Molecular Conformation of Inosine

INOSINE is one of the rare nucleosides found in nucleic acids. It occurs especially in transfer ribonucleic acid where it appears to form part of a number of anticodons[1,2]. It can form a base pair with any one of the bases adenine, uracil or cytidine in the third codon[1,2] position, and the possibility of atypical base pairs is part of the "wobble" hypothesis[2]. In addition, the action of mutagenic agents on ribonucleic acid can lead to the conversion of adenosine into inosine.

Inosine crystallizes in two distinct crystal forms, both of which are under examination in this laboratory. The results given here were obtained from crystals formed by slow evaporation from aqueous solution at 25° C. The unit cell is monoclinic, with cell dimensions $a = 4{\cdot}82$ Å, $b = 10{\cdot}45$ Å, $c = 10{\cdot}97$ Å and $\beta = 90°\ 43'$. The space group is $P2_1$ with two molecules in the unit cell. In all, 1,306 reflexions were observed using molybdenum Kα radiation and the Hilger and Watts linear diffractometer. The structure was solved by Patterson function interpretation using the $I(\theta\varphi)$ function[3], the rotation function[4] and the Q-functions[5]. The structure was refined by a least-squares process using anisotropic temperature parameters for the non-hydrogen atoms to a final R factor of 0·046, where

$$R = \frac{\Sigma||F_o| - |F_c||}{\Sigma|F_o|}$$

The positions of all the hydrogen atoms were confirmed from a three-dimensional difference synthesis. The details of the structure and the results obtained will be published later.

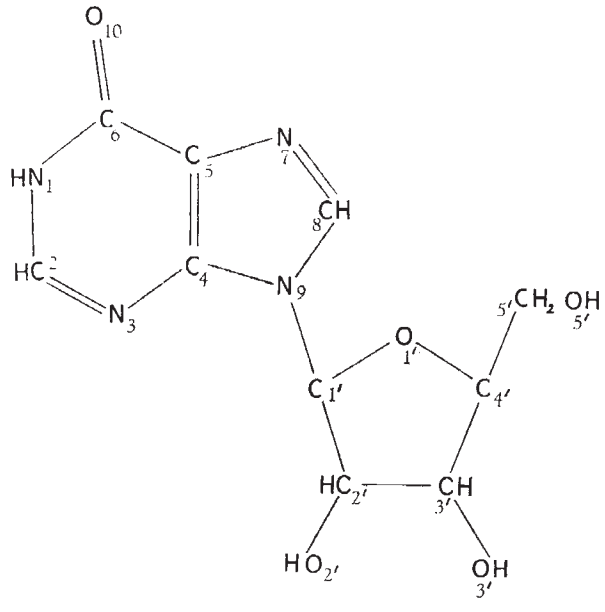

Fig. 1. The structural formula of inosine with the numbering system used.

The molecular formula of inosine and the conventional numbering system for the atoms are given in Fig. 1. The bond lengths and angles obtained from the structure determination are given in Table 1.

The purine ring in inosine is planar, but both O_{10} and C_1' are significantly displaced from this plane, the deviations being 0·16 Å and 0·15 Å respectively. The conformation of the sugar residue relative to the purine plane is best described by the torsion angle φ_{CN}[6], which in this case is −10·6°, the sugar being in the *anti* conformation. Atom C_3' of the ribose rings is displaced 0·63 Å from the plane of the remaining ring atoms and is in the *endo* conformation. The bond C_5'—O_5' is *gauche* with respect to the C_4'—O_1' bond and *trans* with respect to the C_4'—C_3' bond, the φ_{OO} and φ_{OC} angles[7] being 74·7° and 169° respectively. All available groups participate fully in the hydrogen bonding. The fact that the structure is strongly bonded is reflected in the relatively high calculated density of 1·61 g/cm^{-3}. In addition there are a number of close contacts between atoms of different molecules with distances between 3·0 and 3·2 Å. The possibility of a carbon–oxygen hydrogen bond has received some discussion[8,9] and some possible examples have been found[10]. One of the short intermolecular contacts in inosine is a short C—O distance of 3·09 Å between C_2 and an O_5' atom of a neighbouring molecule. The C—H and H—O distances are 0·98 Å and 2·34 Å respectively and the angle between the C—H bond and the line joining C_2 to O_5' is 34°.

