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Deoxyribonucleic Acid Binding Studies on Several New Anthracycline Antitumor Antibiotics. Sequence Preference and Structure–Activity Relationships of Marcellomycin and Its Analogues as Compared to Adriamycin†

Virgil H. DuVernay, Jr.,* Jonathan A. Pachter,‡ and Stanley T. Crooke

ABSTRACT: The deoxyribonucleic acid (DNA) binding characteristics of adriamycin and several new anthracycline glycosides, including marcellomycin, aclacinomycin, rudolfomycin, musettamycin, and pyrromycin, have been studied. The fluorescence spectra were determined for all six anthracyclines, and the fluorescence quenching effects caused by interactions with the natural DNAs poly(dAdT)–poly(dAdT) and poly(dGdC)–poly(dGdC) were characterized. Binding parameters were determined by Scatchard analyses of results obtained by spectrofluorometric titrations of anthracyclines with DNA. Consistent with earlier structure–activity relationship studies of nucleic acid synthesis inhibitory effects, the results demonstrate a correlation between the length of the glycosidic side chain and DNA binding affinity. In addition, the sugar residue 2-deoxyfucose appears to confer greater DNA binding ability than do the sugars rednosamine and cinerulose when present in the terminal position of the glycosidic side chain, also in agreement with earlier studies. The sequence preference of anthracycline–DNA interaction has been examined by using DNAs of varying GC content, including the naturally occurring calf thymus DNA (43% GC), *Clostridium perfringens* DNA (28% GC), and *Micrococcus luteus* DNA (72% GC) and the synthetic double-stranded copolymers poly(dGdC)–poly(dGdC) and poly(dAdT)–poly(dAdT). The results demonstrate that although adriamycin shows an absolute requirement for GC sequences for DNA binding, marcellomycin and its analogues showed no such sequence requirement. Furthermore, an AT preference for DNA binding was demonstrated with marcellomycin and its analogues.

The specificity of interaction of anthracycline antitumor antibiotics with DNA has been the subject of many studies using a variety of techniques, including equilibrium dialysis (Zunino et al., 1972; Arlandini et al., 1977), spectrophotometric methods (Calendi et al., 1965; Zunino et al., 1972; Gabbay et al., 1976), viscometric methods (Arlandini et al., 1977; Zunino et al., 1977), and fluorescence methods (Tsou & Yip, 1976; Zunino et al., 1976; DiMarco et al., 1977; Plumbridge & Brown, 1977; Zunino et al., 1977). As a result it is generally accepted that a major portion of the anthracycline–DNA interaction involves the insertion of the anthracycline ring between adjacent nucleotide bases of the DNA duplex by an intercalation mechanism (Lerman, 1961). This argument has been further substantiated by the studies of Waring (1970) and others [e.g., DiMarco et al. (1975), Gabbay et al. (1976), and Zunino et al. (1977)], who demonstrated that upon intercalation of anthracyclines between

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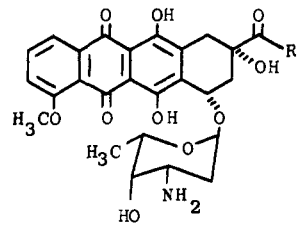
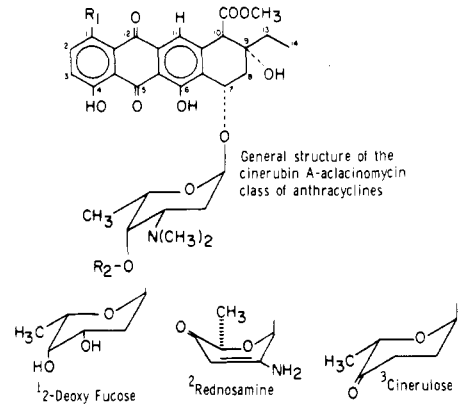


Adriamycin, R = CH_2OH

Daunomycin, R = CH_3

FIGURE 1: Structures of adriamycin and daunomycin.

Table I: Structural Modifications in the Cinerubin A-Aclacinomycin Class of Anthracyclines



anthracycline	R_1	R_2
pyrromycin	OH	H
musettamycin	OH	2-deoxyfucose[1]
rudolfomycin	OH	2-deoxyfucose-rednosamine[2]
aclacinomycin	H	2-deoxyfucose-cinerulose[3]
marcellomycin	OH	2-deoxyfucose-2-deoxyfucose
cinerubin A	OH	2-deoxyfucose-cinerulose

bases of the native DNA duplex, alteration in the secondary structure of DNA occurs. The results include altered sensitivity to nucleases (Tsou & Yip, 1976; Fecchinetti et al., 1978) and decreased template activity (Ward et al., 1965; Barthelemy-Clavey et al., 1976; Momparler et al., 1976; Böhner & Hagen, 1977; Sakano et al., 1977). The studies of Pigram et al. (1972) have demonstrated that in addition to the strong intercalative binding process, the interaction of adriamycin (ADM)[1] with DNA involves weaker electrostatic interactions. This weaker binding process involves electrostatic interactions between the ammonium group of the amino sugar of ADM and the phosphate group of the DNA backbone.

