RajBhandary, U. L., Chang, S. H., Stuart, A., Faulkner, R. D., Hoskinson, R. M., and Khorana, H. G. (1967), *Proc. Natl. Acad. Sci. U. S. 57*, 751.
Richards, E. G., Coll, J. A., and Gratzer, W. B. (1965), *Anal. Biochem. 12*, 452.
Robinson, H. K., and Wade, H. E. (1968), *Biochem. J. 106*, 897.
Sarin, P. S., and Zamecnik, P. C. (1964), *Biochim. Biophys. Acta 91*, 653.
Sarin, P. S., and Zamecnik, P. C. (1965), *Biochim. Biophys. Res. Commun. 20*, 400.
Schleich, T., and Goldstein, J. (1966), *J. Mol. Biol. 15*, 136.
Spahr, P. F., and Tissières, A. (1959), *J. Mol. Biol. 1*, 237.
Takemura, S., Mizutani, T., and Miyazuki, M. (1968), *J. Biochem. (Tokyo) 63*, 277.
Zachau, H. G., Dütting, D., and Felman, H. (1966), *Angew. Chem. 78*, 392.

Chromomycin A_3 Studies in Aqueous Solutions. Spectrophotometric Evidence for Aggregation and Interaction with Herring Sperm Deoxyribonucleic Acid*

Terumi Hayasaka and Yasuo Inoue

ABSTRACT: Chromomycin A_3 is an antibiotic with antitumor activity and is known to exhibit an inhibitory effect on the deoxyribonucleic acid dependent ribonucleic acid and deoxyribonucleic acid synthesis. The effects of environmental factors on the absorption spectrum and optical rotatory dispersion of the antibiotic were examined, and chromomycin A_3 molecules were found to form aggregates of various sizes. Concentration and magnesium ion were found to cause characteristic changes in the absorption spectrum due to the shifts of the equilibrium positions of various molecular forms present in aqueous solutions. The aglycone, chromomycinone, was studied in the same way and found not to exhibit any aggregative properties on spectrophotometric observations. The mode of interaction of chromomycin A_3 with herring sperm deoxyribonucleic acid in the presence of magnesium ion was studied with the aid of spectrophotometric techniques. The data suggest that in agreement with the aggregative tendency of chromomycin A_3 found in this study, the antibiotic molecules bind deoxyribonucleic acid to form an aggregative state through the bridging with magnesium ion. In this state chromomycin A_3 molecules are believed to be preferentially bound to a site adjacent to those already occupied in an antibiotic–deoxyribonucleic acid complex. The comparative study of the binding with deoxyribonucleic acid was made by the use of a series of the antibiotic analogs and the interacting tendency was shown to be completely parallel with their biological activity. The direct involvement of the side-chain sugar moieties in the binding to deoxyribonucleic acid was indicated. The interaction study was also extended to transfer ribonucleic acid, mononucleotides, amino acids, and bovine serum albumin, and the cooperative binding suggested for deoxyribonucleic acid was found not to occur with transfer ribonucleic acid.

Chromomycin A_3 is one of the antibiotics produced by *Streptomyces griseus* No. 7 (ATCC 13273) and is known to have antitumor activity. Chromomycin A_3 has been used as a clinic medicine under the name of "Toyomycin." Miyamoto *et al.* (1967) elucidated the chemical structure of chromomycin A_3 and a number of biochemical studies have been reported in the literature (Kajiro and Kamiyama, 1965, 1967; Hartmann *et al.*, 1964; Ward *et al.*, 1965; Kamiyama and Kajiro, 1966; Koschel *et al.*, 1966; Kersten *et al.*, 1967). They showed that this antibiotic substance inhibits DNA-dependent RNA and DNA polymerase reactions when Mg^{2+} is present in the system. Studies on the mode of interaction of chromomycin A_3 with DNA have also been carried out by means of biochemical and physicochemical techniques. It has been found that addition of native or heat-denatured DNA from various sources to a solution containing chromomycin A_3 and Mg^{2+} induces changes in the electronic absorption spectrum (Behr and Hartmann, 1965; Ward *et al.*, 1965). Other physicochemical data based on hydrodynamic measurements have indicated that the binding mode of this antibiotic to DNA is quite similar to that observed for actinomycins C and D and different from those for anthracyclines (daunomycin, nogalamycin, etc.) or acridine dyes (Ward *et al.*, 1965; Kersten *et al.*, 1966). Concerning the base specificity in the interaction of chromomycin A_3 with DNA, Ward *et al.* (1965) and Kaziro and Kamiyama (1967) have demonstrated that the antibiotic requires the presence of the guanine base. From the comparative studies on the inhibition of RNA polymerase reaction in cell-free system with chromomycin A_3 and the related materials, it was shown that the presence of sugar moieties is essential for the inhibitory effect on RNA synthesis. Mithramycin (Rao *et al.*, 1962) and olivomycin (Gause *et al.*, 1964; Berlin *et al.*, 1966) are chromomycin-like antibiotics, and all of these are closely related to

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each other in their chemical structures and in biological activities.

