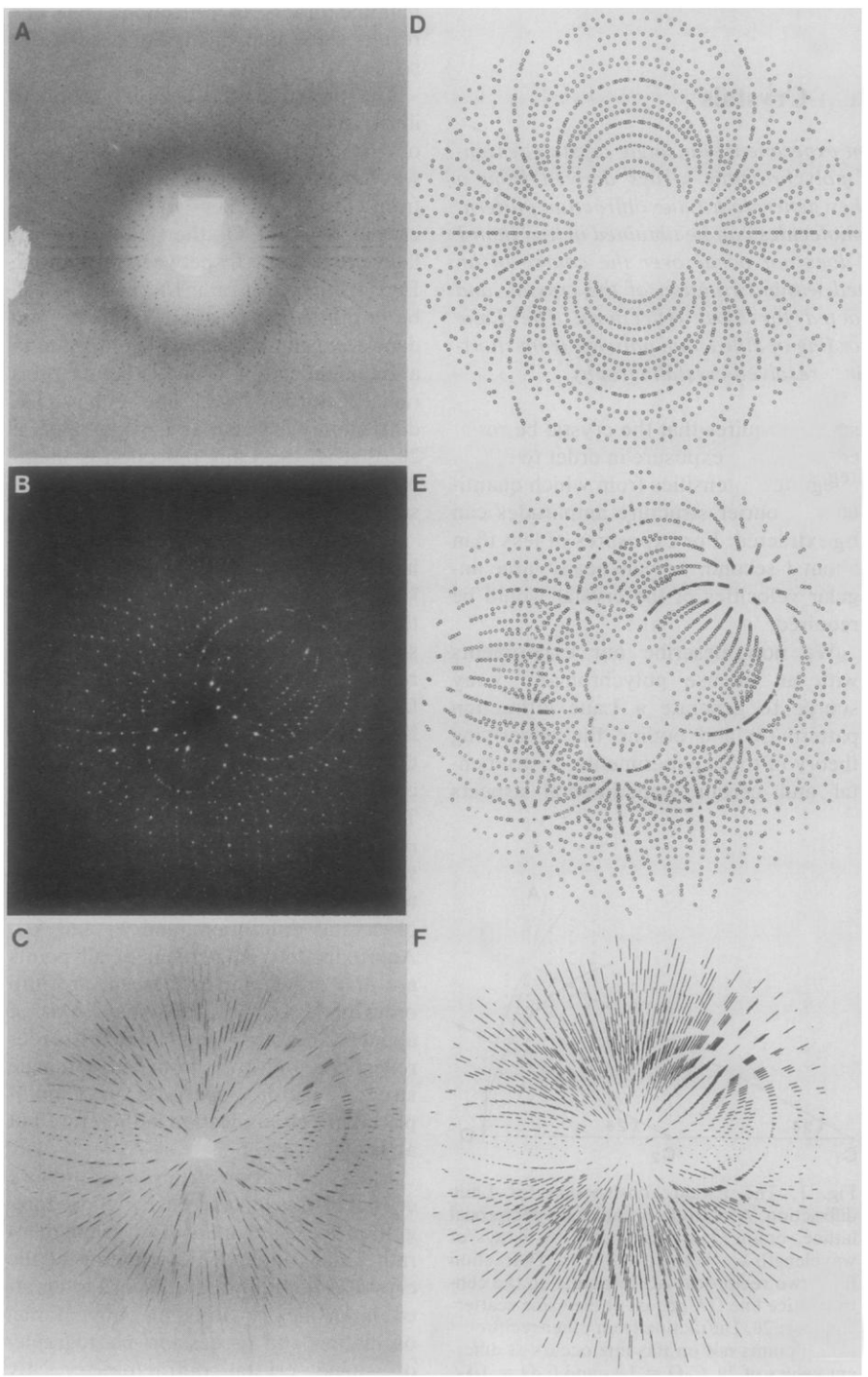

Fig. 2. Radiation from the Cornell High Energy Synchrotron Source (CHESS) (*15*) was modified by absorption and reflection (*4*) to produce a polychromatic spectrum with a bandpass $\Delta\lambda/\lambda$ of about 0.3. Crystals were illuminated through a 0.2-mm collimator, and diffraction data were recorded on Kodak No-Screen or Polaroid Type 57 film. The total x-ray flux through the collimator was estimated to be 3×10^{11} photons per second, two orders of magnitude greater than that obtained with a conventional monochromator at CHESS. Panels A to C show experimental Laue diffraction patterns and panels D to F show corresponding computer simulations of reflection position. (A) Bovine intestinal calcium binding protein crystal, *b* axis 20′ from x-ray beam. Peak intensity of the incident spectrum occurred at 13.9 keV. A 30-second exposure on Kodak film; prior exposure was 30 seconds. The photograph is overexposed. (B) Horse methemoglobin crystal, general orientation. Peak intensity occurred at 9.5 keV. A 1-minute exposure on Polaroid film; prior exposure was 1 minute 15 seconds. (C) Horse methemoglobin crystal, 1°30′ oscillation. A 2-minute exposure on Kodak film; prior exposure was equivalent to 5 minutes 15 seconds. (D) Simulation of (A), using an energy range from 7 to 17 keV, corresponding to full width at approximately one-tenth maximum height. Structure factors beyond 2.3 Å were ignored. The pattern contains 1702 Laue reflections, of which 1636 arise from a single structure factor (○) and 66 arise from two structure factors (+). (E) Simulation of (B), using an energy range of 7 to 14 keV, corresponding to full width at one-tenth maximum height. Structure factors beyond 2.8 Å were ignored. The pattern contains 1941 Laue reflections, of which 1880 arise from a single structure factor (○), 50 arise from 2 factors (+), 8 arise from 3 factors (△), and 1 each arises from 4 (■), 5 (X), and 7 factors (□). (F) Simulation of (C). Each diffraction spectrum is shown by a line whose ends correspond to the lowest and highest wavelengths sampled by that reflection during the oscillation. Other conditions as in (E) except that structure factors beyond 3.1 Å were ignored.

