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SPECTROSCOPIC STUDIES OF THE INTERACTION OF DAUNOMYCIN WITH TRANSFER RNA

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Daunomycin is a glycosidic anthracycline antibiotic with both cytotoxic and antimitotic activity. The drug has been shown to inhibit both RNA and DNA synthesis (1,2). This biological action is most likely related to its ability to bind strongly to double-stranded (ds)DNA and thus inhibit template activity (3). Considerable experimental evidence exists in support of intercalation as the mechanism of strong binding of daunomycin to ds-DNA (4). Clinical activity of daunomycin has been primarily demonstrated in acute leukemias (5).

In this report, the interaction of daunomycin with unfractionated yeast transfer RNA (t-RNA) is examined using UV-visible spectroscopy, thermal denaturation and fluorescence. The binding of daunomycin to ds-RNA of viral origin has been previously investigated by thermal denaturation and viscosity studies (6). The results of those experiments show the secondary structure of the ds-RNA to be irrelevant to the stability of the complex formed with daunomycin. Furthermore, no evidence of intercalation was obtained. The conclusion, thus, was that the native B conformation of DNA is required for intercalation of daunomycin. The T_m and fluorescence studies described below present evidence of a substantially different kind of binding of daunomycin to t-RNA than to ds-RNA. While no means proof, the results are compatible with an intercalative mechanism for the drug-t-RNA interaction.

Unfractionated yeast t-RNA (A grade) was purchased from Calbiochem. Daunomycin (daunorubicin hydrochloride) and calf-thymus DNA (Type I) were

purchased from Sigma Chemical Company. These chemicals were used without further purification. All solutions were made up in 0.01 M phosphate buffer, pH 7 and all measurements were made at room temperature.

Concentrations of t-RNA solutions were determined spectrophotometrically, in moles nucleotide, using $\varepsilon_{260}^{1\%} = 201$. DNA concentrations, in moles nucleotide, and daunomycin concentrations were similarly obtained using the molar extinction coefficients $\varepsilon_{260} = 6600$ and $\varepsilon_{480} = 9870$ respectively (7).

The visible spectra were obtained on a Cary 14 spectrometer. Thermal hyperchromism was measured using a Beckman Acta CIII spectrophotometer with a Beckman temperature programmer. Fluorescence measurements were made on a Perkin-Elmer Fluorescence Spectrophotometer 204 with a Perkin Elmer 150 xenon power supply. All melting profiles at 260 nm were obtained with the reference cell containing daunomycin at the same concentration as the sample cell.

The visible spectrum of daunomycin undergoes a hypochromic, slightly bathochromic shift upon addition of t-RNA. Such behavior is typical of the interaction of planar drugs with nucleic acids, but does not reveal information about the mechanism of binding.

The melting profiles for various solutions of daunomycin with t-RNA are presented in Fig. 1.

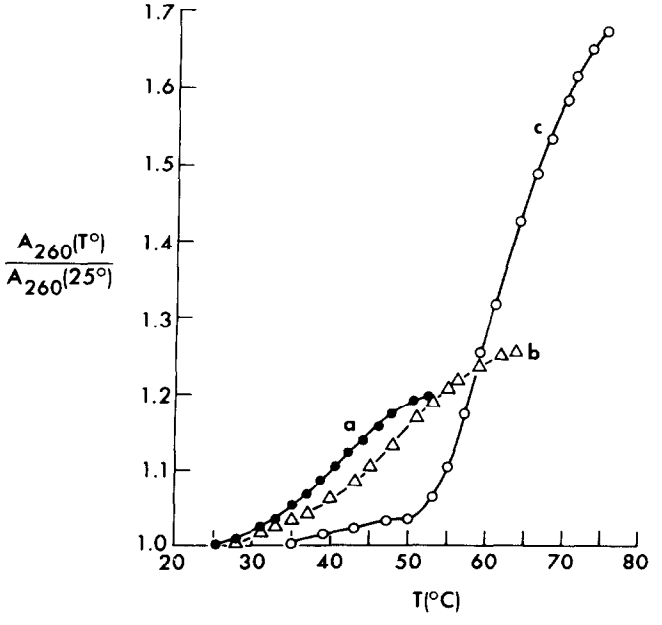

Figure 1. Melting profiles for daunomycin + t-RNA: (a) t-RNA alone; (b) P/D = 2.7; (c) P/D = 0.9. [t-RNA] = 10^{-4} M.

