Evidence for the presence of an essential arginine residue in photoreactivating enzyme from *Streptomyces griseus**

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Butane-2,3-dione inhibits the enzymic activity of *Streptomyces griseus* photoreactivating enzyme (PRE). Some characteristics of the inhibition, notably the enhancement by borate buffer and the reversibility, indicate that arginine residues are modified. From the kinetics of inhibition it can be concluded that a single essential arginine residue is involved. U.v.-irradiated DNA, the substrate for PRE, protects the enzyme against inactivation by butane-2,3-dione. This suggests that the essential arginine residue is situated in or near the u.v.-irradiated-DNA-binding site. Non-irradiated DNA at higher concentrations also protects against inactivation, indicating that PRE can form non-specific complexes. From the ratio of complex constants obtained from protection experiments with non-irradiated and u.v.-irradiated DNA it appears that PRE preferably binds to dimer sites.

Irradiation of cells with u.v. light induces as a major injury the formation of dimers from two adjacent pyrimidine bases in the DNA, causing mutations and killing of the cells. Several repair mechanisms are known through which a cell can cope with these u.v.-induced lesions. Best characterized are excision repair and enzymic photoreactivation. In excision repair, mediated by at least three different enzymic activities, part of the DNA strand containing the lesion is excised and replaced by newly synthesized DNA. In photoreactivation a single enzyme called 'photoreactivating enzyme' (PRE or photolyase, EC 4.1.99.3) binds to the DNA lesion and repairs it through a photochemical process with the help of visible or near-u.v. light. Enzymic photoreactivation specifically repairs pyrimidine dimers, which are monomerized into the constituent pyrimidine bases.

Gradually more knowledge is being obtained about the structure of PRE and its mechanism of action. However, the mechanism and specificity of binding to the dimer regions in u.v.-irradiated DNA is largely unknown. The binding seems to be

mainly electrostatic by nature, since at high ionic strength the complex readily dissociates. It was also found that PRE from the actinomycete *Streptomyces griseus* firmly binds to Blue Sepharose (Eker, 1980). A simple electrostatic interaction with the sulphonate groups of the Cibacron Blue moiety is unlikely, since the enzyme carries a negative net charge at pH 7. The binding can be explained by assuming an interaction between the dye and an anion-binding site in a specific part of the enzyme (e.g. the polynucleotide-binding site). This incited me to investigate the possible presence of essential arginine residues in PRE by means of specific chemical modification.

Experimental

Materials

Butane-2,3-dione was obtained from Merck, cyclohexane-1,2-dione from Aldrich and phenylglyoxal hydrate from Fluka. Borate buffer contained 40 mM-NaCl and 50 mM-boric acid, pH 8.0. Modification buffer was borate buffer containing 5 mM-2-mercaptoethanol and 0.1% bovine serum albumin. Hepes buffer contained 40 mM-NaCl, 10 mM-Hepes, pH 8.0, 5 mM-2-mercaptoethanol and 0.1% bovine serum albumin.

For each experiment a fresh stock solution of 20 mM-butane-2,3-dione in borate buffer was prepared, after which the pH was adjusted to 8.0.

Abbreviation used: PRE, photoreactivating enzyme.

* Part 5 of a series of papers on the topic 'studies on a DNA photoreactivating enzyme from *Streptomyces griseus*'; previous parts are identified as follows: 1, Piessens & Eker (1975); 2, Eker & Fichtinger-Schepman (1975); 3, Eker (1980); 4, Eker *et al.* (1981).

Highly purified PRE was isolated from *Streptomyces griseus* cells as described previously (Eker & Fichtinger-Schepman, 1975). DNA was isolated from *Escherichia coli* B cells by the method of Marmur (1961), with an additional Pronase-incubation step. DNA solutions were irradiated with a General Electric G4T4-1 germicidal lamp emitting mainly 254 nm, at a fluence rate of $92 \text{ J} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$ as measured with a photovoltaic u.v.-meter (Ultraviolet Products).

Methods

Modification

PRE was diluted 100-fold in modification buffer, and, in the case of protection experiments, mixed with (u.v.-irradiated) DNA and preincubated 15 min at 20°C . Modification was started by the rapid mixing of PRE and butane-2,3-dione solutions, followed by incubation in a 20°C water bath. The reactants were previously equilibrated to this temperature. Routinely, modification was carried out in dim light with a black cloth wrapped around the water bath. At indicated times, $20 \mu\text{l}$ samples were taken from the modification mixture, immediately diluted 25-fold with modification buffer and kept in the dark at 4°C until enzyme activity was measured.

