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BINDING OF THE ANTITUMOR DRUG ADRIAMYCIN TO DNA-HISTONE COMPLEXES

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ABSTRACT

Isotherms of the binding of the anthracycline antibiotic, adriamycin (adriblastin), to DNA histone complexes was studied by means of spectroscopic analysis. The results indicated that: (a) binding of adriamycin to histones reduced the interaction of histones with DNA, (b) binding of the drug to DNA did not change the binding affinity of histone to DNA and, (c) in the explored binding range of $r<0.1$ the binding of adriamycin to DNA-histone complex proved to be anticooperative with n values of 0.32 for the interaction of histone with DNA-drug and 0.26 for the binding of DNA to histone-drug complex. The results suggest the possible participation of histones in the DNA-drug complex formation.

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***Key words:** Anthracycline, Chromatin, Histones, Adriamycin*

INTRODUCTION

Adriamycin or adriblastin is the main representative of the anthracycline antibiotics widely used as a potent chemotherapeutic agent in the treatment of certain leukemias and solid tumors.[1,2] The drug is believed to exert its biological activity by intercalation of the planar chromophore into DNA double helix,[3] and by its ability, inhibits both DNA replication and RNA transcription.[4,5] the equilibrium binding studies, the analysis of data led the authors to conclude that adriamycin binds to DNA in noncooperative manner. However, Graves and Krugh demonstrated that the binding is cooperative and ionic strength-dependent.[6]

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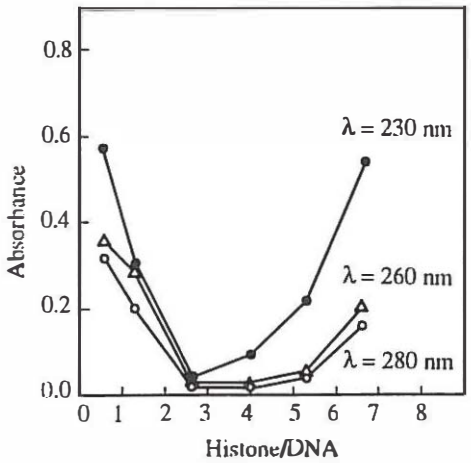

Fig. 1. Complex of histones with DNA at different ratios (mg ml). Absorbances represent the amount of uncomplexed material remained in the supernatants. The data of three independent experiments were averaged.

In the nucleus, DNA is not naked to serve as a binding site for adriamycin, but rather is complexed with histone and other nuclear proteins.[7,8]

This structure has been extensively demonstrated as nucleosome particles in the chromatin.[9] How the presence of proteins on the DNA might affect the interaction of drug is a question of importance in efforts to understand the action of adriamycin. Except for few reports on the accessibility of chromatin or nucleosomes to nucleases by their treatment with adriamycin,[10,11] no evidence exists about the mode of action of drug on DNA-protein complexes. In this study, the possible binding of adriamycin to histones and DNA-histone complexes was investigated.

MATERIALS AND MEHODS

Antibiotic adriamycin was supplied from Sigma Chemical Company. Stock solution at a concentration of 1 mg/ml in distilled water was made and stored in the dark at 40°C or until use. Dilutions of drug stocks into the appropriate buffers were prepared immediately before use and their concentration determined spectrophotometrically. Exinctioncoefficientof7000 M^{-1} cm^{-1} and 11200 M^{-1} cm^{-1} at 480 nm were used for DNA bound and free drug, respectively.

DNA from calf thymus was prepared according to the procedure of Kay et al[12] or supplied from Sigma. It was dissolved in 0.05 M tris-HCl buffer pH 7.5 at a final concentration of 1 mg/ml and kept at 40°C for at least two weeks without any change in its turbidity or absorbance. In some cases DNA solution was dialysed overnight against tris buffer to remove any salt and sonicated. The concentration of DNA in dilute solutions was measured spectrophotometrically by using a molar extinction coefficient of 12824 M^{l} cm^{-1} at 260 nm.

Whole histone was prepared essentially by the method of Johns[13] and further purified by recycling through acetone precipitation. Protein solution was also prepared freshly in tris-buffer immediately before use. The pH of all samples was adjusted if necessary.

