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ORIGINAL ARTICLE

Retrospective analysis of double-strand break rejoining data collected using warm-lysis PFGE protocols

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Abstract

Sample preparation procedures for the pulsed-field gel electrophoresis (PFGE) assay usually involve a lysis step at temperatures as high as 50°C. During this warm-lysis procedure, multiply damaged sites containing heat-labile sites (HLS) can be converted into double-strand breaks (DSB). Once formed, these DSB cannot be distinguished from the DSB formed directly by ionizing radiation. This paper develops a method to correct DSB estimates for the effects of HLS in warm-lysis protocols. A first-order repair model is used to predict the number of HLS available for conversion into DSB as a function of the time available for repair before initiating warm-lysis. A mathematical expression is derived to separate prompt DSB from those formed through the artefactual conversion of HLS into DSB. The proposed formalism only requires the specification of two adjustable parameters, both of which can be estimated from measured data. Estimates of prompt DSB yields obtained by correcting warm-lysis data are in good agreement with estimates obtained using cold-lysis protocols, which do not include the effect of HLS. The retrospective analyses of two published datasets suggest that corrections for HLS have a substantial impact on DSB yields within the first 20 – 30 min after irradiation. Bi-exponential fits to the DSB data for Chinese hamster ovary cells suggest that corrections for HLS reduce the half-time for fast DSB rejoining by about 15%, whereas the half-time for the slow DSB rejoining only decreases by 4%. The total DSB yield and the fraction of fast-rejoining DSB decrease by 24 and 38%, respectively, when the correction is applied. The proposed formalism can be used to characterize trends and uncertainties in DSB rejoining kinetics associated with the artefactual conversion of HLS into DSB. The retrospective application of the methodology to warm-lysis data enhances their relevance and usefulness for studies of DSB rejoining kinetics.

Keywords: *Heat-labile sides, DSB rejoining, PFGE, cell lysis*

Introduction

Double-strand breaks (DSB) are regarded as one of the most critical forms of radiation-induced DNA damage, and pulsed-field gel electrophoresis (PFGE) is an analytical method widely used for the quantification of DSB. Conventional PFGE protocols involve a cell lysis step at a temperature of about 50°C (Rydberg 2000, Stenerlöw et al. 2003). In addition to DSB, ionizing radiation is also known to produce a class of DNA lesions that can be converted into DSB during cell lysis at elevated temperatures (Jones et al. 1994, Rydberg 2000, Stenerlöw et al. 2003, Gulston et al. 2004). Damage sites that can be converted into DNA strand breaks by heat are often referred to as heat-labile sites (HLS).

Although the exact chemical nature of the DNA damage that gives rise to HLS is unknown, damaged sugar moieties, not including single-strand breaks (SSB), are the most likely candidates (Jones et al. 1994). The lesions that are converted into DSB during the lysis step are most likely to be comprised of two closely spaced HLS on opposite DNA strands or a HLS opposite a SSB (Jones et al. 1994, Rydberg 2000, Gulston et al. 2004). Once multiply damaged sites containing HLS are converted into DSB, they cannot be distinguished from the prompt DSB formed directly by ionizing radiation. As a result of the artefactual conversion of HLS into DSB, most of the DSB yields reported in the literature may be overestimated by as much as 40% (Rydberg 2000, Stenerlöw et al. 2003, Gulston et al. 2004). Also, the

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fast DSB rejoining rates often observed in PFGE experiments may be due, at least in part, to the conversion of HLS into DSB (Rydberg 2000, Stenerlöw et al. 2003).

Stenerlöw et al. (2003) have developed a protocol that employs lysis at 0°C to prevent the conversion of HLS into DSB. Others (Jones et al. 1994, Rydberg 2000, Gulston et al. 2004) have also used low-temperature treatments to account for this artefact. Although these cold-lysis protocols are preferable to procedures performed at elevated temperatures for the estimation of prompt DSB yields, many published studies have been based on warm-lysis PFGE protocols. Published datasets are often used in the development and testing of radiobiological models, and a method to correct DSB estimates for the effects of HLS will enhance the relevance and usefulness of studies performed with warm-lysis protocols.

This paper develops a model to account for the conversion of HLS into DSB as an artefact of warm-lysis protocols. The model is then used to derive a mathematical expression to estimate prompt DSB yields from warm-lysis data. The proposed method requires the specification of only two adjustable parameters, both of which can be estimated from measured data. It shows that the proposed methodology produces estimates of prompt DSB yields that are in good agreement with similar data obtained using cold-lysis protocols. The proposed method is used to correct DSB estimates for Chinese hamster ovary (CHO) cells from two published studies (Stackhouse & Bedford 1994, Gulston et al. 2004). The effects of the correction for HLS on the inferred DSB rejoining kinetics are discussed.