Reversal of modification

In order to study the reversal of inhibition of enzyme activity, $150 \mu\text{l}$ of a modification mixture was chromatographed on a column ($1 \text{ cm} \times 7.7 \text{ cm}$) of Sephadex G-25 equilibrated and eluted with Hepes buffer. The fraction containing PRE (1.5–3.5 ml elution volume) was collected and tested for enzyme activity after a suitable period of incubation.

Assay of photoreactivating activity

The activity of PRE was measured with a microbiological assay (for details, see Piessens & Eker, 1975). To the diluted samples withdrawn from the modification mixture u.v.-irradiated *Haemophilus influenzae* transforming DNA was added. This mixture was divided into two equal parts: one part was incubated in the dark, while the other was illuminated with blue fluorescent lamps (Philips TL ADK 30W/03) for 30 min at 35°C . During this illumination the PRE repairs u.v.-induced pyrimidine dimers in the transforming DNA, restoring the biological activity of the streptomycin-resistance marker which was inactivated by u.v. irradiation. Subsequently, to both 'dark' and 'light' samples, competent streptomycin-sensitive *Haemophilus influenzae* cells were added, followed by transformation to streptomycin resistance. Suitable samples were plated on strep-

tomycin-containing agar and, after 48 h of incubation, transformants were counted. The photoreactivating activity is calculated as:

$$(N_L/N_D - 1) \cdot \text{DF}$$

where N_D and N_L are the number of transformants in dark-incubated and illuminated samples respectively and DF is the enzyme dilution factor (Muhammed, 1966).

Kinetics of inactivation

We assume the following simplified reaction scheme:

in which E is the PRE, M the modifying agent (butanedione), P the protecting agent (u.v.-irradiated DNA), E_i the inactive modified enzyme, EP the enzyme-protector complex, and n and m are the reaction order of M in the inactivation reaction of E and EP respectively. The total amount of active enzyme, E_a , is defined as:

$$[\text{E}_a] = [\text{E}] + [\text{EP}] \quad (1)$$

(a) *No protection.* The kinetics of the inactivation process can be described by:

$$\ln[\text{E}_a]/[\text{E}_{a0}] = -k_{+1} \cdot [\text{M}]^n \cdot t \quad (2)$$

in which t is the inactivation time and $[\text{E}_{a0}]$ is $[\text{E}_a]$ at zero time, if it is assumed that the back-reaction (reversal of modification) is negligible under normal conditions. Provided that an excess of M is used, experimentally first-order kinetics will be found with the pseudo-first-order rate constant:

$$k_{\text{app}} = k_{+1} \cdot [\text{M}]^n \quad (3)$$

which yields, after taking logarithms:

$$\log k_{\text{app}} = n \cdot \log [\text{M}] + \log k_{+1} \quad (4)$$

Therefore the reaction order, n , can be obtained from the slope of a double-logarithmic plot (Levy *et al.*, 1963).

(b) *Protection.* When a protector is present the kinetics of inactivation can be described by:

$$\ln[\text{E}_a]/[\text{E}_{a0}] = - (k_{+1} \cdot K_d \cdot [\text{M}]^n + k_{+2} \cdot [\text{P}] \cdot [\text{M}]^m) \cdot t / (K_d + [\text{P}]) \quad (5)$$

in which K_d is the dissociation constant of the enzyme-protector complex. Experimentally first-order kinetics will be found provided that $[\text{P}]$ and $[\text{M}]$ are large enough to be considered constant

during the reaction, with a pseudo-first-order rate constant:

$$k_{app}^p = (k_{+1} \cdot K_d \cdot [M]^n + k_{+2} \cdot [P] \cdot [M]^m) / (K_d + [P]) \quad (6)$$

After rearrangement and combination with eqn. (3), eqn. (7) is obtained:

$$k_{app}^p / k_{app} = (k_{+2} \cdot [M]^{m-n} / k_{+1} + K_d \cdot [1 - (k_{app}^p / k_{app})] / [P]) \quad (7)$$

in which k_{app}^p and k_{app} are the pseudo-first-order rate constants with and without protection respectively (Scrutton & Utter, 1965). A plot of k_{app}^p / k_{app} against $[1 - (k_{app}^p / k_{app})] / [P]$ will give a straight line with slope K_d . If the line goes through the origin, $k_{+2} \cdot [M]^{m-n} / k_{+1} = 0$, meaning that the enzyme-protector complex EP is fully protected against inactivation by M.