The aggregative nature of this substance in aqueous solutions has been demonstrated by the studies of (1) concentration effects on absorption and optical rotatory dispersion spectra, (2) bindings with magnesium ion, (3) sedimentation equilibrium, and (4) gel filtration. Some of these studies were made simultaneously with the aglycone of chromomycin A_3 (chromomycinone) for the purpose of comparison. The results of these studies suggest that chromomycin A_3 could form distinguishable aggregation states, which can only be characterized as equilibrium mixtures of various molecular species. The formation of such aggregation states is unique for chromomycin A_3 and could not be observed for chromomycinone, indicating that two sugar side chains are in part responsible for the aggregation mechanism of chromomycin A_3.

We will present further information concerning the mode of interaction of chromomycin A_3 with DNA which has been obtained from the spectrophotometric measurements.

Experimental Section

Materials. Chromomycin A_3 and the related compounds were kindly provided from Takeda Chemical Industries, Ltd., Osaka. Most of the mononucleotides used in this study were also supplied through the courtesy of Dr. M. Honjo, Takeda Chemical Ind. The sodium salt of highly polymerized herring sperm DNA was kindly given by Dr. S. Inoue, the University of Tokyo. Yeast tRNA was prepared from baker's yeast according to Holley's (1963) method. Other chemicals are of analytical grade and water was glass distilled. Chromomycin A_3 solutions were made up freshly just before every use. All aqueous solutions used in this study contain approximately 0.4% dioxane.

Preparation of Single-Stranded DNA. A solution of DNA in 0.01 M Tris buffer at pH 7.0 was heated in a boiling-water bath for 20 min and then rapidly cooled in ice. The sample was brought to room temperature just before the binding experiments.

Buffer Solutions. Since the spectrum of the antibiotic has been noticed to be affected by buffer, Tris-Cl buffer was used at low concentration (0.008 M final concentration) but high enough to maintain the constancy of pH.

Binding Experiments. A stock solution of DNA was prepared in Tris-Cl buffer containing a desired amount of NaCl, and the solution was adjusted to a desired DNA phosphorus concentration as determined at 260 mμ (ϵ(p) = 6300). A stock solution of chromomycin A_3 was also prepared by dissolving approximately 22 mg of the sample in a minimum amount of dioxane, usually 0.9–1.0 ml, and made up to 50 ml with Tris-Cl buffer containing $MgCl_2$ and sodium chloride. This solution was combined with a DNA solution and further diluted to give final concentrations of approximately 4.5×10^{-5} M in the antibiotic, 6×10^{-4} M in a DNA phosphorus, 0.008 M in NaCl, and 0.001 M in Mg^{2+} for the measurements of difference spectra. For other measurements concentrations of all the components varied slightly from the above values as specified in the text. The solution of chromomycin A_3 was prepared just before use, since this has been demonstrated to be considerably labile to light. All of the binding experiments were carried out at room temperature (about 20°) and at pH 7.0, where chromomycin A_3 exists as more than half-ionized form.

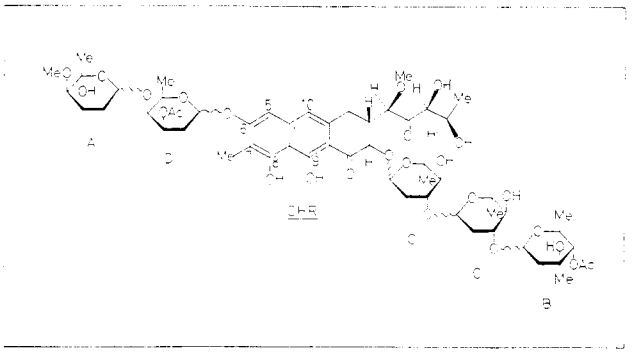

FIGURE 1: Structure of chromomycin A_3. CHR, A, B, C, and D denote chromomycinone, D-chromose A, L-chromose B, D-chromose C, and D-chromose D, respectively.

Instrumentations. Light absorption spectra were measured on a Hitachi recording spectrophotometer Model EPS-3 or a Hitachi-Perkin-Elmer spectrophotometer 139 with 0.2-, 1.0-, 2.0-, 5.0-, and 10.0-cm quartz cells. The pH of the solutions was measured with a pH meter Model TTT1b (Radiometer, Copenhagen) which was calibrated with the 0.05 M sodium borate and 0.05 M potassium hydrogen phthalate solutions. The optical rotatory dispersion measurements were made on a Jasco spectropolarimeter Model ORD/UV-5. To ascertain the reproducibility, measurements were repeated at least twice for each sample. Base lines were recorded just before and after each measurement. The optical rotatory dispersion data thus obtained were reproducible within experimental errors of ±0.002°. Rotations were expressed in molar rotation based on the molecular weight of chromomycin A_3.

