Acta Cryst. (1988). A44, 443-448

Sequential Radiation Damage in Protein Crystallography

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(*Received* 31 *July* 1987; *accepted* 2 *February* 1988)

Abstract

Radiation damage in protein crystals is described in terms of a sequential process of protein disordering. A new radiation-damage model has been tested against data from several protein crystals and can describe radiation damage corresponding to loss of the original intensity in excess of 80%. The model is an extension of previous models which characterize radiation damage in terms of successive conformational transitions of the protein from an undamaged to a spatially disordered to finally an amorphous state. The proposed model provides a more-general positional characterization of the disordered protein and includes, prior to the disordered state, a new dose-dependent state in which the protein conformation resembles the undamaged protein. Comparison of this model with the best previous model shows that the proposed model provides an improved fit to radiation-damage data.

Introduction

Radiation damage in protein crystals as manifested by deterioration in the diffraction pattern is a common phenomenon occurring during data collection of diffracted intensities. Exposure to X-rays by protein crystals diminishes the diffracted intensity with exposure times and does so most markedly at high scattering angles. The severity of radiation-induced damage can vary considerably among protein crystals; myoglobin crystals exhibit relatively little damage upon prolonged irradiation (Blake & Phillips, 1962) whereas crystals of glycolate oxidase by comparison display extreme sensitivity to X-ray irradiation (Lindqvist & Brändén, 1985). Recovery of the reflection intensity corresponding to the protein prior to radiation-induced damage depends upon the model employed for describing radiation damage in protein crystals.

The study by Blake & Phillips (1962) on radiation-induced decay of intensities in myoglobin crystals characterized radiation damage in a protein crystal in terms of a linear combination of three states; an irradiated protein crystal would consist of a relatively undamaged fraction A_1, a disordered fraction A_2 which contributes predominantly to low-angle scattering and an amorphous fraction A_3 which is no longer capable of coherent scattering. Provided the spatial disordering of the protein is random in the crystal and has a Gaussian distribution then scattering from the disordered fraction relative to the undamaged fraction is modified by a smearing function, $\exp(-Ds^2)$, where $D/8\pi$ represents the mean squared atomic displacement of the protein in the disordered fraction about its initial position and $s = (\sin\theta)/\lambda$. The diffracted intensity I after irradiation of the protein crystal for time t is then described by

$$I(t)/I(0) = A_1(t) + A_2(t)\exp(-Ds^2). \quad (1)$$

The validity of this radiation-damage model derived from myoglobin crystals was tested on crystals of lamprey hemaglobin and was shown to be capable of describing radiation damage in these crystals (Hendrickson, Love & Karle, 1973). Hendrickson (1976) subsequently derived an analytical expression for radiation damage based upon rate constants for all possible paths in the Blake & Phillips (1962) model including a transition representing the conversion of the undamaged protein directly to the amorphous state. Analysis of the myoglobin data with this model showed that the extra aforementioned transition made only a very minor contribution and suggested that radiation damage is best described by successive irreversible transitions of the kind $A_1 \rightarrow A_2 \rightarrow A_3$. A very similar radiation-damage model derived also on the basis of a sequential process of radiation damage was used to describe radiation damage in crystals of glycogen phosphorylase A (Fletterick, Sygusch, Murray, Madsen & Johnson, 1976). Application of this model to the myoglobin data provided the best possible fit up to moderate levels of radiation damage (Hendrickson, 1976). However, none of these models provided an adequate description of radiation-induced damage in myoglobin crystals at high doses of irradiation. One possible explanation for the inadequate description of radiation damage at high exposure levels by these models resides in their simplified assumption that the physical state of the protein capable of contributing to the diffracted intensity

0108-7673/88/040443-06$03.00

can be adequately described in terms of only two fractions A_1 and A_2.

