0026-895X/88/020225-06$02.00/0

MOLECULAR PHARMACOLOGY, 33:225-230

Daunomycin-DNA Dissociation Kinetics

DON R. PHILLIPS, PETER C. GREIF, and RAY C. BOSTON

Department of Biochemistry, La Trobe University, Bundoora, Victoria, 3083, Australia (D.R.P.), Laboratory of Mathematical Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892 (P.C.G.), and Computer Science Program, Murdoch University, Murdoch, Western Australia,6008, Australia (R.C.B.)

Received August 11, 1987; Accepted November 10, 1987

SUMMARY

The dissociation of daunomycin from DNA was examined using a sodium dodecyl sulfate-sequestered stopped flow procedure. Two dissociation processes were observed with calf thymus and bacterial DNA, with approximately 45% of the amplitude associated with the faster process. Both processes were largely independent of DNA sequence for bacterial DNA, comprising 30–70% (G + C) content. The rate of both processes increased by a similar amount with increasing ionic strength. The faster process decreased with decreasing drug loading, whereas the slower process was independent of drug loading. Only one dissociation event was observed for the dissociation of daunomycin from four different synthetic polydeoxynucleotides. All observations are consistent with a parallel model of sodium dodecyl sulfate-induced dissociation of daunomycin from DNA, where the two processes observed reflect two resolvable processes that may be comprised of a series of rate constants for the dissociation of drug from differing environments. The slower process observed with bacterial DNA (0.5–0.7 sec^{-1}) is related to dissociation from preferential 5′-CA DNA-binding sites, whereas the faster process reflects dissociation of drug from lower affinity sites on heterogenous DNA (3.2–4.1 sec^{-1}). Dissociation of daunomycin from four different synthetic polydeoxynucleotides (which did not contain the 5′-CA preferential daunomycin-binding site) exhibited dissociation rates characteristic of low affinity sites (3.3–4.8 sec^{-1}).

Daunomycin (daunorubicin) and Adriamycin (doxorubicin) are members of the anthracycline family of antibiotics. They are effective anti-cancer agents and have been in clinical use for some 15 years (1–3). The chemical and physicochemical aspects of these drugs have been reviewed in detail (1–7). Many potential receptors have been identified, including the cell membrane, proteins, and DNA (5–8). The interaction with DNA is considered to be of major significance because of the inhibition of DNA replication and RNA synthesis by these drugs (3, 5, 6), and the extensive correlation of biological activity with DNA binding and DNA-related events (7, 9, 10). The cardiotoxicity of these drugs (6–8) has prompted extensive efforts to improve their clinical usefulness (1–6, 10–12) and to obtain greater detail of the drug-DNA interaction (7, 9, 10).

There has been an increasing body of evidence regarding the significance of the kinetics of drug-DNA interactions (13–19). If the rate-limiting effect for DNA polymerase action is due to dissociation of the drug from DNA, then the biological effectiveness of the drug will be dependent upon the drug dissociation kinetics. This general mechanism has been demonstrated for chromomycin derivatives (13) and actinomycin D derivatives (14). Although it has been recognized that merely the rate of dissociation of anthracyclines from DNA cannot explain the effectiveness of these drugs (17), the longer a drug resides with its receptor, the greater is the probability of other cellularly disruptive processes (17). Examples of these events could be the induction of topoisomerase activity (20) or DNA damage resulting from free radical damage mediated by the drug itself (21) or drug-metal ion complexes (7, 22).

There have been several studies of DNA-anthracycline dissociation kinetics (23–29) as measured by the SDS sequestering procedures of Muller and Crothers (30). There has been some debate as to the number of kinetic processes discernable using the SDS-sequestered DNA-daunomycin system, with earlier studies indicating only one process (23, 24, 27), whereas more recent studies have clearly documented that two processes occur (26, 28, 29). On the basis of a thorough analysis using both T-jump and stopped-flow studies, Chaires *et al.* (26) were unable to distinguish between a parallel and a sequestial model, while Fox *et al.* (28) and Krishnamoorthy *et al.* (29) support a parallel model to account for the two observed kinetic processes. We present here additional data for SDS-induced dissociation of DNA-daunomycin for DNA containing varying base compositions, and at different drug/DNA ratios where we detect cooperative effects of the drug kinetics, depending on fractional drug saturation levels. A model is presented to account for all daunomycin-DNA kinetics measured to date, irrespective of the source of the DNA (bacterial, mammalian, or synthetic polydeoxynucleotides).

This work was supported by the Australian Research Grants Scheme.

ABBREVIATIONS: SDS, sodium dodecyl sulfate; PIPES, 1,4-piperazinediethanesulfonic acid; EDTA, ethylenediaminetetraacetate; bp, base pair.