Sedimentation Equilibrium. Schlieren optical photographs were recorded for the following two solutions, A and B: solution A, 1.34 mg of chromomycin A_3 was dissolved in 0.05 ml of dioxane to which 1 ml of 0.02 M Tris-Cl (pH 7.0) was added; solution B, 1.245 mg of chromomycin A_3 was dissolved in 0.05 ml of dioxane, and 1 ml of 0.02 M Tris-Cl (pH 7.0) containing 0.002 M $MgCl_2$ was added to make up the ratio of $[Mg^{2+}]/[\text{chromomycin } A_3] = 2:1$. In the present method molecular weight, M, is defined by

$$M = \frac{1}{\bar{r}c_0}\left(\frac{dc}{dr}\right)_{r=\bar{r}} \frac{RT}{\omega^2(1 - \bar{v}\rho)} \qquad (1)$$

where $\bar{r}$, c_0, ω, R, and T are the radial distance to band center of the Gaussian distribution, the concentration of associated chromomycin A_3 at the band center, angular velocity, the gas constant, and temperature. The quantities $(dc/dr)_{r=\bar{r}}$ and $\bar{v}$ are the density gradient at the band center and the partial specific volume of the solvated species, respectively.

Results

Concentration Effects. The absorption spectrum of chromomycin A_3 in aqueous solutions was found to vary with the concentration, indicating that this antibiotic undergoes self-association to form a dimeric or further polymerized molecular species. This phenomenon was not observed for chromomycinone. In Figure 2 the spectra recorded at three different concentrations at pH 7.0 are reproduced. Curves 1–3 represent

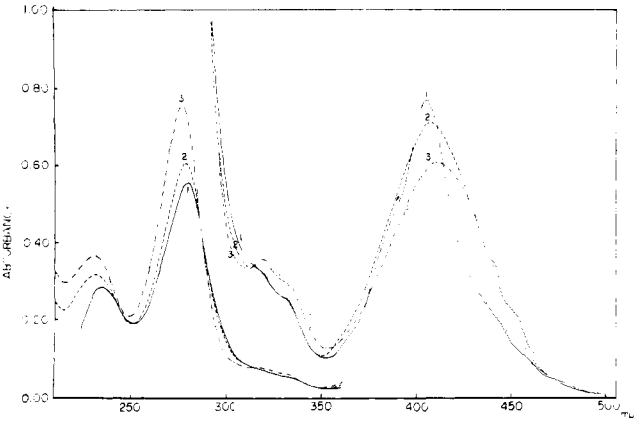

FIGURE 2: Ultraviolet and visible spectra of chromomycin A_3 at different concentrations. All spectra were measured at pH 7.00. (1) 1.50×10^{-5}, (2) 7.47×10^{-6}, and (3) 3.79×10^{-4} M (in visible region). (1) 3.75×10^{-6}, (2) 1.87×10^{-5}, and (3) 9.48×10^{-5} M (in ultraviolet region).

equilibrium mixtures of monomer and aggregates of various sizes at different equilibrium positions. The sharp, intense band at approximately 280 mμ seems to be further enhanced in its intensity on aggregation and this band is named the S band.

Magnesium Ion Effects. Earlier studies by Behr and Hartmann (1965) and Ward *et al.* (1965) showed that the spectrum of chromomycin A_3 was altered by Mg^{2+} and in general by other divalent cations such as Ca^{2+}, Cu^{2+}, Mn^{2+}, Co^{2+}, and Zn^{2+} but not by univalent cations. Furthermore it is known that Mg^{2+} is required for the interaction with DNA. Therefore, with a view to obtaining further information on the action of magnesium ion on the antibiotic solution, which is also considered to be useful to a better understanding of the interaction mode of chromomycin A_3 with DNA in the presence of Mg^{2+}, we have chosen $MgCl_2$ to study its action in aqueous solutions of chromomycin A_3. Figure 3 shows the effect of a stepwise addition of $MgCl_2$ on the absorption of chromomycin A_3 at pH 7.0. Figure 3a shows that at the antibiotic concentration of 7.5×10^{-5} M the absorbancy of the longest wavelength band decreased with the increase of Mg^{2+} concentration until it reached approximately an equimolar concentration. When the concentration of Mg^{2+} became further greater, the absorbancy increased with the Mg^{2+} concentration as shown in Figure 3b, and at the same time the absorption maximum was shifted from 410 to 422 mμ. These spectral changes therefore arise from the 1:1 coordination complex formation followed by the shifts of the equilibrium positions between monomer and aggregates of various forms. The state denoted by curve 5 in Figure 3b represents an extreme molecular state since the spectrum is no longer shifted when the Mg^{2+} concentration is further increased. Spectral behavior shown in Figure 3 indicates that there must be a "transient" state which may be approximated by curve 4 in Figure 3a, although the state characterized by curve 4 is certainly composite in molecular species. Intensity change on the addition of Mg^{2+} is more clearly seen from the relative absorbancy at 410 mμ *vs.* logarithm of the Mg^{2+} concentration profile (Figure 4).

