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# Kinetics of Cleavage of Intra- and Extracellular Simian Virus 40 DNA with the Enediyne Anticancer Drug C- 1027

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Keywords: Rate constants; Kinetic analysis; Intracellular DNA cleavage; Kinetic theory

**Abstract**

A kinetic analysis of cleavage of simian virus DNA (SV40 DNA) inside and outside green monkey BSC-I cells by the enediyne-protein antibiotic C-1027 and its free chromophore is described. Information on rate constants was obtained by fitting populations of forms I (closed circular DNA), II (nicked circular DNA) and III (linear DNA) of SV4f.J DNA as a function of drug concentration to a kinetic model which includes: cutting of form I to give form II with rate constant $k_1$. cutting of form I to give form III with rate constant $k_4$, and cutting of form II to give form III with rate constant $k_2$. Theratio of single-strand (ss) to double-strand (ds) cutting for the holoantibiotic and the free chromophore. $k_1/k_4$, is approximately 1.8 for extracellular SV40 DNA. For intracellular DNA and extracellular DNA which has been post-treated with putrescine, ds cutting is much more probable, with $k_4$, about four times as large as $k_1$. This observation suggests that amine groups present in the cell are able to convert abasic sites opposite an ss break into a ds break in SV40 chkomatin. The overall rate of cleavage of form-1 DNA inside the cell is much larger than the rate outside, the sum $k_1 + k_4$ being about three times as large for intracellular DNA as for extracellular DNA.

## 1. Introduction

Most studies on the mechanism of action of anticancer drugs have focused on the selectivity of the agents for specific sequences and on the details of their binding modes with DNA [l-3]. Since sequence specificities of clinically used anticancer drugs are relatively low [4] and different drugs with different binding modes produce the same end effect, i.e., the death of the cell, sequence specificity and the mode of interaction are probably less important than the rate at which the drug produces lesions in DNA. The cell, which is constantly suffetting damage from numerous sources, has available ‘repair processes for correcting lesions in DNA [5-8]. If the rate of damage by the drug exceeds the rate of repair by the repair systems, the cell will die. Clearly, measuring the rates of damage and repair in cancer and normal cells is an important determinant of the therapeutic efficacy of the drug.

SV40 DNA can exist in three forms: closed circu-**[end of page 201]** lar (form I), nicked circular (form II), and linear (form III). A nick on one strand by the drug C-1027 converts form I to form II. A second nick will convert form II to form III if it is on the opposite strand and is sufficiently close to the first nick. Previously, we studied cleavage of SV40 DNA inside BSC-1 monkey cells [9] and DNA in the nucleus and mitochondria of the episome-containing cell line, 935.1 [10], using the enediyne antitumor drug, C-1027 [11,12]. In this report we present the kinetic theory and measure rate parameters associated with cleavage of SV40 DNA inside and outside BSC-I green monkey cells using the holoantibiotic and the free chromophore of C-1027.

## 2. Materials and Methods

### 2.1. Chemicals and Reagents

The antitumor antibiotic C-1027 was supplied by the Taiho Pharmaceutical Co. (Tokushima, Japan). Stock solutions of the drug in water were stored at -20°C in the dark. SV40 DNA (strain 776) was used as obtained from GIBCO-BRL (Grand Island, NY). All DNA concentrations are stated in base pairs.

