LSD-25 Does Not Intercalate in DNA

APART from its hallucinogenic effect, lysergic acid diethylamide (LSD-25) is believed to cause chromosome abnormalities[1-3], which have been attributed to a direct interaction of the LSD-25 and DNA[4]. The apparent resemblance of the circular dichroism spectra of the DNA–ethidium-bromide complex and mixtures of LSD-25 and DNA has led to the suggestion that LSD-25 might be intercalated in the DNA[5].

A more specific and sensitive method for studying intercalation is provided by the interaction of drugs with twisted, circular, covalently closed duplex DNA[6-8]. By intercalating molecules into the DNA the duplex is unwound and, because the total number of duplex turns and supertwists is a topological invariant, this leads to the unwinding of the supertwists and therefore to the conversion of twisted circles into open circles[9]. This change in form can be followed by sedimentation analysis, or, more conveniently, in a viscometer[15]. We have used this method to verify Wagner's suggestion[5] that LSD-25 intercalates.

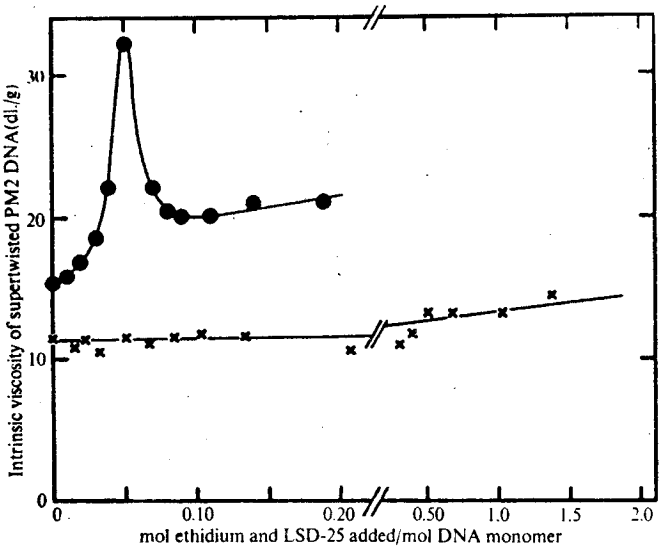

Fig. 1 The intrinsic viscosities of the DNA from phage PM2 as a function of the ethidium (●) and the LSD-25 (×) concentration present. Conditions were: temperature 20° C; DNA concentration, 27 μg ml.$^{-1}$ of 15 mM NaCl, 1.5 mM sodium citrate, *p*H 7.0 (●), and 20.3 μg ml.$^{-1}$ of 10 mM sodium phosphate, *p*H 7.0 (×). The intrinsic viscosities were determined in a rotating cylinder viscometer (Krannich GMbH, Göttingen) according to Zimm and Crothers[10]. Ethidium was a gift from Boots, and LSD-25 was purchased from Sandoz.

Fig. 1 shows the results of an experiment in which we compared the effect of LSD-25 and the intercalating dye ethidium on the closed circular duplex DNA of bacteriophage PM2 (refs. 11 and 12). Whereas the results with ethidium were as expected, no interaction between DNA and LSD-25 was detectable, in the range of concentrations in which strong interaction was found by others[4,5].

We therefore repeated previous experiments, in which interaction between LSD-25 and DNA was detected spectrophotometrically. In contrast to the results of Yielding and Sterglanz[4], the spectra of different mixtures of LSD-25 and DNA between 220 and 600 nm were the exact summations of the two components separately; in contrast to Wagner's results[5], we also found complete additivity for the circular dichroism spectra.

These results indicated that either LSD-25 does not interact directly with DNA, or that our LSD sample contained no LSD-25. The latter alternative was excluded by infrared spectroscopy and NMR studies. We therefore conclude that chromosome damage in the presence of LSD-25 is not a consequence of the intercalation of LSD-25 into DNA.

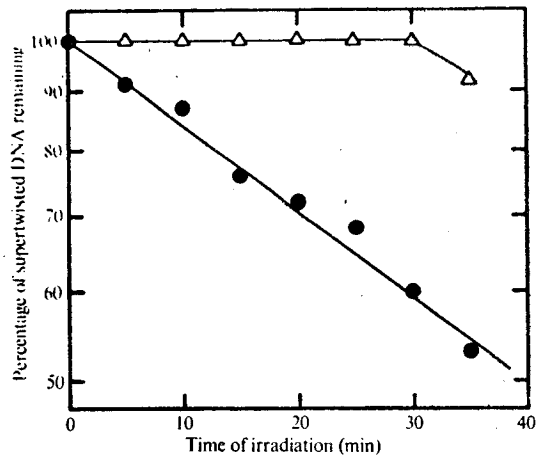

Fig. 2 Breakage of the supertwisted from of PM2 DNA by ultraviolet irradiation in the presence of LSD-25. Samples were irradiated with (●) and without LSD-25 (△) with a germicidal lamp. The radiation below 300 nm was cut off by a glass filter. Conditions were: DNA concentration, 63.5 μg ml.$^{-1}$; LSD-25 concentration, 310 μM; medium 15 mM NaCl +1.5 mM sodium citrate, *p*H 7.0. The conversion of the supertwisted PM2 DNA was followed by band sedimentation of small samples through neutral 3 M CsCl in an MSE analytical ultracentrifuge. The percentage of supertwisted DNA was estimated from scannings at 260 nm, using a Dupont 310 curve resolver.