Materials and Methods

Buffers. All experiments were conducted in PIPES buffer, pH 6.8, ionic strength 0.1 (0.1 PIPES) or 0.01 (0.01 PIPES). A stock solution of 0.04 M PIPES, 1 mM EDTA, 0.9 M NaCl was adjusted to pH 6.8 with HCl, and was diluted 10-fold or 100-fold (with no significant change of pH) to yield 0.1 and 0.01 PIPES, respectively. Type I water from a four-bowl Millipore Milli-Q system was used for all solutions.

DNA. Calf thymus DNA (Worthington Biochemical Corp., Freehold, NJ) was dissolved in PIPES buffer, filtered through a 3-μm mixed cellulose acetate and nitrate Millipore filter, sonicated, and then refiltered. DNA nucleotide concentrations were determined spectrophotometrically, using an extinction coefficient of 6600 M^{-1} cm^{-1} at 260 nm. Synthetic polydeoxynucleotides were obtained from P-L Biochemicals, Inc. (Milwaukee, WI) and were dissolved in 1 mM PIPES buffer containing 0.15 M NaCl, pH 6.7.

Daunomycin. The daunomycin used was a gift from Farmitalia Carlo Erba and was used without further purification. High performance liquid chromatographic analysis on a Waters reverse phase Z-pak C-18 column with a mobile phase of 65% methanol/35% water, containing 3% (w/w) ammonium acetate (31), indicated a purity of >99%, with the only significant contaminant being daunomycinone. The concentration of drug was determined from its absorbance at 480 nm, using an extinction coefficient of 11,500 M^{-1} cm^{-1}. Drug solutions were freshly prepared for each experiment and were kept for a maximum of 5 days in the dark at 4°.

Stopped flow experiments. Stopped flow studies were performed at 20.0° using a Durrum apparatus (Palo Alto, CA), using equal volumes of drug-DNA and SDS solutions (both in the same PIPES buffer), a path length of 5 mm, and monitoring the reaction of 480 nm. The dead time of the instrument was less than 5 msec. Data were initially stored on a Datalab DL901 transient recorder (scaled as 0–256) and subsequently transferred to a Digital Equipment Corp. VAX 11/780 computer. Typically, 1024 data points were recorded in a time span of 5, 10, or 20 sec, and then averaged to a total of 250 points, a constraint of subsequent software. This data aquisition process limited maximum signal slope to around 30 sec^{-1}.

The data were analyzed using the computer program CONSAM (32) on a Digital Equipment Corp. VAX 11/780 computer. This is an interactive version of SAAM, the simulation analysis and modelling program developed at the National Institutes of Health (33). The program simulates the kinetic response based on a multiple exponential model. For a model involving two relaxation processes, the equation used was

$$A_t - K_3 = K_1 e^{-p_1 t} + K_2 e^{-p_2 t}$$

where A_t is the absorbance at time t, p_1 and p_2 are the exponential coefficients associated with each process (related inversely to the time constant for each relaxation process), K_1 and K_2 are the respective amplitudes associated with each process, and K_3 is the calculated absorbance at infinite time. The elements, K_i and p_i lead to a matrix pair, respectively, the eigenvector and eigenvalue of the "system" describing the data, and under certain assumptions they can be construed as microrate constants (see following discussion).

Results

Effect of SDS concentration. It has previously been established that the dissociation kinetics of preformed DNA-daunomycin mixtures are independent of SDS concentration in the range 0.1–1.0% SDS (23) and 0.2–0.6% prior to mixing (25). However, these earlier studies lacked sensitivity since the analysis simply relied upon apparent linearity of a first order logarithmic plot. The current procedure for the analysis of data is significantly more objective. It was therefore necessary to reexamine the SDS concentration dependence of the dissociation kinetics using this more stringent analysis, and also because of recent observations of a dependence of relative amplitudes at low SDS concentrations (28).

For all combinations of drug loading, ionic strength, and SDS concentration, the absorbance change was described by two relaxation processes, as noted by others (26, 28, 29). Several earlier studies using salmon sperm DNA reported a single exponential process, and these studies relied upon subjective assessment of linearity of a semilogarithmic plot (23, 24). A small curvature of such a plot (resulting from similar exponentials or a small amplitude of one of the processes) is difficult to detect by this procedure.

The calculated time constants and amplitudes exhibited a standard deviation of less than 2%. An example of the fit of the calculated values to the experimental values is shown in Fig. 1a. The residual of the fit is shown in Fig. 1b. A small systematic error (less than ±1% of the signal value) was often observed which varied between individual traces and was shown to be due to baseline oscillation of approximately 0.05 Hz associated with the photomultiplier detection system. The larger deviation of ±3% in the initial 100 msec was due to the latter stages of a more rapid dissociation event previously detected by T-jump kinetics (26), and was inconsequential to the present analysis. The deviation of the experimental data from the fitted line was routinely less than 1% after the first 100 msec (Fig. 1b).

