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CIRCULAR DICHROIC PROBE OF THE COMPETITION FOR DRUG AND DYE BETWEEN ANIONIC POLYMERS DNA AND HEPARIN. I.

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ABSTRACT

The changes in the absorption and CD spectra of daunomycin caused by DNA and heparin (Hep) are characteristically different for the two biopolymers. These changes have been utilized to study the competition for the drug between DNA and Hep. The results show that DNA can totally take away the drug bound to Hep; the intercalating ability of DNA being the decisive factor. The spectrophotometric and dichroic probes also show that the same intercalating ability helps DNA to strip off acridine orange bound to Hep, a much stronger polyelectrolytic chromotrope than DNA. It is essential that the concentration of DNA be at least four times the concentration of the ligand to overcome the competition with Hep.

INTRODUCTION

When a drug is administered to a living body with the expectation that it will reach the target, the drug may bind to other biomolecules as well. The question of competition for a drug between anionic biopolymers like DNA and heparin (Hep) arises particularly if the drug is cationic. It is known that a polymer containing sulfate or sulfonate as the anionic groups binds a cationic ligand more strongly than a polymer containing phosphate groups [1, 2]. However, DNA has two potential modes of binding, namely, outer electrostatic and inner intercalation, unlike Hep which lacks the second mode. Consequently, the competition for a cationic drug cannot always be predicted from the nature of the anionic groups of the polymers. We have chosen daunomycin (DMC) as a representative cationic drug. This drug is a glycosidic anthracycline antibiotic which inhibits nucleic acid synthesis in both a cell-free system [3] and *in vivo* [4]. This is an intercalating drug with preference for the A-T base pair [5]. Since the spectral changes of drugs induced by a polyanion may often be minor and noncharacteristic, absorption spectroscopy would not always be a useful monitoring technique for studying such a competition; we used the dichroic probe also. Our results show DNA to be the winner in the competition; obviously, the intercalating ability of DNA is the pertinent factor. To ascertain whether intercalation can also be used by DNA in winning over a dye from Hep, we made a parallel study of the competition between DNA and Hep for the intercalating dye acridine orange (AO). Although Hep is one of the strongest chromotropes in inducing metachromasia in dyes [6], our results surprisingly show that DNA can strip off all dyes bound to Hep.

EXPERIMENTAL

Calf thymus DNA, daunomycin, and heparin of Sigma Chemical Co. were used as such. Acridine orange [3,6-bis(dimethylamino) acridinium hydrochloride] of Searle Scientific Services was purified by converting it to the dye base, extracting with chloroform, and finally recrystallizing as dye hydrochloride.

Stock solutions of nucleic acids were stored in the cold and diluted just before use. Stock solutions of the dye were stored in the dark. The pH's of the experimental solutions were maintained at 7.4 ± 0.2 without using a buffer.

Absorption and CD spectra were recorded with a PYE UNICAM SP-8-100 UV/Vis spectrophotometer and a Jasco J-500 C spectropolarimeter, respectively, using 1.00 cm circular cuvettes with water as the blank.

RESULTS AND DISCUSSION

Figure 1 shows the absorption spectra of 2.00×10^{-5} *M* DMC in water (A), in the presence of 1.00×10^{-4} *M* DNA (B) and 1.00×10^{-4} *M* Hep (C), and in the presence of Hep and the DNA mixture at the same respective concentrations with Hep pipetted to DNA-DMC (D) and DNA pipetted to Hep-DMC (E). (The molarity of a polyanion is in terms of its equivalent weight, defined as the average weight of the polyanion containing one anionic group. Equivalent weights of DNA and Hep

