COMPLEX FORMATION OF DAUNOMYCIN WITH DOUBLE-STRANDED RNA

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1. Introduction

Daunomycin, a glycosidic anthracycline product of *Streptomyces peuceticus*, is an antibiotic with cytotoxic and antimitotic activities. This action is probably due to the ability of forming an intercalation complex with DNA [1]. A complex with RNA was also found, but its character seemed to be different from that of DNA [2]. This communication is concerned with complex-forming ability of daunomycin with double-stranded RNA (replicative form of f2 phage RNA) in comparison with ribosomal RNA and both native and denatured DNA. In contrast with DNA, the secondary structure of RNA was found irrelevant for the stability of the complex and no evidence of intercalation was obtained.

2. Methods

Double-stranded RNA was isolated from cultures of *E. coli* K 38 infected with phage f2 sus 11 [3], using the method of Billeter and Weissmann [4]. The RNA had a sedimentation coefficient of 8.1 S, its T_m was 86°C (in 10 mM NaCl with 5 mM trisodium citrate) and the hypochromicity was 24.9%). The absence of contamination with single-stranded RNA was evident from the fact that there was no change in absorbance between 25 and 72°C, followed by a sharp increase of absorbance above 75°C. The absence of DNA was checked by diphenylamine reaction. For viscosity measurements this RNA was chromatographed on a column of Sephadex G-200 to obtain a fraction with mean sedimentation coefficient 12.9 S.

DNA was prepared from *E. coli* according to Marmur [5]. Ribosomal RNA was extracted as described earlier [6] and separated from tRNA and fragments of DNA by repeated precipitation with 2M-NaCl [7, 8]. Daunomycin hydrochloride A grade (Calbiochem) was used.

The substances were dissolved in 10 mM NaCl with 5 mM trisodium citrate, pH 7.0 Thermal hyperchromic spectra were obtained using a Zeiss spectrophotometer, adapted for measurements at different temperatures [9]. Viscosity was measured at 30°C using a six-bulb Ubbelohde viscosimeter and extrapolating the measured values of specific viscosity to zero shear stress. Circular dichroic spectra were determined with a Roussel-Jouan CD 185 Dichrograph in a cell of 1.0 cm optical path length at approx. 22°C. The values of molar ellipticity are given in $deg.cm^2.dmol^{-1}$ and were calculated using a molar concentration of daunomycin 8.9×10^{-5} M.

Results and discussion

When single- or double-stranded RNA was added to aqueous neutral buffered solutions of daunomycin, a hypochromic effect and a shift of the absorption maximum to longer wavelengths was observed on the visible spectrum of daunomycin. A macromolecular structure of the polynucleotide was required for the alteration of spectrum, since the effects disappeared upon treatment with RNAase (rRNA) or by heating

and RNAase treatment (double-stranded RNA). More RNA than DNA was required to produce maximum hypochromic effect and single-stranded polynucleotides (denatured DNA or rRNA) were capable of binding slightly more antibiotic than double-stranded DNA or RNA, respectively (fig. 1). The stronger binding capacity of DNA, especially of denatured DNA, is probably connected with the ability of precipitating the antibiotic at higher concentrations of both components. No such tendency was ever observed with RNA.

The thermal stability of the complexes in 10 mM NaCl with 5 mM trisodium citrate, pH 7.0, was determined by measuring hyperchromic profiles at 480 nm and visible spectra of daunomycin at critical temperatures (fig. 2). The behaviour of the complex of native DNA of *E. coli* (fig. 2A) was similar to that observed by Zunino et al. [1] using calf thymus DNA; in our experiment the dissociation of the complex, as indicated by the sharp increase of A_{480}, took place at about 93°C, while the T_m of DNA alone in the same buffer was 79°C. The cooling curve of the dissociated complex showed two distinct steps, the first one, between 95 and 85°C, indicating the complex formation with reversibly-renaturing DNA, in consequence of the stabilization of nucleotide pairs by daunomycin [1]; the second hypochromic effect, observed between 70 and 50°C, was apperently due to the formation of a complex with denatured DNA and was fully reversible as shown by second heating. It may be noted that the hypochromic effect of denatured DNA is larger than that of native DNA before heating.