Free and bound enzyme

The complex-dissociation constant of the PRE-DNA complex is defined as:

$$K_d = [E] \cdot [D] / [ED] \quad (8)$$

where $[E]$ and $[ED]$ are the concentrations of free and bound enzyme and $[D]$ is the DNA concentration expressed as molar nucleotide concentration. Since in our experiments $[D] \gg [E]$, the following derivation is possible:

$$[ED] / [E_a] = [D] / ([D] + K_d) \quad (9)$$

permitting the calculation of the relative amounts of free and bound enzyme.

Distribution over specific and non-specific binding sites

When PRE is complexed to u.v.-irradiated DNA, some of the enzyme molecules will be complexed to the dimer sites whereas others will be non-specifically bound to the DNA. The complex-dissociation constant of the specific complex is defined as:

$$K_s^d = [E] \cdot [U] / [EU] \quad (10)$$

where $[EU]$ is the concentration of specific complexes and $[U]$ the concentration of dimer sites. Combination of eqns. (8) and (10) yields:

$$[ED] / [EU] = (K_s^d \cdot [D]) / (K_d \cdot [U]) \quad (11)$$

The ratio of the dissociation constants of non-specific and specific complexes is called the 'equilibrium specificity ratio' (ESR) (Lin & Riggs, 1975; Strauss *et al.*, 1980). Substitution in eqn. (11) yields:

$$[EU] / [ED] = \text{ESR} \cdot [U] / [D] \quad (12)$$

With eqn. (12) the distribution of enzyme molecules over specific and non-specific sites can be

readily calculated, provided that the enzyme concentration is low compared with the concentration of specific sites.

Results

Choice of arginine-modifying reagent

PRE from *Streptomyces griseus* was treated with different arginine-modifying agents. Straight lines were obtained in a semi-logarithmic plot of residual enzyme activity, measured with the *H. influenzae* transformation assay, as function of incubation time (Fig. 1), indicating that inactivation follows pseudo-first-order kinetics. By using the same reagent concentration (8 mM) butane-2,3-dione (pseudo-first-order rate constant, $k_{app} = 0.163 \text{ min}^{-1}$) inactivated PRE more rapidly than phenylglyoxal ($k_{app} = 0.022 \text{ min}^{-1}$), whereas cyclohexane-1,2-dione had no appreciable influence.

Since butane-2,3-dione in borate buffer is probably the most selective reagent for arginine residues in proteins (Riordan, 1979), it was preferred to other reagents for further use, particularly in view of the fact that diluted PRE is less stable under the prolonged-incubation conditions needed when less active reagents are used. The same applies for the more alkaline pH values used to increase the rate of inactivation in the case of phenylglyoxal or cyclohexane-1,2-dione (Riordan, 1979).

Characteristics of inactivation by butane-2,3-dione

Incubation of PRE with different concentrations of butane-2,3-dione yielded straight lines in

Fig. 1. Time course of inactivation of PRE by different arginine-modifying agents

S. griseus PRE was treated with 8 mM-butane-2,3-dione (○), phenylglyoxal (□) or cyclohexane-1,2-dione (△) in borate modification buffer, pH 8.0.

the semi-logarithmic plot down to 25% of residual activity (Fig. 2). From the slope of the plots the following pseudo-first-order rate constants $k_{app.}$ (in min^{-1} at the indicated butane-2,3-dione concentrations) were obtained: 0.176 (10mM), 0.139 (7.5mM), 0.099 (5mM), 0.049 (2.5mM) and 0.021 (1mM).

The inactivation was slower when Hepes buffer was used instead of borate (Fig. 2): compare $k_{app.} = 0.036 \text{ min}^{-1}$ in Hepes with 0.099 min^{-1} in borate. This is characteristic for the modification of arginine residues (Riordan, 1979). Another characteristic of arginine modification by butane-2,3-dione in borate is the reversibility of inactivation after removal of borate and reagent (Riordan, 1973). Indeed, we found restoration of enzyme activity after removal of butane-2,3-dione and exchange of borate with Hepes buffer by gel filtration (Table 1). On the other hand, when the modification mixture was diluted to the same extent with Hepes buffer, decreasing the borate and butane-2,3-dione concentration to one-tenth of the original value, no reversal of inactivation was found, indicating that the reversal is not a dilution effect. These results strongly suggest that inactivation of the enzyme is due to modification of one or more arginine residues.