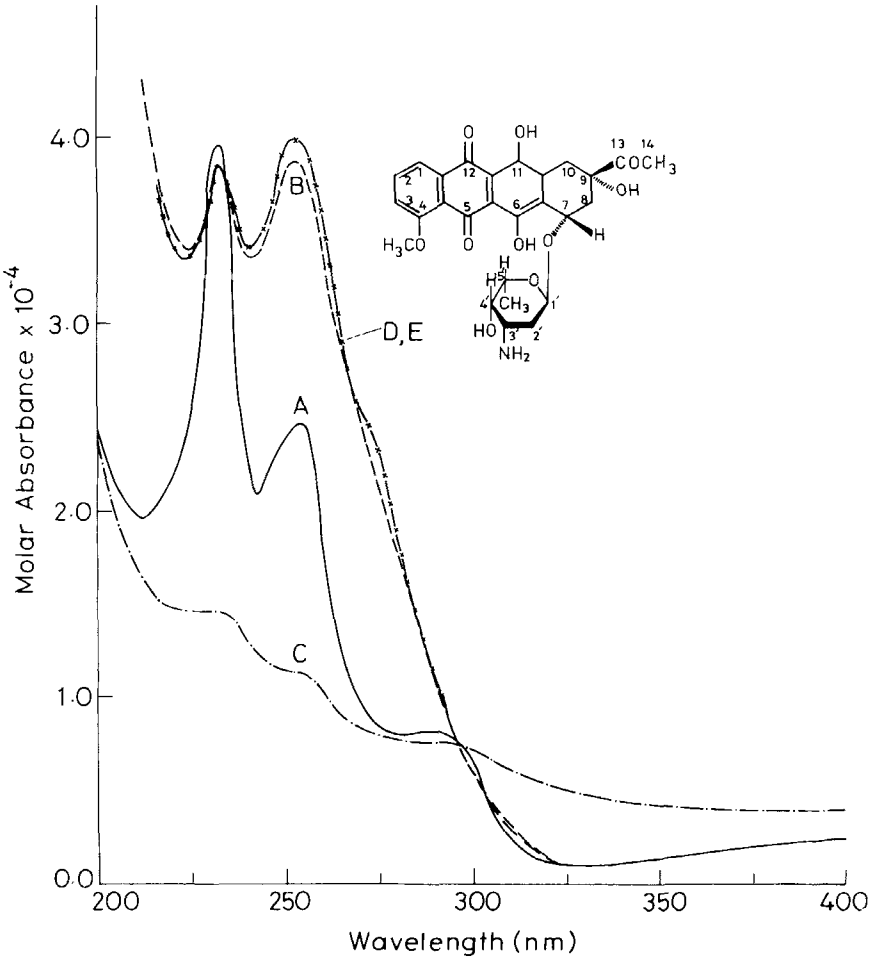

FIG. 1. Absorption spectra of DMC in water (A), in the presence of DNA (B), in the presence of Hep (C), in the presence of Hep pipetted to DNA-DMC (D), and DNA pipetted to Hep-DMC (E). The concentration of DMC in each solution is 2.00×10^{-5} *M*; the concentration of Hep as well as DNA, whenever present, is 1.00×10^{-4} *M*.

are 330 and 203, respectively.) DMC has two peaks and a shoulder at 232, 254, and 291 nm, respectively, in addition to a broad and weak band in the visible range around 490 nm (not shown). Spectrum B of DNA-DMC apparently shows equal absorbances at 232 and 254 nm; taking account of the UV absorption by DNA itself, it seems that DNA enhances the 254 nm band of DMC and reduces the absorbance of its 232 nm band. The Hep-DMC spectrum (C) is characteristically different with much reduced absorbances at λ below 280 nm and somewhat enhanced absorbance above 300 nm. However, when DNA is pipetted to Hep-DMC, Spectrum C dramatically changes to E, which is almost identical with the Spectrum B of the DNA-DMC system. This indicates without a doubt that DNA takes away all DMC from Hep and forms its own complex with the drug. When Hep is pipetted

to DNA-DMC, the absorption spectrum remains unchanged, characterizing DNA-DMC.

