

Radiolysis of *lac* Repressor by γ -Rays and Heavy Ions: A Two-Hit Model for Protein Inactivation

Michel Charlier,* Séverine Eon,* Édouard Sèche,* Serge Bouffard,[†] Françoise Culard,* and Mélanie Spothheim-Maurizot*

*Centre de Biophysique Moléculaire, CNRS, 45071 Orléans Cedex 2, France; and [†]Centre Interdisciplinaire de Recherche Ion Lasers, CEA-CNRS-ISMRA, 14070 Caen Cedex 5, France

ABSTRACT Upon γ -ray or argon ion irradiation of the *lac* repressor protein, its peptide chain is cleaved and the protein loses its *lac* operator-binding activity, as shown respectively by polyacrylamide gel electrophoresis and retardation gel electrophoresis. We developed phenomenological models that satisfactorily account for the experimental results: the peptide chain cleavage model considers that the average number of chain breaks per protomer is proportional to the irradiation dose and that the distribution of the number of breaks per protomer obeys Poisson's law. The repressor inactivation model takes into account the quaternary structure (a dimer of dimer) and the organization of the repressor in domains (two DNA binding sites, one per dimer). A protomer is inactivated by at least two different radiation-induced damages. A dimer is inactivated when at least one of the two protomers is inactivated. A tetramer is inactivated when both dimers are inactivated. From the combination of both models, we can deduce that chain cleavage cannot account for the protein inactivation, which should mainly result from oxidation of amino acid side chains. Indeed, particularly oxidizable and accessible amino acids (Tyr, His) are involved in the DNA binding process.

INTRODUCTION

The *Escherichia coli* *Lac* repressor-*lac* operator complex is considered to be the paradigm of the protein-DNA interacting systems (Barkley and Bourgeois, 1978). The repressor is a homotetrameric protein of 4×360 amino acids (Fig. 1) (Farabaugh, 1978). Two dimers are associated by the C-terminal parts of the protomers (Lewis et al., 1996). Each dimer bears a DNA-binding site formed by the two N-terminal parts of the protomers, the headpieces (≈ 60 amino acids). Thus the repressor is organized in domains: the headpieces, which can be enzymatically cleaved in particular conditions, and the tetrameric core formed by the C-terminal parts of the protomers (Fig. 1). (Weber and Geisler, 1978).

The role of the repressor is to bind the operator, a quasi-palindromic sequence of 30 basepairs (Gilbert and Maxam, 1973), located a few bases upstream of the *lac* promoter. This prevents the binding of the RNA polymerase on the promoter, and consequently the expression of the structural genes of the *lac* operon. The repressor is an allosteric protein: binding of a metabolite of the lactose to the core induces the disruption of the complex, and the enzymes involved in the lactose catabolism can be synthesized (Miller, 1978).

In vitro and at 200 mM K^+ , the binding constant of the repressor-operator system is very high, of the order of

magnitude of 10^{11} – 10^{13} M^{-1} , depending on the length of the operator-bearing DNA (Tsodikov et al., 1999). Repressor can also bind non-operator DNA, with a reduced affinity constant (10^4 M^{-1} in 200 mM Na^+), by a process strongly ionic strength-dependent (Kao-Huang et al., 1977).

The repressor-operator couple seems to us a good example for studying the effects of radiolysis on the behavior and on the functioning of a protein-DNA system. In previous papers we have shown that the repressor protects the operator against radiolytic damage, and leaves a footprint at the site of interaction (Franchet-Beuzit et al., 1993). We have also shown that irradiation of the repressor prevents operator binding. The irradiation of the complex disrupts the association, but at doses largely higher than those inactivating the repressor irradiated alone. This shows that the operator can also protect some determinant sites on the repressor against radiolytic damage (Eon et al., 2001).

The radiolysis of proteins in aerated dilute solution occurs essentially, similar to DNA radiolysis, by means of the oxidizing OH radical, produced by the radiolytic decomposition of water (Ferradini and Jay-Gerin, 1999). The primary damages due to OH radical attack are hydrogen abstraction, either from the peptide chain ($H\alpha$) or from the amino acid side chain, the addition to the rings of the aromatic residues, and the reaction with sulfur (Davies, 1987; Garrison, 1987; Mee, 1987; Stadtman, 1993; Maleknia et al., 1999). Some of the resulting damages, such as chain breaks, amino acid modifications, or release of amino acid side chain, can be responsible for the dysfunction of the binding process: the loss of DNA-binding activity or the disruption of preexisting complexes.

The shape of the experimental curves of the fraction of repressor still able to bind DNA (active repressor) as a function of the irradiation dose are sigmoidal. For low

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Address reprint requests to Dr. Michel Charlier, Centre de Biophysique Moléculaire, CNRS, rue Charles-Sadron, 45071 Orléans Cedex 2, France. Tel.: 33-2-38255549; Fax: 33-2-38631517; E-mail: micharli@cnrs-orleans.fr.