Sequential model of radiation damage

Solution irradiation studies of transferrin proteins at concentrations comparable to those employed for crystallization purposes suggest that the initial effects of irradiation perturb surface residues and disulfide bonds and only at higher doses of irradiation do nonspecific changes occur in the secondary and/or tertiary structure (Miller & Bezkorovainy, 1973). This interpretation is consistent with the results of solution inactivation studies on ribonuclease A where doses required to inactivate the enzyme in solution are 100 times less than doses for the lyophilized enzyme (Gunther & Jung, 1967). Loss of catalytic activity in the wet state of ribonuclease A is attributed to a surface attack of the active site by the radiolytic species produced in the solvent which is only then followed by radical processes leading to the loss of the molecular conformation (Dertinger & Jung, 1970). This type of damaging process in the wet state of a protein is also consistent with observations from crystals of rabbit skeletal muscle aldolase where although enzymatic activity is completely lost at the end of the data collection period, denaturing electrophoretic gels show no apparent evidence of polypeptide backbone scission (unpublished data). Since protein crystals commonly contain 40 to 60% aqueous buffer, the radiation-damage mechanism most likely operating initially is surface damage of the protein due to solvent radical attack. Moffat, Bilderback, Schildkamp, Szebenyi & Loane (1986) have recently pointed out in hemoglobin crystals that until a critical dose has been absorbed by the crystal lattice, radiation damage appears to be delayed.

A minimal description of radiation damage in protein crystals would entail that protein disordering in the crystal lattice be preceded by a dose-dependent step involving some kind of surface modification that does not appear to alter significantly the protein conformation in the lattice. Solvent radical attack, however, on the surface of a protein also implies a directional dependence in the subsequent protein disordering process. Anisotropy in the disordering process is due to non-uniform solvent accessibility of the protein surface which can be a consequence of the packing of the protein into a crystal lattice. The conservation of space-group symmetry throughout the crystal irradiation period, however, limits the degree of anisotropy possible in describing the directional changes of protein disordering. The constraint imposed by space-group-symmetry conservation requires the directional anisotropy to be invariant under the corresponding Laue-group-symmetry operations. In this paper a radiation-damage model is proposed which takes into account the aforementioned considerations. The resultant sequential model of radiation damage has provided an improved description of radiation damage in crystals of rabbit skeletal muscle aldolase (Sygusch, Boulet & Beaudry, 1985), rabbit liver aldolase (Sygusch & Beaudry, 1985) and F_1 ATPase (Amzel, McKinney, Narayanan & Pederson, 1982) and does so to even very high levels of radiation damage.

Model considerations

Incorporation of the proposed modifications into a quantitative description of radiation damage resides upon several simplifying assumptions. It shall be assumed that radiation damage in a crystal lattice is solely a function of the physical state of the protein in the lattice and does not depend upon any cooperative transition by the crystal lattice. Protein modification by ionizing processes will be assumed to be essentially irreversible and it shall also be assumed that initial protein damage does not seriously perturb the protein conformation in the lattice. It is only when the protein crystal has absorbed a sufficient dose that radiation damage will become apparent. According to Moffat *et al.* (1986) this dose corresponds to approximately one ionization event per protein molecule. Irradiation by ionizing radiation is known to create radical species in solution as well as in proteins which can persist even after irradiation has ended (Dertinger & Jung, 1970). In the context of the model, these long-lived radical species would modify the evolution of the populations of fractions A_2 and A_3. However, provided diffracted intensities are measured continuously, damage by these radical species cannot be distinguished from damage mechanism due to short-lived radicals and as such will not be considered separately. It will also be assumed that the distribution of the atomic displacements characterizing the disordered protein does not change as a function of time.