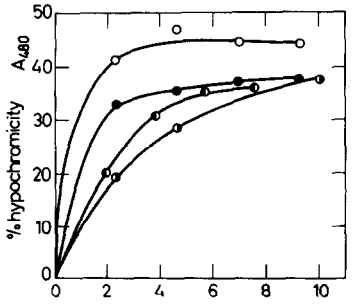

Fig. 1. The hypochromic effects of nucleic acids on daunomycin. To 22.5 uM daunomycin in 10 mM NaCl with 5 mM trisodium citrate, pH 7.0, increasing amounts of nucleic acids were added and the spectra were recorded correcting the absorbance for dilution. The hypochromicity at 480 nm was plotted as a function of molar ratio of nucleotides to daunomycin. (○–○–○), denatured DNA; (●–●–●), native DNA; (◐–◐–◐), rRNA; (◑–◑–◑), double-stranded RNA.

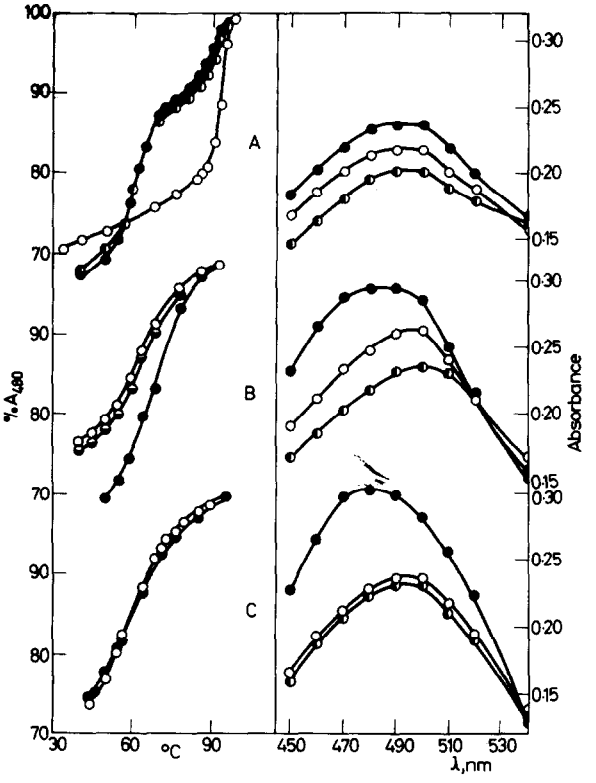

Fig. 2. Spectral changes of complexes of daunomycin with nucleic acids upon heating and cooling. The concentration of daunomycin was 44.5 μM. Nucleic acids were added in amounts required to produce maximum hypochromic effect. Left, A_{480} in % absorbance at 95°C. Right, visible spectra, recorded at different temperatures, before or after heating. A, DNA, 2.8 nucleotides/daunomycin; B, double-stranded RNA, 11.6 nucleotides/daunomycin; C, rRNA, 8.75 nucleotides/daunomycin. (○–○–○) left, heating curve; right, spectrum at 35°C before heating; (◉–◉–◉) cooling curve after heating to 80°C; (●–●–●) left, cooling curve after heating to 95°C; right, spectrum at 75°C; (◐–◐–◐) left, second-heating curve; right, spectrum at 35°C after heating to 95°C and cooling.

Double-stranded RNA, however, behaved in quite a different manner. A hyperchromic effect was observed when heating the complex between 50 and 80°C concurrently with the change of the shape of the spectrum to that of pure daunomycin. If the solution was cooled from 80°C downwards, the cooling curve was found to coincide with the heating curve, indicating the reversible formation of the complex with double-stranded RNA. If, however, the solution was heated to 96°C and cooled afterwards, the hypochromicity

was larger than before heating, which means that the RNA in denatured state has caused a stronger hypochromic effect than the same RNA in the double-helical form. (fig. 2B). The thermal profile of ribosomal RNA (fig. 2C) indicated a reversible dissociation of the complex with the T_m about 61°C.

When the thermal melting profile of double-stranded RNA was measured at 260 nm, the T_m value of melting of the double-helix in the presence or absence of daunomycin was the same, namely 86°C. The cooling curve of thermally denatured RNA showed the same hysteresis of renaturation with and without daunomycin, indicating that no stabilization of nucleotide pairs has taken place.

Although the bonds between daunomycin and single- or double-stranded RNA dissociate at the same temperature, circular dichroic spectra indicate that the structure of the daunomycin molecule is altered more profoundly by interacting with the latter (fig. 3).

