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**An improved background correction for protein X-ray intensities using the partial $\omega$-scan method.** By EDWARD J. HILL and LEONARD J. BANASZAK, *Washington University School of Medicine, Department of Biological Chemistry, St. Louis, Missouri* 63110, *U.S.A.*

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An improved background correction for protein X-ray intensities which have been measured by the partial $\omega$-scan method is described. The application of this correction to the determination of X-ray data from single crystals of supernatant malate dehydrogenase is discussed.

The use of the partial $\omega$-scan in conjunction with a 4-circle diffractometer has been described by Wyckoff *et al.* (1967). This method significantly reduces the time required to measure single-crystal X-ray data because the background is not measured for each reflection. The method has been used successfully for structural studies on ribonuclease-*S* (Wyckoff *et al.*, 1970), cytochrome $B_5$ (Mathews, Levine & Argos, 1972) and malate dehydrogenase (Tsernoglou, Hill & Banaszak, 1972).

The relationship used to correct the X-ray intensities obtained by the partial $\omega$-scan method is given in equation (1).

$$|F_{hkl}|^2 = K^*(I_{hkl} - B_{2\theta})^* A_{2\theta,\chi,\varphi}{}^* \mathrm{Lp}_{2\theta} \quad (1)$$

$|F_{hkl}|^2$ is the square of the corrected structure factor; $K$ is a scale factor used to normalize intensities from different crystals; $I_{hkl}$ is the measured intensity; $A_{2\theta,\chi,\varphi}$ is the semi-empirical absorption correction described by North, Phillips & Mathews (1968); $\mathrm{Lp}_{2\theta}$ is the Lorentz and polarization correction factor. $B_{2\theta}$ is the background count linearly interpolated for the appropriate $2\theta$ value. The background is measured in equal intervals (*e.g.* 2° intervals) along $2\theta$ with $\omega$, $\chi$ and $\varphi$ angles held constant. The correction shown in equation (1) assumes that the background count is spherically symmetric in reciprocal space.

In high-resolution studies of the enzyme supernatant malate dehydrogenase, we have found that the method of equation (1) introduces significant systematic errors, particularly for the weaker reflections. The background count rate is not spherically symmetric in reciprocal space, but has a definite $\varphi$ dependence shown in Fig. 1. The crystal and its mounting to the capillary absorb background radiation with the same $\varphi$ dependence as they absorb the incident and diffracted beams. Measurements indicate that the $\varphi$ dependence is similar for all values of $\theta$ and $\chi$.

The accuracy of the background correction is improved if the relationship shown in equation (2) is used.

$$|F_{hkl}|^2 = K^*[(I_{hkl}{}^* A_{2\theta,\chi,\varphi}) - B_{2\theta}^{A\min}]^* \mathrm{Lp}_{2\theta} \quad (2)$$

The background profile $B_{2\theta}^{A\min}$ is measured with the crystal at the diffractometer $\varphi$ value which has the minimum absorption ($A_{\min}$).

The effect of choosing a single mean value of background and applying it to malate dehydrogenase intensities between 3·0 and 2·5 Å resolution typically underestimates the corrected intensities by about 5% when the longest dimension of the crystal is parallel to the incident and diffracted rays, and overestimates intensities parallel to the short crystal dimension by about 5%. The percentage error is greater for flat crystals with large absorption corrections or weak reflections. These are just the conditions often encountered in high-resolution protein work where one is trying to use the largest possible crystals.

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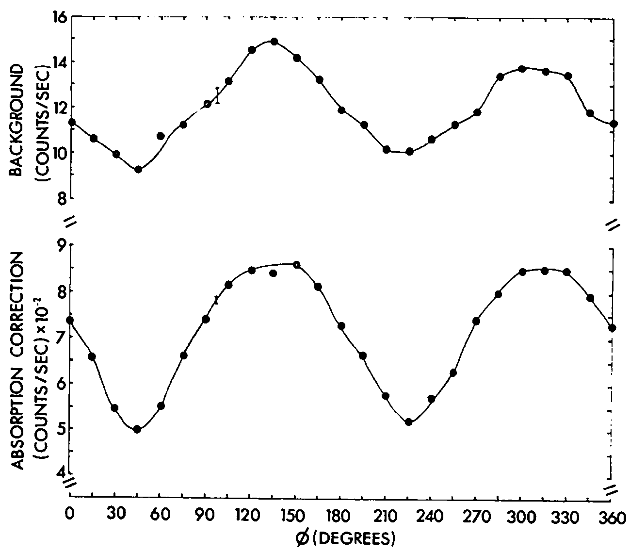


Fig. 1. The $\varphi$ dependence of the background count rate and semi-empirical absorption correction count rate for a crystal of supernatant malate dehydrogenase. The absorption correction count rate was measured on the 400 reflection with $\chi = 270$ and $2\theta = 2\cdot54°$. The background count rate was measured with $\chi = 271\cdot4$ and $2\theta = 23\cdot7°$. The error bars represent the statistical uncertainty of one standard deviation.

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