Figure 2 shows the corresponding CD spectra of the systems. DMC itself is chiral, giving the biphasic CD Spectrum A with peak, trough, and crossover at 222, 270, and 248 nm; the spectrum crosses over again at 297 nm to an extremely broad and shallow positive band in the longer wavelength region [7]. The CD spectra of DMC in the presence of 1.00×10^{-4} *M* DNA (B) and in the presence of 1.00×10^{-4} *M* Hep (C) show that the two spectra are characteristically different. While the DNA-induced dichroism has two peaks at 256 and 208 nm and a negative peak at 295 nm, the Hep-DMC system shows a positive peak at 230 nm and a weak

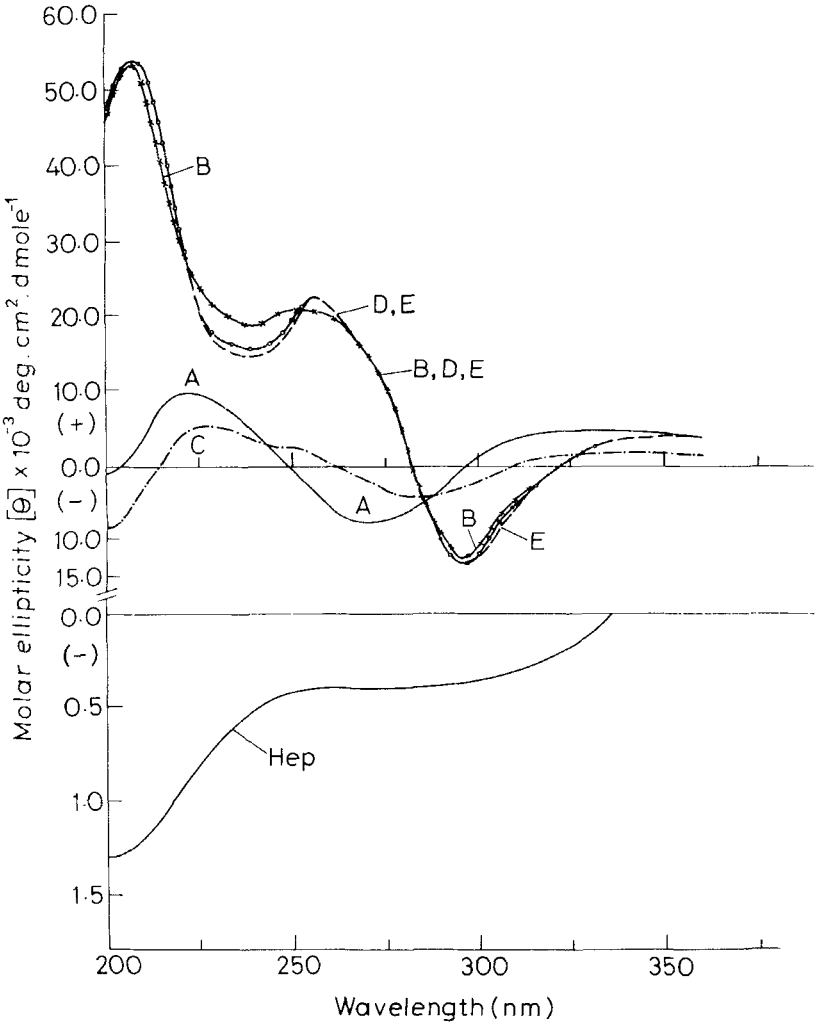

FIG. 2. The CD spectra of the solutions A to E of Fig. 1. Molar ellipticity values $[\theta]$ in degree·cm^2·dmol^{-1} calculated with respect to DMC concentration. The curve at the bottom is the UV-CD of 1.00×10^{-4} *M* Hep in water.

negative peak at 280 nm. It may be pointed out that the shape of this CD spectrum of Hep-DMC is rather insensitive to changes in the polymer/dye (P/D) ratio from 1.5 to 10 at least, as well as to the replacement of Hep by other glycosaminoglycans like hyaluronate and chondroitin sulfates A, B, and C. The sharp and characteristic peak at 208 nm of DNA-DMC was not recorded by Dalgleish et al. [8], and the concentration of DNA used by these authors was abnormally high. We preferred not to use their experimental conditions since at this high concentration of DNA the experimental solutions become extremely viscous and other complications might arise from the exclusion volume factor and also from the strong CD by the polymer itself. When DNA is added to the Hep-DMC system, the CD spectrum changes completely to one absolutely characteristic of DNA-DMC, except for some minor differences in the 225 to 265 nm wavelength region, indicating that DNA robs Hep completely of its bound DMC, as inferred also from the respective absorption spectra (Fig. 1). Mere electrostatic competition would not result in such an exchange; DNA uses its intercalation ability. This is possible because DMC is an intercalating drug [9]. To give DNA a fair chance of intercalating DMC, we have kept the P/D ratio at 5.0 since every four bases are normally capable of intercalating one ligand [10]. Though daunomycin prefers the A-T pair [5], the drug may intercalate at the G-C pair also. In fact, Chaires et al. [11] are of the opinion that the affinity of DMC increases with increasing G-C content of native DNA. Otherwise a larger amount of DNA than was used by us would be required for the complete snatching of the drug from Hep if the drug totally avoided G-C pairs.