The corresponding effects of Mg^{2+} on the absorption of *chromomycinone* could not be observed; instead a rather small change was found and this can be understood simply by the formation of a coordination complex without any further molecular transformations.

Sedimentation Equilibrium. Determination of the partial specific volume of chromomycin A_3 was made for the two solutions, A and B, and found to be 0.77 and 0.82 ± 0.02, respectively. The average molecular weights determined for the chromomycin A_3 aggregates present in solutions A and B are 5500 ± 440 and 6800 ± 830 (*cf.* molecular weight of chromomycin A_3 for $C_{57}H_{82}O_{26}$ is 1182).

Optical Rotatory Dispersion Spectra. Since the side chains of chromomycin A_3 are optically active, it is expected that the optical rotatory dispersion of this substance should depend upon the state in which the antibiotic molecules exist. Chromomycin A_3 exhibits anomalous optical rotatory dispersion with multiple Cotton effects. The Cotton effects associated with the longest wavelength absorptions. Chromomycinone shows also anomalous optical rotatory dispersion spectrum but the rotational strength is far less than that for the parent compound. Figure 5a shows the concentration effects on visible optical rotatory dispersion of chromomycin A_3 at pH 7.0. In accordance with the previously observed effects on the absorption spectrum, the position of the longest wavelength composite Cotton effect is shifted to a longer wavelength with the increase of concentration. Although it is impossible at the present stage to see how the arrangement of the chromomycin A_3 aggregates could affect the magnitude of the Cotton effects, the observed increase in the magnitude on the increase of concentration may be understood as a general consequence of the molecular association of chromomycin A_3 in a stacked configuration.

The magnesium ion effects on the optical rotatory dispersion of chromomycin A_3 are shown in Figure 5b. The addition of Mg^{2+} at a lower level resulted in a slight increase in the rotatory power of the longest wavelength Cotton effects whereas further addition of Mg^{2+} to the solution caused a marked decrease of the Cotton effects, indicating that molecular arrangements of the species constructing the transient coordination complex seem to have an ordered configuration, but molecular constitutions formed at higher levels of Mg^{2+} are probably formed by aggregation in a random fashion. In randomly arrayed aggregates, the dissymmetric fields arising from the side chains of chromomycin A_3 molecules are presumably arranged in such a way as to counteract the strengthening of the rotatory power.

Interaction of Chromomycin A_3 with DNA and Its Component Monomers. The stepwise addition of native DNA to a solution of chromomycin A_3 at pH 7 caused a spectral shift of the antibiotic. This change occurred only when divalent cations were present. The requirement of Mg^{2+} for the inhibitory action of chromomycin A_3 in the RNA synthesis and for the spectral shift is the most distinct property of this antibiotic. With a depression of the absorption maximum at 409 mμ a new broad peak appeared at longer wavelengths and this spectral shift reached a limit where the spectrum no longer showed any change, that is, the antibiotic is presumably bound completely to DNA molecules. The effect of heat-denatured DNA on the absorption spectrum of the antibiotic is shown in Figure 6. Although heat-denatured DNA is less effective than native DNA in producing the spectral shift as demonstrated previously by Ward *et al.* (1965), the general shape of the difference spectrum is similar to that produced on complexing with native DNA, and completely different from those obtained for tRNA and other macromolecules.