Table 1. THE BOND LENGTHS AND ANGLES FOR INOSINE

N_1–C_2	1·355 Å	C_2–N_3	1·308 Å
N_3–C_4	1·365	C_6–C_5	1·374
C_4–N_9	1·372	C_5–C_6	1·433
C_6–N_1	1·397	C_6–O_{10}	1·233
C_5–N_7	1·371	N_7–C_8	1·307
C_8–N_9	1·372	N_9–C_1'	1·477
C_1'–O_1'	1·417	C_1'–C_2'	1·530
O_1'–C_4'	1·459	C_2'–O_2'	1·420
C_2'–C_3'	1·525	C_3'–O_3'	1·413
C_3'–C_4'	1·522	C_4'–C_5'	1·506
C_5'–O_5'	1·428		
N_1–C_2–N_3	124·6°	C_2–N_3–C_4	111·9°
N_3–C_4–C_5	128·5	C_4–C_5–C_6	118·3
C_5–C_6–N_1	111·0	C_6–N_1–C_2	125·4
N_1–C_6–O_{10}	120·6	C_5–C_6–O_{10}	128·4
C_6–C_5–N_7	130·1	C_4–C_5–N_7	111·6
N_7–C_8–N_9	113·6	C_5–N_7–C_8	103·8
N_9–C_4–C_5	105·4	C_8–N_9–C_4	105·7
C_8–N_9–C_1'	128·1	N_9–C_4–N_3	126·1
N_9–C_1'–O_1'	108·4	C_4–N_9–C_1'	126·0
O_1'–C_1'–C_2'	106·8	N_9–C_1'–C_2'	111·5
C_1'–C_2'–O_2'	109·6	C_1'–O_1'–C_4'	109·6
O_2'–C_2'–C_3'	107·5	C_1'–C_2'–C_3'	100·6
C_2'–C_3'–O_3'	115·9	C_2'–C_3'–C_4'	101·5
C_3'–C_4'–O_1'	104·0	O_2'–C_2'–C_4'	114·2
O_1'–C_4'–C_5'	110·0	C_3'–C_4'–C_5'	114·1
		C_4'–C_5'–O_5'	112·0

Estimated standard deviation in bond lengths is 0·004 Å, and in bond angles is 0·4°.

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In vitro Interaction of LSD with Purified Calf Thymus DNA

THE observation of broken chromosomes in test animals and humans treated with the hallucinogen lysergic acid diethylamide (LSD) has been well documented[1–8]. Although there has been some speculation about the mode of action of the drug, no specific studies of the mechanism of LSD-induced chromosomal damage have, as yet, been presented. Such studies would be most useful in determining whether LSD interacts directly with the chromo-

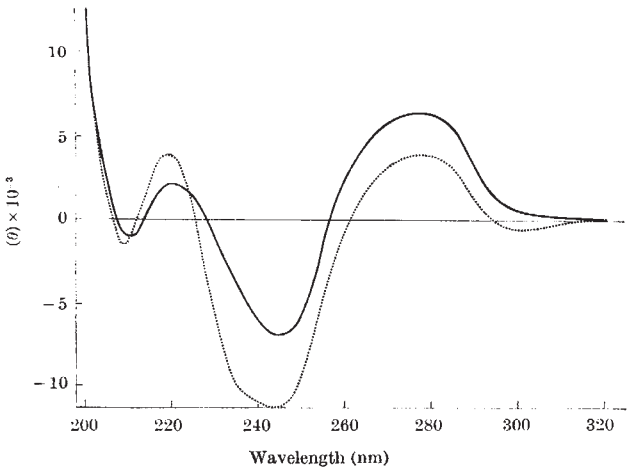


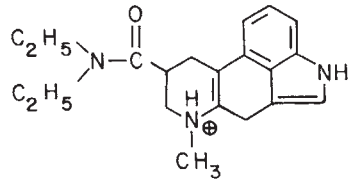
Fig. 1. Circular dichroism spectra of calf thymus DNA separated from ——— and in contact with .... LSD-25. Concentrations: DNA, 0·02 mg/ml., and LSD-25, $3·3 \times 10^{-6}$M. 0·1 M NaCl, *p*H 7·0, 25°. A mean residue weight of 336·84 for calf thymus DNA was used in calculating the molar ellipticity (θ).

some which becomes broken or whether chromosomal breakage is the result of a series of biochemical events begun by LSD at some site distant from the chromosome. This communication describes circular dichroism experiments which indicate that LSD interacts directly with purified calf thymus DNA, probably by intercalation, causing conformational changes in the DNA. It is suggested that this observed interaction may serve as a model for the action of LSD on the chromosomal material of intact cells.

Using a Cary model 60 spectropolarimeter equipped with a Cary model 6002 circular dichroism attachment, I recorded circular dichroism spectra of calf thymus DNA solutions (Sigma Chemical Co., 0·02 mg/ml. in 0·1 M NaCl) in the presence and absence of LSD (Sandoz Batch LSD–25 65002). The effect of LSD on the circular dichroism spectra of DNA was observed using a double sector cell (two fused 1 cm cells) so that whether the DNA and LSD are in contact (both in the same compartment with 0·1 M NaCl in the other compartment) or separated (DNA in one compartment and LSD in the other) the concentrations of LSD and DNA in the incident beam of polarized light are identical. In a similar way, I measured the effects of ethidium bromide (a molecule known to interact with DNA by intercalation[9]) on the circular dichroism spectra of calf thymus DNA.