Binding assays were performed at 20-23°C. The ratio of DNA to protein for the highest degree of complex formation was determined by mixing DNA and histones at different ratios. The samples were then incubated for 45-60 min by occasional shaking, centrifuged at 2000g for 15 min and the amount of DNA or protein released into supernatants were measured. Interaction of adriamycin with DNA-histone complex was carried out in two ways: (1) drug was first interacted with DNA and then histones were added. (2) Drug was incubated with whole histone and then DNA added to the solution. In both cases the binding was assayed using UV-260 spectrophotometer using wave length at 480, 260 and 230 nm. Data were cast

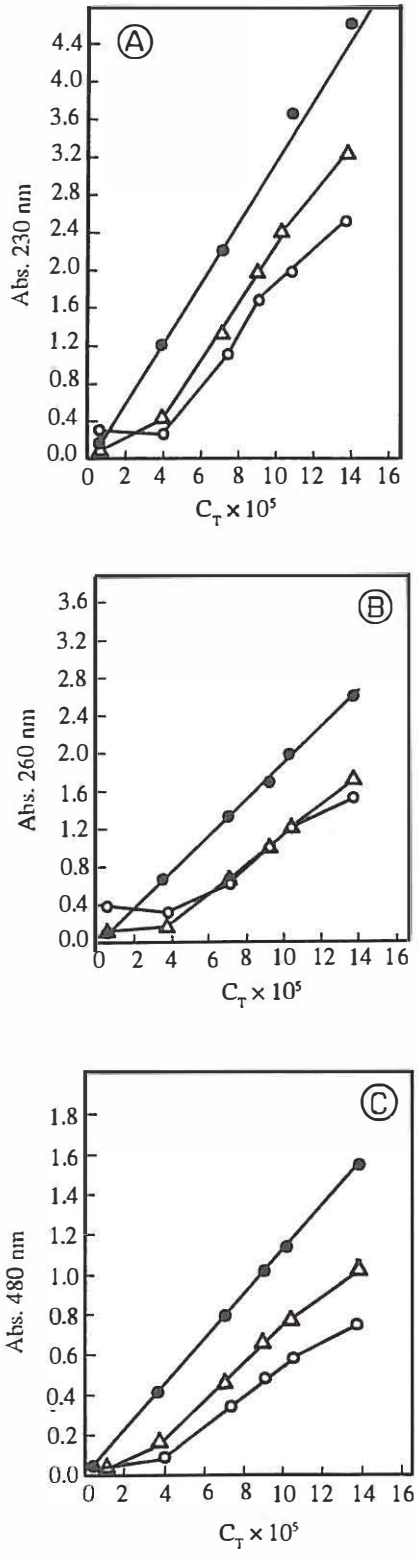

Fig. 2. Spectroscopic measurements of the binding of adriamycin to DNA-histone. C_T is total molar concentration of ●—● drug; drug in 50 mM tris buffer (control); ○—○ interaction of histone with DNA-drug and; ▲—▲ interaction of DNA with histone-drug complexes. The results are average of at least eight independent experiments.