Damage kinetics

The proposed sequential model for radiation damage is

$$\text{native}\,(A_1) \xrightarrow{k_0} \text{dose dependent}\,(A_1')$$

$$\text{dose dependent}\,(A_1') \xrightarrow{k_1} \text{disordered}\,(A_2)$$

$$\text{disordered}\,(A_2) \xrightarrow{k_2} \text{amorphous}\,(A_3)$$

such that for a given reflection $\mathbf{h}$

$$I(t)/I(0) = A_1(t) + A_1'(t) + A_2(t)\exp(-\mathbf{h}^t U\mathbf{h}) \quad (2)$$

where the exponential distribution function characterizing the disordered protein is anisotropic with respect to crystallographic direction and depends on

the second-rank tensor U whose components $U_{ij}/8\pi$ describe the average mean squared displacement of the disordered protein with respect to its initial position in the unit-cell reference frame and which must be invariant under the Laue-symmetry-group operations of the space group. The components of **h** are expressed in reciprocal-lattice units. Kinetically the transition $A_1 \rightarrow A_2$ has been modified to take into account the transition of the protein in the undamaged fraction A_1 to a dose-dependent state A_1' before its passage to the disordered fraction A_2. The dose-dependent fraction A_1' will be characterized at the molecular level in terms of protein that has undergone only a limited number of ionization events and as a result has been negligibly modified to a first-order approximation in terms of its scattering power with respect to the undamaged fraction A_1. With the exception of the transition $A_1 \rightarrow A_1'$ which is assumed to be dose dependent, the transitions $A_1' \rightarrow A_2$ and $A_2 \rightarrow A_3$ will be considered as successive conformational transitions of the protein induced by the radiation damaging process (Fletterick *et al.*, 1976) and which are therefore kinetically first-order processes (Tanford, 1968). The evolution of the various protein fractions as a function of time may be obtained by solving the rate equations

$$dA_1/dt = -k_0 I_0 \tag{3a}$$

$$dA_1'/dt = k_0 I_0 - k_1 A_1' \tag{3b}$$

$$dA_2/dt = k_1 A_1' - k_2 A_2 \tag{3c}$$

$$dA_3/dt = k_2 A_2, \tag{3d}$$

subject to the constraints that

$$A_1(t) + A_1'(t) + A_2(t) + A_3(t) = A_0 \tag{4a}$$

$$A_1(0) = A_0, \quad A_1'(0) = 0, \quad A_2(0) = 0, \quad A_3(0) = 0 \tag{4b}$$

where I_0 represents the intensity of the incident radiation and A_0 is the quantity of protein in the irradiated volume. Substitution of the constraints into (3) yields the following radiation-damage factor $R = I(t)/I(0)$ for the sequential model:

(i) $k_1 \neq k_2, k_2 \neq 0$

$$\begin{aligned} R(t, \mathbf{h}) = {} & 1 - k_0' t + (k_0'/k_1)[1 - \exp(-k_1 t)] \\ & + k_0' \{1/k_2 + [1/(k_1 - k_2)] \\ & \times [\exp(-k_1 t) - (k_1/k_2)\exp(-k_2 t)]\} \\ & \times \exp(-\mathbf{h}^t U \mathbf{h}) \end{aligned} \tag{5}$$

if $t < 1/k_0'$, where $k_0' = k_0 I_0 / A_0$. According to this definition the zero-order rate constant k_0' will vary inversely with the irradiated volume or crystal size. Since the transition $A_1 \rightarrow A_1'$ is zero order, irradiation beyond a certain time will convert all of the undamaged fraction A_1 into the dose-dependent fraction A_1'. Consequently the decay model simplifies to the rate model $A_1' \rightarrow A_2 \rightarrow A_3$ when $t \geq 1/k_0'$.