Figure 1 shows the structure of ADM and its analogue daunomycin (DNM). Table I shows the general structure of the class II group of anthracyclines (Crooke et al., 1978) as well as that of pyrromycin (PYM). Also shown are the structural modifications present in the various analogues. Previous studies from this laboratory (Crooke et al., 1978) have demonstrated that anthracyclines may be divided into two classes based on their effects on nucleic acid synthesis. Although many studies have been reported which examine the DNA interactions of ADM and DNM, no such studies have been reported for pyrromycinone-based anthracyclines, shown in Table I. This paper reports the results of studies examining the interactions of MCM and its analogues with DNA. DNAs of varying base compositions were employed in an effort to characterize the sequence preference of these interactions.

Experimental Procedures

Materials. The synthetic copolymers poly(dAdT)–poly(dAdT) and poly(dGdC)–poly(dGdC) were purchased from PL-Biochemicals, Inc., Milwaukee, WI. Calf thymus DNA (43% GC), salmon sperm DNA (41% GC), *Cl. perfringens* DNA (28% GC), *Micrococcus lysodeikticus* (*M. luteus*) DNA (72% GC), and ethylenediaminetetraacetic acid disodium salt (EDTA) were purchased from Sigma Chemical Co., St. Louis, MO. The ratios of the absorbance at 260 nm (A_{260}) to the absorbance at 280 nm (A_{280}) for all of the DNA preparations varied from 1.8 to 1.9. All spectrophotometric determinations were made on a Zeiss PMQ-3 spectrophotometer by using 1-cm path length quartz cuvettes. The ultraviolet (UV)–visible spectra of these drugs were determined on a Cary 14 recording spectrophotometer or on a Beckman Acta III recording spectrophotometer. The anthracycline antibiotics ADM, PYM, musettamycin (MSM), rudolfomycin (RDM), aclacinomycin (ACM), and marcellomycin (MCM) were all generously supplied by Bristol Laboratories, Syracuse, NY. Aqueous stock solutions of anthracyclines were prepared by wetting the drug crystals with dimethyl sulfoxide, followed by addition of water. Stock drug solutions were stored at −20 °C and used within 2–4 weeks. Working stock solutions of each drug were prepared by dilution of aqueous stock solutions with DNA binding buffer (0.05 M sodium phosphate buffer, pH 6.2, 0.05 M NaCl, and 0.001 M EDTA). Concentrations of anthracycline solutions were determined spectrophotometrically in methanol by using molar extinction coefficients determined previously (see Table II). DNA concentrations of solutions of calf thymus DNA, salmon sperm DNA, *Cl. perfringens* DNA, and *M. luteus* DNA were all determined spectrophotometrically at 260 nm by using a molar extinction coefficient with respect to nucleotide residues of 6600 M^{-1}. Similarly, DNA concentrations for solutions of poly(dAdT)–poly(dAdT) and poly(dGdC)–poly(dGdC) were determined by using molar extinction coefficients of 6600 M^{-1} at 262 nm and of 8400 M^{-1} at 254 nm, respectively. Spectrofluorometric measurements were made on an Aminco Bowman 4-8106 spectrophotofluorometer by using 1-cm quartz cuvettes.

Fluorescence Spectra and Fluorescence Quenching Effects of DNA. Fluorescence spectra of each of the anthracycline antibiotics were determined by exciting a 1-mL solution of 5 μM drug at the visible absorption maximum of each drug (see Table II) and varying the emission wavelength in the lower energy region of each spectrum. Quenching of drug fluorescence by DNA was measured by addition of microliter volumes of concentrated DNA solutions to the cuvette. The dilution effect caused by addition of DNA solution was negligible.

Binding Measurements. All measurements were carried out for 1 h at 25 °C in DNA binding buffer. A minimum of two determinations of binding parameters was made for each anthracycline–DNA combination. Identical preparations of each type of DNA were used for all anthracyclines. Each binding determination consisted of a minimum of 11 different DNA concentrations, each of which consisted of duplicate or triplicate samples. All solutions were maintained on ice until the binding reaction was initiated.

