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SHORT COMMUNICATION

Binding and Circular Dichroism Studies on Interactions of Lysergic Acid Diethylamide with Deoxyribonucleic Acid

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SUMMARY

The interactions of *d*- and *l*-lysergic acid diethylamide (LSD) with DNA were studied by means of equilibrium dialysis and circular dichroism. There was no significant difference between the binding of *d*- and *l*-LSD to DNA. Circular dichroism data do not favor intercalation, and it is suggested that LSD is probably bound outside the DNA helix, perhaps to the phosphate groups of DNA by some ionic mechanism.

There is considerable controversy regarding the teratogenic and mutagenic properties of lysergic acid diethylamide (1–4). The evidence that the drug produces embryonic malformations and chromosomal damage in human users and animals is inconclusive. Elsewhere (5, 6) we have reported on the cytogenetic effects of LSD[4] (100 μg/kg) on mother rats, their embryos, and mature offspring when the drug was given early in pregnancy (gestation day 4 or 8). Exposure to LSD elicited no statistically significant alterations in the distribution of chromosome number (aneuploidy, diploidy, or polyploidy) and no significant increase in structural chromosomal aberrations in the cells from the pregnant animals or their offspring. In another study (7), pregnant rats were given LSD (100 μg/kg) at different periods during gestation and were allowed to deliver and raise their offspring. We did not observe any gross malformations in the progeny, nor did we note any increased incidence in resorption, fetal death, or small fetuses. However, the incidence of infant deaths was increased in all the drug-exposed groups. A significant suppression of the normal developmental increase in body weight and of the ontogenesis of certain biochemical systems in brain and liver was also observed in the drug-exposed animals.

Similar controversy also surrounds studies

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[4] The abbreviation used is: LSD, lysergic acid diethylamide.

of DNA and LSD at the molecular level. Fluorescence studies have been interpreted as evidence for strong binding and intercalation (8, 9). Modification of the circular dichroism bands by added LSD was also interpreted by Wagner (10) as evidence for intercalation. On the other hand, Brady *et al.* (11), in their studies of circular dichroism, found no effect of LSD on DNA conformations. In addition, viscosity measurements of closed circular DNA as a function of added LSD failed to show the behavior characteristic of intercalating drugs (12).

Our experiments were designed to answer the following questions. (a) Does LSD bind to DNA, and are there differences between the *d*- and *l*-forms in this regard? It has been suggested in popular literature (13) that *l*-LSD complexes with DNA while the psychotomimetic *d*-form does not, implicating *l*-LSD as the mutagenic agent. We have discovered some anomalies in the fluorescence method which may be connected with photodegradation observed by Smit and Borst (12). Therefore we chose equilibrium dialysis for the binding studies. (b) Under conditions of binding, does intercalation occur? This question may be answered by studying the circular dichroism bands of the LSD chromophore. The effects of the DNA helix in inducing Cotton effects in intercalated chromophores is well known.

d- and *l*-LSD were obtained from the National Institute of Mental Health (Nair, IND 4184). DNA was obtained from Sigma Chemical Company, and was further purified by the procedure of Marmur (14) to yield a high molecular weight compound. The purity of the LSD was verified by spectrophotofluorometric analysis (15). Spectra of solutions of *d*- and *l*-LSD containing various concentrations of DNA were recorded with a Cary 15 spectrophotometer over the range of 240–500 nm. The reaction mixture of Yielding and Sterglanz (8) was employed, which consisted of the drug in 0.01 M potassium phosphate buffer, pH 6.1, and calf thymus DNA. Fluorescence measurements were made in a Farrand mark I photofluorometer. Under these conditions we noted severe artifacts from extraneous quenching and photodegradation, and consequently could not measure the fluorescence yield or hypochromicity with any degree of confidence. Our best data (Fig. 1) show no difference between the spectra of LSD in the presence and absence of DNA. The identity of the spectra suggests that LSD is not intercalated in the DNA molecule. We then employed equilibrium dialysis and estimated LSD by an extraction procedure. Equilibrium dialysis was carried out using Visking 8/32 dialysis tubing. The inner compartment contained 1 ml of DNA solution of the desired concentration, and the outer compartment contained 9 ml of 0.01 M potassium phosphate buffer, pH 6.1. LSD was added to the outer compartment, and dialysis was allowed to proceed for 24–48 hr at 4°. DNA then was determined by absorbance measurements, and LSD, by the extraction procedure of Axelrod *et al.* (15). Recoveries of LSD added to the buffer were on the order of 85%. The data from the equilibrium dialysis experiments were plotted according to Klotz *et al.* (16) (Fig. 2), and the binding constants were calculated. The association constant for binding of *d*-LSD was approximately 1025 Liter/Mole, and that of *l*-LSD, 780 Liter/Mole. The difference between the two values