For daunomycin/DNA bp ratios of 0.1, all four kinetic parameters associated with the two kinetic processes were independent of final SDS concentration in the range 0.25–2.5%, at both low (0.01) and high (0.1) ionic strength. This was also observed at high drug/bp ratios (1.6) studied at high ionic strength.

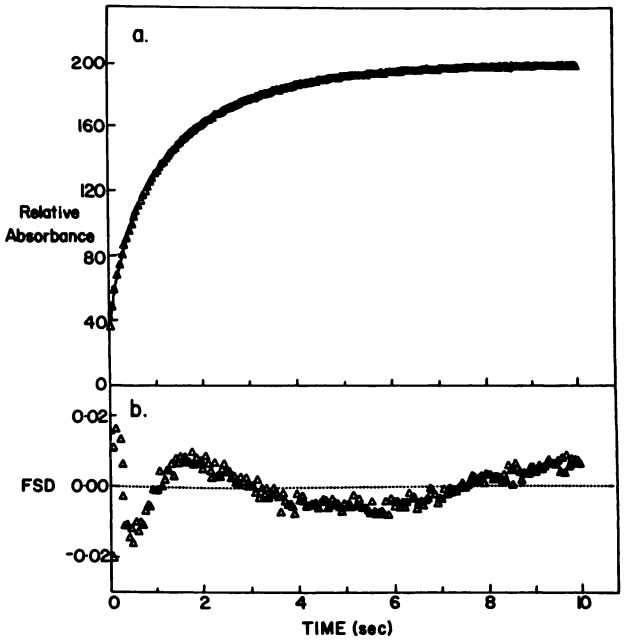

Fig. 1. Dissociation of daunomycin from DNA. a. Absorbance charges at 480 nm (scaled and digitized in the range 0–256 by the transient recorder) are shown (Δ) as a function of time after mixing with SDS. The *continuous line* shows the calculated values fitted according to the exponentials and amplitudes derived from nonlinear simulation using CONSAM. The drug/bp ratio was 0.2, and the final SDS concentration was 2%. The reaction vessel was thermostatted at 20.0°. b. The residual of the fitted curve (a) to the experimental data is shown as a fractional standard deviation (*FSD*) as a function of time after mixing with SDS. All experimental values fitted within 2% of calculated values over the entire time course.

However, at low ionic strength, with this high drug loading level, the kinetics were independent of SDS concentration only above 0.5% final SDS concentration. For this reason, the standard conditions selected used a final SDS concentration of 1% as a compromise between the desire to use the lowest possible SDS concentration (to minimize the ionic strength contribution from the SDS) and the need to add sufficient SDS to ensure that the kinetics were in the range which was unaffected by SDS concentration.

Effect of drug loading and ionic strength. The total daunomycin/DNA bp ratio was varied from 0.1 to 1.6, and the four kinetic parameters (described earlier as two exponentials and two amplitudes) were determined at both low (0.01) and high (0.1) ionic strengths. At both ionic strengths, the slower process was independent of drug loading, and this has also been observed by others (26). The faster process increased with drug loading up to drug saturation levels of approximately 0.4 daunomycin/bp (Fig. 2, a and b). The exponential for the faster process appears to extrapolate to the same value as that of the slower process at diminishing drug loading levels (Fig. 2, a and b). The amplitudes of both processes were largely independent of drug loading at both ionic strengths, and constituted approximately 55% for the slower process (Fig. 2c).

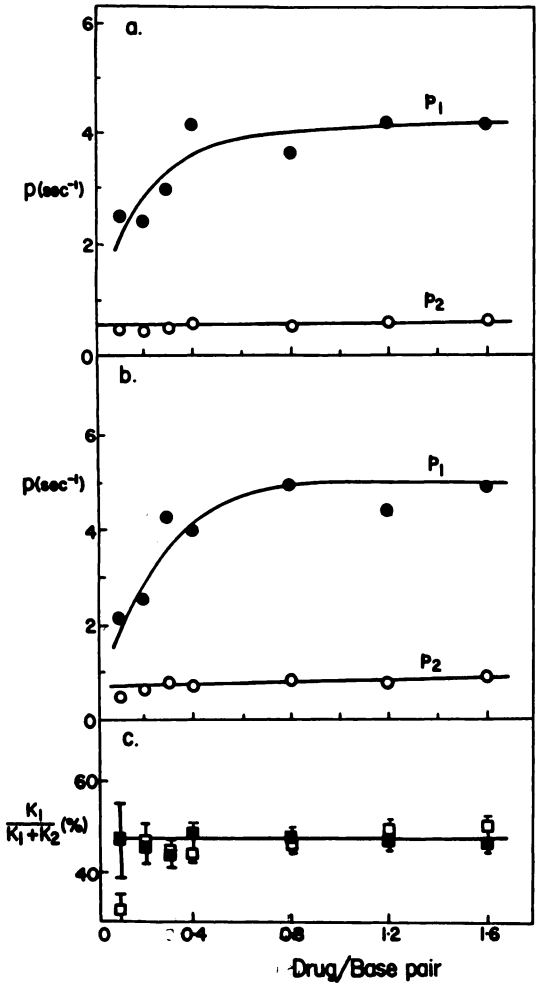

Fig. 2. Effect of drug loading on dissociation parameters. The calculated exponentials (faster process, ●; slower process, ○) are shown as a function of drug loading at low ionic strength (0.01, a), and high ionic strength (0.1, b), for the dissociation of daunomycin from calf thymus DNA at 20°. The amplitude associated with the faster process (expressed as a percentage of the total amplitude) is shown in c at low (0.01, □) and high (0.1, ■) ionic strength, as a function of drug loading levels.