### 2.2. Cleavage of Extracellular SV40 DNA by the Holoantibiotic

Due to the photosensitivity of C-1027 [11], the following cleavage reactions were carried out under subdued light using a photographic safety light (Kodak GBX-2). Stock solutions of the drug in water (66 μM) were stored in the dark at -20°C until needed. The cleavage reactions were at room temperature in a total volume of 40 μ1 of buffer (30 mM Tris-acetate, 0.75 mM EDTA, pH 8.0) containing 63 μM of SV40 DNA. Reactions were performed using total final drug concentrations of (μM): 1.32, 1.19, 1.06, 0.92, 0.79, 0.66, 0.53, 0.40, 0.26, 0.13. After 15 min the DNA was separated by electrophoresis in a 1% agarose gel in 1 X TAE buffer. The gel was prepared by addition of 1 g of low melting agarose to 100 ml of 1 X TAE buffer. After heating to boiling, the solution was poured into the gel casting and cooled to room temperature [13]. Next, the DNA was visualized by soaking the gel in an ethidium bromide solution (0.5 μg $ml^{-1}$) for 2 h. Using these conditions the order of electrophoretic mobility in the gel is: form I (fastest moving) > form III > form II (slowest moving) [13]. The gel, without destaining, was placed on a UV transilluminator and photographed with a Polaroid camera using type-55 film. The exposure time was such that the maximum intensity of all bands was in the linear response range of the film. Linearity of measured intensity with DNA was checked by loading different amounts of DNA into the gel, and after electrophoresis, staining and photography, constructing plots of band intensity versus DNA concentration. The photographic negative was scanned with a Molecular Dynamics (model 300A) microdensitometer and band areas obtained using ImageQuant software. Under these conditions, closed circular form-I DNA stains with the same efficiency as the other two forms of DNA (Shubsda et al., unpublished). All cleavage experiments were performed using drug concentration ranges in which form-III DNA was not degraded into smaller fragments. In order to correct for errors due to loading, non-uniform illumination of the gel and vignetting due to the camera, the total amount of the three DNA forms in each lane were normalized to a constant. The normalization correction was always less than 15% of the total intensity in a given lane. Four separate determinations were performed (experiments A-D) involving cleavage of extracellular SV40 DNA.

Cleavage reactions for digest times of 15, 30 and 60 min were performed using the above DNA concentration for two different drug concentrations, 0.26 and 0.40 μM. For each concentration, the amounts of all three DNA forms were the same at all times, indicating that the cleavage reaction is complete within 15 min (data not shown).

The stability of C-1027 in water (2.64 μM) was examined by allowing the drug to stand at 25°C for 0, 0.25, 0.5, I, 2, 3, 6 and 12 h in the dark. The ability of the drug in each incubation solution to then cleave DNA was measured by adding it to SV40 DNA. The final concentrations in the reaction mixture were: SV40 DNA (25 μM), C-1027 (0.53 μM) in 40 mM Tris-acetate, 1 mM EDTA, pH 8.0. The cleavage reaction was allowed to proceed for 15 min and samples were separated as previously described. **[end of page 202]** Analysis showed that within experimental error, all incubation times produced the same amount of cleavage of SV40 DNA (data not shown).

### 2.3. Cleavage of Extracellular DNA by the C-1027 Chromophore

The chromophore of C-1027 was isolated from the protein under subdued lighting at 4°C. Methanol saturated with sodium acetate (100 μl) was added to 25 μ1 of a stock solution of the antibiotic (132 μM) in water followed by the addition of 25 μl of water. The suspension was vortexed and allowed to stand for 30 min on ice and the precipitated protein was separated from the methanol-water by centrifugation. Dilutions in methanol/sodium acetate of the methanol-water supernatant containing the chromophore (22 μM) were prepared and added to SV40 DNA. In the final reaction mixture of 10 μl were: SV40 DNA (5O μM) in 40 mM Tris-acetate, pH 8.0, I mM EDTA and 20% methanol. Assuming a 100% recovery, the final concentrations of chromophore were (μM): 4.40, 3.61, 3.21, 2.82, 2.42, 2.02, 1.63, 1.23. 0.84. and 0.44. After reaction for 15 min, all subsequent steps were carried out according to the procedure for the hoioantibiotic.

In order to compare the kinetics of cleavage of the holoantibiotic and the chromophore, the former was used to cleave SV40 DNA in solutions containing methanol. The final concentrations of C-1027, in a reaction volume of 10 μl containing 25 μM SV40 DNA. 40 mM Tris-acetate and 20% methanol, were as earlier stated for studies with the holoantibiotic and extracellular DNA (25 μM). After reaction for 15 min, all subsequent steps were carried out according to the procedure for the holoantibiotic in buffer without methanol described above.

### 2.4. Procedure to Reveal Abasic Sites

Cleavage of SV40 DNA was carried out in the manner described for the reaction of intra- and extracellular DNA with the holoantibiotic except that the total volume was 20 μl. Sufficient putrescine to give a final concentration of 90.5 mM was added to 10 μl of each reaction. After incubation at 37°C for 2 h, the putrescine-treated and non-treated samples were analyzed using agarose gel electrophoresis. As a control, drug-cleaved DNA was exposed to

90.5 mM putrescine for 0.5 to 12 h at 37°C. No change in the populations of the three DNA forms was noted, indicating that the amine-catalyzed decomposition of the abasic site is complete in 0.5 h (data hot shown).

