ROLE OF MAGNESIUM ION ON THE INTERACTION BETWEEN CHROMOMYCIN A_3 AND DEOXYRIBONUCLEIC ACID

R. NAYAK and M. SIRSI
Microbiology and Pharmacology Laboratory

and

S.K. PODDER
Department of Biochemistry, Indian Institute of Science, Bangalore-560012, India

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1. Introduction

Chromomycin A_3 (CHR) and related antibiotics have shown anti-tumor properties against experimental and human tumors [1]. A systematic study on the *in vivo* action of these drugs has been undertaken in our laboratory to establish the relationship between their tumor inhibitory and immunosuppressive properties. Simultaneously their interaction with chromatin isolated from normal and neoplastic tissue is being studied with a view to evaluate the relative therapeutic potential of these antibiotics in terms of antibiotic–chromatin interaction constant and to understand the mechanism of drug action. The preliminary results of these studies have been reported recently [2]. During these investigations it was observed that Mg^{2+} ion influences the binding of chromomycin A_3 to chromatin even under conditions where salt induced dissociation of chromatin into DNA and histones does not occur. Similar observation was made earlier in case of interaction between DNA and chromomycin A_3 [3, 4]. Studies [3, 4] concerning the role of Mg^{2+} ion in this interaction are not very systematic and therefore, the influence of Mg^{2+} ion on the binding is not well understood. The study of Waring [5] shows that chromomycin A_3–DNA complexes are different from the intercalated dye–DNA complexes in their ultracentrifugation behaviour, but the details are lacking to draw any definite conclusion regarding the nature of these complexes. Thus a reinvestigation is felt necessary to find out i) how specifically Mg^{2+} ions are involved in binding, and ii) the nature of chromomycin A_3–DNA complex.

An attempt has been made to resolve these problems by spectrophotometric investigation of DNA–chromomycin A_3 complexes under varying pH and concentrations of Mg^{2+} ions. From the data presented here it is concluded that Mg^{2+} ion is directly involved in binding to counteract electrostatic repulsions between negatively charged phosphate group of DNA and anionic chromomycin A_3.

2. Materials and methods

DNA isolated from mouse tumor mouse fibrosarcoma (MFS) following the procedure of Kay et al. [6] and calf thymus DNA from Sigma Chemical Company were used. Chromomycin A_3 (Calbiochem, Switzerland) and heparin (Sigma Chemical Company, St. Louis) were used directly. Spectrophotometric titrations were carried out at room temp. (29°) using Cary-14 spectrophotometer.

3. Results and discussion

The structural formula [7] of chromomycin A_3 (CHR), shown in fig. 1 suggests that one of the (OH) groups attached to the chromophore should dissociate between pH 6–8. To find out whether CHR exists as an anion at neutral pH, pKa was determined from the pH-induced changes in the spectrum of CHR (shown in