The kinetic parameters were not dramatically affected by ionic strength. The amplitudes (normalized with respect to total amplitude) were independent of ionic strength (Fig. 2c), while both exponentials were increased some 10–20% at the higher ionic strength (Fig. 2, a and b). An increase of both apparent rate constants with increasing ionic strength has also been observed by others (29).

The kinetic parameters are in general agreement with the observations of Chaires *et al.* (26), who also detected two processes by this method, with apparent rate constants of 5.5 ± 1.2 sec^{-1} and 1.25 ± 0.25 sec^{-1} for drug loading in the range 0.05–0.2 drug/bp at 21°, ionic strength 0.2 (cf. 4.7 ± 1 sec^{-1} and 0.8 ± 0.1 sec^{-1} at 20°, ionic strength 0.1 for drug/bp ratios of 0.3–1.6 in Fig. 2b of this work). Two dissociation processes, with similar apparent rate constants, have also been noted by others (28, 29).

Effect of G + C content. Two processes were detected for each of the four bacterial DNAs studied at low ionic strength (Table 1). Both were essentially independent of G + C content (Fig. 3, a and b), although there is a suggestion that both processes may be slightly slower with increasing G + C content. Interpolated values for the exponentials at 42% G + C content were 0.54 sec^{-1} and 3.4 sec^{-1}, in agreement with averaged values detected under the same conditions (Fig. 2a) for calf thymus DNA (0.54 and 3.5 sec^{-1}). The amplitude associated with each process was also independent of G + C content (Fig. 3c), with approximately 45% associated with the faster process.

Synthetic polydeoxynucleotides. In terms of the mechanism of SDS-induced dissociation of daunomycin from DNA, it is important to establish unambiguously the number of kinetic processes existing for the dissociation of daunomycin from DNA polymers containing only one repeating unit. Previous data for dissociation of daunomycin from four synthetic polymers were analyzed originally by conventional semilogarithmic first order plots, and all indicated only one dissociation process (25). These data were reanalyzed using more data points and a more sensitive approach involving computer simulation utilizing CONSAM (32). All four synthetic polydeoxynucleo-

TABLE 1

Kinetic parameters for the dissociation of daunomycin from DNA

All solutions were in 0.01 PIPES, pH 6.8, at 20°. The daunomycin/bp ratio was 0.2, and the drug concentration in the drug/DNA solution, prior to mixing with an equal volume of 2% SDS, was 5 μM. All samples were analyzed in duplicate over 5, 10, and 20 sec. The kinetic parameters were evaluated from a two-compartment model, using CONSAM (32), and were found to be largely independent of sampling duration. The tabulated data were obtained for a sampling duration of 10 sec. This represented the optimal sampling interval for 5 msec between averaged data points and yielded the errors (shown in parentheses) associated with each parameter.

DNA	(G + C)	P_1	P_2	K_1	K_2	K_3
Micrococcus lysodeikticus	71	3.17	0.53	94	144	233.4
		(0.08)	(0.01)	(2)	(2)	(0.4)
Escherichia coli	50	3.8	0.493	64	99	231.7
		(0.1)	(0.007)	(2)	(2)	(0.3)
Bacillus subtilus	44	3.57	0.58	107	118	205.8
		(0.09)	(0.01)	(2)	(2)	(0.3)
Calf thymus	42	3.50	0.539	57	112	198.2
		(0.09)	(0.006)	(1)	(1)	(0.2)
Clostridium perfringens	31	4.1	0.647	83	124	244.3
		(0.1)	(0.009)	(1)	(2)	(0.2)