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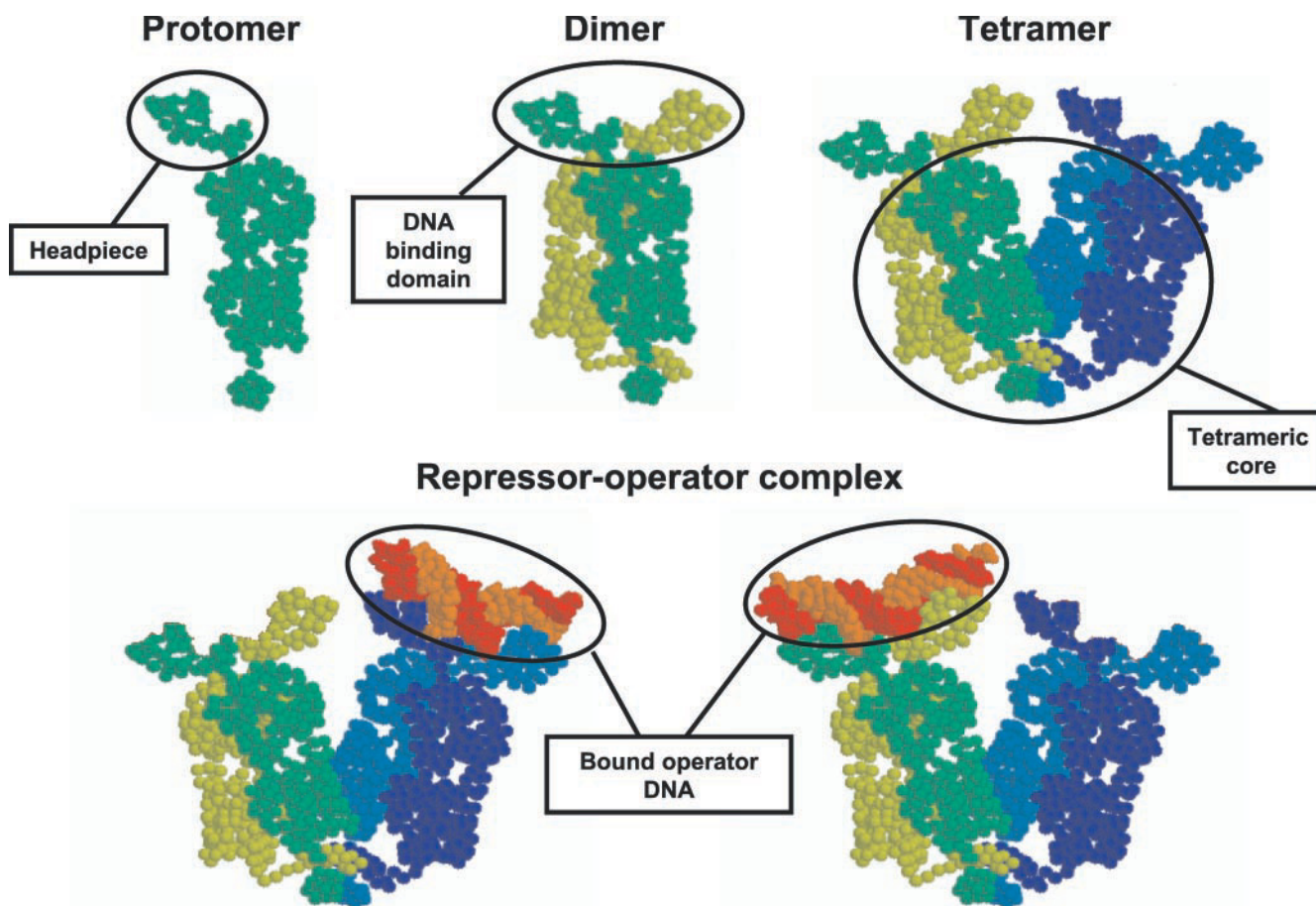


FIGURE 1 Quaternary structure of the *lac* repressor showing one protomer (green), with the headpiece, one dimer (green and yellow-green protomers) with the DNA-binding domain, and the tetramer. With long DNA fragments bearing the operator, as those used for the experiments, the repressor binds only one DNA fragment through one or the other of the two dimers. The repressor-operator complex structure was extracted from the PDB databank (<http://www.rcsb.org/pdb/>, 1LBG entry).

doses, the protein does not seem to be affected by the radiation and abruptly, at a given dose, the DNA-binding activity drops and vanishes. Such a behavior is very different from that of another DNA-binding protein that we are studying, the MC1 chromosomal protein extracted from a *methanosarcina*, and whose loss of activity is monotonic from 100% to 0 (F. Culard, manuscript in preparation). However, the latter protein is monomeric, and bears only one binding site for DNA. This difference in behavior led us to draw a model of inactivation that takes into account the special feature of the repressor protein, i.e., the tetrameric quaternary structure, the organization in two dimers, each of them bearing a DNA-binding site involving the N-terminal 60 amino acids of both protomers.

This model of repressor inactivation, and a model of peptide chain cleavage are not based on chemical investigations concerning the damages, but only on the phenomenological analysis of the two dose-response curves: fraction of remaining active repressors versus dose, and fraction of remaining intact protomers versus dose. In coupling the

repressor inactivation model with the peptide chain cleavage model, we are able to evaluate the contribution of the peptide chain breakage to the protein inactivation.