The DNA binding of anthracycline antibiotics was measured by spectrofluorometry in a manner analogous to previous studies (Daniel & Weber, 1966; Pesce et al., 1971; DiMarco et al., 1977; Zunino et al., 1977). Briefly, fixed concentrations

[1] Abbreviations used: ADM, adriamycin; DNM, daunomycin; PYM, pyrromycin; MSM, musettamycin; RDM, rudolfomycin; MCM, marcellomycin; ACM, aclacinomycin; EDTA, ethylenediaminetetraacetic acid disodium salt; *r*, moles of ligand bound per nucleotide; *C*, the molar concentration of free ligand.

Table II: Spectral and Fluorescence Parameters of Several Anthracyclines

drug	absorbance, λ_{max} (nm)[a]	extinction coefficient (E) (at absorbance λ_{max})	excitation wavelength (nm)	fluorescence wavelength (nm)
adriamycin	477	12 200	480	560
marcellomycin	490	13 400	490	555
musettamycin	490	12 500	490	555
pyrromycin	490	6 900	490	555
rudolfomycin	490	13 900	490	555
aclacinomycin	434	12 000	435	550

[a] Absorbance maxima were obtained from absorption spectra of each anthracycline: adriamycin (Calendi et al., 1965), pyrromycin (Brockmann & Lenk, 1959a,b; Keller-Schierlein & Richle, 1971), musettamycin (Nettleton et al., 1977), marcellomycin (Nettleton et al., 1977), aclacinomycin (Oki et al., 1975), and rudolfomycin (Doyle et al., 1978; T. W. Doyle, D. E. Nettleton, R. E. Grulich, D. M. Balitz, D. L. Johnson, and A. L. Vulcano, unpublished experiments).

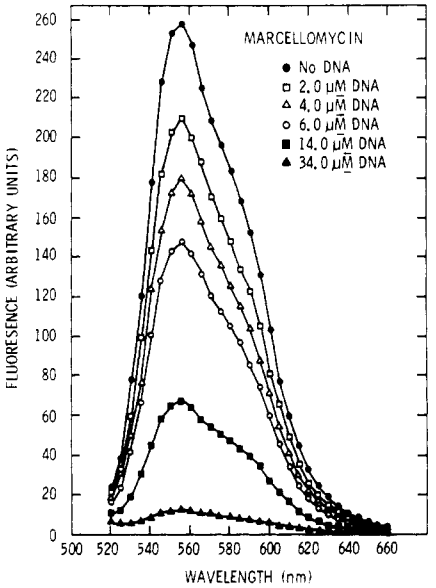


FIGURE 2: Fluorescence spectral changes of marcellomycin upon interaction with salmon sperm DNA. Spectra were taken of 5 μM anthracycline solutions in 0.05 M sodium phosphate buffer, pH 6.2, 0.05 M NaCl, and 0.001 M EDTA. Spectra were obtained by using the specific excitation wavelength indicated in Table II and recording drug fluorescence at wavelengths and in the low-energy region of the spectrum (longer wavelengths). Increasing concentrations of DNA were obtained by addition of microliter volumes of a concentrated solution of salmon sperm DNA to the above buffer.

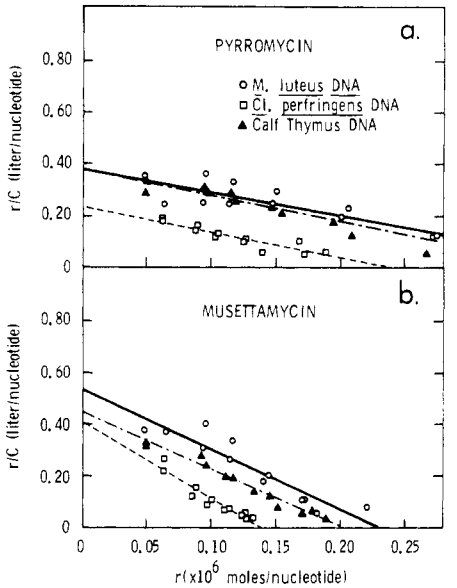


FIGURE 3: Scatchard plots of the binding data for the interaction of anthracyclines with calf thymus DNA, *M. luteus* DNA, and *Cl. perfringens* DNA. A fixed concentration of anthracyclines was titrated with increasing concentrations of DNA in binding buffer, 0.05 M sodium phosphate, pH 6.2, 0.05 M NaCl, and 0.001 M EDTA, and incubated at 25 °C for 1 h. Fluorescence measurements were taken by using the fluorescence parameters indicated in Table II. The Scatchard parameters r (moles of ligand bound per nucleotide), C (moles per liter of free ligand), and r/C were calculated as indicated under Experimental Procedures. The results of duplicate experiments, each of which contained duplicate or triplicate values at each DNA concentration, are shown: (a) pyrromycin; (b) musettamycin.

of anthracycline (3, 4, 5, or 10 μM) were titrated with increasing concentrations of DNA, thereby varying the DNA/drug ratios from 0 to 200. The DNA/drug ratio of 200 is taken as the end point in this titration assay with the drug considered totally bound. Total reaction volume was 1.0 mL. Fluorescence determinations were made for each anthracycline by using the fluorescence parameters shown in Table II.