### 2.5. Cleurlage of intracellular DNA b!, the holoatrtibiotic,

Maintenance of BSC-I (African green monkey kidney) cells was in Minimal Essential Medium, 10% calf serum (MEM). Infection with 5-15 plaque-forming units of SV40 virus per 6 X $10^5$ cells was as described elsewhere [14]. BSC-1 cells at 6 X $10^5$ per 35 mm plate were infected as described above with SV40 virus. Forty hours after infection, the plates were rinsed once and incubated for 30 min with fresh medium. Cells were treated for 2 h (37°C. 5% CO,) with C-1027 at 5O nM and 150 nM in 1 .O ml (total volume) of medium and then rinsed twice with phosphate-buffered saline (PBS). Preliminary experiments found that maximal C- l027-induced damage to intracellular supercoiled SV40 DNA was achieved within 2 h after treatment. In addition, 1% SDS completely inhibited C- l027-induced SV40 DNA strand damage. Reactions were terminated by adjusting samples to a final concentration of 1% sodium dodecyl sulfate (SDS) and 5O μg $ml^{-1}$ Proteinase K, incubating for 2 h at 37°C followed by the addition of 40 μg $ml^{-1}$ RNAse and incubating for an additional 2 h at 37°C. The cancentrations used in the 5O nM experiment were (nM): 0 (control). 1, 5, 1O, 15, 20, 25, 30, 35, 40, 45, 5O. In the 150 nM experiment, the concentrations were (nM): 0 (control). 5, 25, 5O, 62.5, 75, 87.5, 100, 112.5, 135, 137.5, 150.

### 2.6. Cleavage of Intracellular SV40 DNA by the C-1027 Chromophore

The chromophore of C-1027 was isolated from the protein as described above. Dilutions of the chromophore were made in the water-methanol mixture, and incubation and lysis of the SV40-infected BSC-1 cells was as with the holoantibiotic. The concentrations used for the chromophore assay were (nM): 0 (control), 1, 5, 10, 25, 5O, 100, 200, 250. **[end of page 203]**

## 3. Results

### 3.1. Kinetic theory

We fitted the fractional populations of forms I, II and III of SV40 DNA to the kinetic model described below by minimizing *D*, the sum of the squares of the differences between measured fractional populations and fractional populations calculated from the model. The Simplex or other search method was used to find the values of parameters (initial populations of the three species and rate constants) which minimize *D*.

The chromophore portion of C- 1027 is capable of causing both single (ss) and double (ds) strand cleavage of DNA [15- 17]. The rate processes considered are: form I → form II (ss), form II → form III (ss and ds) and form I → form III (ds). All rate expressions are assumed to be second order. In fact, the ss cleavage of form II to produce form III is not a single second-order reaction, but is instead many second-order reactions with different rate constants, corresponding to cleavage of form II with an assortment of nicks in it. It is apparently sufficient, for the experiments analyzed here, to represent all of them by using a single reaction with a single effective rate constant which is an average of the true rate constants. The rate expressions can then be solved as long as [C-1027] = $[C\text{-}1027]_o$ $f(t)$ with $f(t)$ independent of the initial drug concentration $[C\text{-}1027]_o$. The expressions for the amounts of the three forms of DNA after digest are similar to those from the pseudo-first-order equations, except that where the

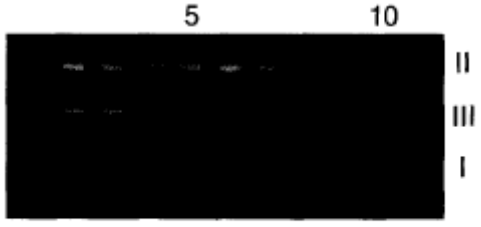


Fig. 1. Agarose gel of C-1027 cleavage of extracellular SV40 DNA. Lane 11: control (no drug). Lanes 1–10: drug concentrations (lane 10, lowest; lane 1, highest) and other conditions are as stated in Section 2. The forms of SV40 DNA are: form I, closed circular; form II, nicked circular; form III, linear DNA.

latter contain $k_i\tau$, the former contain $k_iG(\tau)$, where $k_i$ is a second-order rate constant and $G$ is a function of $\tau$, the digest time. The pseudo first order equations correspond to $f(t) = 1$.