During these experiments, we noted that irradiation of DNA with ultraviolet light in the presence of LSD-25 led to phosphodiester bond breakage, even if most of the radiation below 300 nm was cut off with a glass filter. This phenomenon was illustrated by the experiment in which phosphodiester bond breakage in DNA was measured by the conversion of the closed circular duplex form of PM2 DNA to slower sedimenting material (Fig. 2). Electron microscopy confirmed that the irradiation led to the formation of open circles rather than linear molecules. There was no conversion when DNA was irradiated in the absence of LSD-25 or when DNA–LSD mixtures were kept in the dark. Although the conditions used in this experiment were very different from those prevailing *in vivo*, it should be realized that the molecular weight of PM2 DNA is only 6×10^6, whereas a human diploid nucleus contains 4×10^{12} daltons of DNA[13]. LSD-25-dependent photo-destruction of DNA could therefore contribute to chromosome damage in tissue culture experiments and possibly *in vivo*, and photo-sensibilization of an *Escherichia coli* mutant, deficient in repair, by high concentrations of LSD-25 has been reported[14].

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Induced Optical Activity of 2,4-Dinitrophenyl-lysine Specifically Bound to Mouse MOPC-315 Myeloma Protein

THE optical activity generated by the interaction of small ligands with proteins, visible as extrinsic optical rotatory dispersion (ORD) Cotton effects or circular dichroism (CD) bands, has been used extensively to study the binding of ligands to non-antibody proteins[1,2]. We describe here the use of circular dichroism to monitor the binding of DNP-lysine to a mouse IgA myeloma protein with high affinity for dinitrophenylated haptens[3-5].

The MOPC-315 mouse tumour line of Potter and Lieberman[6] was maintained in 5–8 month old BALB/c mice, and the IgA myeloma protein was purified from ascitic fluid by affinity chromatography[5]. DNP-lysine (ε-N-2,4-dinitrophenyl-L-lysine)-'Sepharose' was prepared by the method of Porath, Axen and Ernback[7]. We washed a 10 ml. DNP-'Sepharose' column with 200 ml. of 0.15 M NaCl–50 mM sodium borate buffer (*p*H 8.4), 10 ml. of 1% (w/v) bovine serum albumin, 200 ml. of 1 M acetic acid and 200 ml. of borate-buffered saline immediately before use. MOPC-315 ascitic fluid was dialysed against borate-buffered saline, and the protein was reduced at 25° C with 10 mM dithiothreitol for 1 h, and alkylated for 30 min at 4° C with a 10% molar excess of iodoacetamide. The reduced and alkylated protein was dialysed against borate-buffered saline and applied to a DNP-'Sepharose' column, which was washed with the same solvent, and the MOPC-315 myeloma protein was eluted with 1 M acetic acid. The recovered protein was immediately dialysed against borate-buffered saline (*p*H 8.4) at 4° C, and insoluble materials were removed by centrifugation. Examination of the purified protein by immunoelectrophoresis with rabbit anti-mouse serum and anti-MOPC-315 protein sera yielded only a single component.

Circular dichroism was measured at 25° C with a JASCO ORD/UV-5 spectropolarimeter equipped with a CD attachment, and standardized with d-camphor-10-sulphonic acid. Cell path lengths of from 1.0 cm to 0.1 mm, and protein concentrations between 0.8 and 4×10^{-3} g per 100 ml., were used to determine the CD spectrum (600 to 200 nm) of the MOPC-315 protein. The data are given in molar ellipticities, $[\theta]_\lambda$, in degrees cm^2 dmol^{-1}, where $[\theta]_\lambda = \frac{3{,}300}{lc}(A_L - A_R)_\lambda$, and l = the path length (cm), c = the molar concentration and $(A_L - A_R)_\lambda$ is the observed difference in absorption between left and right circularly polarized light at wavelength λ. The average residue weight, M_0, taken as 110 (rather than the molecular weight) for the MOPC-315 protein was used to obtain the protein $[\theta]_\lambda$ values. We determined optical density difference spectra with a JASCO ORD/UV-5 spectrophotometer. The quenching of MOPC-315 protein fluorescence by DNP-lysine (Sigma)[3] was measured with a Zeiss double M4 QIII monochromator spectrofluorometer with a Farnell stabilized EHT power supply and a Keithley 610 B electrometer, in a thermostatically controlled cell compartment at 25° C. The data were corrected for dilution and internal quenching as before[8]. Protein concentrations were determined from the optical density at 280 nm (corrected for light scattering), measured with a Zeiss PMQII spectrophotometer, with an extinction coefficient, $E^{1\ \text{mg ml.}^{-1}}_{1\ \text{cm}}$, of 1.44 for the MOPC-315 IgA protein[3,5]. The molar extinction coefficient, ε, at 362 nm for DNP-lysine was taken as 1.75×10^4 (ref. 5).