intensity I_L. If the polychromator (*8*) wavelength range $\Delta\lambda$ is set to span the absorption edge of a heavy element in the crystal (such as the Fe K edge in hemoglobin or the Au L_{III} edge of an Au–heavy atom derivative of a protein), the maximum variation in $|\mathbf{F}(\mathbf{k},\lambda)|^2$ is then obtained as an aid in phase determination (*2*) through the sharp variation of the anomalous scattering factors f' and f'' in the immediate vicinity of the absorption edge. Variation in the absorption component of I_L, namely $T(\lambda,\mathbf{r})$, is also at a maximum, but this is an unavoidable complication of all multiple wavelength techniques (*9*).

The x-ray Laue technique apparently has not been used for protein crystal analysis, although polychromatic x-rays have been advocated for low-angle scattering measurements of macromolecules in solution (*10*). A related neutron Laue technique (*11*) has been applied to a myoglobin crystal (*12*); however, the full spectrum of thermal neutrons was used with a $\Delta\lambda/\lambda_1$ value of approximately 3, so that many Laue reflections contained contributions from multiple structure factors. Although it was believed (*11*) that accurate individual structure factors could be isolated by deconvolution or Fourier chopping (*12*), the technique has not been widely used (*13*).

The x-ray Laue diffraction technique for macromolecule analysis has four advantages over monochromatic radiation techniques: (i) optimal use of the naturally polychromatic synchrotron radiation spectrum, (ii) reduction in exposure time, (iii) direct production of integrated diffraction intensities with a stationary crystal (*14*), and (iv) simultaneous recording of many thousands of reflections. These advantages apply to both static and kinetic experiments. The latter could provide information (*4*) on the structural changes that occur during biochemical reactions—that is, for time-resolved crystallography.

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8. The term "polychromator" describes the arrangement of mirrors and absorbers making up a wide bandpass filter of the synchrotron x-radiation.
9. An alternative approach to the collection of diffraction data at multiple wavelengths has been described [U. W. Arndt, T. J. Greenhough, J. R. Helliwell, J. A. K. Howard, S. A. Rule, A. W. Thompson, *Nature (London)* **298**, 835 (1982)]. The energy resolution of our technique is described in (*4*); that of U. W. Arndt *et al.* may prove superior.
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12. A. C. Nunes, *J. Appl. Crystallogr.* **8**, 20 (1975).
13. In their structure refinement of a small inorganic molecule from x-ray Laue photographs, Wood *et al.* (*5*) also used a wide spectrum with $\Delta\lambda/\lambda_1 \sim 2.5$, and they did not attempt to extract individual structure factors.
14. Use of a stationary crystal and a monochromatic source does not yield integrated intensities and may introduce serious errors [H. D. Bartunik, *Nucl. Instrum. Methods* **208**, 523 (1983)].
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Association of Parvoviruses with Rheumatoid Arthritis of Humans

Abstract. *A small virus resembling parvoviruses in its morphological and physicochemical properties was derived from synovial tissue of a patient with severe rheumatoid arthritis. This virus, designated RA-1, elicits a syndrome in neonatal mice that includes neurological disturbances, permanent crippling of limbs, dwarfism, alopecia, blepharitis, "masking," and a rigid curvature of the thoracic spine. Polyclonal antibodies against RA-1 display high virus neutralizing activity and in immunoassays detect reactive antigen in synovial cells from different rheumatoid arthritis patients but not persons with osteoarthritis. Putative parvoviruses isolated from several other rheumatoid arthritis patients are only weakly pathogenic for newborn mice but can generate RA-1 virus–specific antigens in tissues of these animals. It has not been established that RA-1 and existing parvoviruses of mammalian species are related.*

The etiology of chronic rheumatoid arthritis (RA) of humans has eluded identification since the first description of this insidious disease by Sir Alfred Baring Garrod in the 19th century (*1*). Infectious agents thought to be associated with RA have included bacteria, mycoplasma, viruses, and viroids (*2–4*). Conventional viruses are known to produce arthritides of humans which are usually of relatively short duration, cause no tissue necrosis or permanent disability, and require only symptomatic treatment (*5, 6*). The one example of a viral pathogen causing chronic arthritis of a mammalian host is the caprine arthritis-encephalitis retrovirus that elicits a proliferative synovitis and periarthritis in older goats (*7*). It was recently reported (*8*) that parvovirus-like agents can be isolated from the synovial tissue of patients with severe RA disease including the unidentified agent originally described by Godzeski *et al.* (*9*). Here we report the salient findings of a 3-year collaborative study leading to the recognition of these agents as viruses with unusual properties and a yet undefined link with human RA.

Long-term cultivation of rheumatoid synovial cells with WI-38 human lung fibroblasts was accompanied by the transient appearance of microfoci of piled-up, aggregated cells; electron microscopic examination of these cells revealed rare intracytoplasmic budding particles with long surface projections. Subsequent inoculation of culture extracts into brains of suckling mice led to the emergence of an agent that was lethal in mice and, on the basis of inconclusive evidence, was tentatively regarded as an enveloped RNA virus (*9*). This agent, hereafter called RA-1 virus, is identified in the present report as a DNA parvovirus on the basis of results from completed virological studies and ongoing biochemical work. More than 10,000 newborn mice have been used to date for the production, bioassay, and characterization of mouse lethal RA-1 virus and other putative isolates because a permissive cell culture system has not been found (*10*).