Materials and methods

Model for the conversion of HLS into DSB during warm-lysis PFGE protocols

The formation, post-irradiation repair, and conversion of HLS into DSB during a typical warm-lysis protocol are illustrated in Figure 1.

Assuming for simplicity that HLS are repaired with monophasic kinetics, the expected number of HLS at time T, $N_{\mathrm{H}}(T)$, is related to the initial number of HLS, $N_{\mathrm{H}}(0)$, by:

$$N_{\mathrm{H}}(T) = N_{\mathrm{H}}(0)e^{-\lambda_{\mathrm{hls}}T}, \qquad (1)$$

where λ_{hls} is the probability per unit time that a HLS is removed from the DNA and T is the time available for repair before cell lysis. The repair probability λ_{hls} may be conveniently expressed in terms of a half-time for HLS removal, i.e. $\lambda_{\mathrm{hls}} = \ln(2)/\tau_{\mathrm{hls}}$. Let $N_{\mathrm{P}}(T)$ denote the expected number of prompt DSB per unit genome length at time T. If warm-lysis protocols are used, most of the HLS present within multiply damaged sites are converted into DSB within the standard lysis time of 10–20 h (Rydberg 2000). With the assumption that all HLS-containing multi-

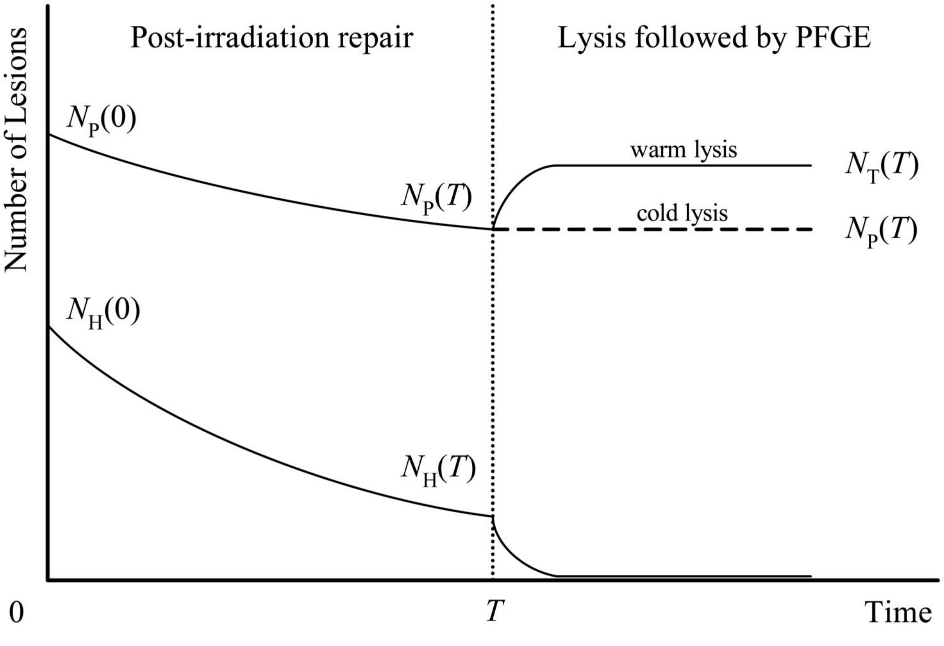

Figure 1. Idealized schematic illustrating the conversion of HLS into DSB during a warm-lysis PFGE protocol (lower curve, HLS; upper curve, prompt DSB). At time T, the cells are lysed at an elevated temperature and all unrepaired HLS are eventually converted into DSB. In the cold-lysis PFGE protocol, HLS are not converted into DSB during cell lysis.

ply damaged sites unrepaired at time T are converted into DSB during cell lysis, the total number of DSB expected after post-irradiation incubation time T is the sum of the prompt DSB at time T and the expected number of unrepaired HLS present at time T, i.e.

$$N_{\mathrm{T}}(T) = N_{\mathrm{P}}(T) + N_{\mathrm{H}}(T). \qquad (2)$$

Substituting the right-hand side of equation (1) into equation (2) and solving for $N_{\mathrm{P}}(T)$ gives:

$$N_{\mathrm{P}}(T) = N_{\mathrm{T}}(T) - f\,N_{\mathrm{T}}(0)e^{-\lambda_{\mathrm{hls}}T}, \qquad (3)$$

where $f \equiv N_{\mathrm{H}}(0)/N_{\mathrm{T}}(0)$ is the fraction of the total number of DSB at $T=0$ that are formed by the conversion of HLS into DSB during warm lysis. Our goal is to use equation (3) to estimate $N_{\mathrm{P}}(T)$ from the total number of DSB expected at the completion of the cell lysis step, $N_{\mathrm{T}}(T)$. To accomplish this goal, we need to estimate two model parameters: f and either λ_{hls} or τ_{hls}.