Let $k_1$ and $k_4$ be the rate constants for ss and ds cutting of form I, producing forms II and III respectively, and let $k_2$ and $k_4'$ be the rate constants for ss and ds cutting of form II, producing form III. Then the solutions to the kinetic equations are as follows

$$[\mathrm{I}] = [\mathrm{I}]_o e^{-(k_1+k_4)[\mathrm{D}]_o G(\tau)} \qquad (1)$$

$$[\mathrm{II}] = [\mathrm{II}]_o e^{-(k_2+k_4')[\mathrm{D}]_o G(\tau)} - \frac{k_1[\mathrm{I}]_o}{k_1+k_4-k_2-k_4'} \times \left[e^{-(k_1+k_4)[\mathrm{D}]_o G(\tau)} - e^{-(k_2+k_4')[\mathrm{D}]_o G(\tau)}\right] \qquad (2)$$

$$[\mathrm{III}] = [\mathrm{III}]_o e^{-k_4[\mathrm{D}]_o G(\tau)} + \frac{k_4[\mathrm{I}]_o(k_1+k_4-k_4') - k_1(k_2+k_4')[\mathrm{I}]_o}{(k_1+k_4)(k_1+k_4-k_2-k_4')} \times \left[1-e^{-(k_2+k_4')[\mathrm{D}]_o G(\tau)}\right] + \frac{[\mathrm{II}]_o(k_1+k_4-k_2-k_4') + k_1[\mathrm{I}]_o}{(k_1+k_4-k_2-k_4')} \times \left[1-e^{-(k_2+k_4')[\mathrm{D}]_o G(\tau)}\right] \qquad (3)$$

In Eqs. (l)-(3), we denote [C- 1027]$_o$, the initial drug concentration, by [D]$_o$. The drug concentration at time $t$ is [D]$_o f$(t). Since $G(\tau)$ is not known, one cannot get actual values of rate constants. However, by varying drug concentration at constant time, one can obtain quantities proportional to the rate constants and ratios of rate constants. The parameters determined are intensities for zero drug concentration (initial intensities) and rate constants multiplied by $G(\tau)$.

### 3.2. Cleavage of Extracellular SV40 DNA with the Holoantibiotic

Four experiments, A-D, involving cleavage of extracellular SV40 DNA by C-1027, were analyzed. A photograph of an agarose gel for one of the experiments is shown in Fig. 1, while fits to observed intensities for a second experiment, using the theoretical expressions, are shown in Fig. 2. The **[end of page 204]**

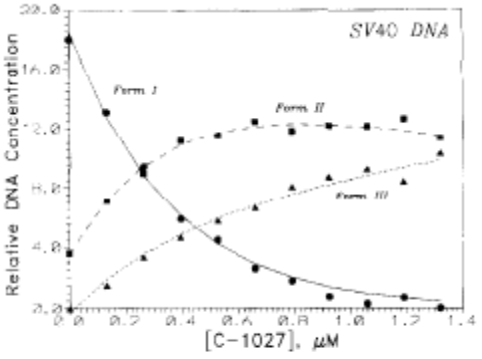


Fig. 2. Cleavage of extracellular SV40 DNA by C-1027. Relative concentrations of three forms of DNA are plotted as a function of drug concentration. Measured values are given by symbols (circles, form I; squares, form II; triangles, form III), and values calculated from the kinetic model are given by lines.

populations of the three forms of DNA as a function of concentration of C-1027 are fit to populations calculated from the theoretical model, which includes ss and ds cutting. There are six parameters: [I]$_o$, the initial population of form I; [II],, the initial population of form II; [III]$_o$, the initial population of form III; $k_1 G(\tau)$, where $k_1$ is the rate constant for the I-II conversion; ($k_2$, + $k_4'$)G($\tau$), where (k, + $k_4'$) is the effective rate constant for the II-III conversion; and $k_4$G($\tau$), where $k_4$ is the rate constant for the I-III conversion. Table 1 shows the values of parameters determined for experiments A-D. The errors are standard errors from the fitting procedure. Averaging the four sets of results, we find $k_1$G = 1.85±t 0.65, $k_2$G = 0.20 ± 0.09, and $k_4$G = 1.05 ± 0.31 $(\mu M)^{-1}$, where the stated errors are now standard deviations.

