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A Simple Method of Absorption and Decay Correction in Intensities Measured by Area-Detector X-ray Diffractometer

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Abstract

A simple numerical method has been developed to correct for absorption and decay effects in the intensities measured by area-detector X-ray diffractometers. Application of this method improves not only the internal consistency of symmetry-equivalent reflections, but also the agreement between the two independent data sets.

Introduction

Correcting for absorption and decay in the intensity data from a single crystal of a protein is important in the determination of the structure. For absorption correction, the conventional straightforward calculation requires an exact knowledge of the crystal shape. With protein crystals which are normally covered by liquid, the determination of the shape is extremely difficult. For this reason, several experimental methods of absorption correction have been developed (e.g. North, Phillips & Mathews, 1968; Lee & Ruble, 1977). These empirical methods require several sets of azimuthal-angle scan data (ψ scan data). In the conventional method of decay correction, several specific reflections are periodically monitored. The relative diminution of the intensities of the reflections is taken as the measure of the relative state of decay of the crystal. The area-detector X-ray diffractometer, a new type of diffractometer, has the extremely useful, if not vital, attribute of data collection at a very high rate; however, the method of data collection usually used with this type of diffractometer has the serious shortcoming that it precludes the use of the above-described conventional procedures for absorption correction and for decay correction. Conventional decay correction and absorption correction cannot be performed if the measurement of monitor reflections and ψ-scan measurement are not carried out by the diffractometer. [The scaling procedure described by Xuong, Freer, Hamlin, Nielsen & Vernon (1978) can be used to carry out the correction without ψ-scan measurements and monitor reflection in a large number of cases, but perhaps not in all cases.] Thus, if a crystal must be large (by virtue of the substance's low tendency to diffract), or if the crystal decays rapidly under irradiation, the absorption and decay effects can render the data difficult or impossible to correct.

A major difference between the single-detector X-ray diffractometer and the area-detector X-ray diffractometer, the rate of data collection aside, is that an area-detector diffractometer can measure a large number of symmetry-equivalent reflections, in the course of measuring the unique data set. The integrated intensities of these symmetry-equivalent reflections should be equal to each other if the anomalous scattering effects are small. By using this relation, we have developed a simple numerical method to correct for the anisotropic absorption effect and the decay effect in the intensities measured by using the multiwire area-detector X-ray diffractometer at the Biotechnology Resource of the University of Virginia.

Method

We define two scale factors $S_1(t, s)$ and $S_2(UVW_I, UVW_D)$. S_1 is a decay correction function which has two parameters, exposure time (t) and $\sin\theta/\lambda$ (s). S_2 is an anisotropic absorption-correction function which has two vector parameters, the direction of the incident beam (UVW_I) and the direction of the diffracted beam (UVW_D). Each function is defined as follows:

$$S_1 = \exp(C_0 t s^2);$$

$$\begin{aligned} S_2 = \tfrac{1}{2}[&C_1(U_I^2 + U_D^2) + C_2(V_I^2 + V_D^2) + C_3(W_I^2 + W_D^2) \\ &+ C_4(UV_I + UV_D) + C_5(VW_I + VW_D) \\ &+ C_6(UW_I + UW_D)]. \end{aligned}$$

The coefficients C_0 through C_6 are determined by minimizing the function

$$Q(C_0, -, -, C_6) = \sum(1/\sigma)^2(K_A F_A^2 - K_B F_B^2)^2,$$

where

$$K_A = S_1(t_A, s_A)S_2(UVW_{I_A}, UVW_{D_A})$$

$$K_B = S_1(t_B, s_B)S_2(UVW_{I_B}, UVW_{D_B})$$

$$\sigma = (\sigma_A^2 + \sigma_B^2)^{1/2}.$$

σ_A and σ_B are the standard deviations of F_A^2 and F_B^2,

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Table 1. *Crystal data of S-adenosylmethionine synthetase*

Space group: $P6_122$ or $P6_422$		
Cell constants: $a = 128{\cdot}8(3)$, $c = 140{\cdot}3(3)$ Å		
Crystal size:	Native crystal (1)	0·7 × 0·7 × 0·3 mm
	Native crystal (2)	0·8 × 0·8 × 0·4 mm
	UO_2Cl_2 derivative crystal (1)	0·8 × 0·6 × 0·3 mm
	UO_2Cl_2 derivative crystal (2)	1·0 × 1·0 × 0·4 mm

respectively, and F_A^2 and F_B^2 are the squares of the structure factors of the reflections hkl_A and hkl_B respectively. hkl_A and hkl_B are symmetry-equivalent reflections.

Every possible pair of F_A^2 and F_B^2 is generated in order to determine the coefficients. The successful approach we found for minimizing the function Q is to determine the coefficient C_0 in S_1 and the coefficients C_1 through C_6 in S_2 independently. Thus, the minimized function is rewritten as

$$Q = \sum(S_1 \text{ term})^2 + \sum(S_2 \text{ term})^2;$$

$$S_1 \text{ term} = (1/\sigma)[S_{1A}(S'_{2A}F_A^2) - S_{1B}(S'_{2B}F_B^2)]$$

$$S_2 \text{ term} = (1/\sigma)[S_{2A}(S'_{1A}F_A^2) - S_{2B}(S'_{1B}F_B^2)],$$

where S'_1 and S'_2 are calculated by using trial values of coefficients. The initial trial values for C_0, C_4, C_5 and C_6 are zero, and the values for C_1, C_2 and C_3 are $1/3^{1/2}$. Thus, the minimization procedure requires several iterations with new trial values. The trial value (C_{trial}) of each C_i for a new cycle is defined as

$$C_{\text{trial}} = \tfrac{1}{3}C_{\text{old}} + \tfrac{2}{3}C_{\text{new}},$$

where C_{old} is the C_{trial} of the previous cycle and C_{new} is the coefficient determined by using C_{old} as the trial values. Since the S_1 term is a non-linear equation, we take the natural logarithm to convert it to a linear expression.