MATERIALS AND METHODS

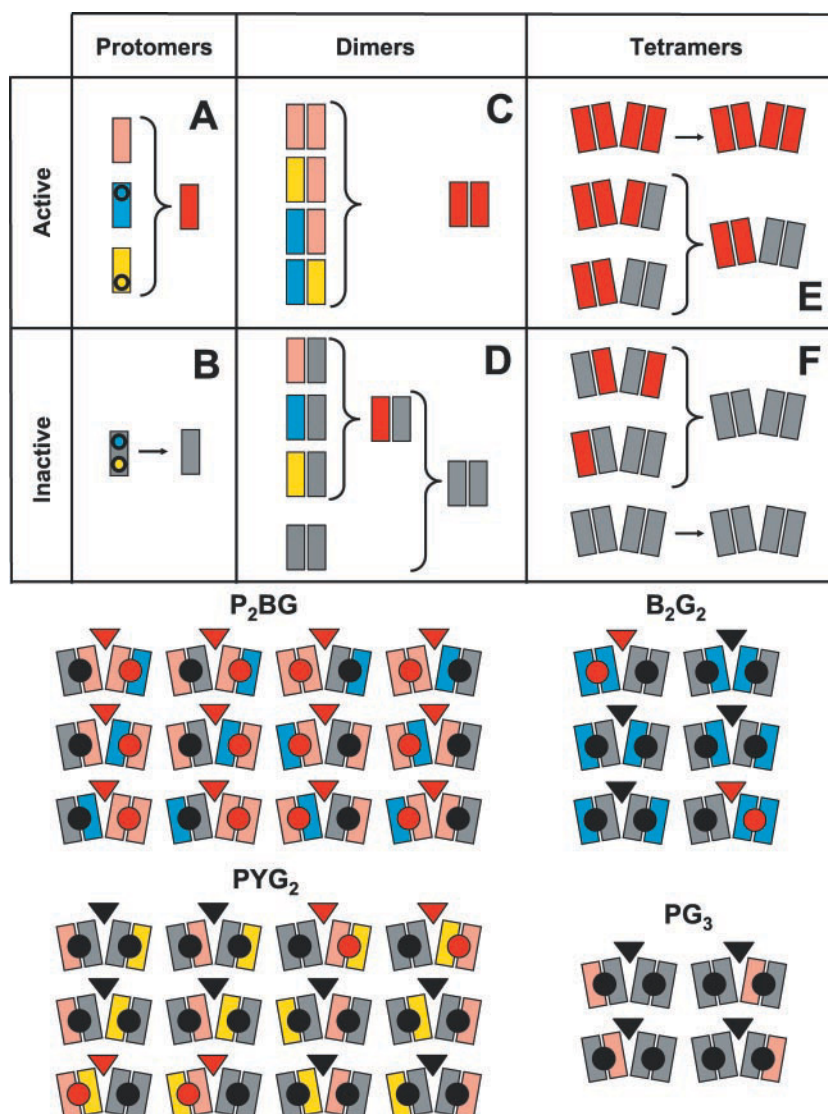
Biochemicals

Lac repressor was prepared from the BMH 493 overproducing strain (a gift from Prof. B. Müller-Hill) as previously described (Culard and Maurizot, 1981). The 80-bp DNA fragment bearing the operator sequence was prepared and labeled as previously described (Franchet-Beuzit et al., 1993).

The measurement of the protein activity was described in a preceding paper (Eon et al., 2001). Shortly, complexes between *lac* repressor irradiated alone (0.34 μ M, in 0.2 M NaCl, 15 mM potassium phosphate, pH 7.25) and 32 P-labeled *lac* operator-bearing DNA fragment (6.8 nM) were analyzed by retardation gel electrophoresis, which separates the free DNA from the DNA-repressor complex.

In our experimental conditions (200 mM Na⁺), we observe only specific repressor-operator complexes, because the ratio between the specific and the nonspecific binding constants is of the order of 10^8 (Kao-Huang et al., 1977; Tsodikov et al., 1999). Because the repressor concentration is

FIGURE 2 Top: Schemes illustrated the hypotheses 2 to 5 used for the two-hit model elaboration. Hypothesis 2: A; 3: B; 4: C and D; 5: E and F. An active protomer (red) can be either intact (pink), or can bear only one damage (blue or yellow). An inactive protomer bearing two damages is gray. An active dimer (both protomers are active, i.e., red) is red. An inactive dimer (at least one of the two protomers is inactive, i.e., gray) is gray. Bottom: Schemes showing four examples of subconfigurations. Red or black circles indicate that the dimer is either active or inactive. Red or black triangles indicate that the tetramer is either active or inactive. For $d = 1$ (P_2BG), $S_{\text{ceff}} = S_c$. For $d = 3$ (PG_3), $S_{\text{ceff}} = 0$. For $d = 2$ (B_2G_2 and PYG_2), $S_{\text{ceff}} = S_c/3$.



much higher than that of DNA, only complexes between one repressor and one 80-bp operator-bearing fragment are observed. No complexes between one repressor and two fragments are formed. Such complexes have been observed only in the presence of a huge excess of 24-bp operator DNA (Fickert and Müller-Hill, 1992). As the concentrations of both DNA and repressor are much larger than the inverse of the specific binding constant (10^{-12} M^{-1}), all 80-bp fragments are bound to repressor. Below, we call inactive repressor a repressor whose binding constant dropped to such an extent that complexes with operator-DNA cannot be detected by retardation gel electrophoresis.

For irradiation of the bound protein in the complex, the complex was directly loaded on the retardation gel. For a given dose of γ -rays, the fractions of radioactivity in the band of the complex and in the band of the free DNA represent the fractions of active and inactive protein, respectively (Eon et al., 2001).

The measurements of the fraction of intact protomers were performed as previously described (Eon et al., 2001). After polyacrylamide gel electrophoresis, on 12% acrylamide gels, the proteins were stained using CyproRed (Molecular Probes, Eugene, OR), and assayed by fluorescence with the STORM (Molecular Dynamics, Amersham, UK). Intact protomers migrate as a well-defined band, whereas broken protomers migrate faster

and as a smear. The percent of intact protomers is equal to the percent of fluorescence in this band.

Irradiation

Repressor or repressor-DNA complexes were irradiated in 0.2 M NaCl, 15 mM potassium phosphate buffer, pH 7.25. Irradiation with γ -rays were performed at 4°C using a ^{137}Cs irradiator (IBL437, CisBio International, Saclay, France) delivering 0.6 MeV γ -rays, at a dose rate of 10 Gy min^{-1} . The linear energy transfer (LET) of the radiation is of the order of $0.5 \text{ keV } \mu\text{m}^{-1}$ (Kiefer, 1990). Irradiation with $^{36}\text{Ar}^{18+}$ ions of 95 MeV per nucleon were performed at Grand Accélérateur National d'Ions Lourds (GANIL; Caen) using the D1 IRABAT beam line. The mean LET through the sample was $247 \text{ keV } \mu\text{m}^{-1}$.