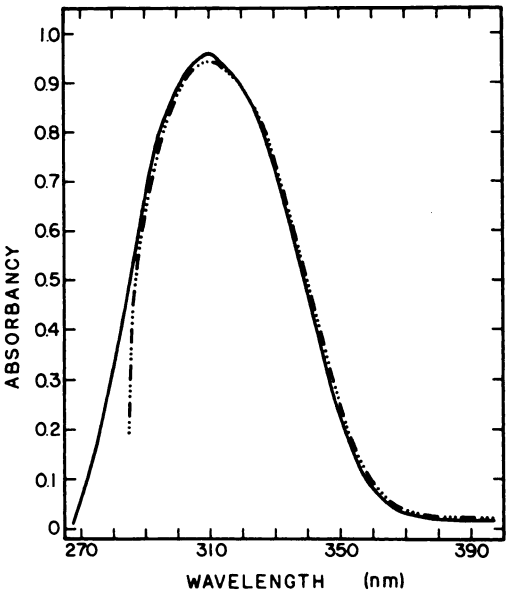

FIG. 1. *Absorption spectra of d-LSD* (—···) *and d-LSD bound to DNA* (——)

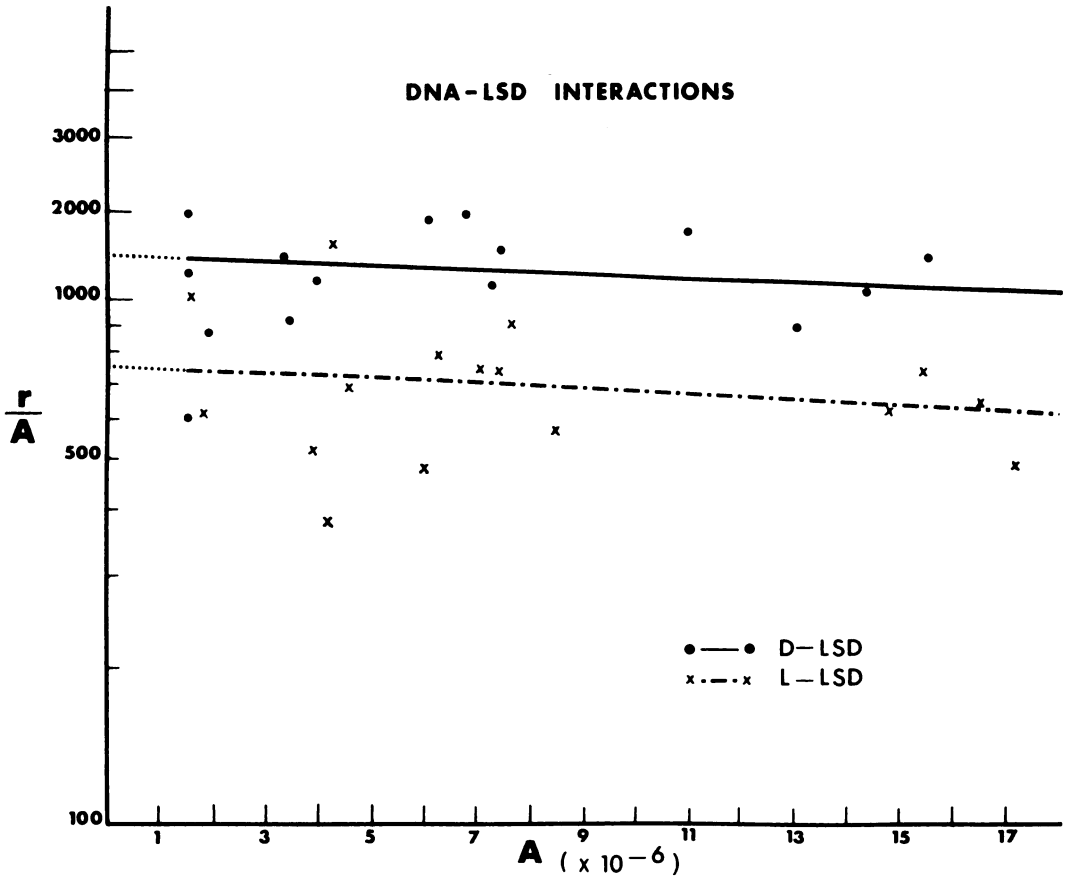

FIG. 2. *Estimation of binding constant of d-LSD* (●) *and l-LSD* (×) *to DNA*
A is the free ligand concentration, and *r* is moles of LSD bound per mole of nucleotide. The lines were calculated by linear regression analysis.

is not statistically significant. The low binding constant and the narrow span of *r* (moles of LSD bound per mole of DNA base) obtained in these experiments precluded extrapolation to obtain *n*, the number of binding sites.

The low binding affinity of the drug for DNA might have resulted from an ionic interaction. To test this hypothesis, the above experiments were repeated for *d*-LSD in the presence of 0.2 M NaCl and 0.01 M potassium phosphate buffer, pH 6.1. No binding was found within the experimental error of the extraction method, also suggesting that the weak binding was ionic.

Circular dichroism experiments were performed with a Cary 60 spectropolarimeter and a 6002 circular dichroism accessory at ambient temperature (19°) in cells of 1-cm path length. The optical density at 310 nm of all solutions was approximately 0.5. As a result of the high DNA concentration, the optical densities were not recorded at wavelengths lower than 295 nm. Both isomers of LSD were