$$S_1 \text{ term} = w'\{\ln[S_{1A}(S'_{2A}F_A^2)] - \ln[S_{1B}(S'_{2B}F_B^2)]\}$$
$$= w'[(f_A - f_B) - C_0(t_Bs_B^2 - t_As_A^2)],$$

where

$$f_A = \ln(S'_{2A}F_A^2), \qquad f_B = \ln(S'_{2B}F_B^2)$$
$$w' = 1/(\sigma_A^2/F_A^4 + \sigma_B^2/F_B^4)^{1/2}.$$

Table 3. *Number of reflections and agreement factors (R)*

Crystal	Number of reflections recorded	Number of independent reflections	R_1*	R_2†
Native (1)	15 858	3217	0·038	0·069
Native (2)	16 876	3260	0·034	0·066
Derivative (1)	12 357	2442	0·056	0·089
Derivative (2)	13 056	1854	0·048	0·082

$$R = \sum_h \sum_i |\langle F(h)^2\rangle - F(h)_i^2| \Big/ \sum_h \sum_i |F(h)_i^2|$$

*Agreement factor (R_1) with absorption and decay correction.
†Agreement factor (R_2) without absorption and decay correction.

For the S_2 term, we determine the eigenvalues and eigenvectors of the 6×6 matrix composed of the partial derivatives of coefficients C_1 through C_6. When the elements of the eigenvector with the smallest eigenvalues are used as the coefficients of S_2, the S_2 term is expected to be at its minimum. We used a second-order polynomial as the anisotropic absorption-correction function. A higher approximation can be obtained by using third- and/or fourth-order polynomials, especially Hermite polynomials.

Results

This method was used to correct for the anisotropic absorption and decay effects in the 5 Å resolution data of native and derivative crystals of *S*-adenosylmethionine synthetase measured by the multiwire area-detector diffractometer at the Biotechnology Resource at the University of Virginia. The crystal data and the final coefficients of the S_1 and S_2 functions are listed in Tables 1 and 2, respectively. The agreement factors among the intensities of symmetry-equivalent reflections are given in Table 3.

Application of this method substantially improved not only the internal consistency of symmetry-equivalent reflections but also the agreement between the two independent data sets. The agreement factor (R) between the two sets of native protein data is 0·068 with correction and 0·107 without, and for the derivative protein data, R is 0·073 with correction and 0·147

Table 2. *The final coefficients in the functions S_1 and S_2*

Crystal	C_0*	C_1	C_2	C_3†	C_4	C_5†	C_6†
Native (1)	0·740	0·766	0·634	0·700	0·105	0·000	0·000
Native (2)	0·609	0·680	0·732	0·706	−0·040	0·000	0·000
Derivative (1)	1·029	0·580	0·753	0·667	−0·311	0·000	0·000
Derivative (2)	0·539	0·732	0·674	0·703	−0·100	0·000	0·000

*Exposure time (t) in S_1 is on an arbitrary scale and $t = 1{\cdot}0$ is approximately 30 minutes.
†Since all the data were measured by the φ-scan mode with $\chi = 0{\cdot}0$, and the center of the detector is located at the incident-beam level, the W components of diffracted-beam vectors are always very small ($< 0{\cdot}15$). Therefore, the coefficients C_3, C_5 and C_6 which are correlated with the W component are not varied. C_3 is reset to $(C_1 + C_2)/2$.

without. Moreover, the difference Patterson map calculated with uncorrected data was uninterpretable, but the map calculated with corrected data clearly showed several heavy-atom–atom vectors. The detailed structure analysis of this protein will be published elsewhere.

The absorption correction method described by Kopfmann & Huber (1968) and further developed by Katayama, Sakabe & Sakabe (1972) is similar in some respects to this method; however, there are three major differences. These are that: (1) the decay correction is taken into account in the minimization procedure; (2) the correction function is in a much simpler form, *i.e.* the number of coefficients to be determined is seven, rather than 27; (3) this method does not require a complete set of symmetry-equivalent data.

The program is available at no charge; interested persons should send a magnetic tape to the author with return postage. This research was supported by NIH grant GM37189 and by the Biomedical Science Support Grant from the NIH administered by the University of Kansas. The excellent facilities and helpful cooperation provided by the Biotechnology Resource at the University of Virginia are gratefully acknowledged.

References

KATAYAMA, C., SAKABE, N. & SAKABE, K. (1972). *Acta Cryst.* A28, 293–295.

KOPFMANN, G. & HUBER, H. (1968). *Acta Cryst.* A24, 348–351.

LEE, B. & RUBLE, J. (1977). *Acta Cryst.* A33, 629–637.

NORTH, A. C. T., PHILLIPS, D. C. & MATHEWS, F. S. (1968). *Acta Cryst.* A24, 351–359.

XUONG, N. H., FREER, S. T., HAMLIN, R., NIELSEN, C. & VERNON, W. (1978). *Acta Cryst.* A34, 289–296.