Marked changes in the ultraviolet circular dichroism spectrum of DNA (Fig. 1) are observed in the presence of LSD at a ratio of LSD to DNA phosphate of 0·5. The LSD-induced changes in the DNA spectrum are characterized by a decrease in the positive band at 276 nm, a shift in the crossover point from 256 nm for DNA in the absence of LSD to 260 nm for DNA in the presence of LSD, a strengthening and broadening of the negative band at 245 nm, and a strengthening and slight blue shift in the positive band at 220 nm. The nature of the apparent LSD-induced conformational change in calf thymus DNA seems to be somewhat different from the anticipated effect of a secondary structure perturbant. As pointed out by Mommaerts[10], any disturbance of the optimally matched helical structure of DNA has been found to lead to a disappearance of the negative and a strengthening of the positive circular dichroism bands. The reverse is the observed effect of LSD on the circular dichroism spectrum of calf thymus DNA, suggesting a helical structure supporting role for the hallucinogen.

LSD, a planar, cationic, aromatic molecule (Fig. 2) displays the molecular characteristics necessary to stack with the phosphate anions. Intercalation into the DNA helix in this fashion has been shown to be the mode of DNA binding exhibited by the dye ethidium bromide[9]. The observed changes in the circular dichroism spectrum of calf thymus DNA in the presence of ethidium bromide are shown in Fig. 3. Although this circular dichroism spectrum is complicated above 280 nm by a dye absorption band, λ_{max} 286 nm, the effect of the dye below 280 nm is markedly similar to that of LSD. The enhancement and broadening of the major negative DNA circular dichroism band at 245 nm shown by ethidium bromide is identical to the effect of LSD in this region of the DNA spectrum.



Lysergic acid diethylamide

Fig. 2.

On the basis of these results, it is suggested that LSD may intercalate within the DNA helix in a manner similar to ethidium bromide causing changes in the conformation of the DNA. It seems unlikely that interaction of LSD with chromosomal DNA in this manner would decrease the internal stability of the DNA helix to a large enough extent to cause breakage.

It is, however, reasonable to suggest that LSD may cause the dissociation of histones from the chromosomal DNA by neutralizing the phosphate anions on the DNA helix backbone in the process of forming an intercalated complex with the DNA. The dissociation of histones, in turn, might well render the chromosomal DNA susceptible to enzymatic attack and breakage. I have observed LSD-induced changes in the circular dichroism spectrum of DNA-poly-L-lysine complexes which would support such a hypothesis and will present them in detail at a later date.

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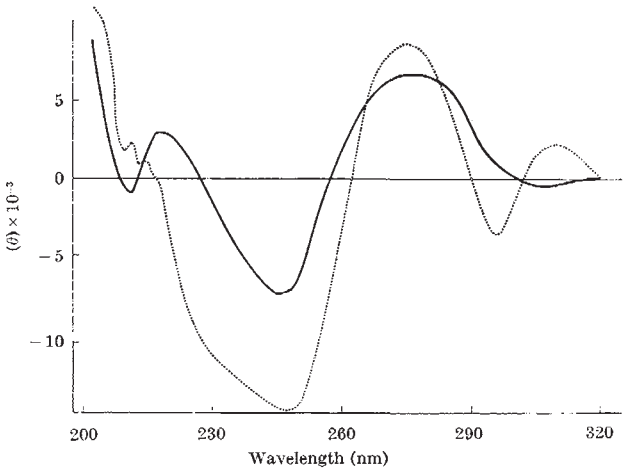

Fig. 3. Circular dichroism spectra of calf thymus DNA separated from ——— and in contact with .... ethidium bromide. Concentrations: DNA, 0·02 mg/ml., and ethidium bromide, $3·3 \times 10^{-5}$M. 0·1 M NaCl, *p*H 7·0, 25°. A mean residue weight of 336·84 for calf thymus DNA

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Stimulation of DNA Synthesis in Resting Stage Human Fibroblasts after Infection with Rous Sarcoma Virus