The binding data were analyzed by the Scatchard method (Scatchard, 1949). The Scatchard variables of r (moles of ligand bound per nucleotide) and C (the molar concentration of free antibiotic) were calculated from the fluorescence data according to the method of Peacocke & Skerrett (1956). Binding parameters were determined from plots of r/C vs. r, where K_{app} (apparent association constant) is the negative slope and n_{app} (the apparent number of binding sites per nucleotide) is the intercept of the curve with the x axis.

Results

The fluorescence spectrum of each anthracycline was determined by using the visible absorption maximum for each compound, shown in Table II. The resultant fluorescence parameters and excitation and emission wavelengths are also listed in Table II. Figure 2 shows the fluorescence spectrum of MCM and the fluorescence spectral changes which occur upon interaction with DNA. Increasing concentrations of salmon sperm DNA progressively decreased the fluorescence throughout the spectrum with no significant enhancement of any portion of the spectrum or peak shift detected. These results are typical of those obtained with the other anthracyclines and with the other DNAs studied. Since the greatest changes in the anthracycline fluorescence spectra upon addition of DNA occurred at the wavelengths indicated in Table II, fluorescence measurements at these wavelengths yielded the most accurate determination of the fraction of the total anthracycline bound.

Figure 3 shows the Scatchard analyses of the interactions of two typical anthracyclines, PYM and MSM, with calf thymus DNA, *M. luteus* DNA, and *Cl. perfringens* DNA. Since Figure 3 represents the typical results of duplicate or triplicate values, p values were obtained to indicate the statistical significance of the derived parameters. The p values, which indicate the probability that the data points describe a straight line, are shown in footnote *a* of Tables III and IV.

Table III: Calf Thymus DNA, *M. luteus* DNA, and *Cl. perfringens* DNA Binding Parameters of Anthracyclines[a]

anthracycline	*Cl. perfringens* DNA (28% GC)		calf thymus DNA (43% GC)		*M. luteus* DNA (72% GC)	
	K_{app}[b] ($\times 10^6$ M^{-1})	n_{app}[c]	K_{app} ($\times 10^6$ M^{-1})	n_{app}	K_{app} ($\times 10^6$ M^{-1})	n_{app}
adriamycin	3.97 ± 0.55[d]	0.10 ± 0.04	3.67 ± 0.42	0.15 ± 0.05	5.46 ± 0.76	0.14 ± 0.08
pyrromycin	1.01 ± 0.13	0.24 ± 0.02	0.98 ± 0.13	0.38 ± 0.02	0.87 ± 0.18	0.43 ± 0.03
musettamycin	2.96 ± 0.28	0.14 ± 0.03	2.21 ± 0.13	0.20 ± 0.02	2.32 ± 0.30	0.23 ± 0.04
rudolfomycin	2.44 ± 0.34	0.16 ± 0.04	1.98 ± 0.16	0.22 ± 0.02	2.02 ± 0.35	0.29 ± 0.05
aclacinomycin	1.75 ± 0.29	0.11 ± 0.02	2.48 ± 0.16	0.18 ± 0.02	2.37 ± 0.34	0.19 ± 0.04
marcellomycin	6.05 ± 1.23	0.13 ± 0.13	5.03 ± 0.23	0.19 ± 0.03	5.25 ± 0.46	0.23 ± 0.07

[a] p values were determined from tables of significance limits for correlation coefficients (Diem & Lentner, 1970). As such, p values were all less than 0.001. The number of averaged values used to construct composite Scatchard curves was no less than 10 and usually 12. [b] K_{app}, apparent association constant, in units of M^{-1}. Values were obtained by linear regression analyses of composite Scatchard curves obtained from two or more separate experiments, each of which contained duplicate or triplicate values at each DNA concentration. [c] n_{app}, apparent number of binding sites per nucleotide. Values were obtained as for K_{app}. [d] Standard deviations of slopes (K_{app}) and x intercepts (n_{app}) were calculated from linear regression analyses as indicated by Diem & Lentner (1970).