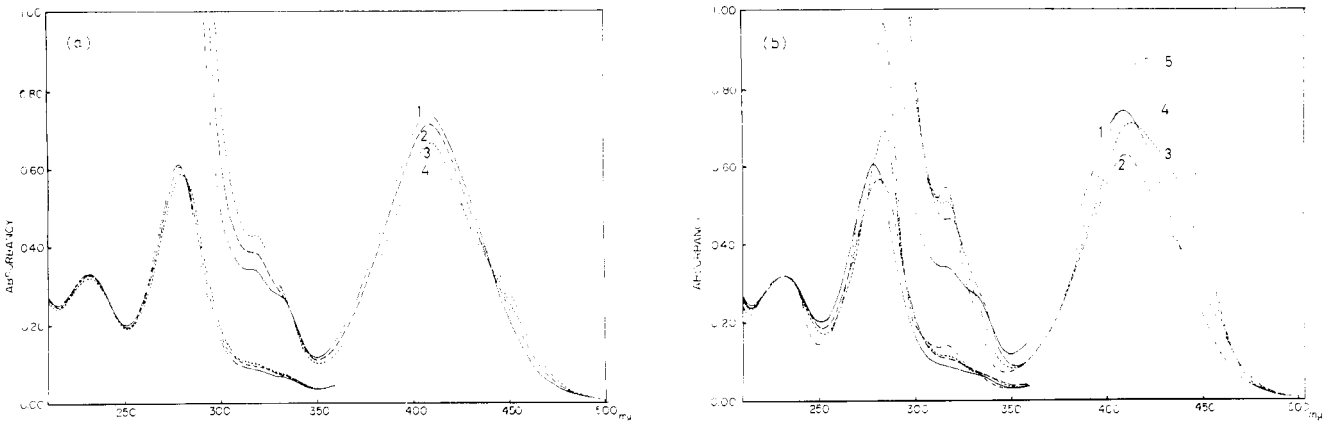

FIGURE 3: Changes induced on the visible (7.50×10^{-5} M) and ultraviolet (1.88×10^{-5} M) spectra of chromomycin A_3 at pH 7.00 by Mg^{2+}: (a) curve 1, 0.0 M $MgCl_2$; curve 2, 1.00×10^{-5} M; curve 3, 2.00×10^{-5} M; curve 4, 7.50×10^{-5} M; (b) curve 1, 0.00 M $MgCl_2$; curve 2, 7.50×10^{-5} M; curve 3, 1.80×10^{-3} M; curve 4, 6.00×10^{-3} M; curve 5, 5.00×10^{-2} M.

From analogy with other study of the determination of the base specificity in the interaction of various antibiotics and metabolites with polynucleotides (Reich, 1964; Clifford and Rees, 1966), it is interesting to see the interaction of chromomycin A_3 with various components of DNA. Addition of deoxyribomononucleotides was found to cause alteration in the spectrum of the antibiotic, although Ward *et al.* (1965) reported that no changes were produced on addition of nucleosides or mononucleotides. In Figure 6b the difference spectra produced on mixing with deoxyribonucleotides are recorded. If a particular base residue is important and the binding mode is similar in a monomer and polymer level, it would be expected that the particular base residue may be deoxyriboguanylic acid. This was not found to occur and the shape of the difference spectrum for 5′-dGMP is completely different from that produced with DNA, but is quite similar to that obtained for 5′-dAMP. This is in contrast with actinomycin (Reich, 1964; Kersten, 1961). Therefore, the fact that the maximum binding capacity observed with the G/C-rich DNA by Ward *et al.* (1965) and by Kaziro and Kamiyama (1967) should be understood as the specificity of chromomcycin A_3 for a special base sequence of DNA molecules rather than simply interpreted as a guanine specificity.

Interaction of Chromomycin A_3 Analogs with DNA. Chromomycin A_3, as shown in Figure 1, consists of an aglycone and five sugars, namely, chromose A, B, C, and D, linked by the glycosidic linkages. Chromomycin A_3 analogs used in this study are those denoted as A-D-*chromomycinone*-C-C-B (chromomycin A_3), D-*chromomycinone*-C-C-B, A-D-*chromomycinone*-C-C (chromomycin A_4), D-*chromomycinone*-C-C, D-*chromomycinone*, and *chromomycinone*. Because of the limited availability of these analogs, we have determined only the difference spectra obtained on mixing with DNA at the

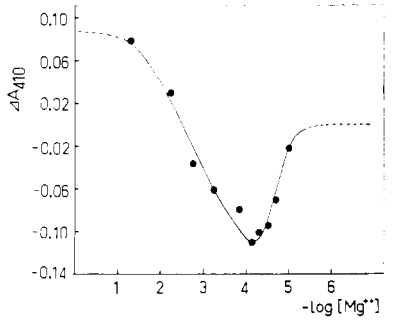

FIGURE 4: Plot of the relative absorbancy changes at 410 mμ against $-\log [Mg^{2+}]$. Chromomycin A_3 concentration was 7.50×10^{-5} M.