The ratio of rate constants, $k_i/k_j$, is obtained by dividing $k_iG(\tau)$ by $k_jG(\tau)$. The ratio $k_1/k_2$ was 10, 12, 9 and 7 for experiments A-D respectively, with very large errors because of the small value of $k_2$. Assuming that, for conversion of II to III, cuts on opposing strands must be within approximately five nucleotides of each other and that forms I and II bind drug equally well, this ratio should be about 500 for a genome the size of SV40 DNA (5.2 kbl). The fact that the ratio is much less indicates that C-1027 exhibits base or sequence specificity. It has recently been shown that the drug most often cleaves at adenine and thymine residues of DNA [16,17].

For the ratio of ss to ds cutting, $k_1/k_4$, we obtain, by averaging experiments A-D. 1.72 ± 0.24. Weighting each value by the reciprocal of the uncertainty, the average of the four values is 1.78. Thus, outside the cell, ss cutting is almost twice as likely to occur as ds cutting.

### 3.3. Cleavage of intracellular SV40 DNA wjith the holoantihiotic

As described in Section 2, BSC-1 cells infected with the SV40 virus were treated with various amounts of C- 1027. and after two hours the DNA was recovered and analyzed using agarase gel techniques. One of these experiments had a maximum drug concentration of 50 nM which reduced the population of form-1 DNA by only half of its initial value. The resulting plots of populations versus [C-1027] were essentially linear. making it impossible to determine three independent rate parameters. A second experiment, using 150 nM as a maximum drug concentration, resulted in loss of approximately 90% of the initial concentration of form-l DNA.

Table 1
Rate constants for cleavage of intra- and extracellular SV40 DNA by C-1027

| | $k_1G(\tau)/(\mu M)^{-1}$ | $(k_2 + k_4')G(\tau)/(\mu M)^{-1}$ | $k_4G(\tau)/(\mu M)^{-1}$ | $k_1/k_4$ |
|---|---|---|---|---|
| *Extracellular* | | | | |
| A | 0.94 ± 0.29 | 0.09 ± 0.72 | 0.66 ± 0.34 | 1.42 ± 0.85 |
| B | 2.20 ± 0.19 | 0.18 ± 0.05 | 1.33 ± 0.35 | 1.65 ± 0.46 |
| C | 2.40 ± 0.21 | 0.27 ± 0.25 | 1.26 ± 0.15 | 1.90 ± 0.28 |
| D | 1.85 ± 0.09 | 0.28 ± 0.05 | 0.96 ± 0.11 | 1.93 ± 0.24 |
| *Intracellular* | | | | |
| 150 nM [a] | 2.6 ± 1.1 | 0.4 ± 2.0 | 10.3 ± 1.1 | 0.25 ± 0.11 |

[a] Refers to the highest concentration used in the experiment.

**[end of page 205]**

For the 50-nM experiment, experimental and calculated populations are given in Fig. 3. The values of the correlation coefficient, initial population of DNA (arbitrary units) and slope $(\mu M)^{-1}$ were respectively: form I, 0.99, 0.82, -8.44; form II, 0.22, 0.20, 0.28; form III, 0.99, - 0.02, 8.16. Theoretically, the slopes for the linear plots for forms I, II and III should be equal, respectively, to $-(k_1 + k_4)[I]_oG$, $(k_1[I]_o - k_2[II]_o)G$ and $(k_2[11]_o + k_4[I]_o)G$, where $G = G(\infty)$. The value of $(k_1 + k_4)G(\infty)$ was 10.3 ± 0.4 $(\mu M)^{-1}$. If we assume that $k_2[II]_o$ is much less than $k_4[I]_o$, $k_4G$ is equal to 8.16/0.82 = 9.3 ± 0.5 $(\mu M)^{-1}$. Alternatively, the slope of form II gives $k_1G = 0.34 + 0.24k_2G$ $(\mu M)^{-1}$, and, since $k_4$ cannot be greater than $k_1$, $k_1G < 0.45$. Thus, $k_4$ is much larger than $k_1$.

The 150-nM experiment (results plotted in Fig. 4) was analyzed according to the kinetic model used for the experiments involving purified DNA. The results of the calculations are given in Table 1. The value of $(k_1G+ k_4G)$, 12.9 ± 1.5 $(\mu M)^{-1}$, agrees with the corresponding value found from the 50-nM experiment. We note that $k_4$ is four times as large as $k_1$. There is much more ds cleavage *inside* than *outside* the cell, Table 1. It also appears that the overall rate of cleavage, $k_1 + k_4$, is higher inside than outside the cell.