MODEL BUILDING

The repressor molecule is a homotetramer, formed by two homodimers. Each dimer can be considered as a domain

able to bind the *lac* operator (Fig. 1, *red* and *orange balls*), through the N-terminal domains of the protomers, the head-pieces. We shall thus consider that two protomers are equivalent in a dimer, but not in a tetramer: two protomers may belong either to the same dimer (*blue* and *cyan*), or to different dimers (*blue* and *green*). However, both dimers (*blue/cyan* and *green/green-yellow*) are equivalent in the tetramer.

Repressor inactivation

A two-hit model

This model deals with the disruption of complexes involving one repressor and one operator. This means that repressor binds only one operator, although two potential binding sites are present on the protein. As one repressor-two operator DNA complexes are highly improbable in our experimental conditions, they are not considered in the model building.

We shall consider the following hypotheses:

1. There are two independent critical targets for radiolytic attack per protomer (Fig. 2, *A* and *B*). These targets, identical for all protomers, could be, for instance, two amino acid side chains implicated in the DNA binding process.
2. The destruction of only one of these targets does not abolish the binding ability of a protomer (Fig. 2 *A*, red protomer).
3. The destruction of both targets completely abolishes the binding ability of the protomer, therefore called inactive protomer (Fig. 2 *B*, gray protomer).
4. The inactivation of at least one protomer abolishes the binding ability of the corresponding dimer (therefore called inactive dimer, Fig. 2, *C* and *D*, gray dimer).
5. The inactivation of only one of the two dimers do not abolish the binding ability of a tetramer; if at least one dimer remains active, the tetramer is considered as fully active (Fig. 2, *E* and *F*). In fact, a tetramer with either one or two active dimers do not have the same operator binding constants. However, in our experimental conditions, due to the high concentrations of partners, to the large excess of repressor, and to the magnitude of the binding constant to both sites of the repressor (Tsodikov et al., 1999), we consider them as being equivalent.

6. The fractions p and q ($0 \leq p, q \leq 1$) of destroyed target (first and second, respectively), are proportional to the dose D :

$$p = k_1 D \quad \text{if} \quad k_1 D \leq 1, \quad p = 1 \quad \text{if} \quad k_1 D > 1$$

$$q = k_2 D \quad \text{if} \quad k_2 D \leq 1, \quad q = 1 \quad \text{if} \quad k_2 D > 1$$

There are four types of protomers: 1) intact ones, called P (pink); 2) with target 1 destroyed, called B (blue); with target 2 destroyed, called Y (yellow); and with both targets destroyed, called G (gray).

One tetramer could be described by a symbol $P_a B_b Y_c G_d$, with $a + b + c + d = 4$ and $0 \leq a, b, c, d \leq 4$. A set of $[a, b, c, d]$ defines a configuration where a protomers are P, b protomers are B, c protomers are Y, and d protomers are G.

For given values of p and q , the probability Cf for a tetramer to have a configuration $[a, b, c, d]$ is:

$$\begin{aligned} \text{Cf}(p, q, a, b, c, d) &= (1-p)^a (1-q)^a p^b (1-q)^b q^c \\ &\quad \times (1-p)^c p^d q^d \quad (1) \\ &= p^{(b+d)} q^{(c+d)} (1-p)^{(a+c)} (1-q)^{(a+b)} \end{aligned}$$

Because of nonequivalence of protomers in the repressor, different subconfigurations $\text{Sc}(a, b, c, d)$ exist for a set of $[a, b, c, d]$ (Table 1):

$$\text{Sc}(a, b, c, d) = C_4^a C_{4-a}^b C_{4-a-b}^c C_{4-a-b-c}^d = \frac{4!}{a!b!c!d!} \quad (2)$$

It can be verified that

$$\begin{aligned} \sum_a \sum_b \sum_c \sum_d [\text{Cf}(p, q, a, b, c, d) \\ \times \text{Sc}(a, b, c, d)] = 1 \quad \forall p, \forall q \end{aligned}$$

For a given configuration, all subconfigurations do not correspond to an active tetramer. The number of efficient subconfigurations $\text{Sceff}(a, b, c, d)$ depends on the number of inactive protomers, and on their repartition in the tetramer. For $d = 0$ or 1 , $\text{Sceff} = \text{Sc}$ because at least one dimer remains active; for $d = 3$ or 4 , $\text{Sceff} = 0$ because no dimer remains active. For $d = 2$, $\text{Sceff} = \text{Sc}/3$, corresponding to the subconfigurations where both gray (inactive) protomers are regrouped in the same dimer (see Fig. 2, *bottom*, and Table 1).

The fraction of active tetramers Tet_{act} is thus equal to:

$$\begin{aligned} \text{Tet}_{\text{act}}(p, q) &= \sum_a \sum_b \sum_c \sum_d [\text{Cf}(p, q, a, b, c, d) \\ &\quad \times \text{Sceff}(a, b, c, d)] \quad (3) \end{aligned}$$

One-hit model

We shall consider the following hypotheses:

7. There is only one target for radiolytic attack on a protomer, whose destruction abolishes the binding ability of this protomer (inactive protomer).
8. The inactivation of at least one protomer abolishes the binding ability of the corresponding dimer (inactive dimer).
9. The inactivation of only one of the two dimers does not abolish the binding ability of a tetramer. If at least one dimer remains active, the tetramer is active.