For $t \geq 1/k_0'$, (5) becomes

$$\begin{aligned} R(t, \mathbf{h}) = {} & (k_0'/k_1)[\exp(k_1/k_0') - 1]\exp(-k_1 t) \\ & + k_0' \left\{ \frac{[1 - \exp(k_1/k_0')]\exp(-k_1 t)}{(k_1 - k_2)} \right. \\ & \left. + \frac{k_1[\exp(k_1/k_0') - 1]\exp(-k_2 t)}{k_2(k_1 - k_2)} \right\} \\ & \times \exp(-\mathbf{h}^t U \mathbf{h}). \end{aligned} \tag{6}$$

(ii) $k_1 = k_2, k_1 \neq 0$

Whenever $k_1 = k_2$, the population of fraction A_2 displays a different form as a function of time. The terms of equations (5) and (6) within the braces must then be modified according to

$$\frac{1}{k_1} - \frac{\exp(-k_1 t)}{k_1}(1 + k_1 t) \qquad \text{if } t < 1/k_0'$$

and

$$\left[\frac{[\exp(k_1/k_0') - 1]}{k_1}(1 + k_1 t) - \frac{\exp(k_1/k_0')}{k_0'} \right] \times \exp(-k_1 t) \qquad \text{if } t \geq 1/k_0'.$$

It should be noted that when $t \ll 1/k_1$, (5) can be simplified to

$$R(t, \mathbf{h}) \simeq 1. \tag{7}$$

That is, provided the transition $A_1' \rightarrow A_2$ is sufficiently slow relative to the time scale of the experiment a delay results in the onset of apparent radiation damage which is consistent with the observation of Moffat *et al.* (1986). It should be noted that this delay could be advantageously exploited by efficient two-dimensional detection systems. The model suggests that it may not always be possible to distinguish adequately between fractions A_1 and A_1'. Both fractions contribute equally to the scattered intensity and thus can only be distinguished kinetically if a significant fraction of the protein in the lattice has undergone disordering. This distinction is best made using data that have decayed significantly with time. The proposed model also predicts that the radiation-damage factor can vary with direction **h** in reciprocal space depending upon the relative magnitudes of the elements U_{ij} of the mean square displacement tensor. Nevertheless, the radiation damage is always more important for reflections at higher than at lower scattering angles given the same direction in reciprocal space. Differential rates of intensity decline have been observed by Ammon, Murphy, Sjolin, Wlodawer, Holcenberg & Roberts (1983). Analysis of the directional dependence in this instance was shown to be consistent with an anisotropic exponential temperature factor. Although this

Table 1. *Refinement results for various radiation-damage models*

Radiation-damage data set	Total exposure time (h)	Model†	Goodness of fit‡	k_0' (h^{-1})	k_1 (h^{-1})	k_2 (h^{-1})	U_{11} (Å^2)	U_{22} (Å^2)	U_{33} (Å^2)	U_{13} (Å^2)	$R(t, \mathbf{h})$ minimum
Rabbit skeletal muscle aldolase*	44	Conventional	26·31	-	0·035 (1)	0·035 (1)	9 (1)§	-	-	-	
		Modified conventional	14·67	-	0·031 (1)	0·032 (1)	9 (2)	74 (11)	15 (3)	11(3)	0·19
		Proposed	3·92	0·031 (1)	0·068 (5)	0·033 (1)	12 (1)	75 (5)	16 (1)	13 (1)	
Rabbit liver adolase*	26	Conventional	5·34	-	0·039 (10)	0·027 (4)	25 (12)§	-	-	-	
		Modified conventional	4·16	-	0·049 (6)	0·020 (2)	14 (2)	34 (8)	23 (8)	-	0·26
		Proposed	2·95	0·069 (7)	0·082 (14)	0·021 (2)	12 (2)	31 (5)	26 (7)	-	
F_1 ATPase*	15	Conventional	1·98	-	0·123 (7)	0·058 (8)	85 (14)§	-	-	-	
		Modified conventional	1·96	-	0·120 (6)	0·066 (14)	80 (13)	-	-	−39 (15)	0·16
		Proposed	1·34	0·189 (34)	0·155 (53)	0·061 (16)	83 (24)	-	-	−4 (1)	

* Constraints on U_{ij} elements required for invariance under appropriate Laue-symmetry-group operations are as follows: rabbit muscle aldolase, $U_{12} = U_{23} = 0$; rabbit liver aldolase, $U_{12} = U_{23} = U_{13} = 0$; F_1 ATPase, $U_{11} = U_{22} = U_{33}$, $U_{13} = U_{23} = U_{12}$.