Table IV: Poly(dAdT)-Poly(dAdT) and Poly(dGdC)-Poly(dGdC) DNA Binding Parameters of Anthracyclines[a]

anthracycline	poly(dAdT)-poly(dAdT)		poly(dGdC)-poly(dGdC)	
	K_{app}[b] ($\times 10^6$ M^{-1})	n_{app}[c]	K_{app} ($\times 10^6$ M^{-1})	n_{app}
adriamycin	no detectable binding		5.54 ± 0.61	0.16 ± 0.06
pyrromycin	1.33 ± 0.21[d]	0.70 ± 0.04	0.88 ± 0.14	0.48 ± 0.02
musettamycin	6.79 ± 0.57	0.28 ± 0.10	2.46 ± 0.23	0.30 ± 0.04
rudolfomycin	6.88 ± 0.72	0.31 ± 0.13	1.74 ± 0.33	0.35 ± 0.05
aclacinomycin	11.81 ± 0.37	0.18 ± 0.05	4.48 ± 0.68	0.18 ± 0.08
marcellomycin	16.13 ± 1.78	0.20 ± 0.25	4.75 ± 0.50	0.25 ± 0.08

[a] All p values were less than 0.001 (see Table III). The number of averaged values used to construct composite Scatchard curves was no less than 11 and usually 12. [b] K_{app}, apparent association constant, in units of M^{-1} (see Table III). [c] n_{app}, apparent number of binding sites per nucleotide (see Table III). [d] Standard deviations (see Table III).

Standard deviations of the slopes (K_{app}) and x intercepts (n_{app}) were calculated from linear regression analyses as described previously (Diem & Lentner, 1970).

The results of the Scatchard analyses of all six anthracyclines studied with respect to calf thymus DNA, *M. luteus* DNA, and *Cl. perfringens* DNA are indicated in Table III. The results obtained for ADM are in the range of values which have been previously published (Zunino et al., 1972; Gabbay et al., 1976; Tsou & Yip, 1976; DiMarco et al., 1977). MCM has a higher affinity for all three DNAs than its structural analogues and approximately equivalent K_{app} values to those of ADM. Some variation in n_{app} is also evident.

To investigate further the sequence preference of anthracyclines, the double-stranded synthetic copolymers poly(dAdT)–poly(dAdT) and poly(dGdC)–poly(dGdC) were employed. Figure 4 shows the fluorescence titration curves of ADM, PYM, and MSM in the presence of increasing concentrations of the two double-stranded synthetic copolymers. The curves obtained for PYM and MSM are typical of the results obtained with MCM, ACM, and RDM. For most of the anthracyclines studied, with the exception of ADM, the fluorescence quench curves obtained for the two synthetic copolymers were similar. For ADM, the fluorescence quenching effect seen at all DNA/drug ratios was more pronounced for poly(dGdC)–poly(dGdC) than for poly(dAdT)–poly(dAdT), suggesting differences in the extent of binding for these two polymers. At a DNA/drug ratio of 200, ADM fluorescence was quenched only to the extent of ~35% by poly(dAdT)–poly(dAdT) whereas the poly(dGdC)–poly(dGdC) effected a quenching of 99%.

Figure 5 shows the Scatchard analyses of the interaction of MCM with poly(dAdT)–poly(dAdT) and poly(dGdC)–poly(dGdC). As indicated, the curve obtained for poly(dAdT)–poly(dAdT) contains a steeper slope than that obtained for poly(dGdC)–poly(dGdC), indicating a higher affinity of MCM for poly(dAdT)–poly(dAdT) than for poly(dGdC)–poly(dGdC). These results are typical of those

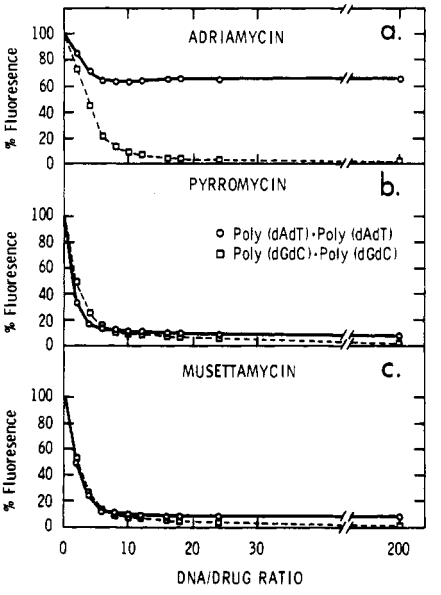


FIGURE 4: Fluorescence quenching of anthracyclines in the presence of increasing concentrations of poly(dAdT)–poly(dAdT) and poly(dGdC)–poly(dGdC). See the legend for Figure 3. (a) Adriamycin; (b) pyrromycin; (c) musettamycin.

obtained with PYM, MSM, RDM, and ACM. However, the Scatchard curve obtained for the interaction of ADM with poly(dAdT)–poly(dAdT) could not be calculated due to the lower levels of binding obtained even at high DNA concentrations (see Figure 4).