Estimating f and τ_{hls} from warm- and cold-lysis data

Estimates of f and τ_{hls} are most likely specific to the biological system and irradiation conditions. Here, we outline a methodology to estimate these parameters from DSB data obtained with the cold- and warm-lysis protocols. The rationale for the parameter estimation method is that HLS are not converted into DSB during cold lysis and therefore cold-lysis measurements produce an estimate of $N_{\mathrm{P}}(T)$, whereas PFGE data obtained using the warm-lysis protocol yield $N_{\mathrm{T}}(T)$. In this paper, estimates for f and τ_{hls} are derived from the studies of Stenerlöw et al. (2003) for normal human GM5758 fibroblasts irradiated with 40 Gy of ^{60}Co γ-rays.

The DSB data reported by Stenerlöw et al. (2003) are given in terms of the fraction of the activity released (FAR) and need to be converted into estimates of the number of DSB per unit genome length in order to use equation (3). For a random distribution of DSB within the DNA, FAR is related to the DSB yield by the following expression (Stenerlöw & Höglund 2002):

$$\mathrm{FAR} = 1 - \left[1 + Nk\left(1 - \frac{k}{X}\right)\right]e^{-Nk}, \qquad (4)$$

where N is the number of DSB per Mbp, k is the threshold value for the size of DNA fragments that are able to leave the well in Mbp (also called the gel exclusion size), and X is the average chromosome size in Mbp. Since equation (4) cannot be algebraically solved for N, an iteration scheme has to be used (Cedervall & Källman 1994):

$$N_0 = 1, \quad N_{n+1} = \frac{1}{k}\ln\left\{\left[1 + N_n k\left(1 - \frac{k}{X}\right)\right]/(1 - \mathrm{FAR})\right\}. \qquad (5)$$

The iteration procedure is terminated when $|N_{\mathrm{n}+1} - N_{\mathrm{n}}| < \varepsilon$, where ε is chosen according to the desired degree of precision in the determination of N, e.g. $\varepsilon = 10^{-10}$.

The Ungraph software by Biosoft® was used to extract the FAR values for GM5758 cells from Stenerlöw et al. (2003, figure 8). Table I shows the FAR values along with their associated uncertainties and the calculated number of DSB for cold- and warm-lysis conditions obtained using equation (5) with $k = 5.7$ Mbp (Stenerlöw et al. 2003) and $X = 130$ Mbp (Stenerlöw & Höglund 2002).

To estimate f and τ_{hls}, we used the non-linear optimization algorithm implemented in Microsoft Excel® to minimize the following figure of merit (FOM):

$$\mathrm{FOM} = \sum_{T}\left[N_{\mathrm{P}}^{M}(T) - N_{\mathrm{P}}^{C}(T)\right]^2, \qquad (6)$$

where $N_{\mathrm{P}}^{M}(T)$ is the expected number of prompt DSB determined using the cold-lysis protocol and $N_{\mathrm{P}}^{C}(T)$ is computed (equation 3) from measured warm-lysis data for a specified set of parameters f and τ_{hls}. The summation is performed over all available post-irradiation repair times including $T = 0$. (The dataset of Stenerlöw et al. (2003) includes six time points.)

Propagation of uncertainties

If experimental uncertainties are reported for the total DSB yields, estimates of the uncertainties associated with calculated prompt DSB yields can be obtained using the rules of propagation of errors (Bevington & Robinson 2003):

$$\begin{aligned} \sigma_{N_{\mathrm{P}}}(T) &= \sqrt{\sigma_{N_{\mathrm{T}}}^2(T) + \left(f\,e^{-\lambda_{\mathrm{hls}}T}\sigma_{N_{\mathrm{T}}}(0)\right)^2}, & T > 0, \\ \sigma_{N_{\mathrm{P}}}(T) &= (1 - f)\sigma_{N_{\mathrm{T}}}(0), & T = 0. \end{aligned} \qquad (7)$$

If data have to be converted from FAR values to DSB yields or vice versa, the following relationship can be used to calculate the propagated uncertainties:

$$\sigma_N = \frac{\sigma_{\mathrm{FAR}}}{\left[kN\left(1 - \frac{k}{X}\right) + \frac{k}{X}\right]ke^{-kN}}, \qquad (8)$$

where σ_N is in units of Mbp^{-1} if k and X are in Mbp, and N is in Mbp^{-1}.