The experiments indicate that the complex of daunomycin with double-stranded RNA dissociates at a lower temperature than the T_m of RNA itself, so that no effect of daunomycin on the thermal stability of the double helix can be observed; secondary structure

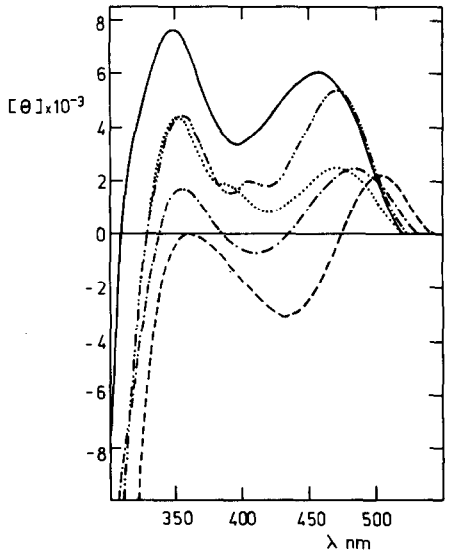

Fig. 3. Circular dichroic spectra of daunomycin and its complexes with different species of nucleic acids. (——) daunomycin (89 μM); (– – –) native DNA, 2.8 nucleotides/daunomycin; (– . – . –) denatured DNA, 2.8 nucleotides/daunomycin; (- - -) double-stranded RNA, 11.6 nucleotides/daunomycin; (— • • —) rRNA, 11.6 nucleotides/daunomycin.

Table 1
Effect of daunomycin on viscosity of DNA and double-stranded RNA

Nucleic acid μM	Daunomycin moles/nucleotide	Specific viscosity η sp.	Coefficient of viscosity increase due to daunomycin
DNA 160	None	0.49	–
DNA 160	0.11	0.87	1.77
dsRNA300	None	0.35	–
dsRNA300	0.10	0.37	1.06
dsRNA300	0.20	0.34	0.97

of RNA seems to be irrelevant for the stability of the complex, in contrast with DNA. It appears that double-stranded RNA is incapable of forming an intercalation complex with daunomycin. Measurements of viscosity [1, 2] confirm this conclusion, indicating that the viscosity of double-stranded RNA remains unchanged upon adding daunomycin, while that of DNA increase markedly under the same conditions (table 1).

The bond linking daunomycin with double-stranded RNA thus appears to be of similar character as with single-stranded RNA or denatured DNA, presumably involving electrostatic attraction between the phosphate group of nucleic acid with amino group of the daunosamine moiety of the antibiotic [1]. The thermal melting profiles presented here indicate that this bond dissociates in a reversible and cooperative manner at about 61°C. Since no major structural change of nucleic acids is known to occur at this temperature, the reasons of the dissociation are not clear at present. In contrast with DNA no evidence of intercalation can be obtained with double-stranded RNA. It appears that the B-conformation of native DNA is a prerequisite for the formation of the intercalation complex. A similar conclusion was obtained studying the complex formation of actinomycin with nucleic acids [10].

References

[1] Zunino, F., Gambetta, R., di Marco, A. and Zaccara, A. (1972) Biochim. Biophys. Acta 277, 489.
[2] Calendi, E., di Marco, A., Reggiani, R., Scarpinato, B. and Valentini, L. (1965) Biochim. Biophys. Acta 103, 25.

[3] Lodish, H.F. and Zinder, N.D. (1966) J. Mol. Biol. 19, 333.
[4] Billeter, M.A. and Weissmann, C. (1966) in: Procedures in Nucleic Acid Research (Cantoni, G.L. and Davies, D.R., eds), p. 498, Harper and Row, New York and London.
[5] Marmur, J. (1961) J. Mol. Biol. 3, 208.
[6] Bolton, E.T. (1966) in: Procedures in Nucleic Acid Research (Cantoni, G.L. and Davies, D.R., eds), p. 437, Harper and Row, New York and London.
[7] Franklin, R.M. (1966) Proc. Natl. Acad. Sci. U.S. 55, 1504.
[8] Cox, R.A., Kanagalingam, K. and Sutherland, E.S. (1970) Biochem. J. 120, 549.
[9] Boublík, M., Pivec, L., Šponar, J. and Šormová, Z. (1965) Collect. Czech. Chem. Commun. 30, 2645.
[10] Haselkorn, R. (1964) Science 143, 682.