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Optical Activity of LSD–DNA Mixtures

REPORTS on the fluorescence-sensitive binding of d-LSD (d-lysergic acid diethylamide), l-LSD and their 2-bromo derivatives to calf thymus DNA[1], and on changes induced in DNA circular dichroism by LDS-25 (ref. 2), have led to the belief that it is the intercalation of the drug in DNA that causes the chromosomal damage[3-6] and abnormalities in human offspring[7] that have been associated with LSD. Our studies of circular dichroism, however, have failed to show that LSD has any effect on DNA conformation.

To begin with, the circular dichroism (CD) of d-LSD, l-LSD and d-2-bromo-LSD at high concentrations does not conform with Beer's law: at concentrations greater than 0.02 mg/ml., for example, the CD bands in the region 260–290 nm are suppressed; at 0.1 mg/ml., the same bands reappear with

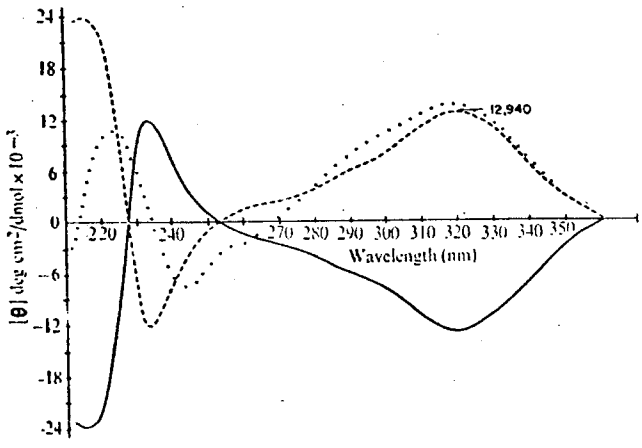

Fig. 1 The circular dichroism of l-LSD (——), d-LSD (- - -) and d-2-bromo-LSD (. . .). CD was measured on a Cary 60 spectropolarimeter with circular dichroism attachment, standardized with an aqueous solution of d-10-camphorsulphonic acid (K and K Laboratories, Plainview, New York), giving $E_L - E_R = 2.20 \pm 0.05$ at 290 nm. Solutions at *p*H 7.0 and 25° C in 0.02 M K_2HPO_4 gave the same spectra with both 0.01 M Tris (hydroxymethyl)aminomethane and 0.15 M NaCl. d-LSD, l-LSD and d-2-bromo-LSD are supplied in an L(+) tartrate buffer at 0.12 mM. Measurements were made at four different concentrations of LSD (maximum 0.01 mg/ml.) to verify conformity with Beer's law (see text). The LSD : tartrate ratio is constant for all of these studies[2,6]. Over the spectral region 215–370 nm L(+) tartrate does not contribute asymmetrically to the optical activity of d-LSD and l-LSD in the conditions studied. In all studies reported here, the l-LSD used was found to be the optical mirror of the d-LSD, as shown in the figure.

