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SHORT COMMUNICATION

Dissociation of Polydeoxynucleotide-Daunomycin Complexes

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SUMMARY

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The first order rate constants for the dissociation of daunomycin from polydeoxynucleotides have been measured at 20° and 37°. The rate constants at a given temperature were similar and were in the order poly(dA)·poly(dT) > poly(dG)·poly(dC) > poly(dA-dT)·poly(dA-dT) > poly(dG-dC)·poly(dG-dC) at both temperatures. A comparison of these data with inhibition studies of DNA polymerase (using the same polydeoxynucleotide templates) indicates that at the DNA level, the mechanism of action of daunomycin is primarily due to disruption of the DNA secondary structure (i.e., dependent on the amount of daunomycin bound to the DNA template) but is not directly dependent on the rate of release of daunomycin from the DNA.

INTRODUCTION

The overall mode of action of the antineoplastic antibiotic, daunomycin, is known to be due to its ability to interact with DNA by an intercalation process (1, 2), resulting in the inhibition of both DNA and RNA synthesis (1, 2). However the details of the complex and its mode of action are still obscure, and without this information, any attempts to develop more effective derivatives of daunomycin must still rely on a "trial and error" basis.

It is well known that the chemotherapeutic index of the anthracyclines is dependent on many factors (solubility, protein binding capacity, membrane permeability, metabolism etc.) (3-5) as well as the amount of the drug bound to DNA (6). However, when considering only the drug DNA interaction, there are two major models for detailed mode of action (7):

i) With different polydeoxynucleotides as templates, the effectiveness of daunomycin in inhibiting DNA polymerase correlates with the affinity of daunomycin for these polymers (6). As the effectiveness of daunomycin is related to the amount of the drug bound to DNA, it indicates that the mode of action is due to disruption of the DNA secondary structure adjacent to the intercalated drug.

ii) The rate constant for the dissociation of derivatives of daunomycin from DNA has been inversely correlated with the ability of the drug to inhibit DNA-dependent RNA polymerase (8, 9). This suggests that the mode of action of daunomycin is due to its slow dissociation rate compared to the less effective derivatives with faster dissociation rates, i.e., the rate-limiting step for progression of the polymerase along the DNA template is the dissociation of the drug-DNA complex.

These two models of the mode of action may appear to be related since the inter-

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action of daunomycin (Da) with DNA can be represented as

$$\text{DNA} + \text{Da} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{DNA} - \text{Da}$$

and therefore the association constant (k_1/k_{-1}) is inversely related to the dissociation rate (k_{-1}). However, recent temperature-jump studies have shown the existence of two bound states of daunomycin at low daunomycin:nucleotide ratios.[3] By analogy with other intercalating systems, these two bound states probably reflect an inside and an outside bound form (10). The DNA-daunomycin interaction should then be represented as

$$\text{DNA} + \text{Da} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{DNA-Da}_{out} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{DNA-Da}_{in}$$

From this two-step binding mechanism it is evident that the two possible modes of action of daunomycin are largely independent, since the first model depends on the overall association constant (which is a function of all four rate constants), while the alternative dynamic model depends on only one rate constant, k_{-2}, for dissociation of the intercalated DNA-drug complex. The question regarding the mode of action of daunomycin can now be restated in a simplified manner. For a range of different DNAs, or a range of daunomycin derivatives, does the inhibition of DNA polymerase correlate with the overall association constant, or just with the off-rate?

In an endeavor to distinguish between these two alternative models of the detailed mechanism of action of daunomycin, we have measured the rate constants for the dissociation of polydeoxynucleotide-daunomycin complexes.

MATERIALS AND METHODS

The polydeoxynucleotides were obtained from P-L Biochemicals Inc., Milwaukee, Wis., U.S.A. and were dissolved in 1 mM PIPES buffer containing 0.15 M NaCl, pH 6.7. The daunomycin hydrochloride was a gift from Farmitalia, Milan, Italy. Fresh solutions were prepared in the PIPES buffer on the day required and concentrations determined using $E_{480} = 11{,}500\ M^{-1}\ cm^{-1}$. The polydeoxynucleotide concentration in the polymer-daunomycin solution was maintained near 4×10^{-4} Mp. The daunomycin:nucleotide ratio was 0.10 for all solutions. This ratio was selected as a satisfactory compromise between the need to have a sufficiently large absorbance change accompanying the dissociation of the complex (11) and the necessity to minimize the extent of weak external binding to DNA (11).

The stopped-flow kinetics were carried out using instrumentation previously described (12).

Sodium dodecyl sulphate (SDS) has previously been used (13) as an agent to disrupt intercalating systems and has also been used in the study of DNA-daunomycin complexes (8, 9). The reactions occurring can be represented (8, 9) as:

$$\text{DNA-Da} \xrightarrow{k_d} \text{DNA} + \text{Da}$$

$$\text{SDS} + \text{Da} \xrightarrow{k_s} \text{SDS-Da}$$

where k_d is a first order rate constant and is the rate controlling step, as the sequestering of daunomycin (k_s) is a diffusion controlled process. However, it is not possible, by analogy with the rate constants for other intercalating systems, to unambiguously determine whether the measured dissociation rate constant, k_d, is identical to k_{-2} of the two-step mechanism. Therefore, we have confined our attention to overall dissociation rate constants.