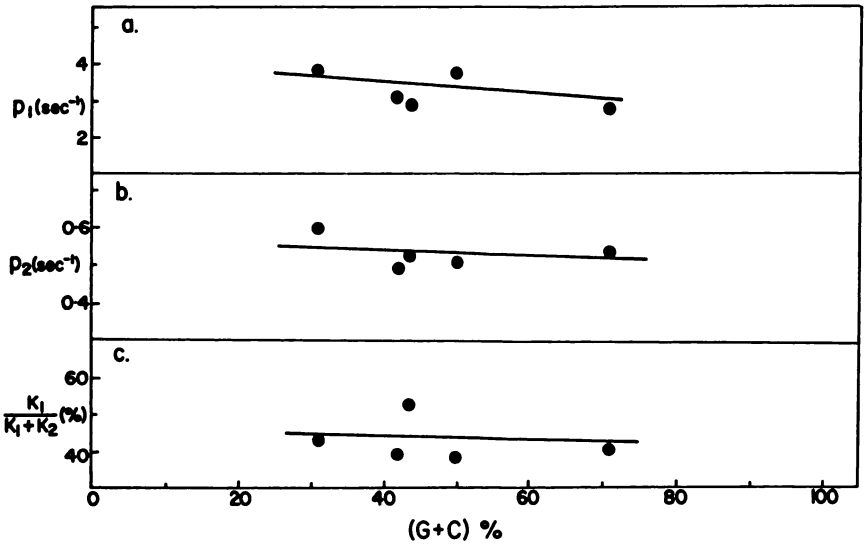

Fig. 3. Effect of (G + C) content on dissociation parameters. The dissociation parameters were calculated at 20° in 0.01 PIPES buffer for calf thymus DNA and four different bacterial DNAs, using a final SDS concentration of 1%. The exponential associated with the faster and slower processes are shown in a and b, respectively. The per cent amplitude associated with the faster processes is shown in c. All values are the average of duplicates of 10- and 20-sec sampling times.

tides [(polydA·poly dT, poly(dA-dT), poly dG·poly dC, and poly(dG-dC)] required only one exponential decay process in order to accommodate the full data range which extended over almost four half-lives. The rate constants calculated by this nonlinear fitting routine were within the error limits of the previously determined values, and were 4.8 ± 0.1, 3.9 ± 0.1, 4.5 ± 0.2, and 3.3 ± 0.2 sec^{-1}, respectively, where the error reflects the maximum difference observed for four kinetic profiles for each polymer/drug system. This result confirms the recent observation that dissociation of daunomycin from poly(dA-dT) and poly(dG-dC) exhibits only one first order dissociation event when examined under SDS-sequestered stopped flow conditions (28, 29).

Discussion

Any model of SDS-sequestered DNA-daunomycin dissociation kinetics must take into account the following four observations. (i) Only one relaxation process is observed for synthetic polydeoxynucleotides containing one repeating unit. (ii) Two relaxation processes are observed with bacterial and mammalian DNA. (iii) Neither of the two relaxation processes are altered appreciably in rate or amplitude by altered G + C content of bacterial DNA. (iv) The faster relaxation processes decreases with drug loading at drug levels less than 0.2 drug/bp.

Because a two-exponential process has been observed for the dissociation of daunomycin from mammalian DNA, two models have been proposed (26, 28), although it has been acknowledged that it is difficult to distinguish experimentally between them (26, 28). The sequential model is shown in Fig. 4a and is fundamentally inconsistent with the fact that the dissociation of daunomycin from four different synthetic polydeoxynucleotides has been totally accounted for by just one first order dissociation event in the time frame of 50 msec–10 sec.

The alternative parallel model, which accounts for the observed two first order dissociation processes, is shown in Fig. 4b. This model proposes that the dissociation profiles can be resolved into two processes which represent averaged, but distinguishable, drug-binding sites with differing microenvironments, represented as environments (*1*) and (*2*). This model is inherently more acceptable because it accommodates the major

(a) Sequential Model

$$DNA + Da \overset{rapid}{\rightleftharpoons} DNA\text{-}Da_{out} \rightleftharpoons DNA\text{-}Da_{in} \rightleftharpoons DNA\text{-}Da^{*}_{in}$$

(b) Parallel Model

$$DNA + Da \overset{rapid}{\rightleftharpoons} DNA\text{-}Da_{out} \rightleftharpoons \begin{cases} DNA\text{-}Da_{in} & (1) \\ DNA\text{-}Da_{in} & (2) \end{cases}$$

Fig. 4. Two models of daunomycin-DNA dissociation. In both models it is assumed that daunomycin (*Da*) binds rapidly to the outside of the DNA as shown by T-jump procedures (26). a. The sequential model (26) envisages a change of conformation of the DNA to enable daunomycin to exhibit altered spectral properties between the fully intercalated form (DNA – Da^{*}_{in}), where the bps have moved apart. b. The parallel model assumes that daunomycin bound to the outside of the DNA can then intercalate into (at least) two different sites distinguished by their differing dissociation rates, which can be resolved as two processes, but which may in fact consist of a multitude of such rates.

observations that one dissociation process is observed when only one repeating binding site is present (as in the synthetic polydeoxynucleotides), whereas (at least) two dissociation processes are observed when more than one drug-binding site is present, as in mammalian or bacterial DNA.

It is important to stress here that the two-site parallel model does not exclude the possibility of a multitude of parallel dissociation events arising from drug binding to a multitude of different binding sites. The model merely reflects that only two kinetically distinguishable processes can be resolved from the data.