† The meaning of the model designations is explained in the text.

‡ The goodness of fit is $\sum_{\mathbf{h},i} w_{\mathbf{h},i}[I_{\mathbf{h}}(t_i)_{obs} - I_{\mathbf{h}}(t_i)_{calc}]^2/(n-m)$ where the sum is over the data points i for each reference reflection $\mathbf{h}$ having measured intensity $I_{\mathbf{h}}(t_i)_{obs}$ at time t_i. The sum is divided by the difference between the total number of observations n and the number of fitted parameters m. The calculated intensity $I_{\mathbf{h}}(t_i)_{calc}$ was defined as $I_{\mathbf{h}}(0)R(t_i, \mathbf{h})$ where the calculated intensity at time $t = 0$, $I_{\mathbf{h}}(0)$, is estimated by the refinement. The weight $w_{\mathbf{h},i}$ is derived solely from counting statistics. In the absence of systematic error, the expected value of the goodness of fit will tend to one provided the damage models are linear in the refinable parameters. For considerable radiation decay, the radiation-damage models can become highly non-linear in terms of the refinable parameters and thus the goodness of fit cannot be strictly normalized by $n - m$ (Hamilton, 1964).

§ Isotropic disordering parameter.

radiation-damage model was elaborated on the basis of radical attack of the protein as the mechanism responsible for radiation damage, other kinds of damage mechanisms cannot be ruled out. For instance, the disorder of the protein resulting from heating of the crystal by absorption of ionizing radiation (Blundell & Johnson, 1976), although a less well studied phenomenon, can represent an alternative but not necessarily exclusive mechanism.

Analysis of radiation-damage data

The applicability of the proposed radiation-damage model was analyzed by fitting both the proposed as well as previous damage models to various radiation-damage data. The reference or conventional damage model chosen was that described by Fletterick *et al.* (1976) which yielded the best overall fit to the myoglobin data (Hendrickson, 1976) and describes radiation damage in terms of transitions $A_1 \rightarrow A_2 \rightarrow A_3$. A modification of the conventional model was also tested which allowed for anisotropic disordering by the disordered fraction A_2. The distribution function characterizing the spatial disordering of the protein in fraction A_2 was the same exponential function as in the proposed model. The parameterized models were fitted to the radiation-damage data by iterative non-linear least-squares refinement. The criterion for judging the quality of the fit to the data was the overall goodness of fit. Weights required for the refinement and various statistics were derived from diffractometer counting statistics. The radiation-damage data represented 9 to 15 reference reflections, depending on the data set, which were repeatedly measured every 1·5 h throughout the data collection period, which lasted 15 to 44 h depending on the protein-crystal sensitivity to Cu $K\alpha$ radiation. The reference reflections were selected to sample uniformly all reciprocal-space directions. Radiation damage was analyzed simultaneously as a function of time and reciprocal-space direction using data collected on an automatic diffractometer from three different protein crystals under very similar experimental conditions: rabbit skeletal muscle aldolase, space group $P2_1$, 15 reference reflections (Sygusch, Boulet & Beaudry, 1985); rabbit liver aldolase, space group $C222_1$, 12 reference reflections (Sygusch & Beaudry, 1985); F_1 ATPase, space group $R32$, nine reference reflections (Amzel *et al.*, 1982). Crystal slippage was continuously monitored and corrected for whenever necessary. Results of these tests are summarized in Table 1.* Additionally, Fig. 1 displays graphically a comparison of the fit of various radiation models as a function of time to the rabbit-muscle data for selected reference reflections **h**.

From both Table 1 and Fig. 1, refinement of the best conventional model to the data clearly yields the least satisfactory fit. Modification of the conventional model to allow for anisotropic disordering in the intermediate fraction A_2 improves the fit to the data.