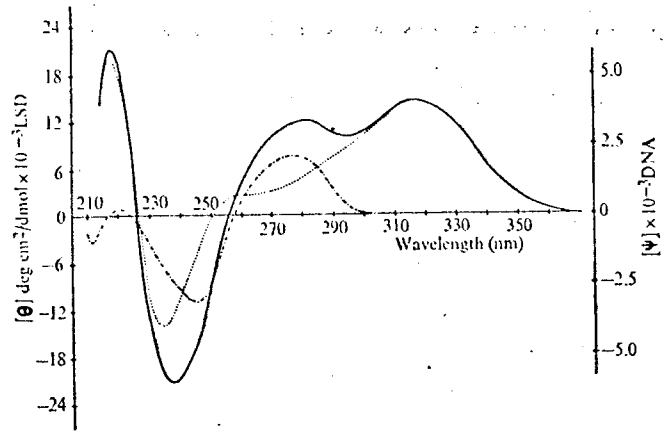

Fig. 2 The circular dichroism spectra of d-LSD (. . . .), DNA (.-.-.-) and their combination (——). The combination is a simple summation of the component spectra. The summed curves are the same in 10–50 mM Tris, 20–50 mM K_2HPO_4 and 100–150 mM NaCl at *p*H 6.5, 7.0 and 8.0. These are the conditions used by Wagner. DNA solutions were measured at concentrations of 20, 30, 60, 75, and 120 μg/ml.; the spectra with added LSD were always the sum of the component spectra. These particular curves were copied from spectra measured on a DNA solution at 20 μg/ml. d-LSD at 3.3×10^{-5} M. Spectra were measured at room temperature. Calf thymus DNA was purchased from Sigma Chemical Co., St Louis, Mo. Other DNA was supplied by Professors Antero So and Sheldon Greer whose DNA (*B. subtilis*) was shown, apart from physical measurements, to possess full biological activity; the biological activity and integrity of the DNA were determined by assaying the capacity of low concentrations of DNA to transform two genetic markers (*his* 31 and *ind* 168) that are linked to the extent of 65%.

inversion of the 220 nm band. These variations have not been explained but it is unlikely that at such high concentrations they are biologically relevant. As a consequence of this unexplained behaviour of LSD, we restricted our *in vitro* studies to solutions of DNA and LSD in which the LSD concentrations were less than 0.01 mg/ml., concentrations at which the results were reproducible and at which the Beer's law dependence of their spectra is verifiable.

In previous work on this subject, the purity of the LSD sample with respect to isomeric forms was not determined. Some have used a racemic mixture (Wagner, private communication). To ensure that our comparisons between d-LSD and l-LSD are valid, solutions were used which gave mirror-image CD spectra of the particular isomer that was used in each case (Fig. 1). Solutions of each isomer are then pure or at worst slightly cross-contaminated to the same degree by the other enantiomer, because they also give characteristic rotations at the sodium D line.

Studying the circular dichroism of DNA in the presence of d-LSD (the psychotomimetic congener) we found no significant changes; the changes were less than 1% of those reported by Wagner[2] in the same conditions; the interaction of d-LSD and DNA, shown by the fluorescence quenching experiments of Yielding and Sterglanz, did not cause any conformational change in either the d-LSD or DNA. In the spectral region 400–200 nm, the circular dichroism of the d-LSD–DNA complex results simply from a combination of the individual d-LSD and DNA spectra (Fig. 2). The calculated LSD + DNA and observed spectra are identical. The DNA was considered to be "native" when they gave CD spectra of native DNA, as defined by Brahms and Mommaerts[8]. The absence of any new optically active contribution to the DNA circular dichroism, when d-LSD was present in the DNA solution, was verified in three different solutions, 0.15 M NaCl, 0.01 M Tris and 0.02 M K_2HPO_4, each at *p*H 6.5, 7.0 and 8.0. Additional annealing of the DNA to close the small open stranded regions and to correct mismatched base-pairing by warming to 50, 60 or 67° C and slow cooling produced no changes in the ability of

d-LSD to "intercalate" with DNA, that is, it produced no changes in optical activity other than those associated with the LSD and DNA themselves. This was true whether the DNA was heated in the presence or absence of any of the LSD forms. Furthermore, DNA from *Bacillus subtilis*, *Escherichia coli*, bovine spleen, sheep bone marrow and phage T4 gave the same negative results, showing the absence of any interaction for a wide range of (A+T)/(C+G) ratios.

