STUDIES ON THE MODE OF INTERACTION OF DAUNOMYCIN WITH DNA

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1. Introduction

Daunomycin inhibits nucleic acid synthesis both in a cell-free system [1, 2] and *in vivo* [3, 4]. This antitumour antibiotic of the anthracycline group binds very strongly to DNA. A considerable body of evidence has been accumulated which points to an intercalative mode of binding [1, 5–7].

Lerman [8] has established that an increase in intrinsic viscosity and a decrease in the sedimentation coefficient are two hydrodynamic criteria for intercalation of ring systems between base pairs of double-helical DNA. In a systematic investigation of drugs believed to intercalate, Waring [9] reported that changes in supercoiling of closed circular DNA may be employed to verify intercalative binding. This variation of supercoils, a result of drug-induced local uncoiling of the double helix, are revealed by changes in the sedimentation coefficient. Recently [10], a viscosimetric method has been developed for following the configurational changes in closed circular DNA. The theoretical and practical aspects of these phenomena are consistent with the model of Lerman [8].

In this paper we present the results of the behaviour of daunomycin on DNA-cellulose column chromatography and of low-shear viscosimetry. These two experimental methods were complementary in that the former provides information regarding the occurrence of different types of interaction, while the latter yields indication of the size and shape of the daunomycin–DNA complex. This dual approach has improved our understanding of the interaction.

2. Materials and methods

Daunomycin hydrochloride (M.W. 563.5) was supplied by Farmitalia, Milano. The antibiotic was stored in the dark in a dessicator at 4°. Solutions in buffer were freshly prepared immediately before use.

Calf thymus DNA was isolated according to the procedure of Zamenhof [11]. Denaturation was accomplished by heating at 90° for 10 min in 0.001 M NaCl, followed by fast cooling. Sonicated DNA was prepared as reported by Doty et al. [12].

Spectrophotometric measurements were carried out in a Zeiss PMQII or a Beckman DU spectrophotometer.

The DNA-cellulose column chromatography was carried out as described by Inagaki and Kageyama [13]. Methods particular to this study will be described in the following.

Viscosities were measured at 20° by means of a Zimm-Crother low shear viscosimeter adjusted to give a shear stress of less than 0.002 dynes/cm^2.

3. Results and discussion

Fig. 1 shows the elution pattern of daunomycin on DNA-cellulose. When 0.2 ml of 0.5% solution was applied to the column, using the stepwise elution technique (fig. 1), about 75% of the drug was eluted by 2 M NaCl and the rest was dissociated by addition of 7 M urea to 2 M NaCl.

Using the gradient elution procedure, the chroma-

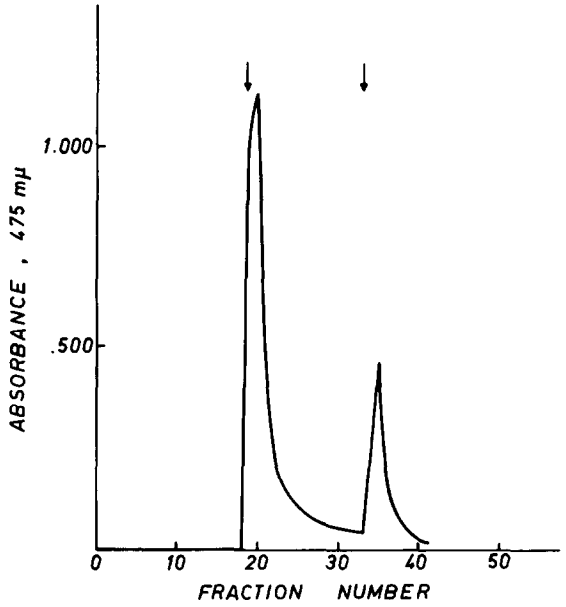

Fig. 1. DNA-cellulose chromatography of daunomycin. 0.2 ml of 0.5% daunomycin solution (in 0.01 M Tris, 0.001 M EDTA, pH 7.0) were loaded on a 0.6 × 4 cm column. The daunomycin loading took place at fraction 0. After loading, the column was washed with the buffer and the bound material was eluted by 2 M NaCl and successively by 7 M urea in 2 M NaCl–0.01 M Tris–0.001 M EDTA, pH 7.0. The stepwise elutions are indicated by the vertical arrows. Fractions of 3.7 ml were collected. Recovery was 100%. Flow rate, 37 ml/hr.

tographic pattern shows two peaks centered at about 0.2–0.4 M in NaCl gradient and at about 2.5–3 M in urea gradient. This finding parallels the results obtained with the stepwise elution technique (fig. 1). When daunomycin samples were loaded at two differing concentrations (table 1), the amount of daunomycin dissociated by urea gradient is not markedly affected by increasing the amount of the sample loaded. The purity of daunomycin sample used was checked by thin layer chromatography. It is unlikely that the chromatographic behaviour can be attributed to a mixture of two different molecular species. In addition, the amount of drug retained by cellulose alone is negligible (table 1).