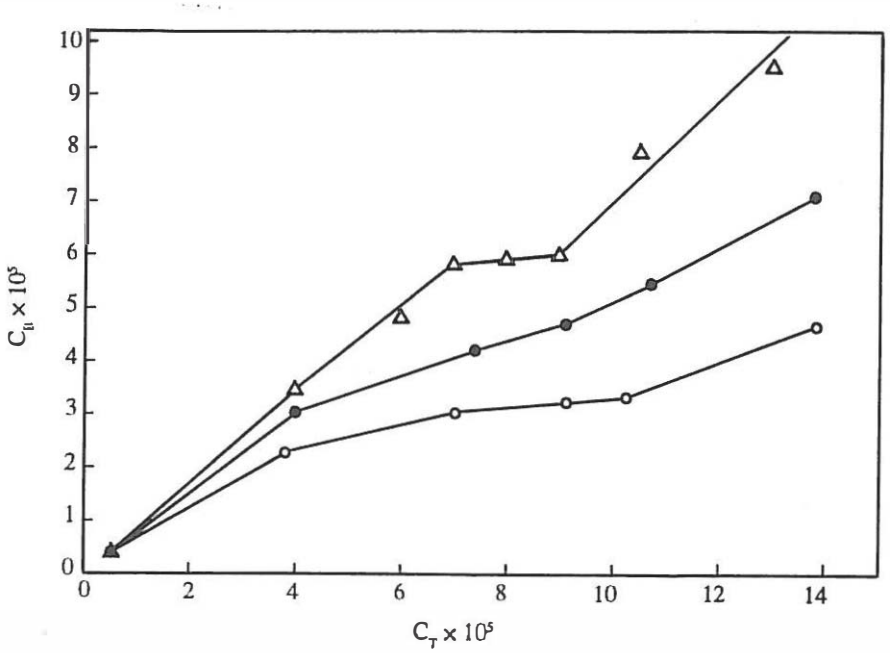

Fig. 3. Drawing C_B (moles of bound drug) against C_T(total molar concentration of drug). ●——● Interaction of histone with DNA - drug. ○——○ Interaction of DNA with histone - drug and ▲——▲ interaction of DNA with drug (control). Number of experiments were as indicated in Fig 2.

in the form of a scatchard plot of r/C_f vs r where r is the number of moles of drug bound per mole of DNA base pairs and C_f is the concentration of free drug.[14]

RESULTS

The binding of adriamycin to histone and histone-DNA complex was monitored by the changes occurred in the absorption of uncomplexed materials remained in the supernatant. Interaction of whole histone with DNA produced an insoluble complex at different ratios. The data illustrated in Fig. 1 shows that the best ratio of histone to DNA for complete complex in solution was 2.5-4:1. Therefore the ratio of 3:1 from histone to DNA was used throughout the following experiments. Adriamycin binding experiments were performed at two states: (1) DNA was interacted with varous concentrations of drug by at least 30 min incubation at room temperature or 37°C and then histone was added to DNA-drug complex (DNA-drug-histone), and (2) drug-histone complex was first made in the same condition and then DNA was added to the samples (histone-drug-DNA). A serial concentration of adriamycin in tris buffer treated in the same way was used as a control. The results are given in Fig 2. In all controls, adriamycin alone, DNA-drug and histone-drug gave a straight line indicating that the binding of drug to both DNA and histone produce a soluble complex. Interaction of histone with DNA-drug complex showed a high decrease in the absorbances which was due to the insolubility of the complex. When DNA was interacted with histone-drug complex, the absorbance patterns were higher than the results obtained above indicating that the binding of drug to histone reduced the interaction of histone with DNA. Drawing C_B (molar concentration of bound drug) versus C_T (total molar concentration of drug) showed that (Fig. 3) the binding is rapid at low concentrations of drug and reached a plateau (at $7 - 9\times10^{-5}$ mole) which was an indication of saturation. At higher concentrations of drug, however, self aggregation of drug occurred giving a sharp upward line. This pattern is more obvious when the binding of drug to free DNA was also considered (Fig 3). Isotherms of the binding of adriamycin to DNA and DNA-histone complex at two different states studied are given in Fig. 4.

As is seen, for DNA, a cooperative scatchard plot was obtained whish showed a positive slope at $r < 0.1$ (positive cooperativity). In the presence of histones, interestingly, each plot showed a pronounced upward curvature, clearly suggesting strong anticooperativity of the binding. Determination of n values (number of binding sites) by the extrapolation of scatchard plots at the point of $r\,C_f = 0$ revealed 0.5, 0.32 and 0.26 for the binding of drug to DNA, DNA-drug-histone and histone-drug-DNA complexes, respectively.