Table I. Comparison of measured and calculated prompt DSB yields.

Time available for repair (h)	FAR^a, warm lysis	DSB yield (Gy^{-1} cell^{-1})^b, warm lysis	FAR^a, cold lysis	DSB yield (Gy^{-1} cell^{-1})^b, cold lysis	DSB yield (Gy^{-1} cell^{-1})^c, this work
0	0.439 ± 0.010	38.06 ± 0.78	0.312 ± 0.029	28.59 ± 2.09	28.61 ± 0.59
0.10	0.311 ± 0.029	28.51 ± 2.09	0.235 ± 0.010	23.07 ± 0.72	23.02 ± 2.09
0.31	0.178 ± 0.011	18.94 ± 0.81	0.154 ± 0.011	17.14 ± 0.83	17.16 ± 0.81
0.61	0.135 ± 0.022	15.69 ± 1.71	0.129 ± 0.010	15.22 ± 0.79	15.34 ± 1.71
1.12	0.097 ± 0.005	12.61 ± 0.42	0.101 ± 0.010	12.95 ± 0.84	12.59 ± 0.42
2.06	0.049 ± 0.014	8.16 ± 1.44	0.047 ± 0.009	7.94 ± 1.02	8.16 ± 1.44

[a] Values were extracted from Stenerlöw et al. (2003, figure 8).

[b] Mean and uncertainty were calculated from FAR values using equations (5) and (8), respectively, and converted into the units of Gy^{-1} cell^{-1} by multiplying by the average genome content for a mammalian cell of 6000 Mbp and dividing by the absorbed dose of 40 Gy.

[c] Mean was calculated from measured warm-lysis data using equation (3) with $f = 0.25$ and $\tau_{\mathrm{hls}} = 7.6$ min. Uncertainty was calculated using equation (7). Both values were converted into units of Gy^{-1} cell^{-1} by multiplying by 6000 Mbp and dividing by 40 Gy.

Results and discussion

Obtaining estimates for f and τ_{hls} from measured data

Using the procedure outlined above and the data reported by Stenerlöw et al. (2003), we obtained $f = 0.25$ and $\tau_{\mathrm{hls}} = 7.6$ min. These estimates can be compared with data reported in the literature. Based on experiments with normal human GM38 fibroblasts irradiated with 150 kVp X-rays, Rydberg (2000) reports that HLS constitute approximately 30% of the initially induced DSB measured using the conventional, warm-lysis procedure. Stenerlöw et al. (2003) report the same value for two normal human cell lines (GM38, GM5758) and one malignant human cell line (M059K) irradiated with various absorbed doses of 320 kVp X-rays, ^{60}Co γ-rays, and ^{137}Cs γ-rays, respectively. Gulston et al. (2004) suggest that f ranges from 0.25 to 0.30 for V79-4 Chinese hamster cells irradiated with ^{60}Co γ-rays in the absorbed dose range of 20 – 100 Gy. Our estimate of $f = 0.25$ derived from the data of Stenerlöw et al. (2003) is thus in good agreement with other estimates reported in the literature. The measured data also suggest that, for low-linear energy transfer (LET) radiation, the values of f are approximately the same for different mammalian cell lines.

Isolated sugar damages, SSB and different types of base damage are efficiently processed by base excision repair (Wallace 1994). Since HLS are hypothesized to be a form of sugar damage (see the Introduction) and both damaged sugars and SSB are processed through the same DNA repair pathway, the estimated half-time for HLS removal (τ_{hls}) can be compared with half-times for repair of SSB following exposure to low-LET radiation. Most SSB are rejoined independently of dose with repair half-times between 2 and 10 min (Frankenberg-Schwager 1990). SSB repair half-times ranging between 5 and 8 min have been observed in *in vitro* experiments performed on several human and rodent cell lines. However, *in vivo* studies suggest longer repair half-times, i.e. between 12 min for mouse testis and 45 min for mouse brain (Olive 1999). SSB are also repaired with slower kinetics in mature cells of the immune system. Repair half-times of 30 and 21.3 min have been determined for human leukocytes (Olive 1999) and rat lymphocytes (Coogan et al. 1992), respectively. Nocentini (1999) reports that about 65% of SSB are removed from the DNA of CHO K1 cells within 15 min of post-irradiation incubation, which yields a repair half-time of about 10 min assuming exponential rejoining kinetics. The 7.6-min repair half-time derived for HLS from the data of Stenerlöw et al. (2003) falls within the discussed range of repair half-times for SSB.

Table I shows experimental warm- and cold-lysis data of Stenerlöw et al. (2003) along with the predicted cold-lysis DBS yields obtained by correcting the warm-lysis data for the effects of HLS using the obtained parameter estimates.