When the $k_{app.}$ values obtained from Fig. 2 are plotted against the butane-2,3-dione concentration in a double-logarithmic plot according to eqn. (4), a straight line is obtained with slope 0.93 (Fig. 3).

Fig. 2. Time course of inactivation of PRE by different concentrations of butane-2,3-dione

PRE was inactivated with butane-2,3-dione concentrations as indicated in the Figure (in mM). Either borate modification buffer (—) or Hepes buffer (---), both at pH 8.0, was used. From the slope of the lines, pseudo-first-order rate constants were obtained.

With the assumption that the stoichiometry of the reaction between butane-2,3-dione and arginine is 1:1 (Riordan, 1973), this indicates that the reaction order, n , is 1, i.e. PRE is inactivated by modification of a single essential arginine residue. From the intercept of the y -axis in Fig. 3, a value for k_{+1} of 12.8 min^{-1} is calculated.

Protection against inactivation

The addition of u.v.-irradiated DNA, the substrate of photoreactivating enzyme, resulted in considerable protection against inactivation (Fig.

Table 1. Reversal of inactivation

S. griseus PRE was inactivated with butane-2,3-dione under the indicated conditions. The butane-dione-containing borate buffer was changed for a Hepes buffer without butanedione by passage over a small Sephadex G-25 column. The eluate was incubated for 1 h at 20°C , followed by determination of the remaining enzyme activity.

[Butanedione] (mM)	Modification time (min)	Enzyme activity (% of initial)	
		After modification	After buffer change
0	30	100	94
10	15	10	115
10	30	3	87

Fig. 3. Determination of reaction order

The pseudo-first-order rate constants, $k_{app.}$, obtained from Fig. 2 were plotted against the butanedione concentration in a double-logarithmic plot according to eqn. (4). A slope of 0.93 was found by linear regression, indicating that the reaction order for butane-2,3-dione is 1.

4). It is therefore reasonable to assume that the essential arginine residue modified by butane-2,3-dione is situated in or near the u.v.-irradiated-DNA-binding site of PRE.

In order to investigate whether non-irradiated DNA also protects against inactivation, DNA from another source had to be used, since small amounts of non-irradiated *H. influenzae* DNA will disturb the transformation assay by giving rise to a high background of transformants not related to photoreactivation. The small amount of u.v.-irradiated *H. influenzae* DNA used in the experiment depicted in Fig. 4 gave no problems. In the following experiments, therefore, *Escherichia coli* DNA was used.

Addition of non-irradiated *E. coli* DNA to the modification mixture resulted likewise in a considerable protection against inactivation, although higher concentrations had to be used compared with u.v.-irradiated DNA. From this it can be concluded that u.v.-irradiated DNA gives better protection than non-irradiated DNA. A plot of pseudo-first-order rate constants against DNA concentration according to eqn. (7) yields a straight line through the origin (Fig. 5), pointing out that the enzyme-DNA complex is completely protected against inactivation. From the slope of the plot a value for the complex-dissociation constant (K_d) of $1.7 \times 10^{-5} \text{ M}$ is calculated.

The same experiment was done with different amounts of u.v.-irradiated *E. coli* DNA. Again the plot according to eqn. (7) yields a straight line through the origin (Fig. 6), indicating full protec-

tion against inactivation. From the slope of the plot a K_d value of $2.5 \times 10^{-9} \text{ M}$ is obtained for the enzyme-u.v.-irradiated-DNA complex. Essentially the same results were obtained when the u.v.-irradiated-DNA concentration was kept constant but different pyrimidine dimer concentrations were used by varying the applied u.v. fluence (Fig. 7); this yielded a K_d value of $2.1 \times 10^{-9} \text{ M}$.

Discussion

Only very few studies have been published concerning the chemical modification of PREs. The presence of an essential thiol group has been established in PRE from *Saccharomyces cerevisiae* (Madden & Werbin, 1974) and *Anacystis nidulans* (Saito & Werbin, 1970). In short reports the modification of tryptophan, histidine and arginine (Hill *et al.*, 1975) and lysine and tryptophan residues (Kim *et al.*, 1977) in *S. cerevisiae* PRE has been mentioned. The interpretation of most of these results, however, was not unambiguous, since some of the reagents used can also react with thiol groups.

Fig. 4. Protection against inactivation by u.v.-irradiated DNA

PRE was incubated with 3.5 mM-butane-2,3-dione in borate modification buffer in the presence of 0 (○), 0.063 (□) or 0.188 (△) µg of *H. influenzae* DNA/ml irradiated with $580 \text{ J} \cdot \text{m}^{-2}$.