In order to check whether the intercalating ability of DNA is the decisive factor, we studied the competition for acridine orange (AO) between DNA and Hep. The chromotropic ability of DNA in inducing metachromasia in a cationic dye is so weak that Michaelis, the pioneer worker in metachromasia, at one time advocated not including DNA in the list of chromotropes [12]; on the other hand, Hep induces very strong and stable metachromasia [6]. One could speculate, based on these informations, that the transfer of AO should have been from DNA to Hep—practically one-way traffic, but our result shows the reverse to be the case. Since the shapes of the metachromatic spectra of AO induced by DNA and Hep are so distinctly different, one could also follow this competition spectrophotometrically; the dichroic probe leads to the same conclusion. Figure 3 shows the metachromatic spectra of DNA-AO (A), Hep-AO (B), DNA-AO-Hep (C), and Hep-AO-DNA (D); for comparison the spectrum of the dye is also included (E). The concentrations of the individual polyanions are five times the dye concentration. It is apparent from (B) that Hep, being a strong chromotrope, induces a distinct metachromatic band at 456 nm, while the DNA-AO spectrum (A) is very similar to that of the dye alone. At the P/D of 5.0 used, probably all the dye molecules are intercalated by DNA and hence do not exhibit distinct metachromasia. On adding DNA to the Hep-AO system, Spectrum B completely changes to that characteristic of the DNA-AO system. On the contrary, Hep added to the DNA-AO system cannot change the spectrum. This definitely shows that in the competitive binding of the dye by Hep and DNA, it is DNA which has again been the winner by using its intercalating ability. That intercalation is the factor is shown by our observation that if the concentration of DNA is reduced by 2.5 times to a P/D of 2.0, so that the concentration of AO is much above the maximum intercalation capacity of DNA, DNA does not remove all dye molecules from Hep.