These experiments agree with the spectrophotometric titration of daunomycin with DNA [14]. The adsorption isotherm ("Scatchard plot") indicates the existence of more than one class of binding sites for daunomycin on DNA. This conclusion is clearly

Table 1
Comparison of the amount of daunomycin dissociated by 7 M urea in 2 M NaCl from a DNA-cellulose column.

	Load (mg)	Amount eluted by urea gradient (mg)	r (molar ratio antibiotic/DNA P)
Control** (cellulose alone)	1.140	negligible (0.005)	–
DNA-cellulose*	1.140	0.162	0.125
DNA-cellulose*	5.560	0.202	0.156

Conditions are as illustrated in legend to fig. 1.
* Elution was carried out with a linear molarity gradient (50 + 50 ml) of NaCl (0–2 M) and successively with a linear molarity gradient (100 + 100 ml) of urea (0–7 M) in 2 M NaCl.
** In this experiment, acid-washed cellulose was used. After loading, the column was washed with the buffer. Elution was carried out stepwise, according to the scheme shown in fig. 1.

supported by the chromatographic behaviour of daunomycin on DNA-cellulose column.

The present results suggest a weak binding mode of interaction, probably electrostatic, between antibiotic molecules and DNA, involving the DNA phosphate groups and daunomycin amino group; and a stronger binding mode requiring urea for the dissociation.

To understand the strong binding of daunomycin to DNA it has been suggested that drug molecules intercalate between successive base pairs of the double helix, a process accomodated by a lengthening and a slight local untwisting of the helix.

This possibility is supported by the marked effects of the binding of daunomycin to DNA on its conformation. Many features of the interaction between daunomycin and DNA [5, 7, 15] are similar to those reported for acridine dyes and similar planar compounds, which were postulated by Lerman [8] to intercalate between base pairs in the DNA complex. Daunomycin-induced increase in viscosity and decrease in sedimentation of native DNA are consistent with the intercalation hypothesis. Other authors reported an increase in the relative viscosity [5, 15] and reduced specific viscosity [7] of native DNA of

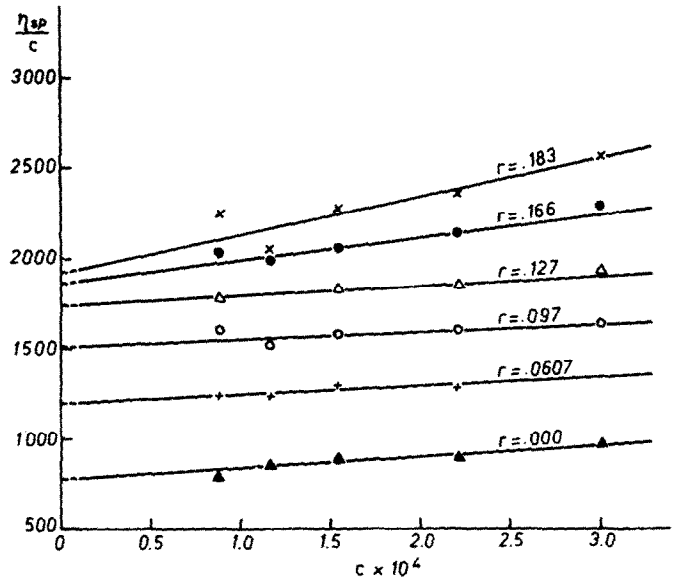

Fig. 2. Determination of intrinsic viscosity of DNA-daunomycin complexes for different values of "r" with sonicated (native) DNA. Conditions of the viscosity measurements; 20°, 0.1 M Tris, pH 7.0. Concentrations (C) of DNA are given in molarities of DNA phosphorus.

high molecular weight. Although a provisional interpretation of this increase was attempted, it could not be securely based, because the points on the plot of the relative (or reduced) viscosity as a function of drug concentration correspond to DNA attached to different amounts (r) of antibiotic. Therefore, a refinement of the viscosity measurements was performed in order to obtain the relation between the intrinsic viscosity of DNA and the amount (r) of antibiotic bound to it.