Fig. 5. Determination of the dissociation constant of the PRE-DNA complex

PRE was inactivated by 3.5 mM-butane-2,3-dione in borate modification buffer in the presence of 0, 2.5, 7.5, 15 or 22.2 µg of unirradiated *E. coli* DNA/ml. Pseudo-first-order-rate constants, k_{app} , in the absence and k_{app}^p , in the presence of protector (DNA), were obtained from semi-logarithmic plots of residual enzyme activity against incubation time and plotted as function of the protector concentration $[P]$ expressed as molar nucleotide concentration according to eqn. (7). The dissociation constant, K_d ($= 1.7 \times 10^{-5} \text{ M}$) was obtained from the slope of the plot.

Fig. 6. Determination of the dissociation constant of the PRE-u.v.-irradiated-DNA complex

PRE was inactivated by 3.5 mM-butane-2,3-dione in borate modification buffer in the presence of 0, 0.0673, 0.225, 0.673 or 2.25 μg of *E. coli* DNA/ml irradiated with $551 \text{ J} \cdot \text{m}^{-2}$. The protector concentration, [P], is expressed as the molar pyrimidine dimer concentration, which was calculated on the assumption that $1 \text{ J} \cdot \text{m}^{-2}$ (254 nm) induces 7×10^{-6} pyrimidine dimers/nucleotide (Rupp & Howard-Flanders, 1968; Unrau *et al.*, 1973; Patrick & Harm, 1973; Patrick, 1977). A value for K_d of $2.5 \times 10^{-9} \text{ M}$ was obtained for the complex-dissociation constant. See also the legend to Fig. 5.

Butane-2,3-dione is known to react under moderate conditions quite specifically with arginine residues in proteins, although in some reports reaction with cysteine, lysine or histidine residues under more drastic conditions has been mentioned (Riordan, 1979). Specificity and reaction rate can be increased by using borate buffer in the reaction mixture. The inactivation of *S. griseus* PRE by both butane-2,3-dione and phenylglyoxal as well as the dependency of the rate of inactivation on the presence of borate buffer and the reversal of inactivation after removal of butane-2,3-dione and borate strongly suggest that the inactivation of PRE by α -dicarbonyl compounds is due to the modification of one or more arginine residues. Direct evidence, for instance from amino acid analysis, could not be obtained, owing to the small amount of PRE available.

Special care was taken to avoid photodynamic inactivation of the enzyme during modification or enzyme assay. This is crucial, because α -dicarbonyl compounds are known to act as photosensitizers, reacting with amino acids other than arginine (Mäkinen & Mäkinen, 1982a). The use of

Fig. 7. Determination of the dissociation constant of the PRE-u.v.-irradiated-DNA complex

PRE was inactivated by 3.5 mM-butane-2,3-dione in borate modification buffer in the presence of 0.673 μg of *E. coli* DNA/ml irradiated with 0, 138, 275, 460, 550, 825 or $1100 \text{ J} \cdot \text{m}^{-2}$. From the slope of the plot, a K_d value of $2.1 \times 10^{-9} \text{ M}$ was obtained. See also the legends to Figs. 5 and 6.

visible light in the wavelength range where photosensitization can occur is unavoidable in the type of enzyme assay we used, since the action spectrum for photoreactivation (Eker, 1983) and the long-wavelength absorption band of butane-2,3-dione (Mäkinen & Mäkinen, 1982b) considerably overlap each other. Therefore modification mixtures were kept in the dark during preparation and incubation. Before the illumination step in the enzyme assay the samples were diluted to keep the butane-2,3-dione concentration low. Moreover, the illumination of PRE/u.v.-irradiated DNA mixtures was performed in the presence of 2.5 mM-2-mercaptoethanol, which is known to protect proteins from photodynamic inactivation by α -dicarbonyls (Mäkinen *et al.*, 1982). Hence it is very unlikely that any photodynamic inactivation had taken place.