RESULTS AND DISCUSSION

The dissociation kinetics were first order for more than four half-lives for all of the polydeoxynucleotides studied, and the rate constants were independent of SDS concentration (in the range 0.2 to 0.6% premixing concentration) in each case. These results confirm that the rate-limiting step is a first order dissociative process and that the sequestering process is diffusion controlled. This has also been concluded by others using daunomycin complexed to salmon sperm DNA (8, 9).

The results are shown in Table 1. A small

[3] Bayley, P., S. Martin and D. R. Phillips, unpublished results.

TABLE 1

Rate constants for the dissociation of polydeoxynucleotide-daunomycin complexes in 1 mM PIPES buffer, 0.15 M NaCl, pH 6.7

Polynucleotide	Rate constant[a]		Inhibition concentration[b]	k^c
	20°	37.1°		
	(sec^{-1})		(μM)	(M^{-1})
poly(dG)·poly(dC)	4.2 ± 0.1	37	6	2.2×10^6
poly(dG-dC)·poly(dG-dC)	3.3 ± 0.15	27	7	1.7×10^6
poly(dA-dT)·poly(dA-dT)	3.9 ± 0.1	29	7	1.7×10^6
poly(dA)·poly(dT)	4.9 ± 0.15	47	26	0.6×10^6

[a] The errors shown for the rate constants measured at 20° represent the standard deviation of at least 15 determinations (three at each of five concentrations of SDS in the range 0.2–0.6%). Three measurements were made at 37°, using 0.6% SDS.

[b] The inhibition concentrations shown are the concentrations of daunomycin required for 50% inhibition of *E. coli* polymerase I when using the appropriate polydeoxynucleotide as a template, and are the values of Phillips *et al.* (6) (Tris HCl buffer, pH 7.4, I = 80 mM, 37°).

[c] The association constants of Phillips *et al.* (6) for the binding of daunomycin to an isolated potential binding site on DNA, as defined by Muller and Crothers (15). These values were determined in 0.15 M NaCl, pH 6.7, 20°.

but reproducible difference exists between each of the polydeoxynucleotides at 20° and also at 37°. The polydeoxynucleotide-daunomycin complexes have rate constants at 20° in the order poly(dG-dC)·poly(dG-dC) < poly(dA-dT)·poly(dA-dT) < poly(dG)·poly(dC) < poly(dA)·poly(dT) and the same sequence was obtained at 37°. The additional measurements were taken at 37° to ensure that the order is not changed at physiological temperatures due to differences of the energy of activation for the different complexes. Most of the reactions were studied at 20° because this enabled better stability and reproducibility of the data than at 37°.

The sequence of the polydeoxynucleotides, with respect to their rate constants, is similar to the sequence of polydeoxynucleotide templates with decreasing ability of daunomycin to inhibit DNA polymerase (Table 1). However, the correlation becomes tenuous if the relative values obtained for the homopolymers are compared, i.e., the relative inhibition concentrations for poly(dA)·poly(dT) compared to poly(dG)·poly(dC) is 4.3 whereas the rate constants are quite similar (a ratio of 1.2–1.3 depending on the temperature). These values should be compared with the ratio of 3.7 obtained for the relative affinity of daunomycin for these polymers (6) under conditions (0.15 M NaCl, pH 7, 20°) similar to those employed for determining the rate constants. Furthermore, there is complete sequence agreement between the inhibition studies and the binding studies (6). The implication then is that of the two sets of comparative data available, the relative affinity of daunomycin for these polymers correlates much better with the inhibition data than do the dissociation rate constants. Similar results have also been obtained by Gabbay *et al.* (9), who studied the inhibition of DNA dependent RNA polymerase with a variety of daunomycin derivatives.

These results suggest that the mode of action of daunomycin, at the DNA level, is due to the affinity of the drug for DNA. Presumably, the more drug bound to the DNA, the greater the disruption of the DNA secondary structure adjacent to the intercalated drug. The mode of action of daunomycin is therefore different from some other intercalating drugs, such as actinomycin, for which a slow dissociation rate appears to be essential for biological activity (7, 13).

It is interesting to speculate on the significance of the rate constant, k_d. By analogy with the magnitudes of the rate constants involved in other two-step intercalating systems (10, 14), it seems likely that the rate-limiting step for the dissociation is the DNA-Da_{in} to DNA-Da_{out} step, with rate constant k_{-2}. However, a completely unequivocal answer to the question of the

extent of a kinetic contribution to the mode of action of daunomycin must wait until all of the rate constants involved in the interaction have been determined, not only for daunomycin but also for some of the biologically active derivatives. These studies are currently in progress.

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