Table IV summarizes the results obtained for the interaction of the six anthracyclines with the two synthetic copolymers. The results indicate the clear preference of the pyrromycinone-based anthracyclines for poly(dAdT)–poly(dAdT), in contrast to the results obtained for ADM. The n_{app} values obtained with these two synthetic copolymers were not significantly different.

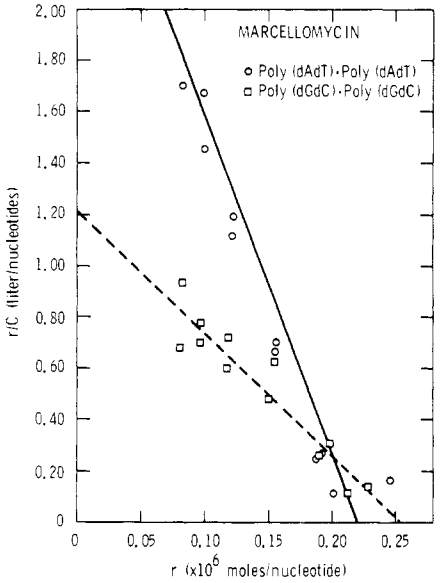


FIGURE 5: Scatchard plots of the binding data for the interaction of marcellomycin with poly(dAdT)–poly(dAdT) and poly(dGdC)–poly(dGdC). See the legend for Figure 3.

A comparison of the K_{app} values of the six anthracyclines for poly(dGdC)–poly(dGdC) shows that the K_{app} for ADM is only slightly greater than those for MCM and ACM, but significantly greater than those for PYM, MSM, and RDM. The values of K_{app} for poly(dGdC)–poly(dGdC) were similar to values obtained with *M. luteus* DNA.

Discussion

Studies employing spectral (Peacocke & Skerrett, 1956; Gellert et al., 1965; Hyman & Davidson, 1971; Gabbay et al., 1976) as well as fluorescence (Tsou & Yip, 1976; DiMarco et al., 1977; Plumbridge & Brown, 1977) titration techniques have been used to examine the binding interactions of ligand chromophores with nucleic acids. These techniques depend upon the demonstration of spectral alterations of the ligand upon interaction with nucleic acids (Peacocke & Skerrett, 1956). Such alterations have been previously reported for DNM (Calendi et al., 1965) and have been suggested to occur for ADM (DiMarco et al., 1975). No studies involving PYM, MSM, RDM, ACM, and MCM have been reported. We have demonstrated that upon interaction of the six anthracyclines studied with nucleic acids, the fluorescence spectral alterations which occur resemble those of DNM (Calendi et al., 1965). Similar alterations in the visible absorption spectra of these compounds also occur (unpublished results). These changes allowed the quantitation of DNA–anthracycline interaction and thus facilitate their use in DNA binding studies.

Previous studies from this laboratory have demonstrated that anthracyclines can be divided into two classes based upon their nucleic acid synthesis inhibitory specificity (Crooke et al., 1978; DuVernay et al., 1979). Accordingly, class I anthracyclines, which include ADM, PYM, and carminomycin, were shown to be nonselective with respect to nucleolar RNA synthesis inhibition; i.e., the inhibition of both DNA and nucleolar RNA syntheses occurs at approximately equivalent drug concentrations. Class II anthracyclines, which include MSM, RDM, ACM, and MCM, inhibit nucleolar RNA synthesis at concentrations 200–1300-fold lower than those required to inhibit DNA synthesis. Structure–activity relationships suggested a correlation with the length and composition of the glycosidic side chain of these compounds (see Table I). One possible interpretation of these results is that class II anthracyclines

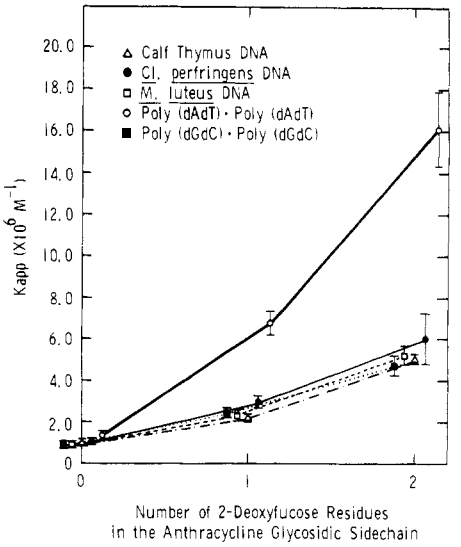


FIGURE 6: Effect of varying numbers of 2-deoxyfucose sugar residues in the anthracycline glycosidic side chain on the apparent association constants for several DNAs. Results are obtained from Tables III and IV. The variation in the number of 2-deoxyfucose residues in the side chain of 0, 1, and 2 corresponds to the compounds PYM, MSM, and MCM, respectively.