The excellent agreement between predicted (column 6) and measured (column 5) DSB yields suggests that the proposed methodology can be used to correct existing warm-lysis PFGE data for the artefacts due to the detection of HLS as DSB.

Figure 2 shows the sensitivity of the DSB estimates to uncertainties in f and τ_{hls}. The horizontal axes of the graphs were cut off at 1 h since parameter estimates obtained for the dataset of Stenerlöw et al. (2003) have little impact on the predicted values for repair times exceeding 1 h.

Values of $f > 0.30$ and < 0.20 ($\tau_{\mathrm{hls}} = 7.6$ min) result in DSB estimates that fall outside the range indicated by the maximum deviation from the mean associated with the measured data. Similarly, $\tau_{\mathrm{hls}} > 10$ min and < 6 min ($f = 0.25$) produce DSB estimates that fall outside the range of uncertainties associated with the measured DSB yields. The results

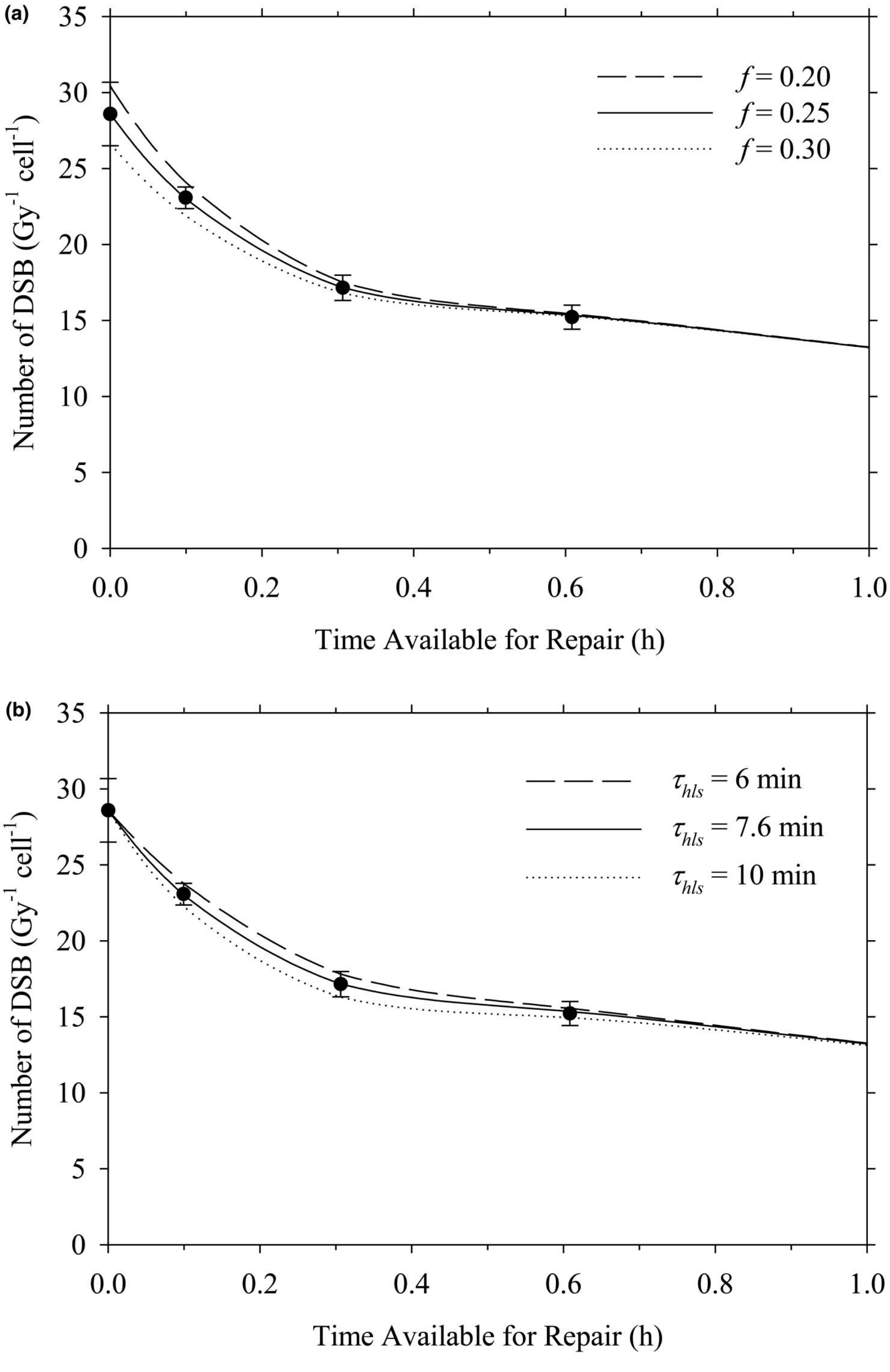

Figure 2. Sensitivity of calculated prompt DSB yields to uncertainties associated with parameter estimates. Symbols represent measured prompt DSB yields (Stenerlöw et al. 2003; GM5758, cold lysis) and their associated uncertainties. (A) Sensitivity to f ($\tau_{\text{hls}} = 7.6$ min); (B) sensitivity to τ_{hls} ($f = 0.25$). Note that prompt DSB yields were calculated only for the time points at which warm-lysis measurements are available; calculated values are connected by smooth curves to guide the eye.

of these sensitivity studies suggest that parameters values $f = 0.25 \pm 0.05$ and $\tau_{\text{hls}} = 8 \pm 2$ min yield DSB predictions that are included within the experimental uncertainty of the data, and therefore, the parameter values within the specified ranges are appropriate for GM5758 cells irradiated by low-LET radiation. Our analysis (data not shown) of the data reported by Stenerlöw et al. (2003) for a human malignant glioma cell line, M059K, produced the estimates of $f = 0.28$ and $\tau_{\text{hls}} = 9.6$ min, which also fall within these ranges.

Application of the proposed methodology to warm-lysis PFGE data

Figure 3 shows the effect of removing HLS on the DSB rejoining kinetics in CHO 10B2 cells following a 20 Gy dose of ^{137}Cs γ-rays delivered at 356.1 Gy h^{-1}. To quantify the effects of HLS on estimates of DSB rejoining kinetics, we performed least-squares fits of the following bi-exponential equation to the corrected and uncorrected DSB yields:

$$\mathrm{DSB}(t) = \sum\nolimits_{\mathrm{dsb}} \left[g_{\mathrm{fast}} e^{-\lambda_{\mathrm{fast}} t} + (1 - g_{\mathrm{fast}}) e^{-\lambda_{\mathrm{slow}} t} \right], \quad (9)$$

where Σ_{dsb} is the total DSB yield (Gy^{-1} cell^{-1}) and g_{fast} is the fraction of the total DSB that are rejoined with fast kinetics. Parameters λ_{fast} and λ_{slow} are related to the corresponding repair half-times as $\lambda_{\mathrm{fast}} = \ln(2)/\tau_{\mathrm{fast}}$ and $\lambda_{\mathrm{slow}} = \ln(2)/\tau_{\mathrm{slow}}$.

When the time available for post-irradiation repair is greater than about 0.4 h (about three times the half-time for HLS removal), the corrected and uncorrected DSB estimates become nearly the same ($<$ 5% difference) in the CHO 10B2 cells. For the slow rejoining DSB, the estimated repair half-time for the corrected dataset is higher by 4% than the corresponding value for the uncorrected data. For the fast rejoining DSB, subtracting the HLS sites reduces the half-time for repair by 15% (4.7 instead of 5.5 min). The total DSB yield and the fraction of fast rejoining DSB decrease by 24 and 38%, respectively, when the correction is applied. The effect of the correction tends to decrease as f and τ_{hls} decrease. For $f = 0$ or $\tau_{\mathrm{hls}} = 0$, the bi-exponential parameters describing corrected and uncorrected DSB rejoining kinetics are identical. If $f = 0.2$ and $\tau_{\mathrm{hls}} = 6$ min (the minimums suggested by sensitivity studies shown in Figure 2), the estimates of Σ_{dsb}, g_{fast} and τ_{fast} derived from the corrected DSB yields are lower by 18, 31 and 2%, respectively. The value of τ_{slow} is higher by 0.4% for the corrected data compared with uncorrected data.

The results shown in Figure 3 indicate that HLS have a substantial impact on the overall DSB rejoining rate within the first 20–30 min after irradiation but have little impact on DSB estimates at later times. The effects of HLS are even more evident for the Gulston et al. (2004) dataset shown in Figure 4. Stenerlöw et al. (2003) have shown that the fraction of HLS converted into DSB decreases when