We have assumed that during the two hour incubation in the cell studies, cutting is completed be-

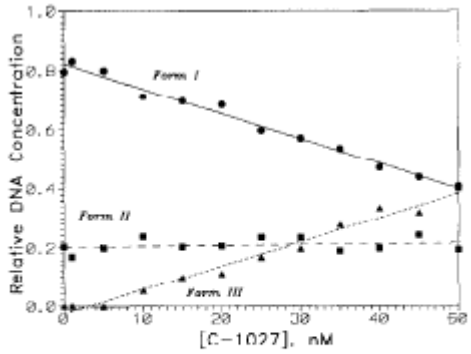


Fig. 3. Cleavage of intracellular SV40 DNA by C-1027 (the 50-nM experiment). Relative concentrations of three forms of DNA are plotted (circles, form I; squares, form II; triangles, form III) as a function of drug concentration. A best-fit straight line is plotted for each form.

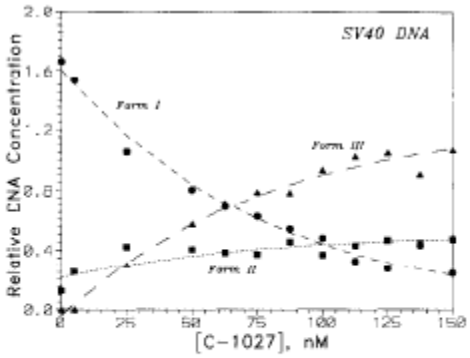


Fig. 4. Cleavage of intracellular SV40 DNA by C-1027 (the 150-nM experiment). Relative concentrations of three forms of DNA are plotted (filled circles, form I; filled squares, form II; filled triangles, form III) as a function of drug concentration. Curves are values calculated from the kinetic model.

cause drug has been exhausted. If this were not the case, the reported parameters represent $k_i G(\tau)$, where $G(\tau) < G(\infty)$, so that the actual values of $k_i$ are *higher* relative to the extracellular values than appears from the model. Furthermore, $G(\tau)$ may be a different function of time inside the cell than outside. If more or faster processes which degrade the drug are available inside the cell, the effective drug concentration at time $\tau$ is lower than outside. Then the ratio of values of $k_i$ inside the cell to $k_i$ outside is actually higher than what we measure. On the other hand, if the drug is protected from degradation inside the cell, its effective concentration at time $\tau$ is larger than outside, and the ratio is actually smaller.

### 3.4. Cleallage of extra- and intracellular DNA with the C- IO2 7 chromophore

Cleavage experiments were performed on purified extracellular SV40 DNA using the chromophore of C-l 027 instead of the holoantibiotic. There was no evidence of cleavage of form-III DNA to produce smaller fragments, so the data were normalized to constant total DNA. Then, assuming $k_3$ (rate constant for cleavage of form-III DNA) to be 0, we fitted amounts of forms I, II and III as functions of chromophore concentration to the model specified by Eqs. (1)-(3). **[end of page 206]**

The resulting values of $k_1G$, $k_2G$, and $k_4G$ for the extracellular SV40/ C-1027 free chromophore experiment are given in Table 2. As can be seen, the ratio of ss/ds cleavage is nearly identical to that obtained using the holoantibiotic. The ratio $k_1/k_4$ may actually be *larger* than that for the holoantibiotic, approaching the value reported by Xu et al. [16] of $\approx 4$ for cleavage of pBR 322 DNA with the chromophore. However, the errors are large because quantitative studies with the isolated chromophore were difficult to carry out. First, the chromophore is unstable when not bound to the apoprotein [18]. Also, since the free chromophore was obtained using a methanol precipitation procedure, its concentration was not accurately known in the cleavage reactions (a 100% recovery was

assumed). The values of $k_iG$ for the chromophore are much smaller than those in Table 1 for the holoantiobotic, perhaps because the actual concentration of chromophore is lower than that stated in Section 2.

We also performed extracellular experiments using the holoantibiotic in the presence of methanol, which was used in the precipitation procedure for the chromophore, and so was present in these cleavage experiments for comparison. Analysis of these data using the kinetic model led to the following values of rate constants: $k_1G$ = 1.01 ± 0.18, $k_2G$ = -0.17 ± 11. $k_4G$ = 0.56 ± 0.30 $(\mu M)^{-1}$. Rate constants are indeed lower in the presence of methanol. but $k_1/k_4$ is I .80 ⊥ 1 .0l . the same as in the absence of methanol. The alcohol probably exerts its effect by slowing the processes leading to the activated chromophore and/or quenching the drug-diradical after it is formed. As is evident from Tables 1 and 2, the kinetic parameters for the chromophore and the holoantibiotic cleaving intracellular DNA are comparable.