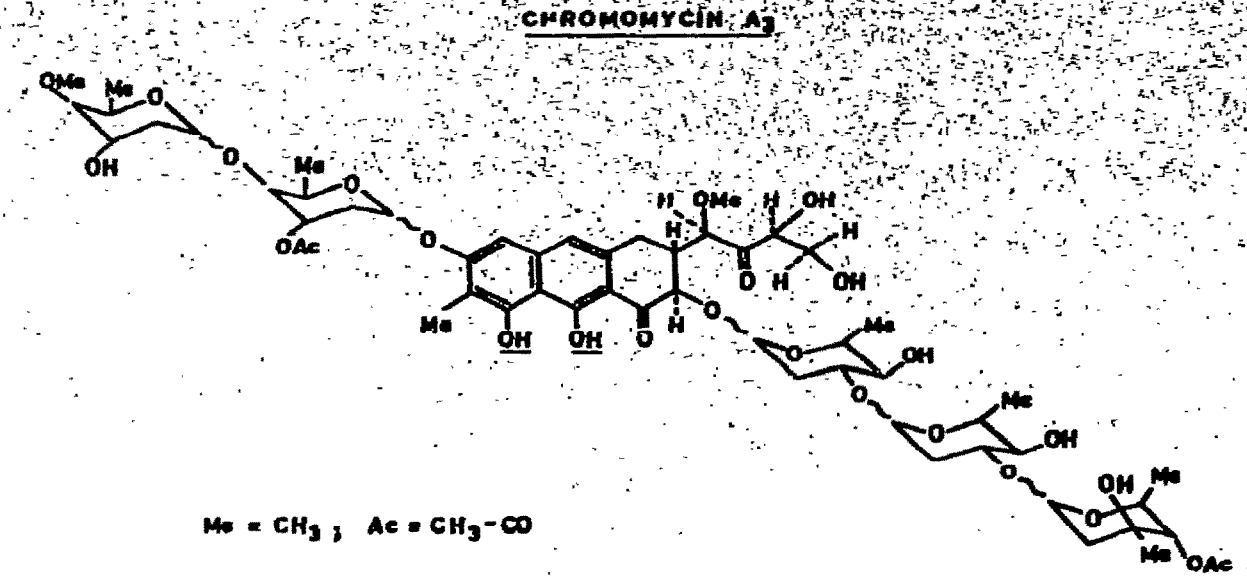


Fig. 1. The structural formula of chromomycin A_3.