TABLE 1 The 35 possible configurations PaBbYcGd of a tetramer in the two-hit model

	P4	P3B	P3Y	P3G	P2B2	P2BY	P2BG	P2YG	P2Y2
P	4	3	3	3	2	2	2	2	2
B	0	1	0	0	2	1	1	0	0
Y	0	0	1	0	0	1	0	1	2
G	0	0	0	1	0	0	1	1	0
Sc	1	4	4	4	6	12	12	12	6
Sceff	1	4	4	4	6	12	12	12	6

	P2G2	PB3	PB2Y	PB2G	PBY2	PBYG	PBG2	PY3	PY2G
P	2	1	1	1	1	1	1	1	1
B	0	3	2	2	1	1	1	0	0
Y	0	0	1	0	2	1	0	3	2
G	2	0	0	1	0	1	2	0	1
Sc	6	4	12	12	12	24	12	4	12
Sceff	2	4	12	12	12	24	4	4	12

	PYG2	PG3	B4	B3Y	B3G	B2Y2	B2YG	B2G2	BY3
P	1	1	0	0	0	0	0	0	0
B	0	0	4	3	3	2	2	2	1
Y	1	0	0	1	0	2	1	0	3
G	2	3	0	0	1	0	1	2	0
Sc	12	4	1	4	4	6	12	2	4

	BY2G	BYG2	BG3	Y4	Y3G	Y2G2	YG3	G4
P	0	0	0	0	0	0	0	0
B	1	1	1	0	0	0	0	0
Y	2	1	0	4	3	2	1	0
G	1	2	3	0	1	2	3	4
Sc	12	12	4	1	4	6	4	1
Sceff	12	4	0	1	4	2	0	0

Bold letters: configurations for which $Sceff \neq Sc$ ($d = 2, 3$, or 4).

10. The fraction p ($0 \leq p \leq 1$) of destroyed target is proportional to the dose D :

$$p = kD \quad \text{if } kD \leq 1, \quad p = 1 \quad \text{if } kD > 1$$

There are two types of protomers: 1) intact ones, called P, and 2) inactive ones, called G. One tetramer could be described by a symbol P_aG_d , with $a + d = 4$, and $0 \leq a, d \leq 4$. A set of $[a, d]$ defines a configuration where a protomers are P, and d protomers are G.

For a given values of p , the probability for a tetramer to have a configuration $[a, d]$ is:

$$Cf(p, a, d) = (1 - p)^a p^d \quad (4)$$

Because of nonequivalence of protomers in the repressor, different subconfigurations $Sc(a, d)$ exist for a set of $[a, d]$ (see Table 2):

$$Sc(a, d) = C_4^a C_{4-a}^d = \frac{4!}{a!d!} \quad (5)$$

It can be verified that, for $a + d = 4$,

$$\sum_a \sum_d [Cf(p, a, d) \times Sc(a, d)] = 1 \quad \forall p.$$

For a given configuration, all subconfigurations do not correspond to an active tetramer. As for the two-hit model,

for $d = 0$ or 1 , $Sceff = Sc$; for $d = 3$ or 4 , $Sceff = 0$; and for $d = 2$, $Sceff = Sc/3$ (Table 2).

The fraction of active tetramer Tet_{act} is thus equal to:

$$Tet_{act}(p) = \sum_a \sum_d [Cf(p, a, d) \times Sceff(a, d)] \quad (6)$$

Peptide chain cleavage

We shall consider the following hypotheses:

11. All peptide bonds have the same probability to be broken upon radiolysis. For small numbers of breaks, the average number r of breaks per protomer is proportional to the dose:

$$r = k_c D$$

12. As r remains smaller than 359 (the number of peptide bonds per protomer), the distribution of the breaks obeys

TABLE 2 The five possible configurations PaGd of a tetramer in the case of the one-hit model (see Table 1)

