STRONG BINDING OF DITRISARUBICIN B TO DNA

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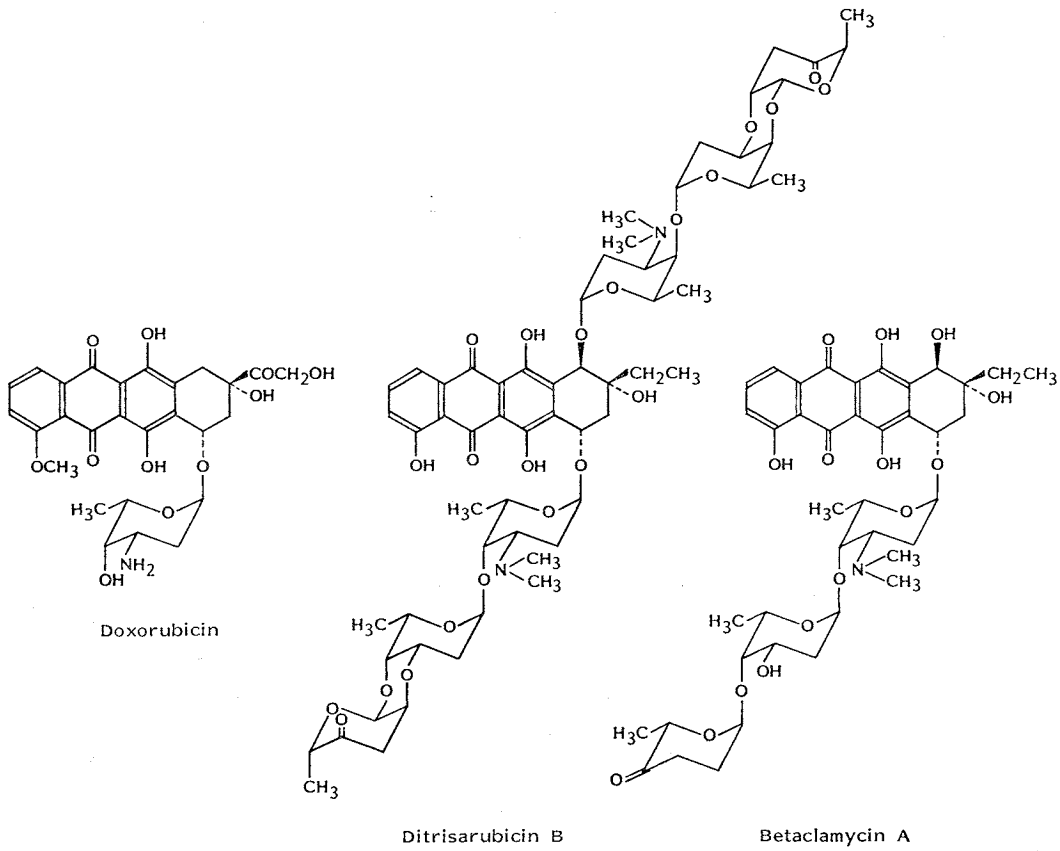
DNA binding characteristics of ditrisarubicin B were studied by the fluorescence titration technique. Ditrisarubicin B bound to calf thymus DNA with an affinity higher than any we have ever seen among anthracyclines. The apparent association constant (K_{app}) of ditrisarubicin B was 2.36×10^8 M^{-1}, which is 22.7 times larger than that of doxorubicin. The apparent number of binding sites (n_{app}) of ditrisarubicin B per nucleotide of DNA was 0.164, and this value is identical with that of doxorubicin. Betaclamycin A, which has a trisaccharide chain at C-7 but no carbohydrate at C-10 in the aglycone, interacted with DNA to give a K_{app} of 5.92×10^6 M^{-1} and n_{app} of 0.178. These results suggest to us that the high affinity of ditrisarubicin B for DNA is caused by the existence of a glycosidic chain at C-10.

Ditrisarubicin antibiotics[1] are new members of the anthracycline family which have three hexoses at each of C-7 and C-10. They prolong the survival period of mice bearing leukemia L1210[1] and

Scheme 1.



are effective against leukemia P388 and also doxorubicin-resistant leukemia P388, as described elsewhere[2]. Concerning their mechanism of action, they have been shown to inhibit nucleic acid synthesis, especially RNA synthesis, at low concentrations[1]. For these reasons we decided to examine the DNA binding activity of ditrisarubicin B. This ditrisarubicin is shown to bind extremely tightly to calf thymus DNA. Structure-activity relationships are discussed based on the comparative study of an analogue, betaclamycin A, and a space-filling model of the drug-DNA complex.

Materials and Methods

Chemicals

Calf thymus DNA was purchased from P-L Biochemicals, Milwaukee, Wis., U.S.A. Ditrisarubicin B was prepared by us as described previously[1]. Betaclamycin A was a gift from Sanraku Incorporated Central Research Laboratories, Fujisawa, Japan.