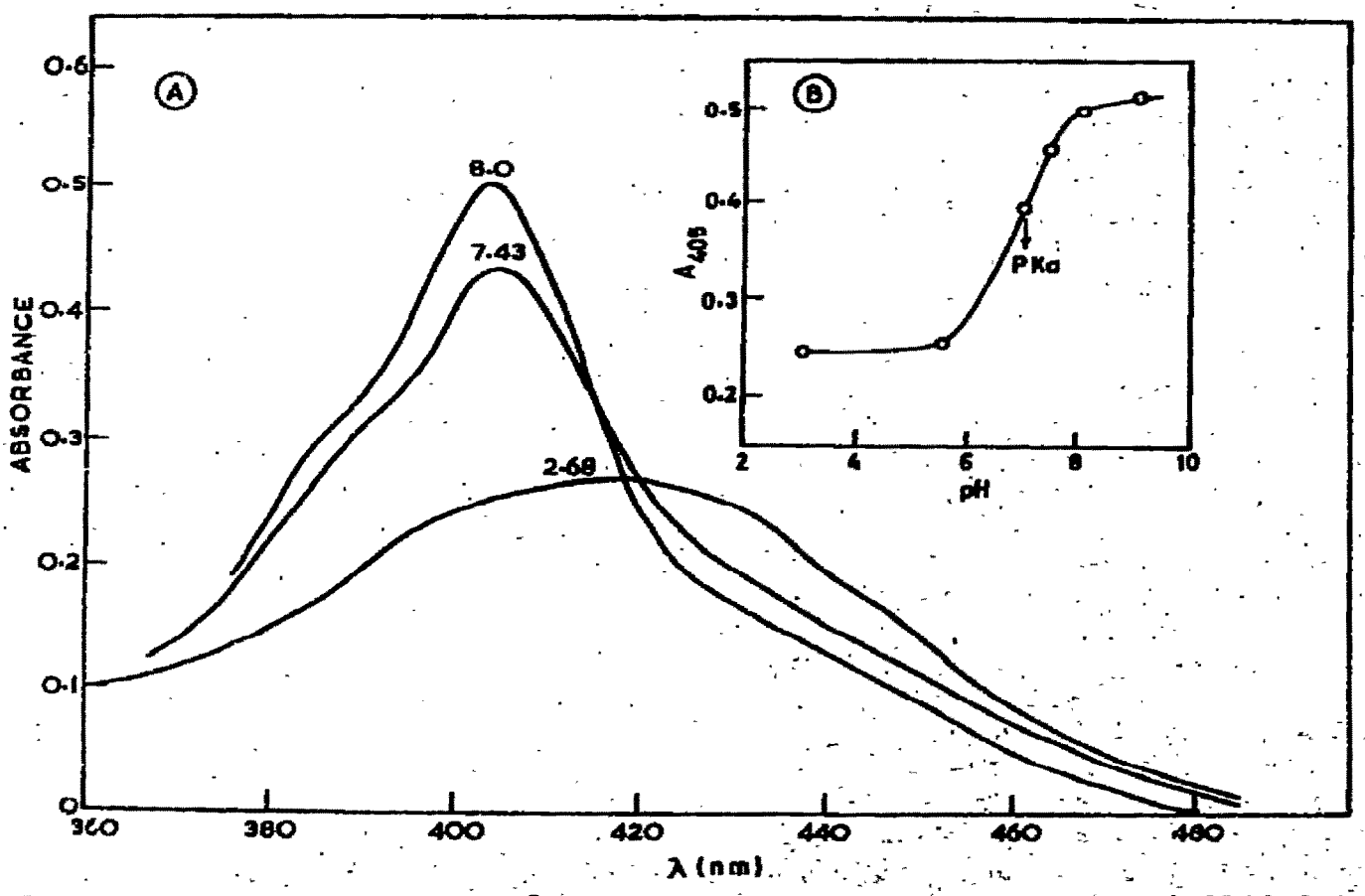
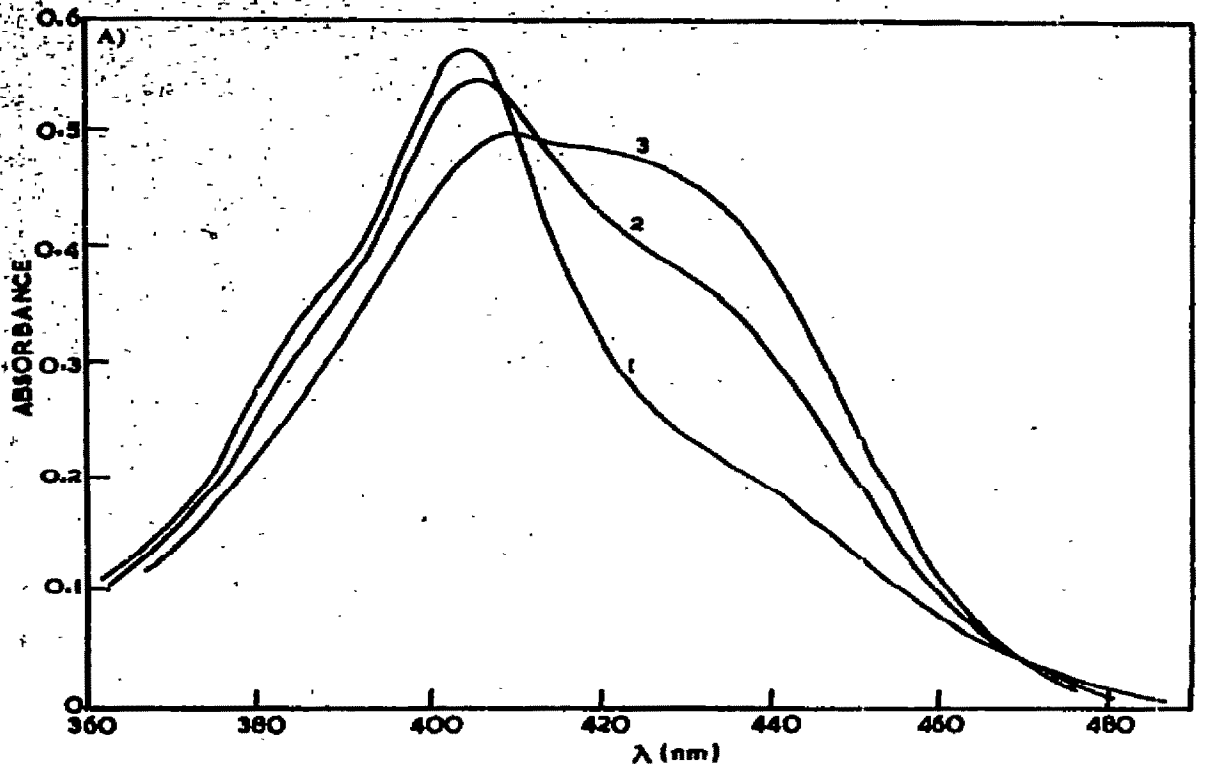


Fig. 2. (A) Absorption spectra of CHR (5.7×10^{-5} M) at three different pH's (indicated in the parenthesis) of 0.1 ionic strength. (B) pH-dependence of absorbance of CHR at 405 nm.