	P4	P3G	P2G2	PG3	G4
P	4	3	2	1	0
G	0	1	2	3	4
Sc	1	4	6	4	1
Sceff	1	4	2	0	0

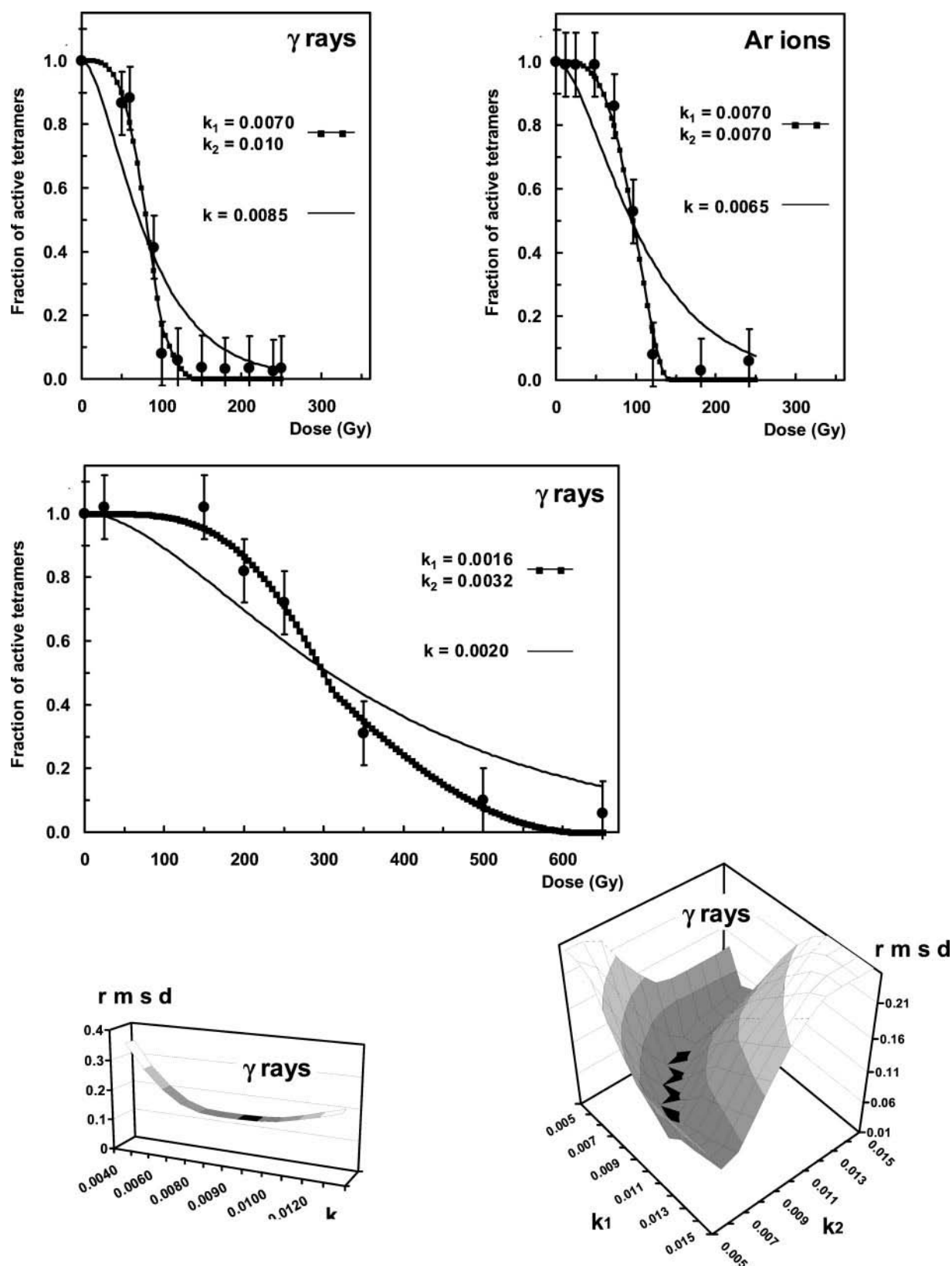


FIGURE 3 Top: Fraction of active repressor molecules (tetramer) as a function of dose, when the protein is irradiated alone in solution either with γ -rays (left) or argon ions (right). Filled circles: experimental data; solid line: best fit using the one-hit model; line and squares: best fit with the two-hit model. Middle: Same as the top, for repressor irradiated in the complex with the operator DNA. Bottom: root-mean-square deviations (rmsd) between experimental and calculated data for the repressor irradiated alone with γ -rays, as a function of k (left, one-hit model) or k_1 and k_2 (right, two-hit model).

Poisson's law, i.e., the fraction of intact protomers P_0 is equal to:

$$P_0 = e^{-r} = e^{(-k_c D)} \quad (7)$$

The fractions P_1 and P_2 of protomers bearing either at least one or at least two chain break(s) are:

$$P_1 = 1 - e^{-r}$$

$$P_2 = 1 - e^{-r} - re^{-r}$$

If we consider that a chain break located at any place is a dramatic damage sufficient to inactivate a protomer, chain breakage is relevant to the one-hit model, with $p = P_1 = 1 - e^{-r}$, and the fraction of tetramers that keeps their full ability to bind DNA is given by Eq. 6, where $p = P_1$:

$$\text{Tet}_{\text{act}}(P_1) = \sum_a \sum_d [\text{Cf}(P_1, a, d) \times \text{Sceff}(a, d)] \quad (8)$$

If we consider that two chain breaks per protomer, located anywhere, are needed to inactivate a protomer, chain breakage is still relevant to the one-hit model, but with $p = P_2 = 1 - e^{-r} - re^{-r}$, and the fraction of protomer that keeps a full ability to bind DNA is:

$$\text{Tet}_{\text{act}}(P_2) = \sum_a \sum_d [\text{Cf}(P_2, a, d) \times \text{Sceff}(a, d)] \quad (9)$$

RESULTS

Repressor inactivation

Fig. 3 (*top* and *middle*) shows the experimental results concerning either repressor irradiated alone with γ -rays and argon ions, and repressor irradiated with γ -rays in the complex with one operator-bearing fragment. In the three cases the experimental data fit a sigmoidal curve.

Varying k_1 and k_2 for the two-hit model, and k for the one-hit model, we have calculated the root-mean-square deviation (rmsd):

$$\text{rmsd} = \sqrt{\frac{\sum (y - y_{\text{exp}})^2}{N}}$$

between the N experimental points (y_{exp}) and the calculated values (y) at the same doses using Eqs. 3 and 6. The values are plotted against k_1 and k_2 , or against k , to obtain the best fit with the proposed models (Fig. 2, *bottom*).

Considering the one-hit model, the best fits were obtained for $k = 0.0085$, 0.0065 , and 0.0020 for repressor irradiated alone with γ -rays, with argon ions, and irradiated in the complex with γ -rays. Table 3 shows the values of the rmsd.

Considering the two-hit model, the best fits were obtained for $k_1 = 0.007$ and $k_2 = 0.010$ for the *lac* repressor irradiated alone with γ -rays, $k_1 = k_2 = 0.007$ for the *lac* repressor irradiated alone with argon ions, and $k_1 = 0.0016$

TABLE 3 Values of the constants for either k (one-hit model) or k_1 and k_2 (two-hit model), corresponding to the best fit of the experimental data of the repressor inactivation by both models

Radiation	Repressor	Model	rmsd	k	
				k_1	k_2
γ	alone	1 hit Eq. 6	0.147	0.0085	
		2 hits Eq. 3	0.049	0.0070	0.0100
	in the complex	1 hit Eq. 6	0.125	0.0020	
		2 hits Eq. 3	0.040	0.0016	0.0032
Ar	alone	1 hit Eq. 6	0.155	0.0065	
		2 hits Eq. 3	0.044	0.0070	0.0070

and $k_2 = 0.0032$ for the protein irradiated by γ -rays in the complex. The rmsd are also given in Table 3.

We immediately observe that the two-hit model better describes the experimental data because the rmsd are three or four times smaller than for the one-hit model.

Peptide chain cleavage

Fig. 4 (*top*) shows the experimental results concerning the remaining intact protomers after either γ -ray or argon ion irradiation.

Calculations to fit the experimental data were performed using Eq. 7. The best fits are obtained for $k_c = 0.0060$ and 0.0095 for γ -rays and argon ions, respectively (Fig. 4, *top*). The values of the rmsd for the best fits are given in Table 4.