ROUS sarcoma virus (RSV) suspensions can induce the synthesis of deoxyribonucleic acid (DNA) and mitosis in resting stage human fibroblasts[1]. The virus can also induce DNA synthesis in undividing myotubes[2] and DNA synthesis and mitosis in resting stage chick embryo fibroblasts[3,4]. The stimulatory activity may not be identifiable with the virus particle, for when the virus suspension was treated with RSV anti-serum the focus forming capacity was inhibited but the stimulatory action was not eliminated[1]. We have now extended these findings. The WI-38 human embryonic line[5] was used maintained in Eagle's minimal essential medium[6] supplemented with 10 per cent calf serum and aureomycin (50 μg/ml.). RSV suspensions were obtained from the supernatants of infected chick embryo fibroblastic cultures, and had a titre of 10^5 FFU/ml. DNA synthesis was estimated by adding 0·1 μCi/ml. of labelled thymidine (^{3}H-TdR) (specific activity, 14 Ci/mmole) to the cultures. The cells were fixed 24 h later, DNA was extracted[7] and the radioactivity incorporated was measured in a liquid scintillation counter.

The mucopolysaccharide used was isolated from *Mycobacterium tuberculosis* Penrois by a technique described earlier[8]. WI-38 cells were pooled and subcultured in new 60 mm plastic Petri dishes and used in the experiment 7 days later when mitotic activity was negligible. Medium was removed, kept at 37° C and 0·5 ml. of the following suspensions were added to duplicate cultures: RSV, RSV + 25 U/ml. of hyaluronidase, 100 μg/ml. of mucopolysaccharide and 100 μg/ml. of mucopolysaccharide + 25 U/ml. of hyaluronidase. All these suspensions were kept in a water bath at 37° C for 30 min before the experiments were carried out. To other cultures was added 0·5 ml. of RSV suspension, and the mixture was kept frozen until use. All cultures were kept for 1 h at 37° C in a humidified atmosphere with 5 per cent CO_2. The medium in which the cultures had been grown (used medium) was then added, supplemented with tritium-labelled thymidine. Fresh medium, supplemented with ^{3}H-TdR, was added to sister cultures, some which had been treated with hyaluronidase and some which had not. Finally, ^{3}H-TdR was added to two untreated cultures which were kept as controls.

The results in Table 1 show that significantly more radioactivity was incorporated into DNA after the addition of RSV suspensions, thawed or kept at 37° C for 30 min before adding to the cultures, than in the controls. The same virus suspension, treated with hyaluronidase, did not stimulate the incorporation of radioactive precursor into DNA compared with the DNA of the controls. More labelled precursor was incorporated after the cultures had been treated with a mucopolysaccharide but not if the same suspension was also treated with hyaluronidase. The enzyme itself was not responsible for the diminished incorporation on ^{3}H-TdR because a change of medium which is known to induce DNA synthesis in resting stage fibroblasts[9] had a stimulatory effect in spite of the treatment of the cultures with hyaluronidase. Thus the results indicate that the stimulation of DNA synthesis after infection with RSV may be caused in part by mucopolysaccharides present in the suspensions. To find out whether any other polysaccharides can induce DNA synthesis, used medium was removed and 0·2 ml. of Eagle's medium without serum (EE) containing 100 μg/ml. of DEAE cellulose was added to ten cultures. Another ten cultures received 0·2 ml. of EE. The cultures were incubated at 37° C for 0·5 h and the used medium was replaced with 0·1 μCi/ml. of ^{3}H-TdR. As Table 2 shows, cultures treated with DEAE contained significantly more radioactivity than the controls.

Table 1. TOTAL C.P.M. AFTER 24 h OF LABELLING IN DUPLICATE CULTURES TREATED AS INDICATED

Controls	11,460 (±115)
RSV thawed immediately before the experiment	29,400 (±294)
RSV kept at 37° C for 30 min before the experiment	30,660 (±307)
RSV + Hy	12,300 (±123)
mps	21,660 (±217)
mps + Hy	9,570 (±96)
New medium	93,060 (±930)
Hy + new medium	115,380 (±1,160)

Hy, hyaluronidase; mps, mucopolysaccharide.
The 95 per cent confidence limits are indicated in parentheses.

Table 2. AVERAGE C.P.M. FOUND AFTER 24 h OF LABELLING IN CONTROLS AND CULTURES TREATED WITH DEAE

Cultures treated with	c.p.m.
EE	28,650 (±2,578)*
EE with DEAE	46,740 (±4,206)*

* ($t = 8{\cdot}52$; $P < 0{\cdot}001$).
The 95 per cent confidence limits are indicated in parentheses.

Our results show that treatment of resting stage cultures with RSV suspensions stimulates DNA synthesis during the following 24 h. A similar effect is obtained with a mucopolysaccharide extracted from a bacterium and with a synthetic polysaccharide. Hyaluronidase eliminates the stimulatory action of the former and of RSV suspensions. Cell division is necessary for the successful infection by RSV[10], and so the stimulation of cell division by RSV suspensions might be brought about by some factor present in the suspensions but not identical with the virus, which influences viral replication.

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