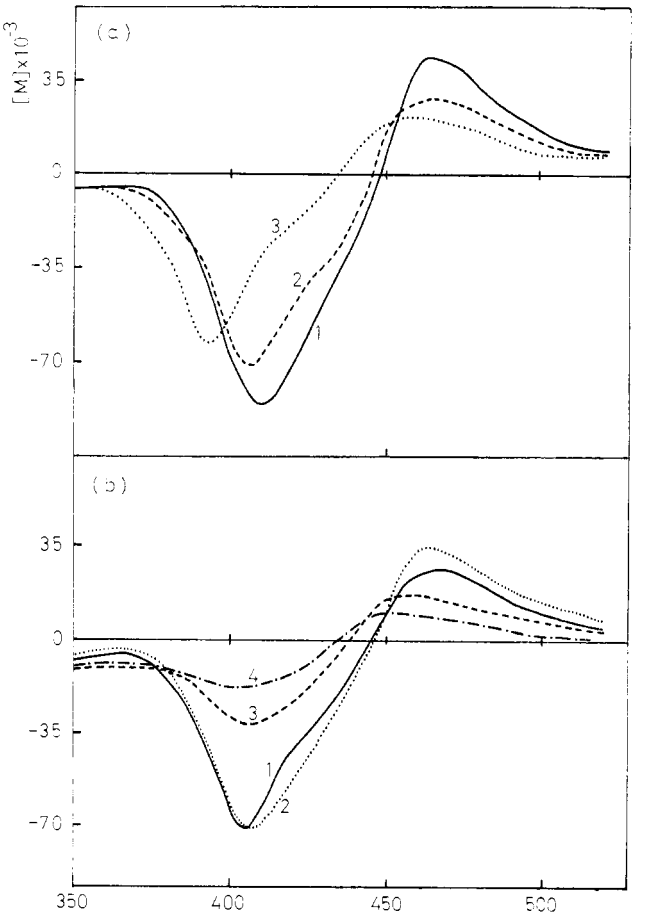

FIGURE 5: Concentration studies. (a) Concentration effect on the visible optical rotatory dispersion of chromomycin A_3 at pH 7.00: curve 1, at 4.25×10^{-4} M; curve 2, at 8.5×10^{-5} M; curve 3, at 1.7×10^{-5} M. (b) Effect of Mg^{2+} on the visible optical rotatory dispersion of chromomycin A_3 (8.50×10^{-5} M) at pH 7.0: curve 1, $MgCl_2$ none; curve 2, 8.0×10^{-5} M; curve 3, 2.0×10^{-3} M; curve 4, 5.0×10^{-2} M.

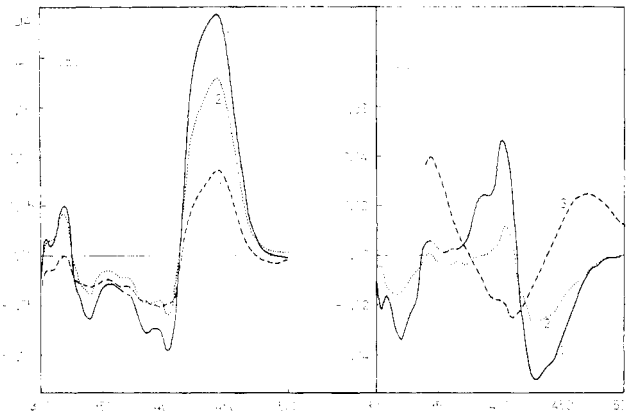

FIGURE 6: Difference spectra. (a) Of chromomycin A_3 (4.459×10^{-5} M in 0.008 M Tris-Cl buffer, pH 7.0, containing 8×10^{-5} M $MgCl_2$ and 10^{-3} M NaCl) with DNA (6.18×10^{-4} M (p)): curve 1, chromomycin A_3–native DNA; curve 2, chromomycin A_3–heat-denatured DNA; curve 3, the chromomycin A_3–native DNA complex was heated. (b) Of chromomycin A_3 with deoxyribonucleotides at the ratio of [phosphorus]/[antibiotic] = 20:1. curve 1, 5′-dAMP; curve 2, 5′-dGMP; curve 3, 5′-dCMP.

[phosphorus]/[chromomycin analog] ratio of 13.2 in the presence of 1.5×10^{-4} M of Mg^{2+}. The results are shown in Figure 7.

Binding of Chromomycin A_3 to Other Polymers and Their Component Monomers. Chromomycin A_3 was found to bind tRNA and even bovine serum albumin to produce spectral shifts in the presence of Mg^{2+}. However, the spectral changes induced by these polymers are much smaller and the shape of the difference spectra are not the same as those resulting from the complex formation with DNA. The results are shown in Figure 8a. The complexing of chromomycin A_3 with DNA exhibits the residual spectrum characterized by a positive intense peak at around 442 mμ and negative bands in the wavelength range of 325–412 mμ, while the difference spectrum ob-

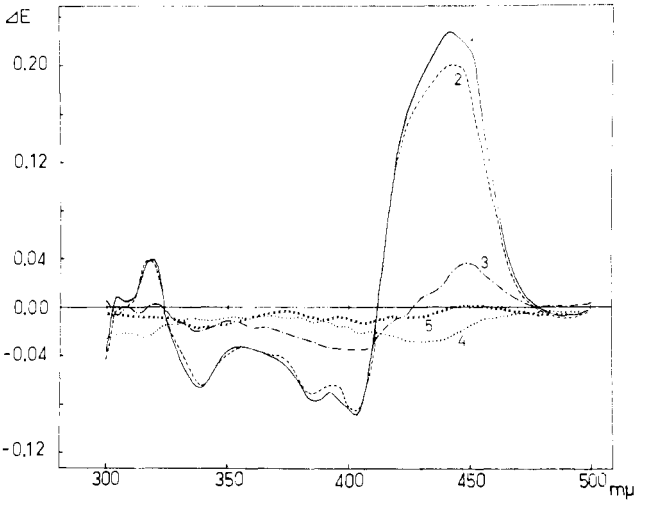

FIGURE 7: Difference spectra of chromomycin A_3 (4.0×10^{-5} M) and its analogs (4.0×10^{-5} M) with native DNA (5.27×10^{-3} M (p)) in 0.01 M Tris-Cl buffer containing 1.5×10^{-4} M $MgCl_2$ and 1.5×10^{-4} M NaCl: curve 1, chromomycin A_3; curve 2, D-chromomycinone-C-C-B; curve 3, A-D-chromomycinone-C-C; curve 4, D-chromomycinone-C-C; curve 5, D-chromomycinone.