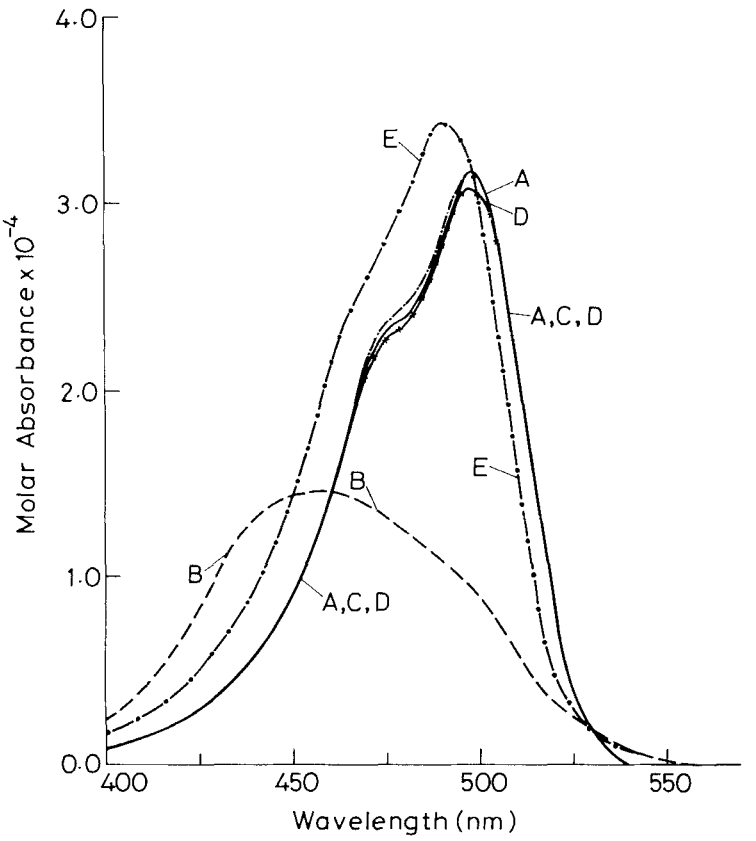

FIG. 3. Absorption spectra of 1.20×10^{-5} *M* AO in water (E) and in the presence of DNA (A); Hep (B); in the presence of Hep pipetted to DNA-AO (C); and DNA pipetted to Hep-AO (D). Concentration of Hep as well as DNA, whenever present, is 6.00×10^{-5} *M*.

Figure 4 summarizes the results of the dichroic probes of this competition for AO. The CD spectra (A) and (B) of DNA-AO and Hep-AO, respectively, are distinctly different. When DNA is added to the Hep-AO system, Spectrum B changes to D, which is very similar to Spectrum A of DNA-AO, thus confirming that DNA deprives Hep completely of AO. On the contrary, when Hep is added to the DNA-AO system, this CD spectrum (C) remains almost unchanged, characteristic of DNA-AO. Thus, in spite of being a much stronger chromotrope, Hep cannot dislodge any dye from DNA. Presumably, intercalation is again the decisive factor. That intercalated dyes are more strongly bound than outer bound dyes is also shown by the fact that the native DNA protects a cationic dye like thionine from photobleaching by EDTA better than the protection given by denatured single stranded DNA [13], though denatured DNA is a stronger chromotrope than native DNA in inducing metachromasia in a cationic dye [14]. When the concentration of DNA is reduced to a P/D of 2.0, the CD spectrum of DNA pipetted to Hep-AO has an intermediate character characteristic of both Hep-AO and DNA-AO systems,

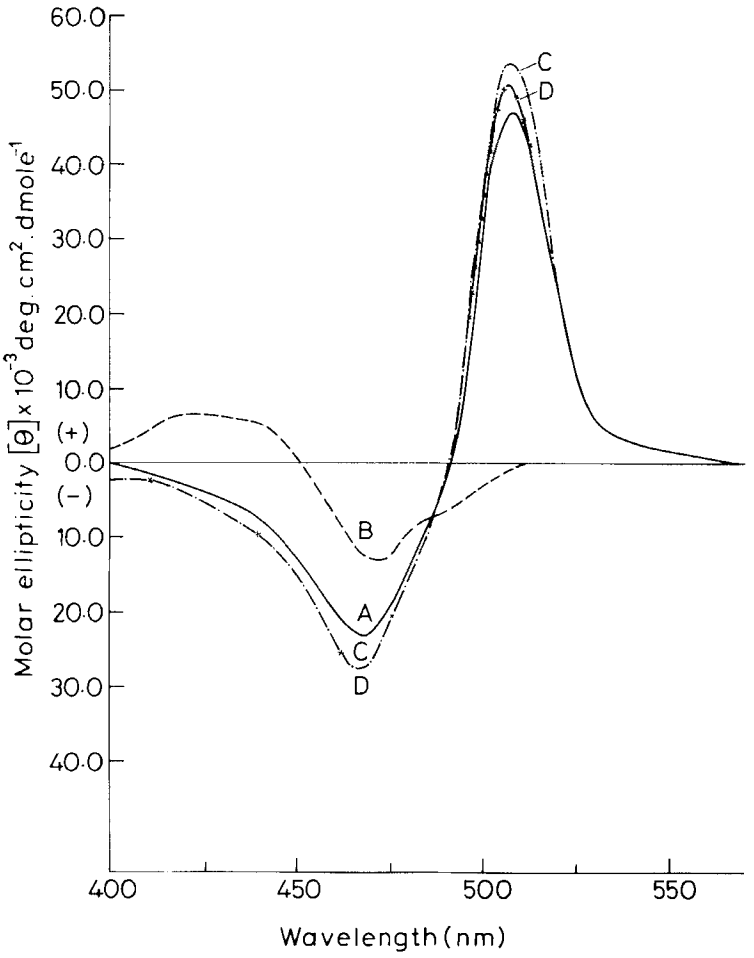

FIG. 4. Visible CD spectra of solutions A to D of Fig. 3; [θ] values calculated with respect to the dye concentration.

indicating that this concentration of DNA is inadequate to intercalate all the dye, and the dye is distributed between the two polyanions.