The increased hyperchromicity as more drug is added is most likely due to hyperchromicity in the strong absorption of daunomycin at 260 nm, resulting from dissociation of the drug-t-RNA complex. The secondary structure of t-RNA is significantly stabilized by the presence of the drug. The results for t-RNA are similar to those obtained in studies of ethidium binding to t-RNA (8). For example, at a nucleotide to drug ratio, P/D, of 2.7, daunomycin increases the T_m of t-RNA by about nine degrees. At a similar value of P/D and in a similar buffer, ethidium stabilizes t-RNA by ten degrees.

Measurement of the absorbance at 480 nm as a function of temperature showed the hyperchromism of the drug to occur in the same temperature range as the hyperchromism of the t-RNA measured at 260 nm. Similar behavior has been observed for the DNA-daunomycin complex (4) and suggests that binding of the drug depends on the integrity of the nucleic acid secondary structure. Furthermore, the interaction is reversible as evidenced by superimposable heating and cooling curves, as shown in Fig. 2.

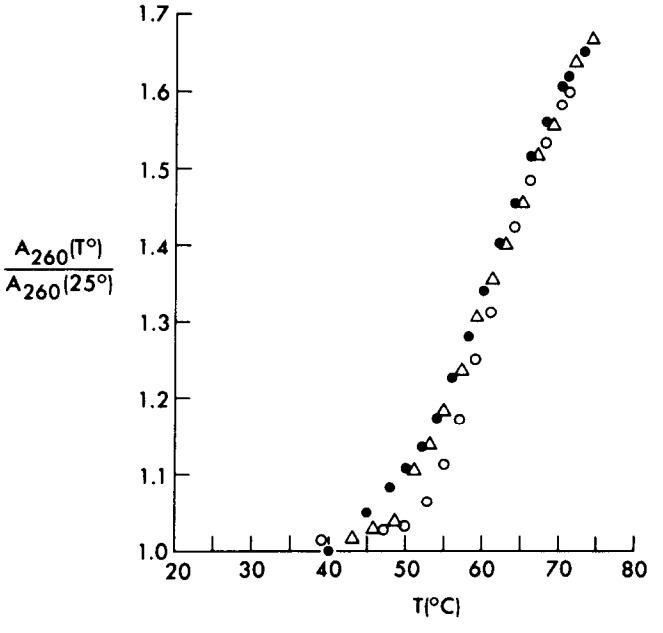

Figure 2. Melting and cooling profiles for daunomycin + t-RNA at P/D = 0.9; (O) first heating; (●) cooling; (▲) second heating. [t-RNA] = 10^{-4}M.

It has been shown that ds-DNA quenches the fluorescence of daunomycin (3). Such quenching is typical for intercalation (in some cases, e.g. ethidium, intercalation results in enhancement of fluorescence). We have measured the fluorescence of daunomycin at a fixed wavelength in solutions of varying concentrations of t-RNA and calf-thymus DNA. The results are displayed in Fig. 3. The results for DNA closely parallel those obtained previously in Mg^{++}-free buffers (3). Addition of t-RNA results in a significant amount of quenching, but somewhat less than addition of DNA. While the emission spectrum was not recorded in these experiments, the addition of nucleic acid did not shift the spectrum significantly.