### 3.5. C-1027 Cleavage of DNA Following Post-Treatment with Putrescine

In order to check for the presence of abasic sites created by C-1027. DNA cleaved by the drug was exposed to excess putrescine. Neocarzinostatin (NCS) and bleomycin (BLM) are known to produce abasic DNA sites which can be converted into a strand break using putrescine [19]. Previous studies with C- 1027 indicated that the drug can abstract a hydrogen from the 4' position of the sugar producing a 4' hydroxylated abasic site [16,17]. For SV40 DNA which has been treated with C-1027. there are a number of possibilities. If the drug attacks form-l DNA by eliminating a base without producing a strand break. form I would not be converted into form-II DNA. However, subsequent treatment with putrescine would break the strand at the abasic site causing conversion of form I to form II. If the drug produced two abasic sites sufficiently close to one another but on opposing strands, exposure to amine would cause form 1 to be directly converted into form-III DNA.

Quantitative analysis of agarose gel data with and without post-treatment with putrescine was carried out. The results of linear regression analysis on the fractional populations of forms I, II and III for the extracellular studies are shown in Fig. 5. Analysis of the data gave the following values for rate constants: $k_1G$ = 1.07 ⊥ 0.61. $k_2G$ = 0.92 ± 0.26. $k_4G$ = 5.35 ± 0.61. The $k_1/k_4$, ratio (0.2) differs drastically from the ratio for extracellular cleavage without putrescine (Table 1). The ratio is similar to that obtained from the intracellular data, 0.25 ± 0.11. This may be a result of the ability of putrescine to mimic the chemistry occurring inside the cell, wherein lysine groups on histones or polyamines like

Table 2
Cleavage of intra- and extracellular SV 40 DNA using the chromophore of C-1027

| | $K_1G(\tau)/(\mu M)^{-1}$ | $k_2G(\tau)/(\mu M)^{-1}$ | $k_4G(\tau)/(\mu M)^{-1}$ | $k_1/k_4$ |
|---|---|---|---|---|
| *Extracellular* | | | | |
| | 0.49 ± 0.09 | 0.088 ± 0.015 | 0.184 ± 0.046 | 1.7 ± 0.8 |
| *Intracellular* | | | | |
| | 1.65 ± 0.25 | 0.12 ± 0.22 | 5.07 ± 0.33 | 0.33 ± 0.05 |

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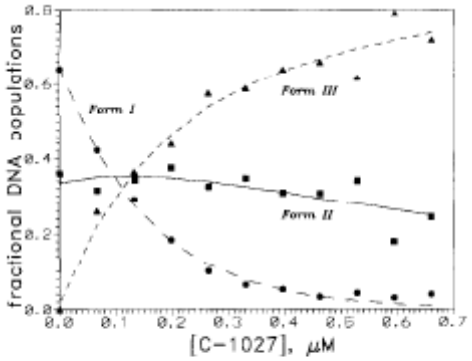


Fig. 5. Cleavage of extracellular SV40 DNA by C-1027, followed by treatment with putrescine (90.5 mM). Relative concentrations of three forms of DNA are plotted as a function of drug concentration (filled circles, form I; filled squares, form II; filled triangles, form III). Curves are values calculated from the kinetic model.

spermidine can cause abasic sites proximal to ss DNA breaks to become strand breaks through $\beta$ elimination. Comparison of the curves in Fig. 2 and Fig. 5 demonstrates that putrescine has practically no effect on the form I

population, while converting form II with abasic sites into form III. In the case of putrescine post-treatment of the intracellular samples, changes in the relative populations of SV40 DNA were not observed (data not shown).

## 4. Discussion

By fitting the measured intensity data to a kinetic model, quantities proportional to rate constants for cleavage of SV40 DNA by C- 1027 were determined. The fits can be seen in Figs. 2-5, and the measured rate parameters and their errors are given in Tables 1 and 2. The striking result is that the apparent rate constant for ds cleavage in the cell is 5-10 times higher as compared to extracellular experiments. As stated in the results section, $G(\tau)$ may be a different function of time inside the cell than outside due to various processes inside the cell which may degrade the drug. If this is the case, it would be reflected in the $k$ values inside versus outside the cell. However, the ratio of single- to double-strand cleavage inside the cell is much lower than outside, and this is unaffected by different $G(\tau)$ values. Furthermore, putrescine treatment of extracellular samples cleaved by C-1027 yielded a ratio of $k_1/k_4$ similar to that obtained in the intracellular assay. The most likely reason for the high number of ds breaks in the cell is caused by the ability of proximal amine functions on histones or polybasic amines like spermidine to induce a break at an abasic site. This argument has been used to explain the increased amount of ds breaks observed for neocarzinostatin in intracellular studies [19] and it may apply to C-1027 as well.