Fig. 4 (*bottom*) shows the calculations of the fraction of active tetramers according to Eqs. 8 and 9, considering that either one or two chain breakage(s) inactivate a protomer. For these calculations we used the k_c values determined in the top of the figure, i.e., the values that give the best fits for the chain breaks induction. We observe that the fit is very bad (see rmsd in Table 4). Thus, considering the chain breaks as the unique cause of repressor inactivation (one or two per protomer) is not suitable.

DISCUSSION

As shown in Fig. 3 and Table 3, the two-hit model of repressor inactivation very satisfactorily accounts for the experimental results, for both γ and heavy ion irradiation, and for repressor irradiated either free or complexed to its operator DNA. Two damages on a protomer, and not only one damage, are necessary to account for the experimental results.

The proposed model based on Poisson's law for the mean number of breaks per protomer also accounts very well for the experimental results (Fig. 4, *top*, and Table 4). In fact, not all peptide bonds are equally susceptible to breaking (hypothesis 11), because of differences of accessibility to

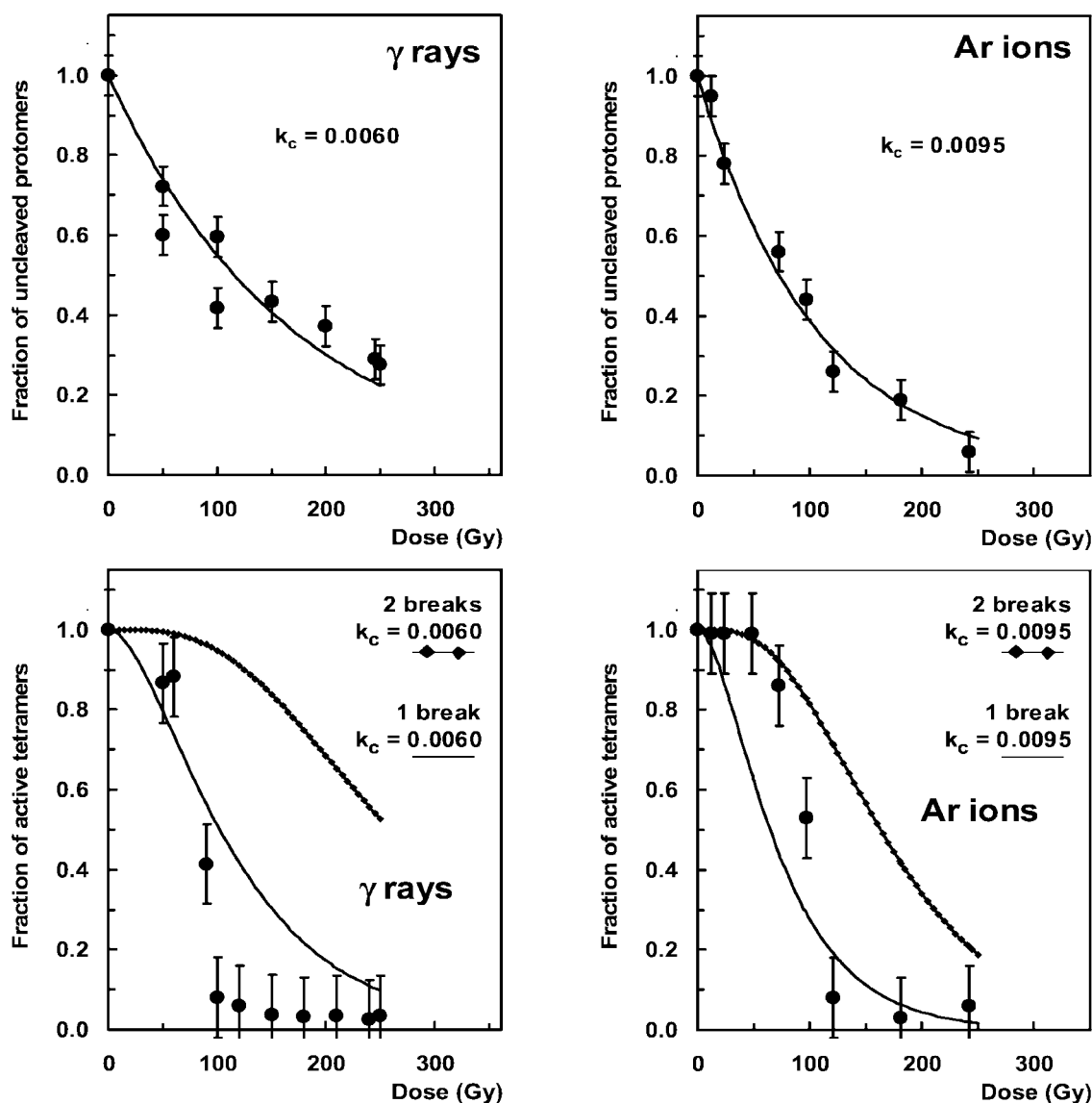


FIGURE 4 Top: filled circles, fraction of uncleaved protomers measured by polyacrylamide gel electrophoresis, as a function of dose. In both cases, the repressor was irradiated alone (free molecules). Solid lines, calculation of the nonbroken protomers according to Eq. 7 using k_c values determined by minimizing the rmsd. Bottom: filled circles, experimental values of the fraction of active tetramers as a function of dose. Solid lines, fraction of active tetramers calculated using Eq. 8 (one-hit model, one break) and the k_c values deduced from the experiments (top curves). Lines and diamonds, the same using Eq. 9 (one-hit model, two breaks).

radical attack due to the folding of the protein (Maleknia et al., 2001). The number of breakable bonds is smaller than 359, probably of the order of several tens. At the dose that totally inactivate the repressor (150 Gy), the average number of breaks per protomer, r , is equal to 0.9 for γ -rays and 1.4 for argon ions. These numbers remain smaller than the number of possible chain breaks per protomer. Therefore, the use of Poisson's law is justified (hypothesis 12).