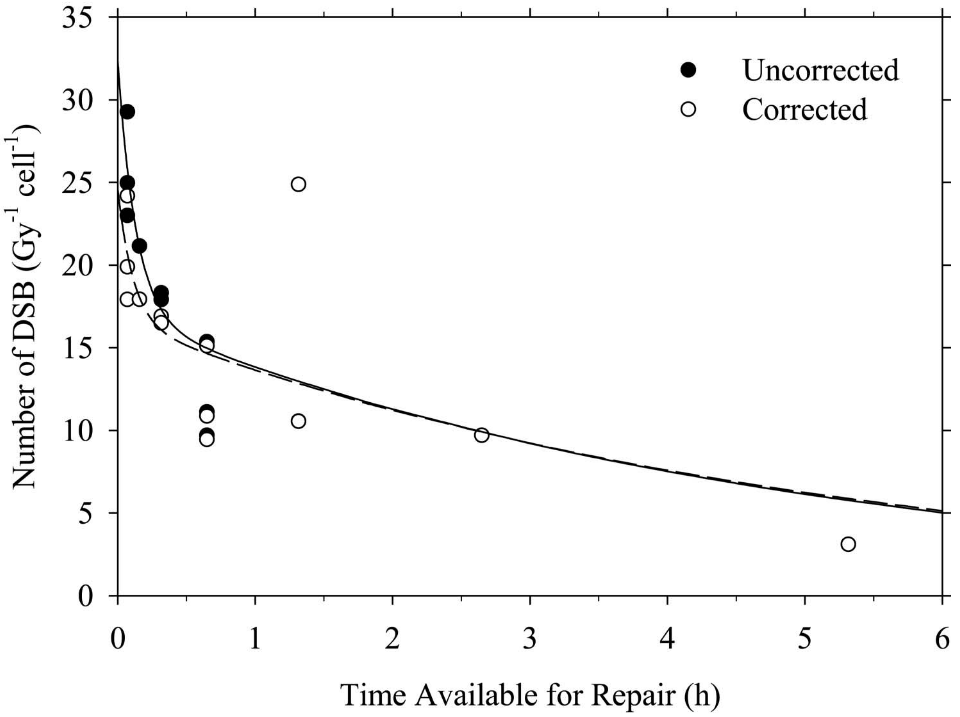

Figure 3. Effect of subtracting HLS from the DSB data reported by Stackhouse & Bedford (1994) for CHO 10B2 cells irradiated by γ-rays from a ^{137}Cs source (20 Gy delivered at 356.1 Gy h^{-1}). Warm-lysis FAR values were converted into DSB Gy^{-1} cell^{-1} estimates using equation (5) with $k = 5.7$ Mbp and $X = 245$ Mbp (Cedervall & McMillan 2002). The data were corrected for HLS using equation (3) with $f = 0.25$ and $\tau_{\mathrm{hls}} = 8$ min. Solid line: bi-exponential fit to uncorrected PFGE data ($\Sigma_{\mathrm{dsb}} = 32.4$ Gy^{-1} cell^{-1}, $g_{\mathrm{fast}} = 0.523$, $\tau_{\mathrm{fast}} = 0.092$ h, $\tau_{\mathrm{slow}} = 3.41$ h). Dashed line: bi-exponential fit to corrected PFGE data ($\Sigma_{\mathrm{dsb}} = 24.6$ Gy^{-1} cell^{-1}, $g_{\mathrm{fast}} = 0.326$, $\tau_{\mathrm{fast}} = 0.078$ h, $\tau_{\mathrm{slow}} = 3.54$ h). For times larger than about 1 h, the correction for HLS becomes negligible and the filled circles are masked by the open circles.

the lysis temperature decreases from 50 to 20°C. For lysis at temperatures below 20°C, few, if any, HLS are converted into DSB. Corrections for the effects of HLS are shown in Figure 4 for $f = 0.14$ (open triangles) and 0.25 (open circles), which correspond to lysis at 37 and 50°C, respectively. The estimate of $f = 0.14$ is based on a linear extrapolation from 50 to 37°C using $f(50°\mathrm{C}) = 0.25$ and $f(20°\mathrm{C}) = 0$ to compute the slope of the line. Because Gulston et al. (2004) used a lysis temperature of 37°C, the DSB yields corrected using $f = 0.14$ are more appropriate than the results obtained using $f = 0.25$.

When the effect of HLS is subtracted from the warm-lysis PFGE data of Gulston et al. (2004), a pronounced peak in the DSB estimates becomes evident approximately 20 min after irradiation. This delayed DSB formation process has been attributed to the conversion of multiply damaged sites into DSB through the enzymatic processing of radiation-induced damage (Gulston et al. 2004). Other studies of DSB rejoining kinetics also suggest that the aborted base excision repair of multiply damaged sites can produce delayed (enzymatic) DSB after low-LET irradiation (Blaisdell & Wallace 2001, Yang et al. 2004). Because some of the DSB observed in this experiment are evidently due to the conversion of multiply damaged sites into DSB through aborted excision repair, the quantity denoted $N_P(T)$ in equation (2) should be interpreted as the yield of prompt and enzymatic DSB at time T rather than the yield of prompt DSB at time T.