Fluorometric Titration of DNA Binding of Anthracyclines

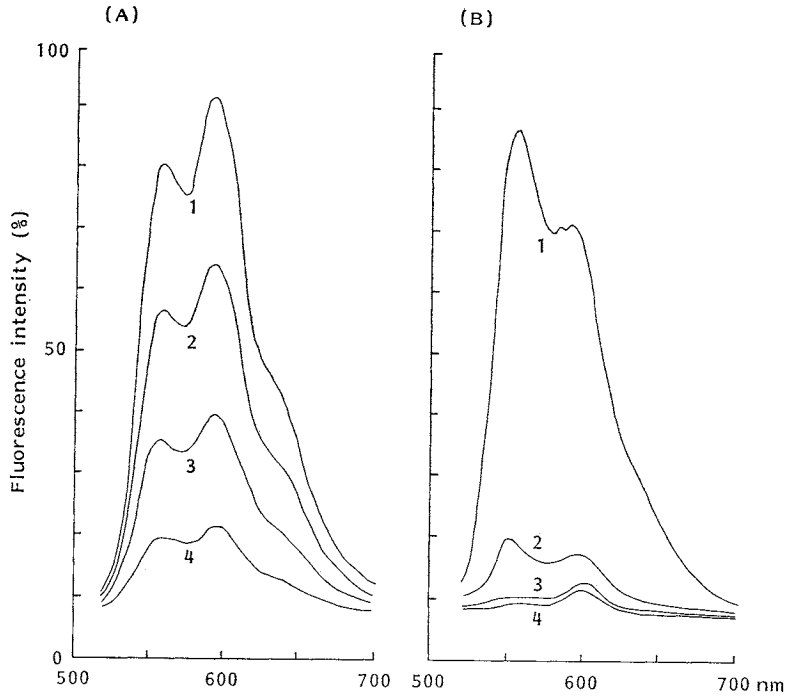
The quenching of anthracycline fluorescence by DNA was measured in 0.05 M phosphate buffer, pH 7.0, containing 2.5×10^{-7} M doxorubicin or ditrisarubicin B and various concentrations of calf thymus DNA. In the case of determination of binding constants ditrisarubicin B (2.59×10^{-7} M), doxorubicin (2.59×10^{-6} M), or betaclamycin A (2.59×10^{-6} M) was mixed with calf thymus DNA varying in amount between 5×10^{-7} M and 2.5×10^{-6} M for ditrisarubicin B and 2×10^{-6} M and 2×10^{-5} M for doxorubicin or betaclamycin A. DNA concentration of 6.66×10^{-4} M was taken as the end point of quenching. A Hitachi MPF-4 spectrofluorometer with thermostat set at 20°C was used. Excitation wavelength was 500 nm, and fluorescence emission was determined at 550 nm for ditrisarubicin B and betaclamycin A or at 590 nm for doxorubicin. DNA concentration was determined spectrophotometrically at 260 nm by using a molar extinction coefficient with respect to nucleotide residues of 6,600 M^{-1}.

The binding data was analyzed by the SCATCHARD method[3].

Results and Discussion

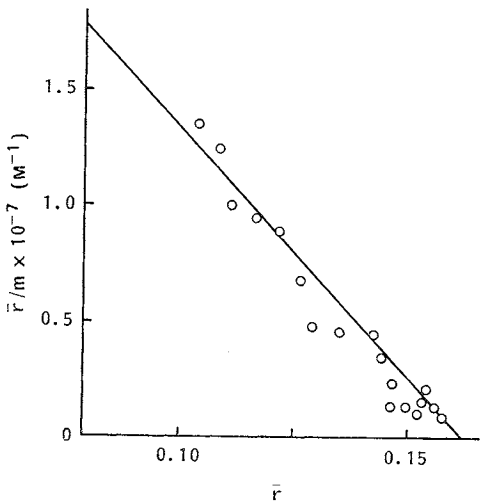
Fluorescence spectral changes of ditrisarubicin B and doxorubicin upon interaction with calf thymus DNA are shown in Fig. 1. Fluorescence of ditrisarubicin B was much more quenched at lower DNA concentrations than that of doxorubicin. Therefore, ditrisarubicin B was demonstrated to have higher affinity for DNA than doxorubicin. The binding parameters of the former were determined by SCATCHARD plot[3] as shown in Fig. 2. The apparent binding constant (K_{app}) and apparent number of binding sites (n_{app}) were obtained from the negative slope and the intercept of the curve with the $\bar{r}$ axis, respectively. Results of similar analysis for doxorubicin and betaclamycin A, which was carried out to clarify the structure-activity relationship for ditrisarubicin B, are shown in Fig. 3; and the mean values of K_{app} and n_{app} from several experiments are given in Table 1. Ditrisarubicin B was equivalent to doxorubicin in the apparent number of binding sites per nucleotide of DNA; however, the K_{app} of ditrisarubicin B was 2.36×10^{8} M^{-1}, which is the highest value ever reported for the binding of any anthracycline to calf thymus DNA. As the K_{app} of doxorubicin was 1.04×10^{7} M^{-1} for the same DNA solution, ditrisarubicin B has apparent affinity for DNA 22.7 times higher than doxorubicin. From comparative studies with another anthracycline, betaclamycin A, we propose the strong binding properties of ditrisarubicin B to be attributable to the trisaccharide at C-10 in the aglycone. Structure of betaclamycin A was determined to be 7-(cinerulosyl-2-deoxyfucosyl-rhodosaminyl)-β-rhodomycinone by YOSHIMOTO *et al.*[4]. This anthracycline thus differs from ditrisarubicin B in the absence of a trisaccharide at C-10 and the displacement of cinerulose B at the end of