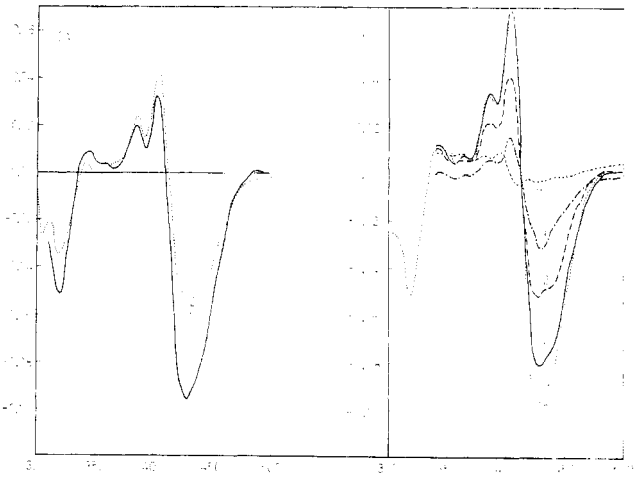

FIGURE 8: Induced changes. (a) On the absorption of chromomycin A_3 (4.2×10^{-5} M) by tRNA (8.4×10^{-4} M (p), curve 1) and bovine serum albumin (26.25 mg/10 ml, curve 2) in the presence of 10^{-4} $MgCl_2$ and 10^{-3} M NaCl in 0.01 M Tris-Cl, pH 7.0. (b) On the visible spectrum of chromomycin A_3 solution (4.3×10^{-5} M, 0.01 M Tris-Cl, pH 7.0) containing 10^{-4} M $MgCl_2$ and 10^{-3} M NaCl by: curve 1, 5′-GMP (8.62×10^{-4} M); curve 2, 5′-UMP (8.62×10^{-4} M); curve 3, 5′-CMP (8.62×10^{-4} M); curve 4, 5-AMP (8.62×10^{-4} M); curve 5, tyrosine (8.6×10^{-4} M).

tained on mixing the antibiotic with tRNA has almost a reverse shape. It is of interest to note that, as judged from the shape of the difference spectra in Figure 8a, tRNA appears to bind chromomycin A_3 in a similar manner as does bovine serum albumin. When ribonucleotides and ribonucleosides were added to chromomycin A_3 solution at the level of [phosphorus]/[chromomycin A_3] = 20, the largest difference in absorption was observed for 5′-GMP and the next for 5′-UMP, probably indicating that chromomycin A_3 reacts preferentially with these nucleotide residues in tRNA. The requirement of properly spaced anionic site seems to be important, since only tyrosine produced a large difference spectrum and other amino acids tested showed no appreciable affinity toward the antibiotic.

Discussion

The optical properties of chromomycin A_3 in aqueous solutions were studied, and a possible mode of association of chromomycin A_3 at pH 7.0 in the presence and absence of Mg^{2+} is considered. At low concentrations of chromomycin A_3, the antibiotic molecules could exist in a dispersed form. However, in general, chromomycin A_3 in aqueous solutions exists as an equilibrium mixture of monomer and aggregates of various sizes. The sedimentation equilibrium by ultracentrifugation showed that the average molecular weight of the aggregates is, under the conditions used, 5500 ± 440 which is about 4.7 times larger than that of the monomeric form. We have found that the intensity of a peak at around 280 mμ is sensitive to a degree of aggregation. By addition of increasing levels of Mg^{2+} we found at first a decrease in the absorption at 409 mμ to a minimum at [Mg^{2+}]/[chromomycin A_3] = 1, followed by a marked increase with further addition of Mg^{2+}. This initial change appears to be associated with the formation of coordination complex(es) without much change in polymerization. This was supported by the experimental data based on

sedimentation equilibrium in the presence of Mg^{2+} at the level of $[Mg^{2+}]/[\text{chromomycin } A_3] = 2:1$. Sedimentation equilibrium experiments suggest that, at approximately 1×10^{-3} M of the antibiotic concentration, the average molecular weight is 6800 ± 830, which corresponds to 5.7 ± 0.7 mer of the free chromomycin A_3 molecule. At pH 7.0 the transiently formed 1:1 antibiotic–Mg^{2+} complex is cationic in nature, so that the presence of excess $MgCl_2$ should favor further aggregation from analogy with similar effects observed for cationic dyes (Kay *et al.*, 1964; Blauer, 1965).