may bind preferentially to GC-rich regions of native DNA, similar to the well-characterized agent actinomycin D (Gellert et al., 1965; Muller & Crothers, 1968; Hyman & Davidson, 1971). Therefore, the present series of studies was undertaken in an attempt to determine whether the observed activities correlate with DNA binding affinities and sequence preferences and to determine structure–activity relationships of anthracyclines relative to DNA binding characteristics.

Figure 6 shows a graph of the K_{app} values vs. the number of 2-deoxyfucose sugar residues in the anthracycline glycosidic side chain. Thus, the anthracyclines PYM, MSM, and MCM contain 0, 1, and 2 residues of the sugar 2-deoxyfucose per drug molecule, respectively. As indicated in Table I, these three anthracyclines are structurally identical with respect to the aglycon and the attached amino sugar rhodosamine. As shown in Figure 6, there is a direct correlation between the length of the glycosidic side chain and the affinity for DNA. A similar correlation was observed for the biological structure–activity relationships (Crooke et al., 1978).

The results obtained for MSM, RDM, and MCM can be compared to examine the influence of the terminal sugar residue of the anthracycline glycosidic side chain on the DNA binding characteristics of these compounds. MCM and RDM are basically modifications of the MSM molecule, with MCM having the terminal sugar group of 2-deoxyfucose and RDM possessing the terminal sugar group rednosamine. The results shown in Tables III and IV demonstrate that the presence of sugar residue 2-deoxyfucose is correlated with increased binding affinity for DNA, whereas the sugar group rednosamine appears to confer no apparent enhancement of DNA binding ability. It should be noted that the primary sugar (attached to the aglycon ring) in all of these anthracyclines is the amino sugar rhodosamine (an *N',N'*-dimethyl derivative of 2-deoxyfucose). The results obtained for ACM suggest that the terminal sugar residue cinerulose may confer some DNA binding advantage over the disaccharide MSM. However, comparable structure–activity relationships for the terminal sugar residue cinerulose, as for 2-deoxyfucose and rednosamine, are difficult due to the differences at the aglycon (ACM lacks the 1-OH group as shown in Table I). The anthracycline necessary for this comparison is cinerubin A (Keller-Schierlein

Table V: Correlation of Nucleolar RNA Synthesis Inhibitory Activity with the Terminal Sugar Group of the Anthracycline Glycosidic Side Chain

anthracycline	no. of sugars in side chain	terminal sugar group	IC_{50} No-RNA synthesis[a] (μM)	(IC_{50} DNA)/(IC_{50} No-RNA) ratio[b]
musettamycin	2	2-deoxyfucose	0.014	714
rudolfomycin	3	rednosamine	0.290	240
marcellomycin	3	2-deoxyfucose	0.009	1256

[a] IC_{50} No-RNA synthesis, 50% inhibitory concentration for nucleolar RNA synthesis. [b] Ratio of the 50% inhibitory concentration for DNA to that for No-RNA synthesis.

& Richle, 1971), which contains the 1-OH group.

The DNA binding characteristics of MSM, RDM, and MCM correlate well with their nucleic acid synthesis inhibitory effects (Crooke et al., 1978; DuVernay et al., 1979). Table V shows a comparison of the effects of these anthracyclines on nucleolar RNA synthesis with changes in the terminal sugar group in the glycosidic side chain. Addition of the terminal sugar residue rednosamine to the MSM molecule is correlated with a decrease in nucleolar RNA synthesis inhibitory activity of approximately 21-fold. In contrast, addition of the terminal sugar group 2-deoxyfucose to the MSM molecule is correlated with an increased nucleolar RNA synthesis inhibitory potency. Thus, these activities correlate well with the DNA binding characteristics of MSM, RDM, and MCM.

DNAs of varying GC content were used in order to study the sequence preference of DNA binding by anthracyclines. The synthetic copolymers poly(dAdT)–poly(dAdT) and poly(dGdC)–poly(dGdC) were taken as limits of either extreme of GC content and are not intended to represent defined models of native DNA solutions. The results summarized in Tables III and IV show that DNAs with high AT content offer fewer binding sites and lower binding for ADM than do DNAs with high GC content. In agreement with previous studies (Tsou & Yip, 1976), ADM has an absolute GC sequence requirement for binding to DNA. This trend was not repeated with the other anthracyclines studied. Thus, as indicated in Tables III and IV, PYM, MSM, RDM, ACM, and MCM demonstrated no base composition requirement for DNA binding.