* It should be noted that the data sets collected over shorter periods of time are necessarily less influenced by dimensional instabilities such as slight changes in X-ray source, X-ray reflection mirrors and crystal during the experiment and as such can be expected to yield lower goodness-of-fit values for the various damage models.

However, it is apparent that the proposed model, which not only allows for anisotropic disordering in fraction A_2 but also includes a dose-dependent state, provided the best description of the variation of radiation damage both as a function of time and of reciprocal-space direction. The proposed model is capable of describing high levels of radiation damage in protein crystals. Damage has been satisfactorily accounted for representing a loss of up to 81% of the original intensity in rabbit muscle aldolase crystals (see Fig. 1), up to 74% in rabbit liver aldolase crystals and up to 84% in crystals of F_1 ATPase. Description of radiation damage to even higher levels should be feasible by the proposed model. The damage models based upon the transitions $A_1 \rightarrow A_2 \rightarrow A_3$ were not as satisfactory in accounting for such high levels of radiation damage even when anisotropic disordering of the protein was allowed for, in agreement with similar conclusions drawn by Hendrickson (1976) on his analysis of the myoglobin data. Noteworthy in Table 1 is the significant positional anisotropy exhibited by the disordered protein in fraction A_2 for the two aldolases. Anisotropy is evident in Fig. 1 where the radiation damage for reference reflection $4,11,\bar{8}$ is much greater as a function of time than damage for reference reflection $17,0,\overline{18}$, even though both reflections have nearly identical Bragg angles. It is somewhat surprising yet gratifying that inclusion of simply anisotropic positional disordering of the protein in the lattice can account for this variation in radiation damage.

Discussion of damage model

The analysis of the data strongly argues that radiation damage in protein crystals is consistent with a sequential process of damage and suggests that the damage process involves several intermediate states. It should be noted, however, that the proposed model, although derived from analysis of irradiation experiments, is strongly phenomenological since the existence of the various intermediate states depends critically upon their relative scattering contributions. At present none of these intermediates have been isolated to confirm their existence and thus their postulated scattering power. In this context, empirical models of radiation-damage descriptions such as that of Abrahams & Marsh (1987) can potentially offer a more flexible approach since they do not depend on any *a priori* postulates. However, the fact that the proposed model

