THE ABSORPTION SPECTRUM OF ACTINOMYCIN D. EVIDENCE FOR THREE TRANSITIONS IN THE VISIBLE BAND

Henry E. AUER

Department of Biochemistry and Department of Radiation, Biology and Biophysics, University of Rochester School of Medicine, Rochester, New York, 14642

and

Barbara E. PAWLOWSKI-KONOPNICKI and Thomas R. KRUGH

Department of Chemistry, University of Rochester Rochester, New York, 14627, USA

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1. Introduction

The actinomycins, a class of chromophoric depsipeptides possessing antibiotic and antitumor activity, exert their effect by forming an intercalated complex with DNA, thereby inhibiting its transcription to RNA. In order to gain a more thorough understanding of the interaction between actinomycin and DNA, Krugh and his co-workers have examined simple model systems comprised of actinomycin D (AMD) and selected mono- and dideoxynucleotides, primarily by absorption and NMR spectroscopies [1–4]. We are investigating these same systems using circular dichroism (CD) spectroscopy. This technique should prove of great value in this work due to its inherent sensitivity to the composition and conformation of optically active substances.

Before embarking on this program, however, the optical activity of actinomycin itself must be thoroughly characterized. Previous studies have revealed the excistence of several intense Cotton effects in the ultraviolet region of the spectrum which are considerably more intense in organic solvents than in water [5–9]. The optical activity in the range of the intense visible absorption band, centered at about 440 nm, is less clearly understood. In nonaqueous solvents, weak ellipticity is found above 435 nm, tailing off to zero at about 550 nm. In aqueous buffer, weak negative ellipticity with some evidence for concentration dependence has been reported in this region [5–8].

Whereas actinomycin C_3 is monomeric even at high concentrations in organic solvents [10], dimerization occurs with both actinomycin C_3 and AMD in aqueous buffer [5,10,11]. Crothers et al. [5] correlated changes in specific rotation as the concentration increases with progressive dimer formation. NMR studies are consistent with a dimer in which the two actinocinyl rings are stacked in inverted fashion over one another, with no evidence for intermolecular interactions between the pentapeptide rings [2,4,12]. In addition, a subtle distinction between the properties of the dimer at 6°C and 25°C has been discerned [4,12].

We have examined the CD spectra of AMD over a broad range of concentrations and at several temperatures in acueous buffer. The dimerization of AMD is apparently complicated by the formation of higher aggregates at millimolar concentrations. Accordingly, spectra of the dimer were calculated from the experimental data using known values of the dimerization constant. A temperature-dependent transformation in the structure of the dimer was found, corroborating earlier observations [4,12]. The pattern of CD bands which emerges indicates that the intense visible absorption band of the actinocinyl chromophore is comprised of at least three different electronic transitions. One is centered at about 372 nm under the

short-wave shoulder of the absorption envelope; a second occurs in the range 415–445 nm in the vicinity of the absorption maximum; a third, found at about 490 nm under the long-wave tail of the absorption spectrum, is most likely strongly associated with the quinoid ring of the chromophore.

2. Materials and methods

Actinomycin D from Merck Sharpe and Dohme, Inc. was dissolved in 5 mM potassium phosphate buffer, pH 7.0. Concentrations were determined from absorption spectra [10] recorded on a Cary 14 spectrophotometer. CD spectra were obtained with a Cary 60 spectropolarimeter equipped with the 6003 CD accessory, in fused silica jacketed cells whose path lengths ranged from 0.027–4.00 cm. The temperature of the sample solutions was measured with a YSI Telethermometer.

3. Results and discussion

The CD spectra of monomeric AMD in aqueous buffer at 6°C and 24°C are illustrated in figs.1a and 1b, respectively. It is evident that temperature exerts little effect on the optical activity of the monomer. A weak negative extremum is found at 440–450 nm, with more intense negative extrema at 371–373 nm and at 262–265 nm; there are positive extrema at 296 nm and 242–245 nm. These spectra do not differ significantly from those already published [6–8]. The spectra calculated for the dimer at these temperatures are also shown in the same figures. They are characterized in common by a loss of the 296 nm extremum and an intensification of those at 375 nm, 270 nm and 234 nm, accompanied by slight bathochromic shifts. Unique to these spectra is the appearance of new CD bands in the region of the visible absorption band. At 24°C, positive and negative extrema arise at 416 nm and 452 nm, respectively, and there is a further region of weak ellipticity above 485 nm extending to about 550 nm. At 6°C, the positive extremum at 416 nm has been lost, but a relatively intense new positive peak appears at 490 nm.

