Thermochimica Acta, 163 (1990) 129-132
Elsevier Science Publishers B.V., Amsterdam - Printed in The Netherlands

INTERACTION OF AN ANTHRACYCLINE ANTIBIOTIC, 4'-O-TETRAHYDROPYRANYLADRIAMYCIN (PYRARUBICIN), WITH PLASMID pJL3-TB5 DNA

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SUMMARY

The effect of an anthracycline antibiotic 4'-O-tetrahydropyranyladriamycin (pyrarubicin) on the stepwise melting of plasmid pJL3-TB5 DNA by means of differential scanning calorimetry. The peaks seen at higher temperature ranges of the DSC curve of the DNA were affected by the addition of pyrarubicin in a manner essentially similar to the other anthracycline antibiotics (daunomycin, adriamycin, aclacinomycin A), suggesting that pyrarubicin binds to the 5'-CG-3' sequences of DNA. However, pyrarubicin broadened the peak at the highest temperature and shifted the peak in a manner different from other antibiotics. This suggests that the sugar moiety attached to the chromophores of pyrarubicin play an important role in binding probably to the minor groove of DNA.

INTRODUCTION

Many investigations on the interaction of various drugs with DNA have been carried out to understand the molecular basis of drug action as well as the structure and function of DNA itself. Drug-DNA interaction has been studied by various techniques including spectroscopy, X-ray crystallography (ref. 1-2), chromatography (ref. 3), footprinting, and calorimetry (ref. 4-5). Despite these investigations, the effect of drugs on the stepwise melting of DNA has not been reported.

We have recently demonstrated that differential scanning calorimetry (DSC) is useful for the analysis of stepwise, cooperative melting of double-stranded DNA to single-stranded DNA along the molecular chain (ref. 6-10) and that the DSC method can be applied to study the interaction between DNA and various DNA-binding drugs (ref. 11). In the absence of drugs, the DSC curve of the plasmid pJL3-TB5 DNA (5277 base pairs in length) consists of seven peaks resulting from the stepwise melting of DNA. Various drugs caused positions of these peaks to shift and their heights to vary in a characteristic manner depending upon the mode of binding of drugs to DNA. We have previously reported that anthracycline antibiotics with a monosaccharide (daunomycin and adriamycin) and a trisaccharide (aclacinomycin A) attached to the chromophores shift positions of DSC peaks to higher temperature ranges. In particular, the peak seen at the highest temperature decreased its height with increasing

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temperatures, and reappeared as a clear peak at a higher temperature range depending upon the drugs examined. In these antibiotics, however, we could not specify which radicals of their chemical structures contributed to these differences in the DSC curves, because these antibiotics have several different substitutions of radicals attached to the chromophore rings.

This paper deals with the effect of another anthracycline antibiotic, 4'-O-tetrahydropyranyladriamycin (pyrarubicin) (ref. 12), on the stepwise melting of pJL3-TB5 DNA. Pyrarubicin is useful to examine the effect of the sugar moiety on the melting of DNA, since it has a dissacharide containing an amino sugar attached to 7-O of the chromophore instead of a monosaccharide in adriamycin. We report here that pyrarubicin intercalates into 5'-CG-3' sequences of DNA like other anthracycline antibiotics and exhibits a DSC curve different from those of others, demonstrating that the sugar moiety attached to the chromophore play an important role in binding probably to the minor groove of DNA.

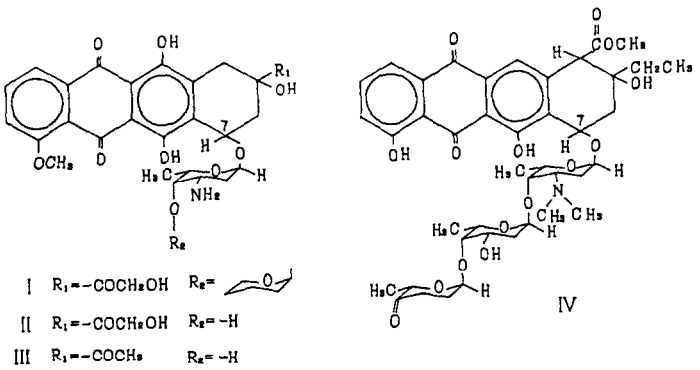


Fig. 1. Structural formulas of pyrarubicin (I), adriamycin (II), daunomycin (III), and aclacinomycin A (IV).

MATERIALS AND METHODS

Pyrarubicin was kindly supplied by Sanraku Co., Ltd., Tokyo. The structural formula of pyrarubicin is shown in Fig. 1 together with those of adriamycin, daunomycin, and aclacinomycin A.