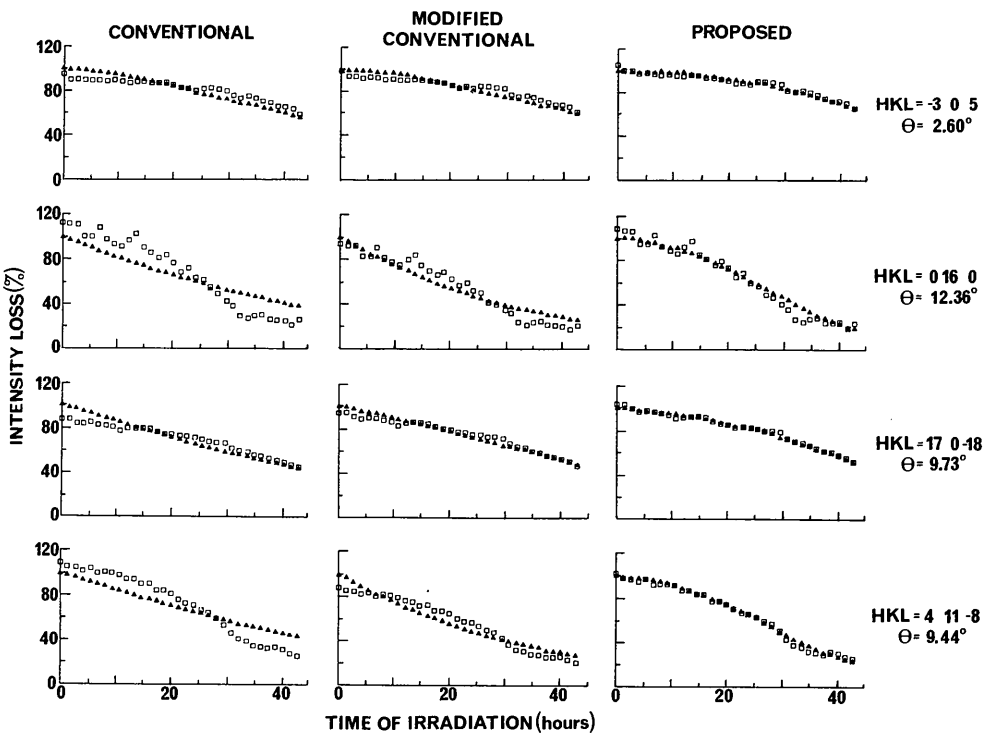

Fig. 1. Comparison of the agreement of various radiation-damage correction models to the same observed data collected from a monoclinic crystal of rabbit skeletal muscle aldolase. The observed intensity data (□) have been normalized by the calculated intensity (▲) at $t = 0$ such that in each model the calculated intensity at $t = 0$ represents 100%. The three panel headings describe the radiation-damage correction models referred to in the text with 'conventional' referring to the model based upon transitions $A_1 \rightarrow A_2 \rightarrow A_3$ and isotropic positional disordering of the protein in fraction A_2, 'modified conventional' to a model described by the same transitions but anisotropic positional disordering in fraction A_2, and 'proposed' to transitions $A_1 \rightarrow A_1' \rightarrow A_2 \rightarrow A_3$ and anisotropic positional disordering in fraction A_2.

fits the data as well as it does suggests a considerable amount of validity in this approach. This approach can thus offer insight into the damage kinetics, which is not possible by empirical models. A consequence of the interpretation of protein damage through initial surface damage would suggest that ligand binding sites at the protein surface are more susceptible to damage than sites that are buried in the protein. For these situations ligand binding studies implicating exposed surfaces sites should be confined to cumulative exposure times of less than $1/k_1$ such that $R(t, \mathbf{h})$ remains close to unity [see (7)]; in the example shown in Fig. 1 this would correspond to less than 14 h. Limiting binding studies to the lag period before the onset of apparent radiation damage, that is $R(t, \mathbf{h}) \simeq 1$, represents, according to Moffat *et al.* (1986), an equivalent dose of approximately one ionization event per protein molecule. It is interesting to note that cumulative exposure times of less than $1/k_1$ would also correspond, according to the calculations of Moffat *et al.* (1986) based upon our experimental conditions, to no more than one ionization event per protein molecule.

Radiation-damage correction

The proposed model is extremely useful in making corrections to intensity measurements to recover the intensity values corresponding to the undamaged protein conformation. Corrections which are derived on the basis of a limited number of reference reflections, even though uniformly dispersed in reciprocal space, could potentially introduce systematic bias. This limited sampling bias if present would tend to be especially pronounced in protein crystals exhibiting radiation damage that is directionally dependent. To investigate potential bias in the corrected intensities, merging residuals were examined of overlapping data sets obtained from crystals of rabbit skeletal muscle aldolase which display radiation damage strongly dependent on reciprocal-space direction (see Table 1). The data base merged represented intensities collected on an automatic diffractometer in shells of constant resolution to 2·7 Å resolution. The data sets were not only overlapping between shells but also the start of one data set was overlapped with the end of another data set within a shell. Especially at the higher resolutions where damage corrections are more significant, the intensities measured at the start of a data set have suffered little radiation damage compared with those measured at the end of a data set. Merging of such overlapping data sets would then provide a suitable test to reveal systematic error. In the presence of bias merging residuals for a given intensity range would tend to increase concomitantly with resolution. The merging of overlapping data sets was carried out on the basis of intensity using the programs of Reeke (1984) with weights employed in the merging procedure based upon counting statistics and various systematic corrections applied in the data reduction procedures. Analysis of the merging residuals showed no systematic variation between data sets nor for any intensity range as successive shells of higher-resolution data were included in the merge. The final overall merging residual based upon intensity (Reeke, 1984) was 0·048 for approximately 61 000 overlapping reflections and 0·075 in the highest shell of resolution, suggesting a data base of sufficient quality to detect potential bias.* Furthermore, the overall goodness of fit for the entire data merge was 1·4, close to the ideal value of one, corroborating that systematic error if present is relatively minor. Consequently, the strategy of monitoring repeatedly a sufficient but limited number, 9 to 15, of reference reflections uniformly dispersed in reciprocal space can provide relatively unbiased estimates of the parameters necessary for radiation-damage intensity correction of diffracted intensities.