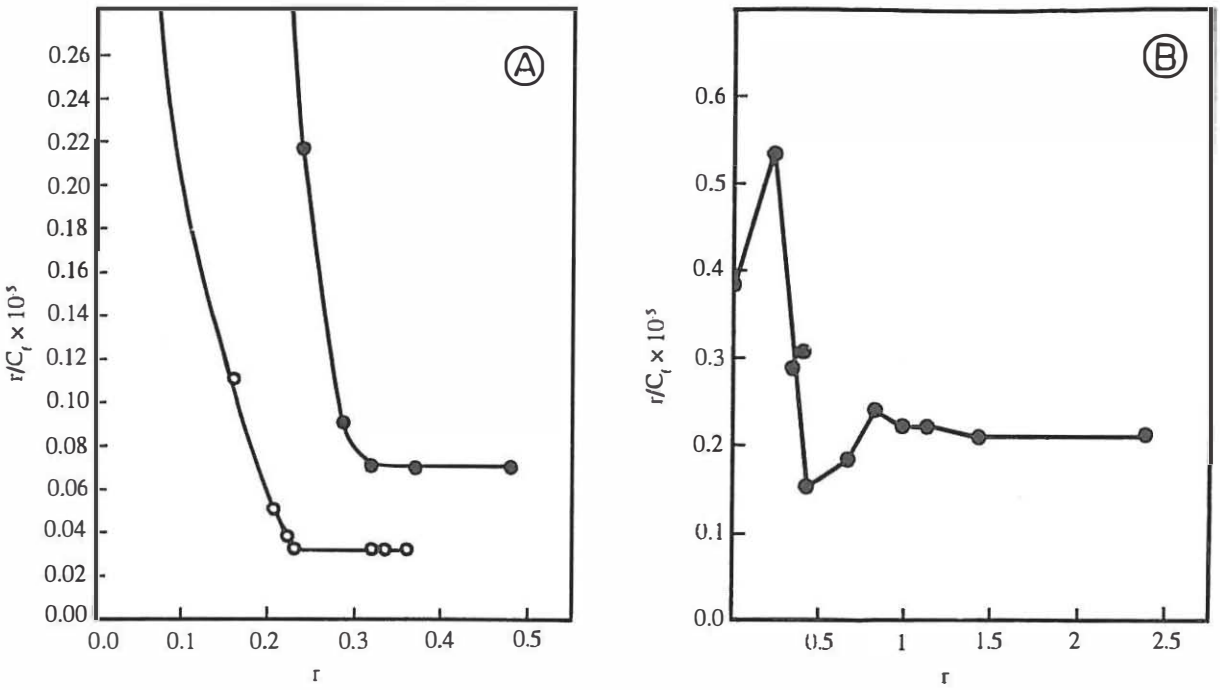

Fig. 4. Scatchard plots for A; the interaction of histones with DNA-drug complex ●——● and the binding of DNA to histone-drug complex. ○——○ B; scatchard plot for DNA-drug complex. The data from three independent experiments were averaged.

DISCUSSION

Adriamycin is one of the most effective antitumor drugs and its binding to DNA by intercalation has been regarded as a critical mechanism for drug action.[3] In eukaryotic cells DNA is not naked but is covered with special sets of proteins mainly histones. Therefore, the elucidation of the certain sites of drug binding at the molecular level might permit progress towards a more rational clinical application. This is only detected if the various chromatin components are taken into account. In this report, as a preliminary work, the binding of adriamycin to DNA in the presence of histone at designed experimental states was perfomed. The data indicated that adriamycin, apart from having a binding site on DNA, also shows high affinity for histones. If drug was first interacted with histone, its binding to DNA was decreased as shown by a considerable change in the spectroscopic measurements. Binding isotherms obtained for the binding of adriamycin to DNA represented a positive cooperativity which is in agreement with the binding isotherms reported earlier.[6] In the presence of histones, scatchard plots showed anticooperativity (negative cooperativity) with n values of 0.32 for the interaction of histones with DNA-adriamycin and 0.2 for the interaction of DNA with histone-adriamycin complex. Although the interaction of drug with DNA has been extensively studied[6,15,16] data about the binding of drug to chromatin is limited. Gyapay and Lapis and also other reports[18] have shown that drug binds to nucleosomes and subsequently enhances DNA fragmentation by nucleases. Induction of considerable increase in the sedimentation rate of isolated chromatin by adriamycin has also been reported.[19] Our finding together with the above mentioned results indicate that the binding of adriamycin to chromatin (DNA and histones) is not a simple process. Although a precise description of a mechanism of drug action is still impossible, it is desirable to study the effect of drug on individual components, histones and nonhistone proteins, to define further the action of adriamycin on DNA and chromosomal proteins.

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