obtained from the National Institute of Mental Health as tartarate solutions, in ampoules, containing 200 μg/ml. However, we observed some variability in the optical densities of the solutions at 310 nm. We chose an average value for the extinction coefficient of 5.5 ± 0.5 mM^{-1} cm^{-1}, and used optical densities to determine concentrations for the circular dichroism measurements. Therefore, although our values for [θ], the molar ellipticity for LSD, may not be absolute, the data should be internally compatible.

The circular dichroism curves for LSD

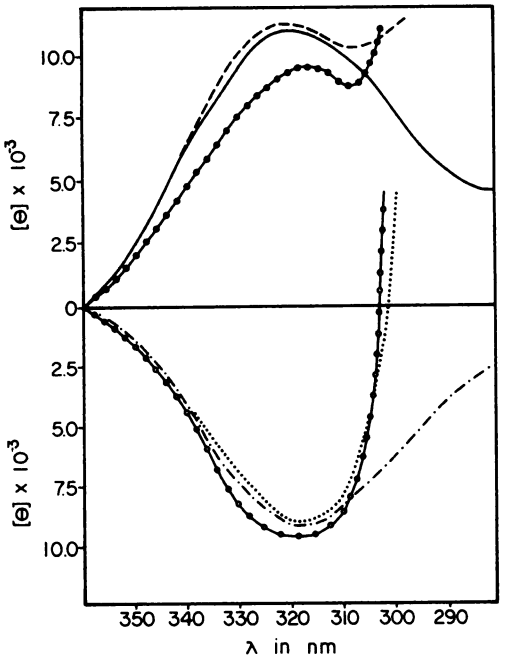

FIG. 3. *Ellipticity per mole of LSD for the drug and its complexes with DNA (3 mM in residues) in aqueous 0.01 M phosphate, pH 6.0*

(——), *d*-LSD; ●—●, complex of *d*-LSD (106 μM) with DNA; - - -, *d*-LSD curve added to that of DNA; -·-·, *l*-LSD; ○—○, complex of *l*-LSD (85 μM) with DNA; ····, *l*-LSD curve added to that of DNA.

and LSD-DNA mixtures are shown in Fig. 3. The ellipticities are reported per mole of LSD in all curves. Equilibrium dialysis experiments indicate that 75% of *d*-LSD and 70% of *l*-LSD were bound to DNA under the conditions used. The circular dichroism for DNA was also measured and added to the LSD curve for comparison with the mixture. The curve in this region is in agreement with that reported previously by Sarkar *et al.* (17).