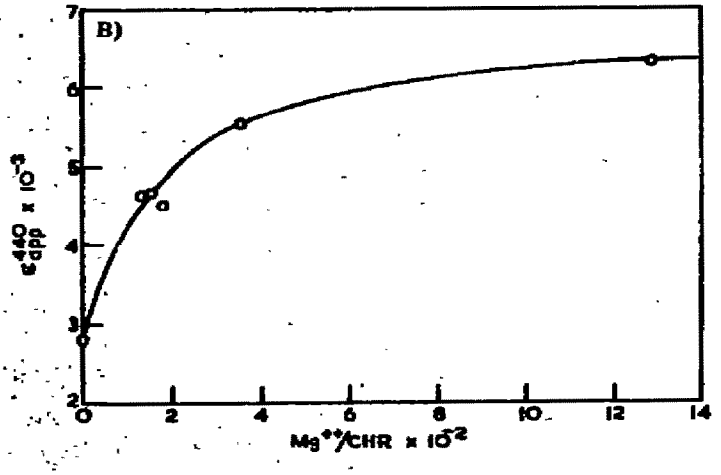


Fig. 3. (A) Absorption spectra of CHR (6.5×10^{-5} M) in 0.01 M Tris-HCl buffer, pH 8, containing different amount of Mg^{2+}: (1) without Mg^{2+} (2) 0.97×10^{-2} M Mg^{2+} (3) 2.38×10^{-2} M Mg^{2+}. (B) Apparent extinction co-efficient of CHR at 440 nm as a function of ratio of Mg^{2+}/CHR.

fig. 2A). The absorbance at 405 nm (A_{405}) vs pH profile is shown in fig. 2B and therefrom pKa of CHR is calculated as 7. This value agrees well with that determined from pH-dependent R_f values of CHR in *n*-amyl alcohol—buffer mixture [8]. The addition of Mg^{2+} in large excess also produces a similar spectral change (fig. 3A). The spectral data represented in fig. 3B yield a value of 2.03 for $pK_{Mg^{2+}}$. Such low value of $pK_{Mg^{2+}}$ suggests that when present in low concentration (10^{-4} M), Mg^{2+} ion does not interact with anionic CHR at pH 8. The addition of DNA to a solution of anionic CHR at pH 8 without any extra Mg^{2+} ion, produces hardly any immediate measurable changes in the spectrum of CHR. The spectral changes, if any, occur very slowly, whereas in presence of small amount of Mg^{2+} (10^{-4} M), spectral changes do occur rapidly and the equilibrium is established in 15—20 min after addition of DNA. Similar rate enhancement was also observed in presence of NaCl. Figs. 4 and 5 show the changes in the spectrum of CHR when increasing amounts of DNA are added to a solution of CHR of constant concentration containing 10^{-4} M and $\sim 10^{-1}$ M Mg^{2+}, respectively. The spectrum was recorded 30 min after each addition of DNA from a micropipette. From the titration curves (figs. 4 and 5), the amount of bound CHR (αCo) (where Co = the total concentration of CHR and α = the fraction bound) is calculated using the relationship:

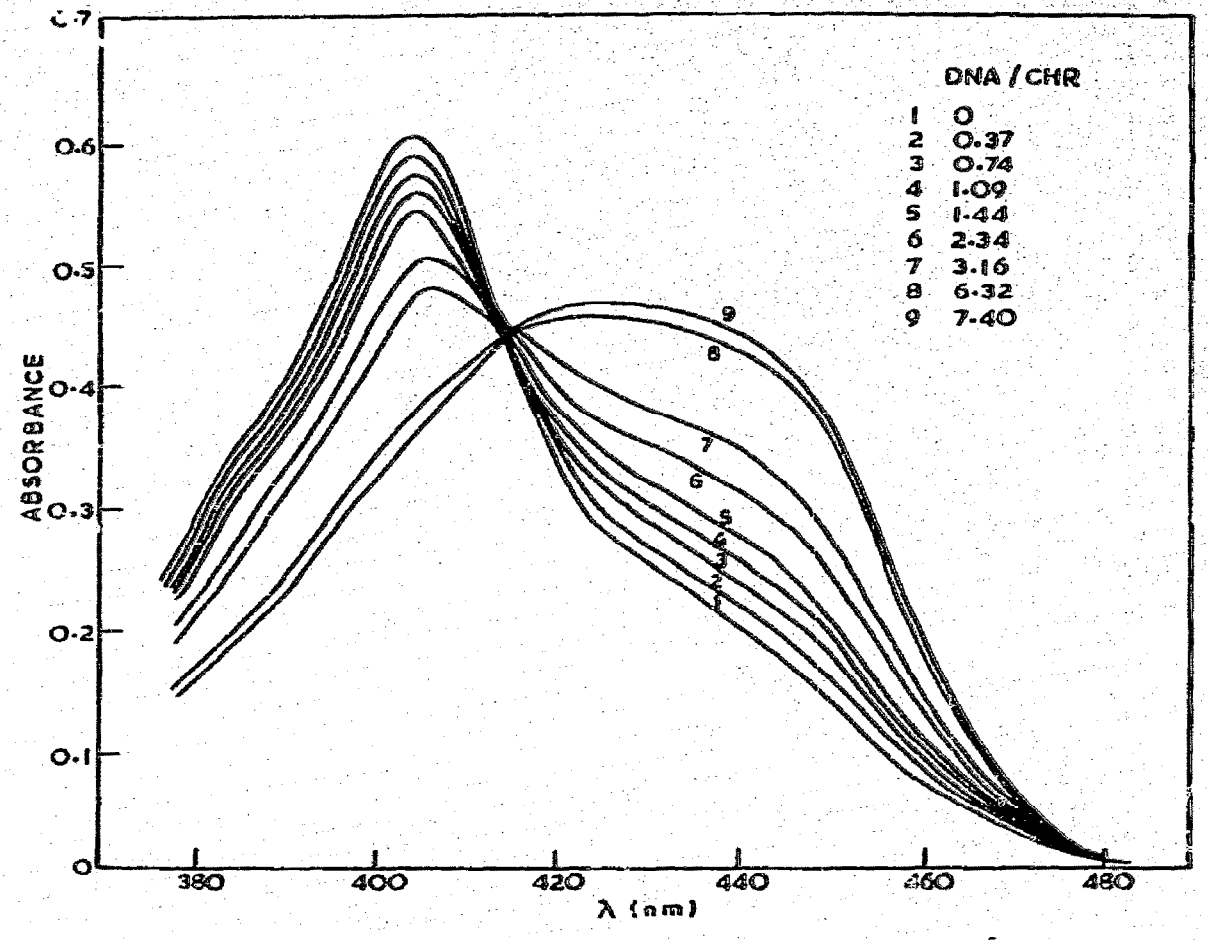


Fig. 4. Spectrophotometric titration of CHR (6.8×10^{-5} M) in 0.01 M Tris-HCl buffer + 23.6×10^{-5} M $MgCl_2$, pH 8 with MFS DNA, numbers indicate different DNA/CHR ratios.

Table 1

Association constants (K_{app} (M^{-1}) and number of binding site/nucleotide (n) for the binding of chromomycin A_3 to DNA at pH 8.00, and 29° under different conditions.

Sample	K_{app} (M^{-1}) (29°)	n (Site/ nucleotide)	Solvent	Mg^{2+}/CHR
MFS DNA	8.5×10^4	0.097	0.01 M Tris 23.6×10^{-5} M $MgCl_2$, pH 8	3.46
Calf thymus DNA	1.36×10^5	0.103	0.01 M Tris 24.4×10^{-5} M $MgCl_2$, pH 8	2.94
Calf thymus DNA	9.7×10^4	0.235	0.028 M Tris 0.098 M $MgCl_2$ pH 8	1.44×10^3
Calf thymus* DNA	2.0×10^5	0.19	—	—

MFS = mouse fibro sarcoma.

* Taken from the literature, [3].