* In particular the data set which is shown in Fig. 1, measured in a shell corresponding to 3·5-3 Å resolution, readily merged with overlapping data sets and corresponded to an overall merging residual of 0·06 with respect to the overlapped data.

References

ABRAHAMS, S. C. & MARSH, P. (1987). *Acta Cryst.* A**43**, 265-269.

AMMON, H. L., MURPHY, K. C., SJOLIN, L., WLODAWER, A., HOLCENBERG, J. S. & ROBERTS, J. (1983). *Acta Cryst.* B**39**, 250-257.

AMZEL, L. M., MCKINNEY, M., NARAYANAN, P. & PEDERSON, P. L. (1982). *Proc. Natl Acad. Sci. USA*, **79**, 5852-5856.

BLAKE, C. C. F. & PHILLIPS, D. C. (1962). *Biological Effects of Ionizing Radiation at the Molecular Level.* IAEA Symposium, Vienna, pp. 183-191.

BLUNDELL, T. L. & JOHNSON, L. N. (1976). *Protein Crystallography*, edited by B. HORECKER, N. O. KAPLAN, J. MARMUR & H. A. SCHERAGA, pp. 251-254. London: Academic Press.

DERTINGER, H. & JUNG, H. (1970). *Molecular Radiation Biology*, ch. 9, pp. 115-133. New York: Springer-Verlag.

FLETTERICK, R. J., SYGUSCH, J., MURRAY, N., MADSEN, N. B. & JOHNSON, L. N. (1976). *J. Mol. Biol.* **103**, 1-13.

GUNTHER, W. & JUNG, H. (1967). *Z. Naturforsch.* **226**, 313-328.

HAMILTON, W. (1964). *Statistics in Physical Science*, p. 160. New York: Ronald Press.

HENDRICKSON, W. A. (1976). *J. Mol. Biol.* **106**, 889-891.

HENDRICKSON, W. A., LOVE, W. E. & KARLE, J. (1973). *J. Mol. Biol.* **74**, 331-361.

LINDQVIST, Y. & BRÄNDÉN, C.-I. (1985). *Proc. Natl Acad. Sci. USA*, **82**, 6855-6859.

MILLER, R. J. & BEZKOROVAINY, A. (1973). *Radiat. Res.* **54**, 212-221.

MOFFAT, K., BILDERBACK, D., SCHILDKAMP, W., SZEBENYI, D. & LOANE, R. (1986). *Structural Biological Applications of X-ray Absorption, Scattering and Diffraction*, pp. 125-133. New York: Academic Press.

REEKE, G. N. (1984). *J. Appl. Cryst.* **17**, 125-130.

SYGUSCH, J. & BEAUDRY, D. (1985). *J. Mol. Biol.* **186**, 215-217.

SYGUSCH, J., BOULET, H. & BEAUDRY, D. (1985). *J. Biol. Chem.* **260**, 15286-15290.

TANFORD, C. (1968). In *Advances in Protein Chemistry*, edited by C. B. ANFINSEN, M. L. ARSON, J. T. EDSALL & F. M. RICHARDS, pp. 122-175. New York: Academic Press.