As shown in Fig. 4 (bottom) and Table 4, the chain breaks ($k_c = 0.0060$ and 0.0095 for γ -rays and argon ions, respectively) cannot account for the repressor inactivation. Such a noninvolvement of the peptide chain cleavage in the inac-

TABLE 4 Values of the k_c constants in the peptide chain cleavage model

	Radiation	Model	rmsd	k_c
A*	γ	Eq. 7	0.072	0.0060^\ddagger
	Ar		0.042	0.0095^\ddagger
B†	γ	1 break Eq. 8	0.212	0.0060
		2 breaks Eq. 9	0.593	
	Ar	1 break Eq. 8	0.208	0.0095
		2 breaks Eq. 9	0.273	

*A, values of the k_c (‡) and rmsd corresponding to the best fit of the experimental data of the noncleaved protomers with the peptide chain cleavage model.

†B, values of rmsd obtained in using the preceding constants k_c^\ddagger to explain repressor inactivation by chain breakage (one break and two breaks).

tivation process is very intriguing, but if we consider that the core is 5 times larger than the headpiece, we may assume that the number of breakable bonds on the surface of the headpiece should be $5^{2/3} = 3$ times smaller than on the surface of the core. After 150 Gy irradiation that totally inactivate the repressor, the headpieces may contain 0.22 and 0.35 chain breaks. Even if they would have an inactivating effect, these chain breaks are not abundant enough to explain the observed inactivation of the repressor. We cannot exclude their influence, but they are obviously not the most important damages in the inactivation process. We have thus to consider damages other than chain breaks to explain the repressor inactivation.

The radiolytic damage to the proteins in aqueous solution (as for other solutes in general) may occur through direct effects, when the protein is directly ionized, and through indirect effects when the protein is attacked by the reactive species (OH and H radicals, hydrated electron, H_2O_2 , H_2 , ...) issued from the radiolytic decomposition of water (Ferradini and Jay-Gerin, 1999). In the present case, for dilute air-saturated aqueous solutions, we assume that direct effects may contribute to the damage for high LET radiation, i.e., argon ions, but do not occur for low LET radiation, i.e., γ -rays (Roots et al., 1990).

The two damages per protomer responsible for the repressor inactivation may result from the oxidation of amino acid side chains, probably located in the DNA-binding domain of the protein, i.e., the headpieces. The sequence of the 59 N-terminal amino acids of the headpiece contains 4 tyrosines, 2 methionines, and 1 histidine:

Met(1)-Lys-Pro-Val-Thr-Leu-**Tyr(7)**-Asp-Val-Ala-Glu-**Tyr(12)**-Ala-Gly-Val-Ser-**Tyr(17)**-Gln-Thr-Val-Ser-Arg-Val-Val-Asn-Gln-Ala-Ser-**His(29)**-Val-Ser-Ala-Lys-Thr-Arg-Glu-Lys-Val-Glu-Ala-Ala-**Met(42)**-Ala-Glu-Leu-Asn-**Tyr(47)**-Ile-Pro-Asn-Arg-Val-Ala-Gln-Gln-Leu-Ala-Gly-Lys-

The reaction rate constants of the OH radicals with Tyr, Met, and His are 1.3×10^{10} , 8.5×10^9 , and $5.0 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, respectively (Buxton et al., 1988). Looking at the structure of the complexes obtained either by NMR (Slijper et al., 1996; Spronk et al., 1999) or by x-ray crystallography (Lewis et al., 1996; Bell and Lewis, 2000), we observe that Tyr(7), Tyr(17), and His(29) have their accessibility to the OH radical significantly reduced when DNA is bound to the protein (Eon et al., 2001). This strongly suggests their implication in the binding process, and marks them out as possible critical targets of the radiolytic attack.

The DNA-binding activity of the repressor is more sensitive to γ -rays than to argon ions. The ratio of the D_{50} (dose at 50% inactivation) is equal to 1.2, and the constants k_1 and k_2 for the protomers inactivation are larger for γ -rays than for argon ions. This could be due to the fact that the OH radical yield is smaller for high LET particles (argon ions) than for γ -rays (Ferradini and Jay-Gerin, 1999): in the

tracks of the heavy ions, the density of ionization, and consequently the radical concentration, is considerably higher than in the tracks of the Compton electrons produced by the γ -rays. Therefore, recombination is more efficient and the concentration of available radicals is smaller. This strengthens the idea that inactivation of the repressor is mainly due to amino acid side-chain oxidation by OH radicals. Because cleavage events can be ruled out of court, the oxidation of amino acids should be the critical event.

However, the sensitivity to chain cleavage is 1.6 times larger with argon ions, as deduced from the ratio of the D_{50} and the k_c constants. Because direct effects could be involved in the damage by high LET radiation (argon ions), one may conclude that the excess of chain cleavage for argon ions may be due to direct effects.

Comparing the D_{50} , the repressor irradiated in the complex with γ -rays appears 3.7 times less sensitive than repressor irradiated alone, as also shown by comparing the two sets of constants (0.0016–0.0032 and 0.0070–0.0010). This protection of the protein by the bound DNA has been discussed in a previous paper (Eon et al., 2001). Tyr(7), Tyr(17), and His(29) are protected against solvent (and radical) accessibility in the complex, and they are particularly sensitive to oxidation by OH radicals. Such a protection of a protein by the bound DNA has been already observed with the CAP protein by Heyduk and Heyduk (1994) and Baichoo and Heyduk (1999).

In conclusion, the elaboration of simple models based on the known functioning and structure of a protein may orient further investigations concerning the radiolytic-induced damages on this protein. Such a phenomenological analysis does not allow one to identify the damages responsible for the protein inactivation, but may establish a hierarchy in some possible chemical modifications.

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