When DNA is added to a solution of chromomycin A_3 in the presence of Mg^{2+}, a DNA–Mg^{2+}–chromomycin A_3 complex is formed. The similarly shaped difference spectra were obtained on complexing with both native and heat-denatured DNA. However, changes induced by deoxyribomononucleotides, tRNA, and ribonucleotides at a higher level on the chromomycin A_3 spectrum were found to differ from these difference spectra in their shapes and magnitudes. The complexing of chromomycin A_3 with tRNA proceeds in a similar manner as is observed for monomeric components and even with bovine serum albumin and certain amino acids, suggesting that the complexing process onto tRNA appears to proceed through depolymerization of the transient complex state; chromomycin A_3 molecule binds to an empty site (preferentially guanylyl residue ?) in a random fashion. From analogy with the formation of the polymerized 1:1 coordination complex in the presence of excess Mg^{2+}, the similar change in the absorption spectrum of chromomycin A_3 produced on addition of DNA can be explained by assuming the further polymerization of chromomycin A_3 molecules on the DNA surface. In other words the fact that the [phosphorus]/[chromomycin A_3] ratio required for the maximum change in the absorption of the antibiotic was much larger than unity does not necessarily mean that the chromomycin A_3–DNA complex is formed by a random distribution of the antibiotic on the DNA surface, but it indicates, when combined with other observations, that only particular segments (probably guanine-rich parts) could react preferentially with chromomycin A_3, and chromomycin A_3 molecules are bound at sites adjacent to those already occupied in a ligand–DNA complex. Therefore, the antibiotic–DNA complex is characterized by relatively close spacing of the antibiotic molecules on DNA. As for the linkages of chromomycin A_3 and DNA through the *bridging* of Mg^{2+}, chromomycin A_3 is unique and should be differentiated from other microbial products such as anthracyclines, actinomycins, and chloramphenicol, and cationic dyes such as acridine orange and carbocyanine dyes.

The amount of Mg^{2+} required to promote maximum interaction between DNA and antibiotic has been found to be a 1:1 molar equivalence with chromomycin A_3 (Ward *et al.*, 1965), indicating that the transiently formed 1:1 antibiotic–Mg^{2+} complex is bound to DNA. Comparative studies on the inhibitory effect on the DNA-dependent RNA polymerase with chromomycin A_3 and its analogs have proved that *chromomycinone* is completely inactive and the order of the inhibitory action decreases with the following sequence: A-D-*chromomycinone*-C-C-B > D-*chromomycinone*-C-C-B > A-D-*chromomycinone*-C-C > D-*chromomycinone*-C-C > D-*chromomycinone* (Koschel *et al.*, 1966; Kaziro and Kamiyama, 1967). The interaction tendency of these analogs with DNA as measured by the difference spectra is in good agreement with the above biological data, indicating that the binding of chromomycin A_3 with DNA is indeed responsible for the inhibitory action on the RNA synthesis. The failure to ascertain the base specificity in the present study using various mononucleotides, when combined with the suggested mode of binding in the chromomycin A_3–DNA complex, may be taken as supporting evidence for the suggestion by Kersten *et al.* (1966), that the specificity of this antibiotic is not simply such as estimated from the interaction at monomeric levels, but is more likely to be base-sequence specific. If it is so, chromomycin A_3 may serve as a possible probe for the correlation of the structure and function of DNA.

References

Behr, W., and Hartmann, G. (1965), *Biochem. Z. 343*, 519.

Berlin, Yu. A., Esipov, S. E., Kolosov, M. N., and Shemyakin, M. M. (1966), *Tetrahedron Letters*, 1643.

Blauer, G. (1965), *J. Phys. Chem. 69*, 89.

Clifford, J. I., and Rees, K. R. (1966), *Biochem. J. 103*, 467.

Gause, G. F., Uklolina, R. S., and Sveshnikova, M. A. (1964), *Antibiotiki 7 (3)*, 34.

Hartmann, G., Goller, H., Koschel, K., Kersten, W., and Kersten, H. (1964), *Biochem. Z. 241*, 126.

Holley, R. W. (1963), *Biochem. Biophys. Res. Commun. 10*, 186.

Kamiyama, M., and Kaziro, Y. (1966), *J. Biochem. (Tokyo) 59*, 49.

Kay, R. E., Walwick, E. R., and Gifford, C. K. (1964), *J. Phys. Chem. 68*, 1896.

Kaziro, Y., and Kamiyama, M. (1965), *Biochem. Biophys. Res. Commun. 19*, 433.

Kaziro, Y., and Kamiyama, M. (1967), *J. Biochem. (Tokyo) 62*, 424.

Kersten, W. (1961), *Biochim. Biophys. Acta 47*, 611

Kersten, W., Kersten, H., Steiner, F. E., and Emmerich, B. (1967), *Z. Physiol. Chem. 348*, 1415.

Kersten, W., Kersten, H., and Szybalski, W. (1966), *Biochemistry 5*, 236.

Koschel, K., Hartmann, G., Kersten, W., and Kersten, H. (1966), *European J. Biochem. 344*, 76.

Miyamoto, M., Kawamatsu, Y., Kawashima, K., Shinohara, M., Tanaka, K., Tatsuoka, S., and Nakanishi, K. (1967), *Tetrahedron 23*, 421.

Rao, K. V., Cullen, W. P., and Sobin, B. A. (1962), *Antibiot. Chemotherapy 12*, 182.

Reich, E. (1964), *Science 143*, 684.

Ward, D. C., Reich, E., and Goldberg, I. H. (1965), *Science 149*, 1259.