THE INTERACTION OF DAUNOMYCIN WITH DNA'S OF VARYING COMPOSITION

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Daunomycin, an anthracycline glycoside antibiotic, has cytotoxic and antimitotic activities (Di Marco et al 1965), and inhibits both enzymatic RNA and DNA synthesis by binding to DNA thus interfering with the DNA template function (Calendi et al 1965). In analogy with the hypothesis of Lerman et al (1964) for acridine dyes, it has been suggested that intercalation of daunomycin between adjacent base pairs on the DNA is responsible for the drug action (Di Marco 1967, Waring 1970). The interaction of daunomycin with DNA has been demonstrated by several methods including ultra-violet and visible absorption spectrophotometry. In the visible region daunomycin shows a peak absorbance at 475 nm. On addition of DNA the absorbance is reduced in proportion to the DNA concentration and no further variations appear after a DNA to daunomycin ratio of about 8.7 to 1 is reached (Calendi et al 1965). Zunino et al (1972) defined two classes of binding site - one being the 'strongly' bound antibiotic molecules intercalated on the DNA double helix, and the other 'weakly' bound molecules probably attached by means of electrostatic interaction involving the phosphate groups and the daunomycin amino group. These workers therefore suggested that the double helical structure of DNA was a necessary condition for the strong binding process. This communication reports the use of visible absorption spectrophotometry to follow the interaction of daunomycin and DNA at different temperatures, and by means of Scatchard plots (Scatchard 1949) to calculate apparent association constants for the strong binding process.

$\log K = \frac{-\Delta H^\circ}{2.303R} \cdot \frac{1}{T} +$ constant, where K is the apparent association constant, and ΔH° is the change in enthalpy. A plot of log K against $\frac{1}{T}$ enables a value for ΔH° to be calculated for the interaction. This has been applied to DNAs of varying guanine-cytosine (G-C) content, and the ΔH° values give an indication of the strength of the interaction involved.

DNA Source	Apparent number of strong binding sites per DNA molecule	K apparent x 10^6 (M^{-1}) at T = 285°	ΔH° (kJ mol^{-1})	Ratio ΔH° to G-C content
Native Calf Thymus (40 mole percent G-C)	0.20	1.214	-18.0	0.45
Denatured Calf Thymus (40 mole percent G-C)	0.22	1.262	-14.0	0.35
Native Salmon Sperm (44 mole percent G-C)	0.22	2.649	-20.0	0.45
Native M.lysodeikticus (72 mole percent G-C)	0.20	5.102	-31.0	0.43

The above results show that the double helical structure of DNA is not essential for the strong binding process since denaturing the DNA does not significantly alter the concentration of binding sites. The interaction is, however, more stable with native DNA as shown by the greater ΔH° value. It is also apparent that increasing the G-C content of the DNA increases the strength of the interaction but not the concentration of 'strong' binding sites.

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