For studying the distribution of such ligands between two polymers, it seems spectroscopy is the best monitoring technique. The method of equilibrium dialysis for the quantitative evaluation of binding constants is not applicable in this case since this method cannot distinguish binding of ligands by DNA and Hep; neither does this method distinguish between intercalation and outer binding by DNA. We made an attempt to determine binding constants from the spectroscopic changes, but the results gave varying values, showing that more than one equilibrium is involved. Although the spectroscopic method has often been used to find the binding constant of a ligand by a polymer, we feel that such a method is applicable only at a particular ligand concentration, since the absorption spectrum (molar absorbance vs λ) changes shape with dilution even for a dye–polymer system (e.g., methylene blue–chondritin sulfate) at a fixed dye/polymer ratio.

This is probably the first report on the dominant role of intercalation, rather than electrostatic binding, in the competition for ligands between different anionic biopolymers. By using DNA-cellulose elution chromatography, DMC was shown to have both weak and strong binding characteristics [15]; the weak binding was assumed to be electrostatic between the protonated DMC and DNA phosphate, and the strong binding resulted from intercalation [9]. The theoretical calculations of Newlin et al. [5], however, suggest the reverse. If one remembers the well-established stoichiometric interactions of dye cations and DNA with one dye per anionic site, taking into account the intercalated as well as outer bound dyes, it is clear that an intercalated dye keeps the nearest anionic phosphate site of DNA engaged and not available for outer binding of a second dye at this site. Thus, an intercalated dye or drug is stabilized further by electrostatic interaction with the nearest vacant phosphate, while an outer bound dye is stabilized primarily by electrostatic interactions. Our results on the competitive binding of dye and drug by DNA and Hep also confirm that intercalation is a stronger mode of binding than the outer electrostatic one as expressed by Tanaka [16].

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REFERENCES

[1] J. B. Lawton and G. O. Phillips, *Makromol. Chem., 183*, 1497 (1982).
[2] M. Shirai, T. Nagatsuka, and M. Tanaka, *Ibid., 178*, 37, 2141 (1977).
[3] G. Hartmann, G. Goller, K. Koshel, W. Kersten, and H. Kersten, *Biochem. Z., 341*, 126 (1964).
[4] J. H. Kim, A. S. Gelbard, B. Djordjevic, S. H. Kim, and A. G. Perez, *Cancer Res., 28*, 2437 (1968).
[5] D. D. Newlin, K. J. Miller, and D. F. Pilch, *Biopolymers, 23*, 139 (1984).
[6] M. K. Pal and M. Schubert, *J. Am. Chem. Soc., 84*, 4384 (1962).
[7] F. Arcamone, *Top. Antibiotic Chem., 2*, 102 (1978).
[8] D. G. Dalgleish, G. Fey, and W. Kersten, *Biopolymers, 13*, 1757 (1974).
[9] S. Neidle, L. H. Pearl, and J. V. Skelly, *Biochem. J., 243*, 1 (1987).
[10] R. W. Armstrong, T. Kurucsev, and U. P. Strauss, *J. Am. Chem. Soc., 92*, 3174 (1970).
[11] J. B. Chaires, K. R. Fox, J. E. Herrera, M. Britt, and M. J. Waring, *Biochemistry, 26*, 8227 (1987).
[12] L. Michaelis, *Cold Spring Harbor Symp. Quant. Biol., 12*, 131 (1947).
[13] M. K. Pal and P. C. Manna, *Makromol. Chem., 183*, 2811 (1982).
[14] D. F. Bradley and M. K. Wolf, *Proc. Natl. Acad. Sci. USA, 45*, 944 (1959).
[15] F. Zunico, *FEBS Lett., 18*, 249 (1971).
[16] S. Tanaka, *Makromol. Chem., 181*, 2175 (1980).

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