If the parallel model is correct, then the exponentials obtained from the simulation analysis are actual rate constants. Because of the evidence supporting the parallel model, all subsequent reference to the kinetic parameters is in terms of rate constants.

The two-site parallel model assumes that daunomycin binds to DNA in kinetically distinguishable sites (or a range of sites).

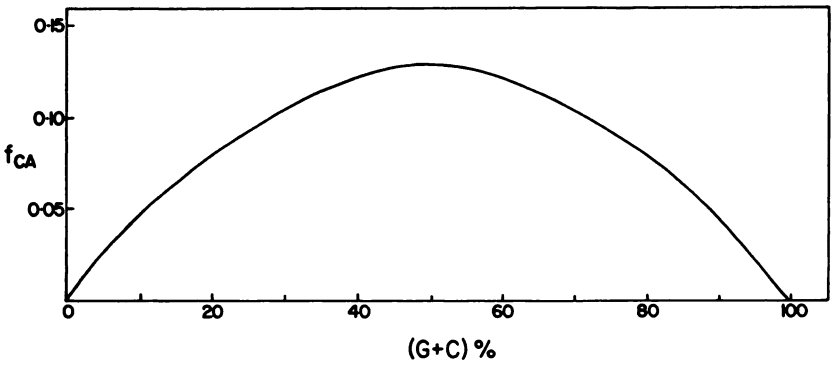

Fig. 5. Probability of existence of CA sequences. The probability of finding a 5′-CA-3′ sequence in DNA (f_{CA}) has been calculated from the relationship $f_{CA} = [f(1 - f)]/2$, where f is the probability of finding a G·C base pair (30, 36), and this is shown as a function of (G + C) content of the DNA.

In order to simplify further discussion, we assume that the more rapid process corresponds to a less favorable binding site(s), and the slower dissociating species reflects a higher affinity binding site(s). The implication, then, is that daunomycin exhibits sequence-specific binding to DNA, and this has recently been revealed by low temperature inhibition of transcription and DNase I footprinting at 5° (34). A 5′-CA-3′ preference was clearly observed, consistent with the observation of a single 5′-CACA-3′ binding site for a bis-daunomycin detected using a transcription assay based on the 203-bp *Escherichia coli lac* L8UV5 promoter (17). This sequence specificity has subsequently been confirmed by the detection of multiple drug sites using a similar transcription system involving a 395-nucleotide transcript.[1] It should be noted that nogalamycin, a related anthracycline, has also recently been shown to exhibit a 5′-CA-3′ preferential binding site (34, 35), and serves to strengthen the notion that all anthracyclines studied to date exhibit a 5′-CA-3′ DNA sequence preference.

The probability of finding a 5′-CA-3′ bp in DNA (f_{CA}) can be calculated as a function of (G + C) content (30, 36), and this dependence is shown in Fig. 5. In the (G + C) range of 30–70%, only a small variation is apparent, with an average value of $f = 0.12 \pm 10\%$. The lack of a major dependence of the dissociation rate constants with (G + C) content of DNA (Fig. 3) is therefore consistent with the preferential sequence specificity of daunomycin, and is also consistent with previous studies which indicated only a minor (G + C) dependence of daunomycin binding to DNA (37).

The amplitude of the slower dissociating processes is approximately 55% of the total absorbance change (Figs. 2c and 3c), and this must be compared to only a 12% occurrence of 5′-CA-3′ dinucleotides in random sequence DNA. The reason for this difference is attributed to several complicating factors. First, the absorbance change (ΔA) accompanying dissociation of daunomycin from "high affinity" (CpA) sites is not known relative to "low affinity" sites. Differences are expected and have already been documented for ApA, TpA, GpA, and GpC sequences, where ΔA for the free and bound form of the drug varies from 51 to 71% (38). Second, it is probable that additional preferential binding sites exist. Low temperature footprinting and transcription inhibition studies have indeed established that other such sites exist, but the data are not yet sufficiently extensive to define their sequence(s) unambiguously, nor their relative affinities (34). Third, nonlinear fitting routines can only resolve with statistical significance rate constants differing by a factor of 2–3, given similar amplitudes. In this context it should be noted that the "averaged" rate constant for the faster process extrapolates to that of the slower process with diminishing drug loading (Fig. 2, a and b), and there is some suggestion of a decrease of amplitude of the faster process at low drug/DNA ratios. These observations are consistent with the notion of a range of rate constants (reflecting dissociation from a range of different binding sites) which are resolvable as two averaged processes at high drug loading levels, but only one process (reflecting dissociation from preferred sites) at limiting drug levels.