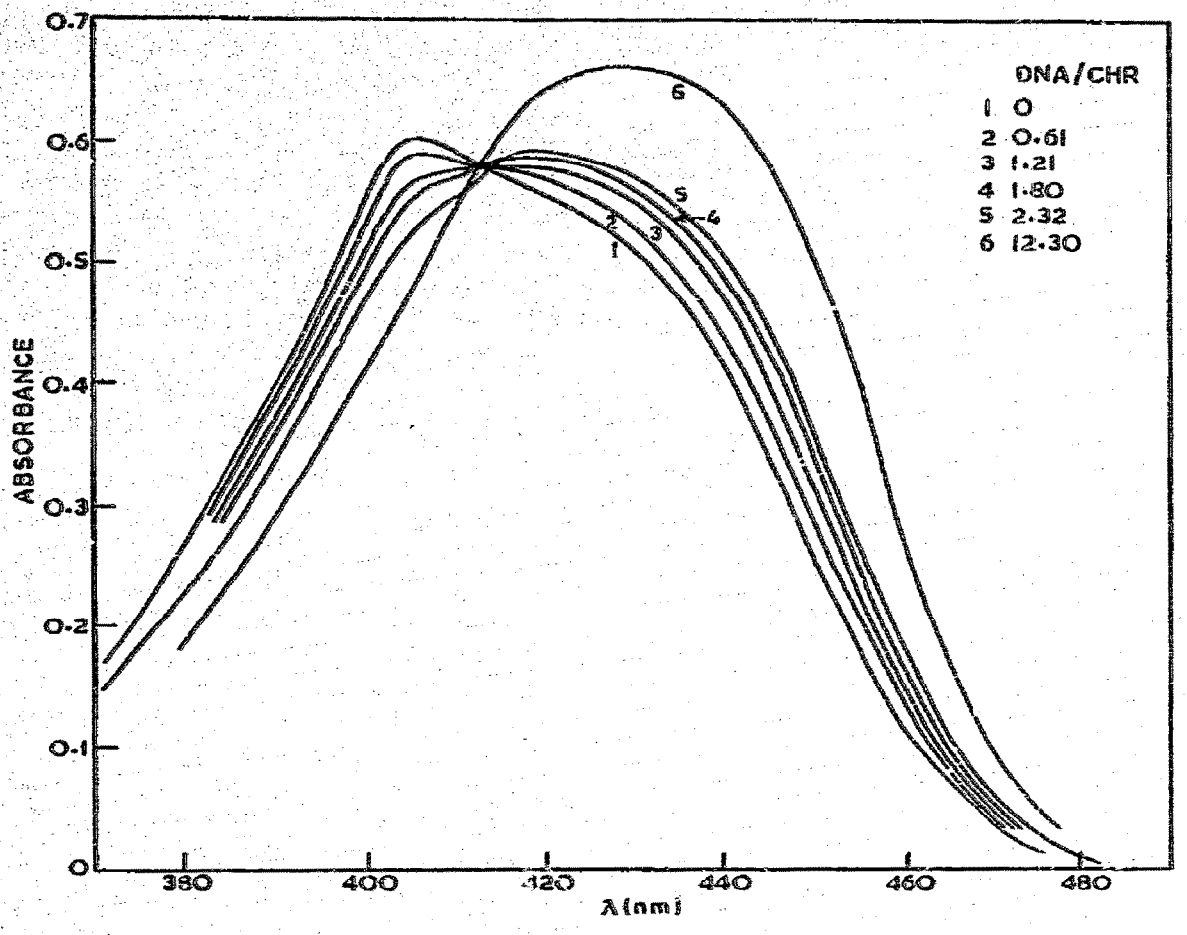


Fig. 5. Spectrophotometric titration of CHR (6.9×10^{-5} M) in 0.028 M Tris-HCl buffer + 0.098 M $MgCl_2$, pH 8 with calf thymus DNA; numbers indicate different DNA/CHR ratios.

$$\alpha = \frac{\epsilon_\alpha - \epsilon_m}{\epsilon_\alpha - \epsilon_0} \qquad (1)$$

where ϵ_m is the measured extinction coefficient of a CHR–DNA mixture. ϵ_0 and ϵ_α are those of free and bound CHR. In these calculations ϵ_α corresponds to ϵ_m at ratio CHR/DNA → 0 and is obtained by extrapolation. The binding data is further analyzed according to Scatchard relationship [9]:

$$r/m = K_{app}(n-r) \qquad (2)$$

where r = the concentration of CHR bound per phosphate, n is the number of binding site per phosphate/nucleotide, K_{app} the association constant, and m = the concentration of free CHR. A typical Scatchard plot is shown in fig. 6. The calculated apparent binding constant K_{app} M^{-1} and number of sites per nucleotide (n) from the slope and intercept of these plots are given in the table 1. The K_{app} thus obtained agree fairly well with those reported in the literature [3] but the value of n differs considerably (see table). The fact that the presence of Mg^{2+} increases not only the number of binding site per nucleotide but facilitates equilibration suggests that Mg^{2+} is required for the binding to counteract the electrostatic repulsions between the negatively charged phosphate group of DNA and anionic CHR at pH 8. If this be the case, one would not expect any interaction between heparin and CHR, both being anionic under this condition. Indeed no spectral change was observed when CHR (9.5×10^{-5} M) and heparin (1.27×10^{-4} M) in 0.05 M Tris-HCl buffer, pH = 8 were mixed together. But in presence of Mg^{2+} (1.9×10^{-3} M) a lowering in

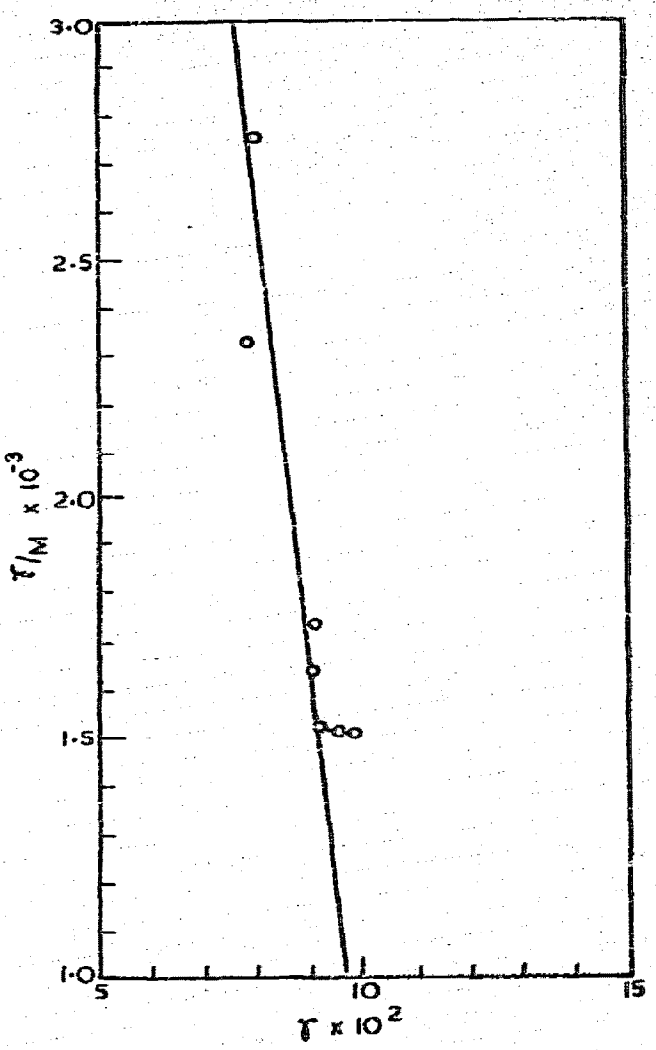


Fig. 6. Plot of r/m vs r for the binding of CHR to MFS DNA in 0.01 M Tris-HCl buffer + 23.0×10^{-5} M $MgCl_2$ pH 8 at 29°.

absorbance above 405 nm was observed without any red shift in the absorbance maximum [10]. This observation agrees well with what one would expect if there is no nearest neighbour interaction between bound CHR. These data together with those in table 1 can be taken to indicate that Mg^{2+} ions are involved directly in the interaction of anionic CHR with DNA. The observed spectral changes shown in figs. 4 and 5 are similar to those found for DNA–proflavin, DNA–actinomycin and DNA–ethidium bromide complexes for which intercalation model is favoured [11–13]. The competition experiments [10] in which ethidium bromide (EB) is gradually added to mixtures of mithramycin and DNA and of CHR and DNA show that the addition of small amount of EB can cause replacement of mithramycin and CHR bound to DNA. This indicates definitely that the binding of EB affects the binding of CHR/mithramycin to DNA, even though the mode of binding may differ in each case as indicated by ultracentrifugation studies [5]. Further studies to decide this point are in progress.

Acknowledgement

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