Conclusions

A method to correct warm-lysis PFGE data for artefacts created due to the detection of HLS as DSB is proposed. The method employs a single mathematical expression (equation 3) to estimate prompt DSB yields from data obtained using warm-lysis protocols. For normal human GM5758 skin fibroblasts irradiated by ^{60}Co γ-rays, analyses suggest that $f = 0.25 \pm 0.05$ and $\tau_{\mathrm{hls}} = 8 \pm 2$ min. Very similar parameter estimates, $f = 0.28$ and $\tau_{\mathrm{hls}} = 9.6$ min, were also obtained from warm- and cold-lysis PFGE studies performed with the M059K human malignant glioma cell line.

The retrospective analysis of two CHO datasets suggests that corrections for HLS reduce the DSB yields observed within the first 20–30 min after irradiation. The effect of the correction gradually decreases as time available for repair increases. Bi-exponential fits to the data reported by Stackhouse & Bedford (1994) suggest that the correction for HLS reduces the half-time for fast DSB rejoining by about

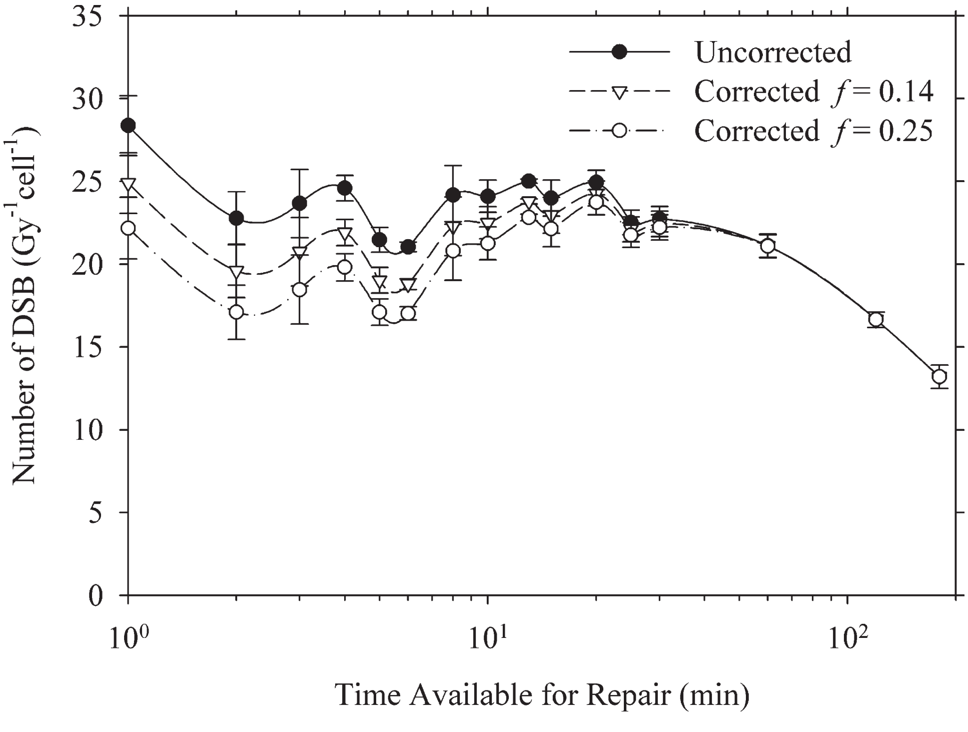

Figure 4. Effect of subtracting HLS from the DSB data reported by Gulston et al. (2004) for CHO xrs-5 cells irradiated by γ-rays from a ^{60}Co source (15 Gy delivered at > 900 Gy h^{-1}). The data were corrected for HLS using equation (3) with $\tau_{\mathrm{hls}} = 8$ min and $f = 0.25$ (open circles) or $f = 0.14$ (open triangles). The latter value corresponds to the estimated value of f for cell lysis at 37°C (see text). Uncertainties in the corrected DSB yields were calculated according to equation (7). Curves are shown to guide the eye. For times larger than about 1 h, the correction for HLS becomes negligible and the three sets of data overlap.

15% whereas the half-time for the slow rejoining DSB increases by 4%. Substantial reductions in the total DSB yield and the fraction of fast rejoining DSB are observed when the correction for HLS is applied, i.e. 24 and 38%, respectively.

The proposed formalism provides a useful method to retrospectively characterize trends and uncertainties in DSB rejoining kinetics associated with the artefactual conversion of HLS into DSB. Application of the methodology to published datasets will enhance the relevance and usefulness of DSB rejoining data obtained with warm-lysis protocols in future modelling studies.

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