The rate constants for the dissociation of daunomycin from the four synthetic polydeoxynucleotides are all similar and encompassed by the range 3.3–4.8 sec^{-1} (25). Since these sequences do not contain known 5′-CA-3′ preferential daunomycin-binding sites (34), it must be concluded that the synthetic polymers contain only the lower affinity sites. It is therefore predicted that the dissociation rate constants observed for the synthetic DNAs should correspond to the faster rate constants observed with the heterogenous DNAs (3.2–4.1 sec^{-1}; Table 1), and this is indeed the case. It should be noted that theoretical gas phase calculations of daunomycin intercalation sites predict other preferred binding sites (39, 40), but these predictions cannot be extrapolated with confidence to the implied behavior in solution.

It has recently been suggested that a parallel model for the dissociation of anthracyclines from DNA implies that a similar dependence of the rate constant should be observed for both processes (29). This has indeed been observed in the present work and by others (29), and provides strong additional evidence in support of a parallel model for the dissociation of daunomycin from DNA.

Conclusions

A two-site parallel model for the dissociation of daunomycin from DNA accommodates all data to date involving synthetic polydeoxynucleotides, and bacterial and mammalian DNA, with the bacterial DNA varying over a wide range of (G + C) content. The model accounts for known preferential binding of daunomycin to defined DNA sequences and for variation of kinetic parameters with drug loading and ionic strength, and predicts a decrease of both rate constant and amplitude associated with the slower processes at drug/DNA ratios significantly less than 0.01/bp.

[1] R. White and D. R. Phillips, unpublished results.

References

1. Crooke, S. T., and S. D. Reich. *Anthracyclines: Current States and New Developments.* Academic Press, New York (1980).