The circular dichroism curves of both the *d*- and *l*-isomers of LSD show single bands centered at 317 nm with a molar ellipticity of approximately 1.0 ± 0.08 mM^{-1} cm^{-1}. Comparison of the *l*-LSD–DNA mixture curve with the sum of the curves for *l*-LSD and DNA reveals their near identity (Fig. 3). The curve for the *d*-isomer was depressed on binding. However, the nearly structureless nature of the difference curve suggests that this depression may be artifactual rather than attributable to binding.

In order to test the hypothesis that LSD is intercalated into DNA on binding, we estimated the magnitude of the circular dichroism expected to be induced in the LSD absorption region by intercalation. Certain dye molecules are known to form intimate and specific complexes with DNA, causing large circular dichroic bands as well as certain modifications of its biological activity. For example, actinomycin D, when bound to DNA at high polymer phosphate to dye ratios, induces changes in the bands in the actinomycin absorption region at 460 nm (18). At phosphate to dye ratios of 15, bands of $[\theta] \cong 25{,}000$ are observed (18). Similar results are obtained for acridine orange–DNA (19) and ethidium bromide–DNA complexes. Although these dyes bind much more strongly to DNA than does LSD, the physical situation should be comparable for high phosphate to drug ratios.

At such high phosphate to dye ratios the dye should be distributed sparsely along the DNA, and circular dichroism induced in the intercalated dye would result from interaction of the dye chromophore with DNA chromophores. When more dye is bound, dye-dye interactions become important and larger Cotton effects are observed. The circular dichroism induced in intercalated dyes results from a coupled oscillator effect between the dyes and the absorption bands of the nucleic acid base chromophore. This effect is analogous to that of interacting chromophores at different wavelengths seen in oligonucleotides of different bases (20). Since the circular dichroism depends on the oscillator strength of the bound dye, much smaller bands should be induced in LSD, since it has a much lower extinction coefficient than acridine orange or actinomycin. In fact (see APPENDIX), under certain assumptions the ratio of the induced circular dichroism bands should approximate the ratio of the extinction coefficients, ϵ_{max}.

For the extinction coefficient of actinomycin, we take 23×10^3 from the work of Yamaoka and Ziffer (18). These considerations suggest that the intercalation of LSD should induce bands of $[\theta] \cong 6000$. If, in contrast to assumptions made in the APPENDIX, the 260 nm bands of DNA are most effective in coupling, the effect in LSD should be even larger. Clearly the effects we

observed are either nil or quite small compared to those expected for intercalation.

Circular dichroism experiments of Wagner (10) on the Cotton effects in the DNA absorption region (240–280 nm) have led to conclusions quite different from ours. On the other hand, the equilibrium dialysis experiments reported here suggest that the experimental conditions of Wagner would produce very little binding of LSD to DNA. Moreover, a recent study of the circular dichroism bands in the DNA region casts doubt on the validity of Wagner's results (11).

It is concluded from these studies that (a) there is no significant difference between the binding of *d*- and *l*-LSD to DNA and (b) the LSD is not intercalated but is probably bound outside the DNA helix, perhaps to the phosphate groups of the DNA by some ionic mechanism.

Chromosomal damage can result from direct or indirect action of the toxicant, through many, and complex, causes. The results of the present study do not support a direct effect of LSD on chromosomes, and are consistent with our earlier observations (6) in LSD-exposed rat embryos and offspring as well as with those of several other investigators (4, 21–28). Our results are also compatible with the conclusions arrived at recently by Dishotsky *et al.* (29), after reviewing 68 studies directly related to this topic, that *pure* LSD in moderate doses does not cause chromosomal damage and is not a teratogen.

APPENDIX

In the text, we stated that the ratio of circular dichroic bands induced in different intercalated dyes, at high phosphate to dye ratios, should be the ratio of the extinction coefficients, ϵ_{max}. This statement is based on a number of assumptions, all of which are reasonable.