Essential arginine residues have now been recognized in a large variety of enzymes (Schneider, 1978; Riordan, 1979) in which an arginine residue functions as an anion-recognition site for carboxylate or phosphate groups of cofactors or substrates (Riordan *et al.*, 1977). Besides, arginine-modifying agents appear to have appreciable preference for arginine residues in anion-binding sites over other exposed arginine residues (Patthy & Thesz, 1980). Essential arginine residues have also been reported in several polynucleotide-binding enzymes like DNA poly-

merase, RNA polymerase, reverse transcriptase and ribonuclease (Takahashi, 1968; Patthy & Smith, 1975; Borders *et al.*, 1975; Salvo *et al.*, 1976; Armstrong *et al.*, 1976; Lee & Chirikjian, 1979; Srivastava & Modak, 1980). In some cases evidence was gained that the arginine residues are situated in the polynucleotide-binding site of the enzyme. Two types of interaction between an arginine residue and a polynucleotide chain can be distinguished. Arginine residues can bind non-specifically to phosphate groups (Cotton *et al.*, 1973; Lange *et al.*, 1974; Furberg & Solbakk, 1974; Salunke & Vijayan, 1981). Arginine residues, together with other amino acids, may also be involved in a specific interaction with certain base sequences of nucleic acids, for instance through hydrogen bonds between guanine and the guanidinium group of arginine (Helene, 1977; Lancelot *et al.*, 1979). For PRE, no specific interaction with DNA base sequences is known, nor to be expected, since the enzyme seems to recognize dimer sites irrespective of the nature of the neighbouring nucleotides. A specific interaction between an arginine residue and a pyrimidine dimer, for instance, through the carbonyl groups at position 2 of the pyrimidine rings, could be possible. On the other hand, PRE does not bind to isolated pyrimidine dimers (Rupert, 1964); binding is only found when the dimer is incorporated in an oligonucleotide of at least nine nucleotides (Setlow & Bollum, 1968). This suggests that, besides the dimer, other nucleotides are involved in binding. From the present results it cannot be decided whether the essential arginine residue is involved in a specific interaction with the dimer or in a non-specific interaction with DNA phosphate groups, although the latter seems to be more likely as non-irradiated DNA also protects against inactivation.

S. griseus PRE turns out to have a substantial affinity for non-irradiated DNA. In living cells the DNA concentration is relatively high: a value of 44 mM can be calculated for *Streptomyces* species if one assumes a cell volume of $0.5 \mu\text{m}^3$ and a genome size of 4 GDa (Gladek & Zakrewska, 1984), and supposes an even distribution along the cell (which, of course, is not the case). Hence it can be estimated from eqn. (9) that nearly all PRE molecules are bound to DNA in cells, even if it is taken into account that, *in vivo*, K_d may have a higher value, owing to differences in ionic strength. Complexation to DNA will greatly enhance the stability of PRE (Eker, 1980).

Dissociation constants for the PRE-u.v.-irradiated-DNA complex have been obtained from separately determined reaction rate constants of association and dissociation reactions of the complex equilibrium. For PRE from *Saccharomyces cerevisiae*, a K_d of 5.3×10^{-11} M was obtained

in experiments *in vitro* (Harm & Rupert, 1970; Madden & Werbin, 1974), whereas for *E. coli* cells a K_d of $(1.7-12) \times 10^{-9}$ M was found, depending on the method of determination used for the dissociation rate constant (Harm, 1970). The value we obtained for *S. griseus* PRE *in vitro* ($K_d = 2.3 \times 10^{-9}$ M) is closer to that found for *E. coli* than to that for *S. cerevisiae* PRE.

For a number of DNA-binding proteins, complex constants have been estimated. In general they are strongly dependent on ionic strength, temperature and pH, so it is difficult to compare them with the values we obtained for PRE. However, the equilibrium specificity ratio is much less dependent on the parameters mentioned above. For *E. coli* RNA polymerase (holoenzyme), an approximate equilibrium specificity ratio of 10^4 was obtained (Strauss *et al.*, 1980), whereas for *EcoRI* restriction endonuclease and for *E. coli lac* repressor values of 2×10^5 (Clare *et al.*, 1982) and 2×10^8 (deHaseth *et al.*, 1977; Barkley *et al.*, 1981) respectively were found. The value of 7×10^3 we calculate for *S. griseus* PRE compares well with that of RNA polymerase, but is certainly lower than that of the *lac* repressor, indicating a poorer specificity. This is not unexpected, since PRE will specifically bind to any site in the DNA as long as it contains a pyrimidine dimer, in contrast with the very specific binding target of the *lac* repressor.

The equilibrium specificity ratio can be used to calculate the distribution of enzyme molecules over specific and non-specific binding sites. For the experiment shown in Fig. 6, it can be calculated that 97% of the PRE molecules bound to DNA is complexed with dimer sites. At low u.v. fluences, however, an appreciable number of the enzyme molecules will be non-specifically bound to the DNA.

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