The high K_{app} values obtained for poly(dAdT)–poly(dAdT), as compared with the natural DNAs and poly(dGdC)–poly(dGdC), for MCM and its analogues, as well as the evidence for altered conformation of this copolymer in solution (Wartell, 1972), indicate the need of a cautious interpretation of the binding parameters obtained for this copolymer. That these results are suggestive of differences in sequence preferences by these anthracyclines is one of several possible interpretations. Futher studies are needed to investigate possible differences in the degree of quenching upon binding to DNA high in GC vs. AT content. Furthermore, fluorescence techniques may be insensitive to nonintercalative binding processes, which may occur for the ADM–poly(dAdT)–poly(dAdT) interaction. Thus, the use of poly(dAdT)–poly(dAdT) as a model for anthracycline–natural DNA interactions is not supported by these results. However, its use in determination of sequence preference of a ligand is of value. As such, these findings, indicating an AT preference for MCM and its analogues, support similar findings for nogalamycin and steffimycin B (two closely related anthracyclines) (Bhuyan & Smith, 1965, 1975; Ward et al., 1965; Zunino et al., 1974; Reusser, 1975) and DNM (Ward et al., 1965; DiMarco et al., 1975). Further studies are required to verify these findings.

The recent demonstration of selective nucleolar RNA synthesis inhibition (Crooke et al., 1978; DuVernay et al., 1979) is difficult to explain in light of the present finding of an AT preference of MCM and its analogues. The electron micrographic demonstration of regional disruption of the nucleolar transcriptional apparatus by RDM (I. Daskal, personal comments) suggests that spacer and/or promotor regions, known to have high AT content, may be targets of anthracycline binding. Definitive studies in this area are needed to examine the involvement of chromatin proteins in these DNA–drug interactions. Further, ADM, which has been shown to prefer GC-rich regions of DNA (Tsou & Yip, 1976; present study), shows no selective inhibition of nucleolar RNA synthesis (Crooke et al., 1978). This is in contrast to actinomycin D, another agent with high GC specificity, which is selective with respect to nucleolar RNA synthesis inhibition.

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Resolution of the Major Components of Human Lung Mucosal Gel and Their Capabilities for Reaggregation and Gel Formation†

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ABSTRACT: Lung mucosal gel is composed mainly of mucin glycoproteins and lower molecular weight (150 000–13 000) proteins. The gel can be solubilized at low concentrations (1 mg/mL) by dissociating agents in the absence of a reducing agent, but the components are aggregated and cannot be resolved by chromatography. Following dissociation of the aggregates by the addition of dithiothreitol, the proteins and glycoproteins can be resolved by chromatography on Sepharose 4B in a buffer containing both $NaDodSO_4$ and dithiothreitol. The mucins elute in the void volume and the proteins in the included volume near the salt peak. Further purification of the mucin glycoprotein fraction on Sepharose 2B and hydroxylapatite resulted in the resolution of major and minor mucin glycoproteins, neither of which contained any detectable serum albumin or lower molecular weight proteins characteristic of lung mucosal gel. The mucin glycoproteins were shown to be capable of forming a gel in the absence of both a dissociating solvent and a reducing agent. Thus, human lung mucosal gels behave differently than another human mucosal system, i.e., the viscid meconium gel—observed in certain cases of cystic fibrosis (meconium ileus)—which results from an interaction of albumin with mucin glycoproteins. Both thiol interactions, as indicated by the effect of dithiothreitol, and nonthiol interactions, as indicated by the effects of guanidine hydrochloride, urea, and sodium dodecyl sulfate, are involved in aggregation and gel formation of lung mucosal gel. Both types of interactions are also implicated in aggregation and gel formation of purified lung mucin glycoproteins. The properties of the carboxymethylated mucins support the conclusion from dithiothreitol experiments, but the results of carboxymethylation are more difficult to interpret because of the apparent effect on nonthiol as well as thiol interactions.

The mucosal layer which coats the surface epithelium of mammalian airways contains macromolecules which interact to form a gel. This lung mucosal gel (LM-gel)[1] is insoluble in physiological saline and provides a protective barrier for the underlying epithelium as well as the viscoelastic properties necessary for the functioning of the mucociliary escalator that

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[1] Abbreviations used: LM-gel, lung mucosal gel; MGP, mucin glycoprotein; $NaDodSO_4$, sodium dodecyl sulfate; DTT, dithiothreitol; Gdn·HCl, guanidine hydrochloride; NANA, *N*-acetylneuraminic acid; HA, hydroxylapatite.

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