2. Khadem, H. S. *Anthracycline Antibiotics.* Academic Press, New York (1982).
3. Neidle, S., and M. J. Waring. *Molecular Aspects of Anti-cancer Drug Action.* The Macmillan Press Limited, London, 93–125 (1983).
4. Arcamone, F. Daunomycin and related antibiotics. *Top. Antibiot. Chem.* **2:**103–239 (1978).
5. Neidle, S. Interaction of daunomycin and related antibiotics with biological receptors. *Top. Antibiot. Chem.* **2:** 242–278 (1978).
6. Brown, J. R. Adriamycin and related anthracycline antibiotics. *Prog. Med. Chem.* **15:**125–164 (1978).
7. Gianni, L., B. J. Croden, and C. E. Myers. The biochemical basis of anthracycline toxicity and antitumor activity. *Rev. Biochem. Toxicol.* **5:**1–82 (1982).
8. Zbinden, G., E. Bachmann, and C. Holderegger. Model systems for cardiotoxic effects of anthracyclines. *Antibiot. Chemother.* **23:**255–270 (1978).
9. Schwarz, H. S. Mechanisms of selective cytotoxicity of Adriamycin, daunomycin and related anthracyclines, in *Molecular Aspects of Anti-cancer Drug Action* (S. Neidle and M. J. Waring, eds.). The Macmillan Press Limited, London, 093–125 (1983).
10. Valentini, L., V. Nicolella, E. Vannini, M. Menozzi, S. Penco, and F. Arcamone. Association of anthracycline derivatives with DNA: a fluorescence study. *Il Farmaco Ed. Sci.* **40:**377–389 (1985).
11. Acton, E. M., G. L. Tong, C. W. Mosher, and R. L. Wolgemuth. Intensely potent morpholinyl anthracyclines. *J. Med. Chem.* **27:** 638–645 (1984).
12. Sikic, B. I., M. N. Ehsan, W. G. Harker, N. F. Friend, B. W. Brown, R. A. Newman, M. P. Hacker, and E. M. Acton. Dissociation of antitumor potency from anthracycline cardiotoxicity in a doxorubicin analog. *Science (Wash. D. C.)* **228:**1544–1546 (1985).
13. Behr, W., K. Henikel, and G. Hartman. Interaction of the RNA polymerase inhibitor chromomycin with DNA. *Eur. J. Biochem.* **9:**82–92 (1969).
14. Crothers, D. M. Kinetics of binding of drugs to DNA. *Prog. Mol. Subcell. Biol.* **2:**11–20 (1970).
15. Mirau, P. A., and D. R. Kearns. The effect of intercalating drugs on the kinetics of the B to Z transition of poly(dG-dC). *Nucleic Acids Res.* **11:**1931–1941 (1983).
16. Feigan, J., W. A. Denny, W. Leupin, and D. R. Kerns. The interactions of antitumor drugs with natural DNA: a ^{1}H NMR study of binding mode and kientics. *J. Med. Chem.* **27:**450–465 (1984).
17. Phillips, D. R., and D. M. Crothers. Kinetics and sequence specificity of drug-DNA interactions: an *in vitro* transcription assay. *Biochemistry* **25:**7355–7362 (1986).
18. Wilson, W. D., and R. L. Jones. Intercalating drugs: DNA binding and molecular pharmacology. *Adv. Pharmacol. Chemother.* **18:**177–222 (1981).
19. Wakelin, L. P. G., G. J. Atwell, G. W. Rewcastle, and W. A. Denny. Relationships between DNA binding kinetics and biological activitiy for the 9-aminoacridine-4-carboxamide class of antitumor agents. *J. Med. Chem.* **30:**855–861 (1987).
20. Ross, W. D. DNA topoisomerases as targets for cancer therapy. *Biochem. Pharmacol.* **34:**4191–4195 (1985).
21. Sinha, B. K., M. A. Trush, K. A. Kennedy, and E. G. Mimnaugh. Enzymatic activation and binding of Adriamycin to nuclear DNA. *Cancer Res.* **44:**2982–2986 (1984).
22. Someya, A., and N. Tanaka. DNA strand scission induced by Adriamycin and aclacinomycin A. *J. Antibiot.* **32:**839–845 (1979).
23. Gabbay, E. J., D. Grier, R. E. Fingerle, R. Reimer, R. Levy, S. W. Pearce, and W. D. Wilson. Interaction specificity of the anthracyclines with DNA. *Biochemistry* **15:**2062–2069 (1976).
24. Wilson, D. W., D. Grier, R. Reimer, J. D. Baumar, J. F. Preston, and E. J. Gabbay. Structure activity relationships of daunomycin and its peptide derivatives. *J. Med. Chem.* **19:**381–384 (1976).
25. Grant, M., and D. R. Phillips. Dissociation of polydeoxynucleotide-daunomycin complexes. *Mol. Pharmacol.* **16:**357–360 (1979).
26. Chaires, J. B., N. Dattagupta, and D. M. Crothers. Kinetics of the daunomycin-DNA interaction. *Biochemistry* **24:**260–267 (1985).
27. Gandecha, B., and J. R. Brown. Dissociation kinetics of DNA-anthracycline and DNA-anthraquinone complexes determined by stopped-flow spectrophotometry. *Biochem. Pharmacol.* **34:**733–736 (1985).
28. Fox, K. R., C. Brassett, and M. J. Waring. Kinetics of dissociation of nogalamycin from DNA: comparison with other anthracycline antibiotics. *Biochim. Biophys. Acta* **840:**383–392 (1985).
29. Krishnamoorthy, C. R., S. Yen, J. C. Smith, J. W. Lown, and W. D. Wilson. Stopped-flow kinetic analysis of the interaction of anthraquinone anticancer drugs with calf thymus DNA, poly[d(G-C)]·poly[d(G-C)] and poly [d(A-T)]·poly[d(A-T)]. *Biochemistry* **25:**5933–5940 (1986).
30. Muller, W., and D. M. Crothers. Studies on the binding of actinomycin and related compounds to DNA. *J. Mol. Biol.* **35:**251–290 (1968).
31. Scourides, P. A., R. T. C. Brownlee, D. R. Phillips and J. A. Reiss. Application of analytical and semi-preparative high-performance liquid chromatography to anthracyclines and bis-anthracycline derivatives. *J. Chromatogr.* **288:**127–136 (1984).
32. Boston, R. C., P. C. Greif, and M. Berman. Conversational SAAM—an interactive program for kinetic analysis of biological systems. *Comput. Programs Biomed.* **13:**111–119 (1981).
33. Berman, M., and M. F. Weiss. *SAAM Manual.* U.S. DHEW Publication No. (NIH) 78–180, Washington, D. C.
34. Skorobogaty, A., R. J. White, D. R. Phillips, and J. A. Reiss. The DNA sequence specificity of daunomycin. *FEBS Lett.*, in press.
35. Fox, K. R., and M. J. Waring. Nucleotide sequence binding preference of noglamycin investigated by DNase I footprinting. *Biochemistry* **25:**4349–4356 (1986).
36. Dougherty, G., and W. J. Pigram. Spectroscopic analysis of drug-nucleic acid interactions. *CRC Crit. Rev. Biochem.* **12:**103–132 (1982).
37. Chaires, J. B., N. Dattagupta, and D. M. Crothers. Studies on interaction of anthracycline antibiotics and deoxyribonucleic acid: equilibrium binding studies on interaction of daunomycin with deoxyribonucleic acid. *Biochemistry* **21:**3933–3940 (1982).
38. Phillips, D. R., A. DiMarco, and F. Zunino. The interaction of daunomycin with polydeoxynucleotides. *Eur. J. Biochem.* **85:**487–492 (1978).
39. Newlin, D. D., K. J. Miller, and D. F. Pilch. Interactions of molecules with nucleic acids. VIII. Intercalation and T.A specificity of daunomycin in DNA. *Biopolymers* **23:**139–158 (1984).
40. Chen, K., N. Gresh, and B. Pullman. A theoretical investigation of the sequence selective binding of daunomycin to double-stranded polynucleotides. *J. Biomol. Struct. Dynamics* **3:**445–466 (1985).

Send reprint requests to: Dr. D. R. Phillips, Department of Biochemistry, La Trobe University, Bundoora, Victoria, 3083, Australia.