Fig. 1. Quenching of fluorescence of doxorubicin or ditrisarubicin B by calf thymus DNA.



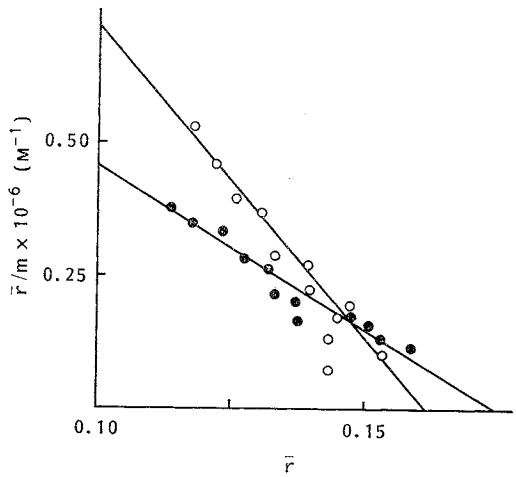
2.5×10^{-7} M doxorubicin (A) or ditrisarubicin B (B) in 0.05 M sodium phosphate buffer, pH 7.0, were titrated with varying concentrations of calf thymus DNA (**1**: free, **2**: 5×10^{-7} M, **3**: 10^{-6} M, **4**: 2×10^{-6} M).

Fig. 2. SCATCHARD analysis of calf thymus DNA binding with ditrisarubicin B.



$\bar{r}$: [Drug]bound/[DNA]total, m: [drug]free.

Fig. 3. SCATCHARD analysis of calf thymus DNA binding with doxorubicin or betaclamycin A.
○ Doxorubicin, ● betaclamycin A.



$\bar{r}$: [Drug]bound/[DNA]total, m: [drug]free.

the C-7 sugar chain by cinerulose. The replacement of cinerulose B by cinerulose is not important for biological activities such as cytotoxicity and inhibition of nucleic acid synthesis, as is also the case for aclarubicin (aclacinomycin A) and aclacinomycin B[5,6] or cinerubins A and B[6]. So betaclamycin

A is considered to represent an analogue of ditrisarubicin B without a C-10 glycosidic chain. The K_{app} of betaclamycin A was 5.92×10^6 M^{-1}, or 39.8 times smaller than that of ditrisarubicin B. However, its n_{app} was 0.178, which was almost equal to the value for ditrisarubicin B. This result suggests that the trisaccharide at C-10 in ditrisarubicin B intensifies the DNA binding ability of the antibiotic. Structure-DNA binding relationships of other anthracyclines have been studied[7~10], but their K_{app}'s are the same order of magnitude as that of doxorubicin or a lower value. Among these anthracyclines none contained a C-10 glycosidic chain. In order to assess the role of the C-10 glycoside in DNA binding a space-filling model of the ditrisarubicin B-DNA complex was built according to the molecular structure of the complex of daunorubicin and DNA fragment resolved by X-ray analysis[11], where rings B~D of the aglycone were stacked in the direction perpendicular to the base plane, the cyclohexene ring A rested in the minor groove of the double helix and was anchored there by hydrogen-bonding interactions, and the amino sugar filled the minor groove of the double helix without hydrogen-bonding. WANG *et al.*[11] expected that the trisaccharide of cinerubin A and aclarubicin could bind to the right-handed B-DNA double helix with their oligosaccharide moiety running along the minor groove to cover several base pairs. Interestingly, it is possible for the two glycosidic chains of ditrisarubicin B to lie along the minor groove upward and downward from an intercalation site. This orientation might contribute to the higher affinity of ditrisarubicin B for DNA compared with the other anthracyclines. Structure-activity relationships for ditrisarubicin B and its comprehensive analogues have been studied systematically and will be described elsewhere[2].

Table 1. Parameters for binding between anthracyclines and calf thymus DNA.

Anthracycline	K_{app} (M^{-1})	n_{app}
Doxorubicin	1.04×10^7	0.164
Ditrisarubicin B	2.36×10^8	0.164
Betaclamycin A	5.92×10^6	0.178

K_{app}: Apparent binding constant.
n_{app}: Apparent number of binding sites per nucleotide.

Acknowledgments

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