The CD spectrum of the MOPC-315 protein is shown in Fig. 1*A*. No circular dichroism was evident in the protein spectrum from 600 to 315 nm. DNP-lysine (up to 1.3×10^{-4} M) in free solution, and in the presence of immunoglobulins lacking DNP-specificity, also had no optical activity in this wavelength range (Fig. 1*B*). In contrast, binding of the DNP-lysine ligand by the MOPC-315 protein generated distinct circular dichroism bands (Fig. 1*B*). A broad positive band centred near 410–420 nm, a sharper positive band at 380 nm and a broad negative band extending from 360 nm to 270 nm and centred near 295 ± 15 nm were observed in the CD spectrum of the MOPC-315–DNP-lysine complex (Fig. 1*A* and *B*). The absorption spectra of DNP-lysine in free solution, and DNP-lysine specifically bound to the MOPC-315 protein, are also recorded in Fig. 1*B*. The redshift and hypochromicity[3] were evident after complexing the ligand with the myeloma protein, the absorption maximum being shifted from 362 nm for free DNP-lysine ($\varepsilon = 1.75\times10^4$) to 368 nm for bound DNP-lysine ($\varepsilon = 1.52\times10^4$).

Hapten-binding curves were constructed by serially adding DNP-lysine (with a Hamilton microsyringe) to a constant amount of MOPC-315 protein, and measuring the circular dichroism at 325 nm and 380 nm. The $(A_L - A_R)_{325\ nm}$ data (uncorrected for dilution) have been plotted as a function of the total DNP-lysine concentration (Fig. 1*C*). The mean value for $(A_L - A_R)_{325\ nm}$ at saturation (that is $(A_L - A_R)_{max}$ at DNP-lysine concentrations greater than 8×10^{-5} M), corrected for dilution, was 5.32×10^{-4}. The $(A_L - A_R)_{325\ nm}$ values were divided by the total DNP-lysine concentration to give the apparent value of $[\theta]_{325\ nm}$, and these values are also plotted in Fig. 1*C* as a function of the total DNP-lysine concentration. The mean of the maximum values for $[\theta]_{325\ nm}$ (apparent) (total DNP-lysine concentration less than 6×10^{-5} M) gave $[\theta]_{325\ nm}$ as -2.10×10^4 degrees cm^2 dmol^{-1}. Similarly, $[\theta]_{380\ nm}$ was calculated to be 1.68×10^4 degrees cm^2 dmol^{-1}. The molar concentration of antibody combining sites was determined from the ratio $((A_L - A_R)_{max}/[\theta]_{325\ nm})$ to be 1.71 mol of sites per 153,000 g of protein.

The intrinsic association constant is an additional parameter for evaluating $[\theta]_\lambda$. The association constant, K_A, obtained for the MOPC-315 protein from the fluorescence quenching data (Fig. 1*D*) was 0.6×10^7 mol^{-1}, consistent with the value reported by Eisen, Simms and Potter[3]. The association constant, $K_A = \frac{r}{(n-r)c'}$, where r is the number of moles of hapten bound per mol of bivalent antibody, $n = 2$, and c' is the free hapten concentration[3,8]. The value of r at each dilution may be obtained from the observed $(A_L - A_R)$ values, corrected for dilution, as the ratio $(2(A_L - A_R)\ \text{observed}/(A_L - A_R)_{max})$. At $r = 1$, $c' = \frac{1}{K_A}$, $c = [(\text{total DNP-lysine concentration}) - c']$ and $[\theta]_\lambda = \frac{3{,}300}{cl}[(A_L - A_R)_\lambda$ at $r = 1]$. The values for $[\theta]_{325\ nm}$ and $[\theta]_{380\ nm}$ calculated by this procedure were -2.13×10^4 and 1.66×10^4 degrees cm^2 dmol^{-1}, respectively, consistent with the values we obtained from the $[\theta]_\lambda$ (apparent) against total DNP-lysine concentration plots (Fig. 1*C*).

For lower affinity antibodies ($K_0 \sim 10^5$ mol^{-1}), circular dichroism is an alternative method to determine directly the association constant.

The optical activity of the inherently symmetric DNP-chromophore is generated by the interaction of the DNP group with asymmetrically placed vicinal groups in the antibody combining site of the MOPC-315 protein. The reciprocal relationship of