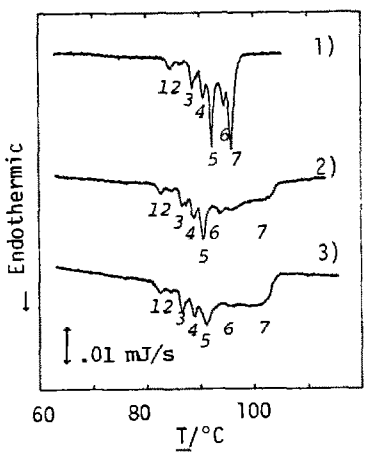
Plasmid pJL3-TB5 (5277 base pairs in length) used was described previously (ref. 4, 9, 13). The covalently closed circular plasmid DNA prepared was linearized by digestion with EcoRI, followed by the successive treatment with phenol, ethyl ether, and ethanol. The plasmid was dissolved in 1 x SSC (saline-sodium citrate) buffer solution (0.015 mol dm^{-3} NaCl and 0.15 mol dm^{-3} sodium citrate, pH 7.0)

Calorimetric measurements were performed using a heat-flux type of differential scanning calorimeter, SSC 560U (Seiko Instruments & Electronics Ltd., Tokyo). About 60 mg of the DNA-drug mixture was put in a calorimeter

vessel at a DNA concentration of 0.5 % (w/v) and subjected to DSC scanning at the heating rate of 0.5 K min^{-1}.

RESULTS AND DISCUSSION

Fig. 2 shows the effect of pyrarubicin on the stepwise melting of the plasmid pJL3-TB5 DNA. Seven peaks in the DSC curve (curve 1 in Fig. 2) seen in the absence of antibiotics were affected by the addition of pyrarubicin (curves 2 and 3 in Fig. 2). This is essentially similar to the effect of adriamycin and aclacinomycin A shown in Fig. 3. This suggests that pyrarubi-



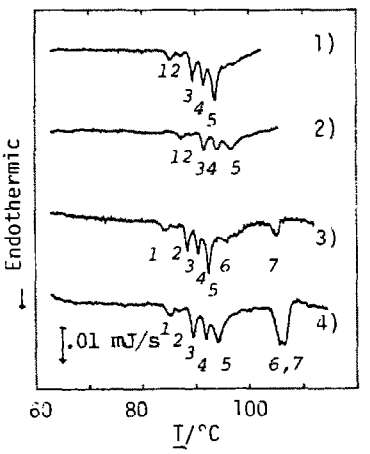


Fig. 2 (left). Effect of pyrarubicin on the stepwise melting of pJL3-TB5 DNA monitored by DSC. The molar ratios of pyrarubicn to base-pairs in the solution are as follows: (1), 0; (2),0.03; (3),0.06.
Fig. 3 (right). Effect of adriamycin and aclacinomycin A on the stepwise melting of pJL3-TB5 DNA monitored by DSC. The molar ratios of antibiotic to base-pairs in the solution are as follows: (1), adriamycin, 0.036; (2), adriamycin, 0.071; (3), aclacinomycin A, 0.03; (4), aclacinomycin A, 0.06.

cin intercalates into 5'-CG-3' sequences of the DNA which occur abundantly in the region contributing to peak 7, as demonstrated previously (ref. 7). However, the effect of pyrarubicin on peak 7 is different from adriamycin and aclacinomycin A. Pyrarubicin broadened peak 7 and shifted the peak to a higher temperature with increasing its concentrations. Adriamycin did not cause such a shift even when scanned up to 115 °C (data not shown), while aclacinomycin A shifted the peak to form a clear peak.

The chemical structure of pyrarubicin is different from adriamycin only in the sugar moiety substituted in the position of 7-O of the chromophore; pyrarubicin possesses a disaccharide, while adriamycin possesses a monosaccharide. Therefore the difference between pyrarubicin and adriamycin in the behavior of peak 7 can be attributed to the sugar moiety, probably due to

binding to the minor groove of the DNA, as previously predicted with the X-ray analysis for daunomycin-oligonucleotide complexes (ref. 1-2). The change of peak 7 observed in the presence of aclacinomycin A is also due to the sugar moiety (trisaccharide) attached to 7-O of the chromophore. The change of peak 7 seems to depend upon the length of the sugar moities attached to the chromophore.

We have previously reported the effects of various drugs other than anthracycline antibiotics (ref. 11): Distamycin A, a minor groove binding antibiotic, shifted positions of all of the seven DSC peaks to a higher temperature range to give rise to a monotonic broad peak, suggesting that distamycin A binds to a minor groove of DNA and stabilizes the A+T-rich regions or A+T-clusters; Peplomycin broadened each peak in the DSC curve and lost the stepwise melting profile of DNA due to cleavage of DNA by peplomycin; cis-dichlorodiammine-platinum (II) (cis-Platin) caused all the peaks to shift to a lower temperature range and to form a broad monotonic peak, due to destabilization of double strands by distorting base-pairs or base-stacking.

Thus we can readily obtain useful information on interaction of anthracycline antibiotics as well as of DNA-binding drugs with DNA by the DSC method.

We thank to Drs. H. Yamaki and Y. Hayakawa for useful discussion. We also thank to Sanraku Co., Ltd. for supplying pyrarubicin. This paper was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

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