First, we assume that circular dichroic bands are induced by electric dipole interaction between the dye and the DNA bases. For electrically allowed transitions, we ignore magnetic coupling effects and static field effects. The coupled oscillator formula is taken from Tinoco (30), whose notation we follow. For the rotational strength R_a of a band at frequency $\nu_{a'}$,

$$R_a = \frac{-2\pi}{c} \sum_{i,j,b} \frac{V_{ioa;job}}{(\nu_b^2 - \nu_a^2)} \mathbf{R}_{ij} \cdot \boldsymbol{\mu}_{job} \times \boldsymbol{\mu}_{ioa} \tag{1}$$

For the present argument, consider a single interaction of group i, the dye, with a single DNA base, group j, which has absorption bands at ν_b. $\mathbf{R}_{ij}$ is the vector distance between the bound drug and the DNA base which couples to it. $\boldsymbol{\mu}$ is the electric dipole transition moment.

Now we calculate the ratio of rotational strengths induced in two different bound drugs [where (AMD) represents actinomycin -D

$$\frac{R_a\,(\mathrm{LSD})}{R_a\,(\mathrm{AMD})} = \frac{\sum_b V_{ioa;job}\,(\mathrm{LSD})\,\nu_a\,(\mathrm{LSD})\,\nu_b \mathbf{R}_{ij} \cdot \boldsymbol{\mu}_{job} \times \boldsymbol{\mu}_{ioa}\,(\mathrm{LSD})/[\nu_b^2 - \nu_a^2\,(\mathrm{LSD})]}{\sum_b V_{ioa;job}\,(\mathrm{AMD})\,\nu_a\,(\mathrm{AMD})\,\nu_b \mathbf{R}_{ij} \cdot \boldsymbol{\mu}_{job} \times \boldsymbol{\mu}_{ioa}\,(\mathrm{AMD})/[\nu_b^2 - \nu_a^2\,(\mathrm{AMD})]} \tag{2}$$

We now ask: What transitions in DNA couple most effectively to the bound dye, the nearby bands at 260 nm or the strong bands of the far ultraviolet region? If we assume that a single transition, b, in the far ultraviolet dominates the sum, all the frequency dependence in the sum over states of Eq. 2 cancels. If the 260 nm bands are more important, the ratio of the frequency differences in Eq. 2 will be larger than unity, and so this assumption places a lower limit on the ratio of the rotational strengths.

Next we introduce a dipole-dipole approximation for the interaction energy, $V_{ioa;job}$. This expression involves the interaction of the electric transition dipole moment of the bound drug, $\boldsymbol{\mu}_{ioa}$, with some far ultraviolet transition moment of the DNA bases, $\boldsymbol{\mu}_{job}$.

$$V_{ioa;job} = R_{ij}^{-3} \left(\boldsymbol{\mu}_{ioa} \cdot \boldsymbol{\mu}_{job} - \frac{3\mathbf{R}_{ij} \cdot \boldsymbol{\mu}_{ioa} \mathbf{R}_{ij} \cdot \boldsymbol{\mu}_{job}}{R_{ij}^2} \right) \tag{3}$$

We must now decide on the geometry of binding. Geometry dependence appears in Eq. 2 in the triple product of R_{ij} with the transition moments, and also in the interaction energy, which we have now approximated by a dipole-dipole formula, Eq. 3. Since there are four different bases in DNA, each having a number of far ultraviolet transitions able to couple with the dye transition moments, most differences in the geometry of binding can probably be averaged out. Allowing for cancelation of all the geometric parts of Eq. 2, we arrive at the simple result,

$$\frac{R_a\,(\mathrm{LSD})}{R_a\,(\mathrm{AMD})} = \frac{\mu_{ioa}^2\,(\mathrm{LSD})\,\nu_a\,(\mathrm{LSD})}{\mu_{ioa}^2\,(\mathrm{AMD})\,\nu_a\,(\mathrm{AMD})} = \frac{f\,(\mathrm{LSD})}{f\,(\mathrm{AMD})} \tag{4}$$

Assuming similar absorption band shapes for the dyes, we may approximate the ratio of the oscillator strengths, f, by the ratio of the absorption coefficients.

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