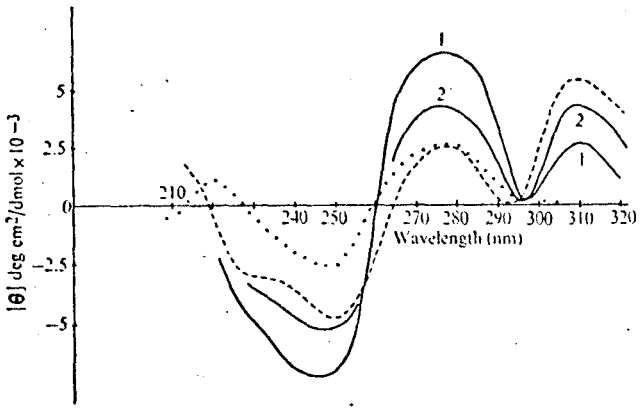

Fig. 3 The CD spectra of ethidium bromide–DNA mixtures. . . ., Curve represents pure calf thymus DNA. The differences in intensities between curves 1, 2 and the broken line relate to the age of the DNA solution. Curve 1 describes the freshest DNA solution; curve 2 was obtained from solutions that had been stored at 4° C for a week, and the dashed curve represents a much older solution of DNA, about 4 weeks old. One can obtain the dashed curve on a freshly prepared solution of old DNA which has been stored as a cold dry powder. The dashed curve is closest to the one published by Wagner[2]. Several samples of calf thymus DNA (Sigma) gave even higher ellipticity values at 272 nm with ethidium bromide, and it may be that they were the most "native" of the more than thirty preparations tested, but the curves reported here are the three which proved to be characteristic of three reproducibly different states of DNA. In spite of the variation in ethidium bromide-induced changes, the DNA alone always gave the same CD spectrum. In all cases the concentration of DNA is 30 μg/ml. and the concentration of ethidium bromide is 3.3×10^{-5} M. The more intense spectra were most consistent for solutions in 10 mM Tris, as compared with phosphate and NaCl solutions.

To eliminate artefacts arising from nicking of the DNA during handling as a complicating factor, experiments were done in two ways: in the first, solutions of fresh DNA were gently poured; and in the second, DNA solutions were extruded through hypodermic syringes with small bore needles. But, in spite of the different degrees of shearing, the results of the two were identical; in both cases, the circular dichroism of the LSD–DNA solution was the sum of the individual LSD and DNA spectra.

The fluorescence changes seen by Yielding and Sterglanz on the binding of LSD to DNA indicated to them that the sort of interaction which occurs is a "general property of this group of drugs", and that the "binding ratio was one drug molecule per base moiety in the DNA chain". It would seem, therefore, that while the drugs of this group may interact with DNA in such a way that their fluorescence emission is suppressed, circular dichroism indicates no conformational change, certainly none similar to that observed in DNA in the presence of the trypanocidal dyes, ethidium bromide or isometamidium chloride. In this respect we are in agreement with Yielding and Sterglanz: the behaviour of dn, ln and d-2-bromo-LSD in the presence of DNA is the same, regardless of the form of LSD.

The CD of ethidium bromide–DNA combinations is shown in Fig. 3. Since ethidium bromide is a good example of the planar compounds believed to intercalate with DNA[9–11], its effect on DNA circular dichroism serves as a useful basis for comparison in assessing the possible effects of LSD. We found, for example, that the character of the ethidium bromide-induced optical activity is influenced considerably by the age of the DNA solution (Fig. 3).

There is no similarity in behaviour between the LSD–DNA solutions (Fig. 2) and the ethidium bromide–DNA complexes (Fig. 3) and it is unlikely therefore that LSD forms this type of complex with DNA. For example, a significant reciprocal behaviour in ellipticity was found (Fig. 3) in the ethidium bromide–DNA interaction between the positive band at 272 nm, which arises from ethidium bromide. It was also observed by Aktipis and Martz[9–11] and it is in accord with Tinoco's[12] general theory of optical activity. It is evidence for a strong interaction between ethidium bromide and DNA which in no way resembles that between DNA and LSD in any of its forms.

We have also studied the CD spectra of combinations of LSD forms with whole tRNA and pure tryptophanyl-$tRNA_{trp}$ of *E. coli*. No additional optical activity was generated beyond the contributions of tRNA and LSD measured singly, and neither d-LSD nor l-LSD affected the capacity of $tRNA_{trp}$ to charge tryptophan.

Recently, it was reported[13] that LSD does not damage chromosomes of *E. coli* except at very high concentrations and high ultraviolet light intensity. Unfortunately, the isometric purity of the LSD used in this work was not determined and a maleate salt of LSD was used whereas others have generally used an L(+)tartrate salt. We emphasize the importance of measuring the relative concentrations of the dn and ln forms and of a uniformity in the preparations used. The conflicting results of studies of LSD-induced chromosomal damage[14] may be largely attributable to differences in isomer concentrations and buffering agents in the LSD preparations. Furthermore, some early LSD–DNA spectra seem to be inaccurate, and do not provide a firm basis for consideration of the mode of action of LSD.

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