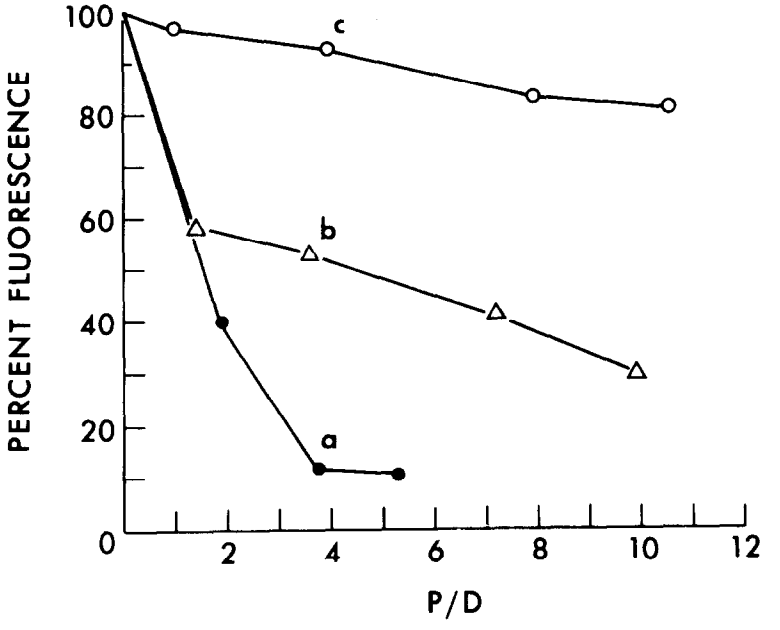

Figure 3. Fluorescence of daunomycin in the presence of various nucleic acids; excitation wavelength 480 nm, emission wavelength 565 nm: (a) drug + DNA; (b) drug + t-RNA; (c) drug + poly(A) - poly(U). [drug] = 4×10^{-6}M.

Data for construction of a Scatchard plot were obtained by spectral titration, at 480 nm, of a 10^{-5}M solution of daunomycin in a 10 cm cuvette. A plot of r/D_f as a function of r, where r is the average number of drug molecules bound per t-RNA molecule and D_f is the free

drug concentration, is presented in Fig. 4. The curve is biphasic, typical of intercalation complexes with nucleic acids (4,8,9). At low values of r, however, the graph is linear and may be analyzed according to the equation $r/D_f = K(n-r)$, where K is the equilibrium (association) constant and n is the number of available binding sites. The resulting value of K is 1.2×10^8 (M^{-1}) and the extrapolated value of n is 0.037. Assuming an average of 75 nucleotides per t-RNA molecule, this is equivalent to 2.8 sites per t-RNA.

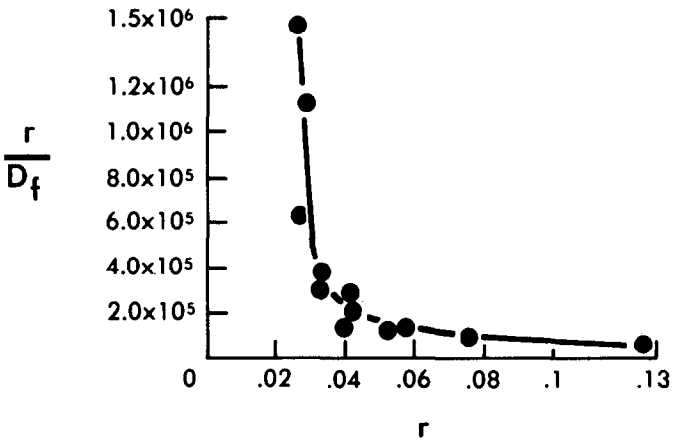

Figure 4. Isotherm for the binding of daunomycin to t-RNA.

The results presented above provide evidence that the interaction of daunomycin with t-RNA is different from that of daunomycin with viral ds-RNA. In the T_m, fluorescence quenching and spectral titration studies, t-RNA interacts with daunomycin in a manner similar to DNA. At the low ionic strength of these experiments, t-RNA is probably in an extended form, but still possessing hydrogen-bonded regions (10). These results are consistent with, but not proof of, intercalation of the drug in the double helical segments of t-RNA, as proposed for ethidium (8).

The question of binding mechanism aside, these studies suggest, in addition to DNA, t-RNA may also be a receptor for daunomycin. This may also be true for adriamycin, which is very similar structurally to daunomycin. In a recent study, adriamycin was shown to inhibit protein

synthesis in cell-free systems (11). This effect may be due to binding of the drug to t-RNA. Work is currently in progress to characterize the anthracycline-t-RNA interaction more completely.

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