The reason for the higher rate of cleavage of intracellular versus extracellular SV40 DNA by C-1027 is unclear. However, both the free chromophore and the holoantibiotic exhibit enhanced cleavage inside the cell, as measured by the disappearance of form-1 DNA ($k_1 + k_4$). It may be that in order for the drug to cleave extracellular DNA, the chromophore must first be released from the protein part of the antibiotic. It is apparent from the studies on the stability of the holoantibiotic in water that the "activator" for C-1027 is DNA itself. Outside the cell, the free chromophore could be partially deactivated by components in the medium before it reaches its DNA target, thus producing lower rates. For intracellular DNA, the holoantibiotic must first bind to the cell membrane at which point it could release the chromophore. Once inside, it may be protected from damage and/or more efficiently delivered to SV40 chromatin.


## Acknowledgements

We wish to thank Dr. T. Otani of the Taiho Pharmaceutical Co. for samples of C-1027. This work was in part supported by a Shannon Award from the NIH (GM459241 to J.C.D. Support for T.A.B. was provided by the American Cancer Society (DHP- 133) and the National Cancer Institute (CA 16056).


## References


[l] E.F. Gale, E. Cundliffe, P.E. Reynolds, M.H. Richmond and M.J. Waring, The Molecular Basis of Antibiotic Action, Wiley, London, 198 I.

[2] B. Pullman and J. Jortner (Eds.), Molecular Basis of Speci **[end of page 208]** ficity in Nucleic Acid-Drug Interactions, Kluwer Academic Publishers, Dordrecht. 1990.

[3] S. Neidle and M.J. Waring. Molecular Aspects of Anti-Cancer Drug Action. Verlag Chemie, Weinheim, 1983.

[4] J.C. Dabrowiak, A.A. Stankus and J. Goodisman, in CL. Propst and T.J. Perun (Eds.), Nucleic Acid Targeted Drug Deatgn. Marcel Dekker. Inc., New York. 1992, pp.93- 149.

[5] A. Sancar, Science. 266 (1995), 1954.

[6] A. Sancar and M.-S.Tang. Photochem. Photobiol.. S7 (1993). 905.

[7] PC. Hanawalt, Science, 266 (1995). 1957.

[8] P. Modrich. Science, 266 (l995), 19.59.

[9] M.M. McHugh, J.M. Woynarowski, L.S. Gawron, T. Otani and T.A. Beerman. Biochemistry. 34 (1995) 1805.

[IO] R.J. Cobuzzi. S.K. Kotsopoulos, T. Otani and T.A. Beerman, Biochemistry. 34 (1995) 583.

[I I] T Otani, Y. Minami. T. Marunaka. R. Zhang and M-Y. XI~, J. Antibiot.. II (1988) 1580.

[12] J. Hu. Y-C. Xue, M-Y. Xie. R. Zhang. T. Otani, Y. Minami. Y. Yamadn and T. Marunaka, J. Antibiot.. 41 (1988) 1575.

[13] J. Sambrook, E.F. Fritsch and T. Mania& Molecular Cloning. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY. 1989.

[14] J.E. Grimwadc. E.B. Cason and T.A. Bccrman, Nucleic Acids Res.. I5 (1987) 6315.

[15] S. Yoshikalu. T. Otani. S. Oie. K. Wterrba and Y. Yumadn. J. Antibiot.. 43 (1990) 417.

[16] Y. Xu. Y. Lhen and I.H. Goldherg. Biochemistry. 33 (1993) 5947.

[17] Y. Sugiura and T. Matsumoto. Biochemistry. 32 (1993) 5548.

[18] K. Yoshida. Y. Minami. R. ATuma. M. Saeki and T. Otant. Tetrahedron Lett.. 34 (1993) 2637.
[I9] L.F. Povirh and C.W. Houlgrnvc. Biochemistry, 27 (1988) 3850. **[end of page 209]**