The development of these new CD bands at high concentrations of AMD is unequivocally related to the

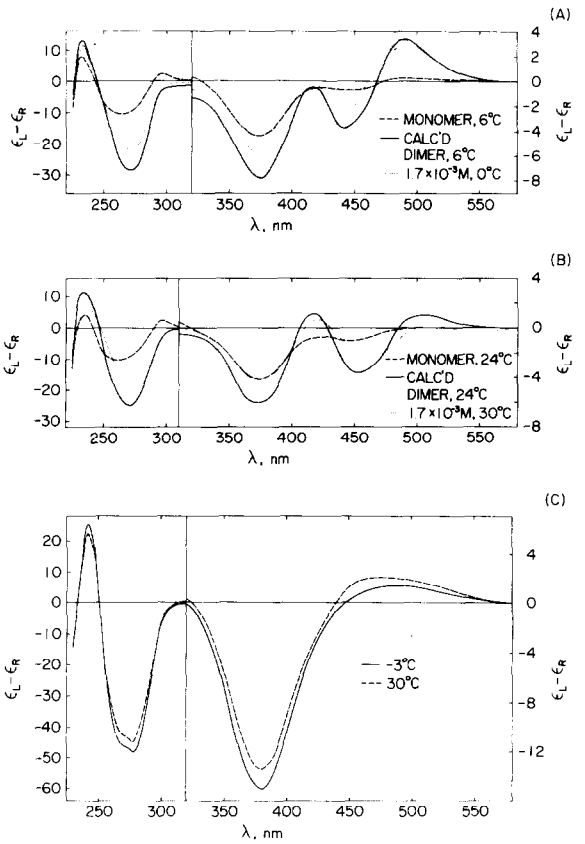
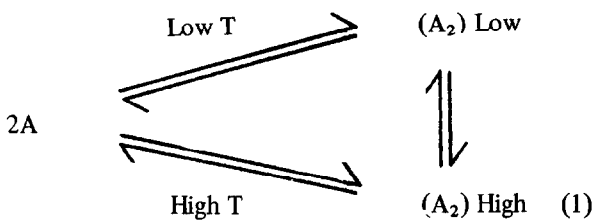


Fig.1. CD spectra of AMD. (A) Aqueous buffer at low temperature. (B) Aqueous buffer at high temperature. (- - -) Monomeric AMD, approximated by solutions of concentration approx. 2.0×10^{-5} M, for which the fraction of monomer (f_M) is 0.94 at 6°C (panel a) and 0.96 at 24°C (panel b). (——) Calculated spectra for dimeric AMD at 6°C and 24°C. Actual concentrations ranged from 1.4×10^{-4} M [f_M (6°C) = 0.74, f_M (24°C) = 0.81] to 2.0×10^{-3} M [f_M (6°C) = 0.32, f_M (24°C) = 0.39]. Values of f_M and $(\epsilon_L - \epsilon_R)_D$ were calculated using values of K_D obtained under identical conditions (ref. [14]). (. . . .) Actual spectra of 1.7×10^{-3} M AMD in aqueous buffer at 0°C (panel a) and 30°C (panel b). (C) CD spectra of 1.5×10^{-3} M AMD in chloroform at −3°C (——) and 30°C (- - -).

formation of the dimer. First, they are absent at high dilution where the monomer prevails (figs.1a and 1b). Second, at constant temperature they increase in amplitude with increasing concentration, such that the values of $(\epsilon_L - \epsilon_R)_D$ (D = dimer) calculated at various concentrations are reproducible. Finally, a concentrated solution of AMD in chloroform, in which

it is quite certain that no aggregation occurs [10], manifests CD spectra having insignificant temperature dependence between −3°C and 30°C (fig.1c) and displaying featureless weak positive ellipticity in the visible region of the absorption spectrum. This is to be contrasted with the behavior at high concentration in aqueous medium.

The difference between the calculated CD spectra at 6°C and 24°C suggests that there is an equilibrium between one structural form of the dimer which is stable at low temperature and another stable at high temperature (eq. 1).



(1)

This is consistent with recent NMR data [4,12]. Support for this view is found in a pseudo-isoelliptic point which develops at about 447 nm in solutions of AMD whose concentrations were 1.95×10^{-3} M and 7.87×10^{-4} M, for example, when the temperature is varied between 0°C and 30°C (not shown; note that the negative extremum in this region falls at about 441 nm at 6°C (fig.1a) and about 452 nm at 24°C (fig.1b). This is presumably made possible in spite of the three species specified in eq. 1 because of the relatively low magnitude of $(\epsilon_L - \epsilon_R)_M$ (M = monomer) at this wavelength (figs.1a and 1b) and because of relatively small changes in the fraction of monomer as the temperature varies.

The finite value of $(\epsilon_L - \epsilon_R)_D$ at 490 nm for 24°C suggests that the structural transformation is incomplete at this temperature. The actual CD spectrum of a solution of 1.7×10^{-3} M AMD at 30°C is shown in fig.1b. It may be seen that this band is entirely absent. Nevertheless the remaining extrema have amplitudes between those of the monomer and the calculated dimer at 24°C, as might be anticipated in a solution containing a mixture of monomers and dimers. (It must be noted that the value of K_D at 30°C is undetermined and that spectra at higher temperatures were not examined. It is thus conceivable that the limiting form has not been attained and that negative ellipticity would develop above 500 nm at yet more elevated temperatures.) Likewise the calculated spectrum of the dimer at 6°C need not represent the low-temperature limit. The actual spectrum of the same solution shown at 30°C in fig.1b is given at 0°C in fig.1a. It overlays the calculated 6°C spectrum for pure dimer above 490 nm, indicating that at 0°C the pure dimer would have an even greater positive amplitude between 460 nm and 550 nm. The remainder of the CD spectrum at 0°C falls between those of the monomer and the calculated dimer at 6°C, as expected for a mixture of the two. The same precautions concerning K_D and the attainment of a limiting form apply for this spectrum as noted above.

