Measurement of Absorption Curves for Protein Single Crystals on the Oscillation Camera with Time Decaying Incident-Beam Intensity and Variable-Wavelength Synchrotron X-radiation

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(Received 22 March 1984; accepted 19 June 1984)

Abstract

The correction of intensity data from protein single crystals for sample absorption effects is important for refinement and interpretation of atomic parameters. Synchrotron X-radiation sources are extensively used to provide high-resolution data and their tuneability utilized to collect data with optimized anomalous dispersion. At longer wavelengths the sample absorption effects become increasingly important. Previous methods of determining the absorption correction using the oscillation camera relied upon film methods, which cannot readily take account of the decay in incident-beam intensity of synchrotron sources. The use of a sensitive ion chamber to measure the transmitted intensity and monitoring of the incident intensity allows an empirical absorption correction to be calculated directly.

Synchrotron X-radiation of high intensity and with a smooth wavelength spectrum from high-energy storage rings is now extensively used for protein crystallographic data collection. The high-resolution data required for protein-structure model refinement and any subsequent analysis of atomic temperature factors to reveal dynamics of protein structure and function are affected in a detrimental way if the X-ray diffraction data are not corrected for sample absorption. Moreover, a major application of tuneable synchrotron X-radiation is the optimization of the anomalous dispersion of specific metal atoms, either to locate their positions within the unit cell or for phase determination. The wavelength range of interest covers $0.5 \leq \lambda \leq 3.0$ Å and over this range the linear absorption coefficient, $\mu_0$, varies as $0.03 \leq \mu_0 \leq 6.4$ mm$^{-1}$ ($33 \geq \mu_0 \geq 0.15$ mm) for a typical protein crystal. At shorter wavelengths, $\lambda \leq 1$ Å, the absorption effects are reduced substantially for protein crystals, which are rarely larger than 1–2 mm. However, data collection at such short wavelengths may not always be desirable for reasons of convenience, the intensity spectral profile of the synchrotron source, the crystal parameters or the absorption edge of interest. At longer wavelengths it is most likely that sample-absorption variations will be significant. Correction of these data for absorption effects will lead to a better determination of the experimental anomalous dispersion changes as well as the refined atomic temperature factors, referred to above.

It is possible to measure a symmetric absorption surface (Kopfmann & Huber, 1968) of a protein single crystal using the Arndt–Wonacott oscillation camera by varying the spindle axis ($\phi$) and the inclination angle ($\mu$) in the ranges $0 \leq \phi \leq 360^\circ$ and $0 \leq \mu \leq 45^\circ$. To date the available devices for measuring an absorption surface using a conventional X-ray source are based on scanning a photographic film in a systematic way as $\phi$ or $\mu$ is varied (reviewed by Bartels, 1977). These are not satisfactory for use with synchrotron X-radiation where the beam intensity incident upon the sample changes with time. Moreover, the efficiency of the detector used to measure the intensity transmitted by the protein crystal is best optimized to the wavelength employed. This is easily achieved by suitable adjustment of the gas mixture in an evacuable ion chamber.

The method described here enables the absorption/transmission surface of a protein crystal to be measured directly with the oscillation camera on a synchrotron source by monitoring both the incident and the transmitted intensities. The principle of the method follows that used for measuring transmission EXAFS spectra except that, here, for each absorption curve $\lambda$ is fixed and instead the $\phi$ or $\mu$ orientation angle of the sample is changed. Fig. 1(a) shows a
The incident monochromatic beam intensity \( I_0 \) is monitored via a small ion chamber placed between the collimator and the sample, similar to the one described by Bartunik, Clout & Robrahn (1981). The monitor takes account of the decay in the circulating beam current and any slight movements of the focused beam with respect to the collimator. The collimation of the beam needed on the oscillation camera restricts the active length of the \( I_0 \) monitor and reduces its accuracy, although by suitable smoothing of the measured decay curve an accurate determination of this effect can be achieved. The collimation is also often such that the beam cross section at the crystal is less than its smallest dimension. This is also an essential pre-requisite for the present method. The intensity of the beam transmitted by the crystal \( I_T \) is measured with an ion chamber having a larger active length. Most importantly, it is capable of being evacuated and filled with a gas more sensitive than air, e.g. argon, the partial pressure of which may be selected according to the wavelength used. There is no restriction on the size of this chamber on the camera (Fig. 1b). The linearity of both monitors is checked by attenuation of the beam by pure-metal foils and/or reducing the geometric acceptance of the optical system in a systematic way using motorized slits.

Fig. 1. (a) A schematic diagram of the experimental arrangement. The symbols are defined in the text. The protein crystal sample is shown mounted in a capillary with a drop of mother liquor enveloping it. Not to scale. (b) A view of the ion chamber used to measure the transmitted beam intensity on the Arndt–Wonacott oscillation camera. The signals from this and the incident-beam monitor are routed to picoammeters, the output of which may be fed into the workstation computer.

Fig. 2. Absorption curves \( \mu_{\text{obs}}/\mu_{\text{calc}} \) as a function of \( \phi \) for a crystal of the TMV disk protein at 1.743, 1.488, 1.040 and 0.620 Å wavelengths measured at the Daresbury SRS protein crystallographic workstations. The plot of the respective SRS stored beam current \( I \) against \( \phi \) illustrates its decay during the experiment.
The relationship between \( I_o, I_T, \mu_0 \) and \( t \) follows the standard relation

\[ I_T = I_o \exp\{-\mu_0 t\} \]  

(1)

where all the parameters have been defined earlier except \( t \), which is the path length of the beam through the crystal sample and is, of course, a function of the angles \( \phi \) and \( \mu \). It is usual to normalize the set of measurements, making up the absorption surface, with respect to the sample orientation that has the maximum absorption corresponding to the maximum path length, \( t_{\text{max}} \), of the beam through the crystal. It is worth noting that in spectroscopy techniques such as EXAFS it is \( \mu_0 \) that is of interest, whereas in crystallography sample absorption corrections are based on \( e^{\mu_0 t} \). We illustrate both below.

In Fig. 2, absorption curves \( \mu_0 t_{\text{max}}/I_0 t \) as a function of \( \phi \) are shown for a crystal of the TMV disk protein measured at the Daresbury Synchrotron Radiation Source (SRS) at several wavelengths together with the respective circulating beam-current decay. Two curves at \( \lambda = 0.620 \) and 1.04 Å were recorded on a new instrument for protein crystallography on the wiggler line of the SRS (Helliwell, Papiz, Moore & Thompson, 1984) and two at \( \lambda = 1.488 \) and 1.743 Å on the existing X-ray beam line (Helliwell et al., 1982). This illustrates the increase in the measured absorption profile as the wavelength is increased.

Fig. 3 shows the empirical absorption factor, \( A \), of a rat liver apoferritin crystal at wavelength 1.488 Å as a function of the angles \( \mu \) and \( \phi \). The form of the correction applied to the observed intensity data is

\[ I_{\text{obs}}^{hkl} = I_{\text{corr}}^{hkl} A(\mu, \phi), \]  

(2)

where \( A(\mu, \phi) \) is defined by

\[ A(\mu, \phi) = \frac{e^{\mu_0 t_{\text{max}}}}{e^{\mu_0 t(\mu, \phi)}}. \]  

(3)

The SERC, Daresbury Laboratory and the University of Keele are thanked for support. The evacuable ion chamber described above was manufactured to a Daresbury specification by CVT Ltd. The TMV single-crystal sample was supplied by Dr A.C. Bloomer and the rat liver apoferritin crystal by Professor P. M. Harrison and co-workers.

References