Fig. 2 shows the curves used to determine intrinsic viscosities. Dilutions were carried out at constant "r" by adding solvent containing a concentration (m) of free daunomycin according to the method of Müller and Crother [16]. The relationships between the intrinsic viscosity of sonicated native DNA and the amount (r) of bound daunomycin are presented in fig. 3. In this figure the intrinsic viscosity of each drug–DNA complex has been divided by the intrinsic viscosity of DNA alone.

The effects on viscosity shown are qualitatively consistent with previous reports [5, 7, 15]. The intrinsic viscosities of the complexes increase with "r" up to "r" values (about 0.16–0.20) corresponding closely both to the apparent number of binding sites per nucleotide ($r = 0.12$), obtained by extrapolation of the curve for small values of "r", in the spectrophotometric titration of daunomycin with DNA [14] and to the molar ratio drug/nucleotides (about 0.12–0.16) recovered by urea gradient, in DNA cellulose column (table 1).

It has been reported [17] that the changes in hydrodynamic properties of sonicated DNA fragments, arising from the intercalation of dye molecules, should be almost exclusively due to an increase in contour length with respect to viscosity. The present results with sonicated DNA could be tentatively explained by a similar mechanism as proposed for acridine dyes. Relevant to this point is the observation that the viscosity increase reported for aminoacridines [18, 19] is very similar to those presented here. In the case of acridine dyes, the change in viscosity, up to an "r" value of about 0.2, corresponds closely to an increase in contour length; for each bound molecule roughly equal to normal spacing (3.35 Å) between DNA base pairs. According to the hypothetical model [8], intercalation of "r" antibiotic molecules would increase the contour length (L) by a factor of $(1 + 2r)$. This consideration suggests a comparison of L/L_0 (the ratio of

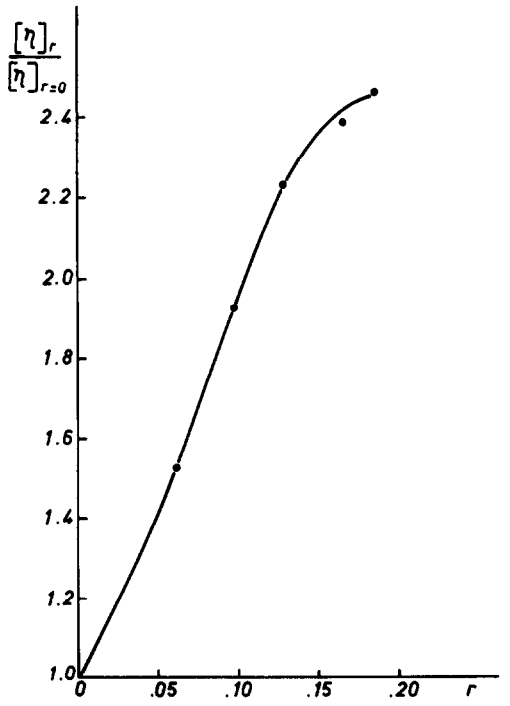

Fig. 3. Intrinsic viscosity of daunomycin-DNA complexes relative to intrinsic viscosity of DNA alone.

length of the complex to that of DNA) as a function of "r", with the theoretical curve $(1 + 2\,r)$.

The relative length increase (L/L_0) could be roughly estimated by the intrinsic viscosity data, by using an equation applicable to rod-like molecules [17] $L/L_0 = \{[\eta]f(p_0)/[\eta]_0 f(p)\}^{1/3}$, where "$p$" is the axial ratio of the particle. The experimental slope of the increase of L/L_0 with r (about $1 + 2.54\,r$) falls rather close to that to be expected $(1 + 2\,r)$, if the length increased by exactly one base-pair spacing when one daunomycin molecule is bound. As this estimation is not exact, alternatively one could compare the viscosity results with those to be expected for pure DNA of corresponding contour length. The relative increase in viscosity $([\eta]/[\eta]_0)$ has been calculated for pure DNA with corresponding contour length by using the method described by Ullman [20] for a wormlike chain model. The experimental viscosity enhancement for daunomycin-DNA complexes is significantly larger than that to be expected according to Ullman's method [we used d (diameter) = 25 Å, α (hydrodynamic parameter) = 10 and λ (degree of extension) = 9.27×10^{-4}].

This consideration suggests that the calculated length increase, larger than one base-pair spacing, could not be of marginal significance. Recent results with daunomycin derivatives [14] indicate that, under identical conditions of "r" and ionic strength, the intrinsic viscosity is enhanced less by some daunomycin derivatives than by daunomycin. The high daunomycin-induced viscosity increase is not completely explained by an increase in contour length of the DNA chain.Other effects, such as decrease in flexibility of the DNA, are possible.

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