At concentrations greater than about 1.8×10^{-3} M, spectral amplitudes begin to decrease in intensity with no change in spectral form, in contrast to the increasing trend apparent at lower concentration. No evidence for aggregation to degrees greater than the dimer has been previously reported for AMD. Nevertheless, it is our feeling that such aggregation, conceivably involving the pentapeptide rings of stacked dimers, is responsible for the phenomenon observed. This prevents attainment of the pure dimeric state.

Three classes of optically active transition may be identified in AMD, based on their responses to changing experimental conditions. First are the CD bands below 400 nm found in chloroform solution and in aqueous medium in the monomer. These bands could arise because the actinocinyl ring itself may be dissymmetric [6,13,14]. Alternatively one-electron or dipole coupling of transitions in the actinocinyl ring with peptide transitions in the pentapeptide rings could be responsible for the bands observed [6,14].

The second class is absent in the monomer but gives rise to a negative extremum in the dimer spectrum in the vicinity of 447 nm which is not particularly sensitive to temperature. Since this band corresponds approximately with the vibrionic maximum observed in the absorption spectrum in organic solvents [6–8] this vibrionic sublevel could be responsible for the CD bands observed [15].

Finally, the third class is comprised of the CD bands which appear with the dimer (a) at 490 nm only at low temperature and (b) at 416 nm only at high temperature. The 490 nm CD band occurs at the long-wavelength tail of the 441 nm absorption band, a region of low intensity. These characteristics

suggest the participitation of a hetero-atom nonbonding orbital, from the 3-carbonyl oxygen for example. The solvent-dependent spectral shifts anticipated for an $n-\pi^*$ transition were sought and not observed. In addition, the absorption spectrum in acidic ethanol exhibits enhanced absorption in this region rather than its disappearance as expected for an $n-\pi^*$ transition [16]. Therefore an appropriate assigment for this band may rather be an intramolecular charge-transfer transition. The proposed participation of an n orbital could confer a finite magnetic transition moment upon this band. Interaction with an electric transition moment in the second actinocinyl ring, upon which it is stacked in the inverted dimer [2,4,12] could lead to the CD band observed at low temperature by the electric–magnetic mechanism [14]. According to this mechanism, the change in juxtaposition of the two chromophores upon passing from low to high temperature could plausibly account for the observed loss of CD intensity.

The 416 nm CD extremum occurs near a vibronic maximum in the absorption spectrum [6–8] just as the 447 nm band treated above. A vibronic coupling dependent on the structure of the dimer is proposed as the origin for this band. Both bands are thought to arise from the same electronic transition.

Acknowledgements

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References

[1] Krugh, T. R. (1972) Proc. Natl. Acad. Sci. 69, 1911–1914.
[2] Krugh, T. R. and Neely, J. W. (1973) Biochemistry 12, 1775–1782.
[3] Krugh, T. R. and Neely, J. W. (1973) Biochemistry 12, 4418–4425.
[4] Krugh, T. R. and Chen, Y. C. (1975) Biochemistry 14, 4912–4922.
[5] Crothers, D. M., Sabol, S. L., Ratner, D. I. and Müller, W. (1968) Biochemistry 7, 1817–1823.
[6] Ziffer, H., Yamaoka, K. and Mauser, A. B. (1968) Biochemistry 7, 996–1001.
[7] Courtois, Y., Guschlbauer, W. and Fromageot, P. (1968) Eur. J. Biochem. 6, 106–113.
[8] Homer, R. B. (1969) Arch. Biochem. Biophys. 129, 405–407.
[9] Ascoli, F., DeSantis, P. and Savino, M. (1970) Nature 227, 1237–1239.
[10] Müller, W. and Emme, I. (1965) Z. Naturforsch. B20, 835–841.
[11] Gellert, M., Smith, C. E., Neville, D. and Felsenfeld, G. (1965) J. Mol. Biol. 11, 445–457.
[12] Angerman N. S., Victor, T. A., Bell, C. L. and Danyluk, S. S. (1972) Biochemistry 11, 2402–2411.
[13] Jain, S. C. and Sobell, H. M. (1972) J. Mol. Biol. 68, 1–20.
[14] Sears, D. W. and Beychok, S. (1973) in: Physical Principles and Techniques of Protein Chemistry (Leach, S. J. ed) Part C, pp. 445–593, Academic Press, New York.
[15] Weigang, O. E., Jr. (1965) J. Chem. Phys. 43, 71–72.
[16] Kasha, M. (1961) in: Light and Life (McElroy, W. D. and Glass, B. eds) pp. 31–64, Johns Hopkins Press, Baltimore.