Conformation of N^6-Methyladenine, a Base Involved in DNA Modification: Restriction Processes

Abstract. *Crystal structures of* N^6,N^9*-dimethyladenine and* N^6*-methyladenine hydrochloride were determined from three-dimensional x-ray diffraction data. The bases assume a conformation in which the N(6)-methyl group blocks one of the hydrogen-bonding sites normally used by adenine to form Watson-Crick pairs with thymine in double-helical DNA. When in this conformation,* N^6*-methyladenine residues might alter the secondary structure of DNA, thereby preventing the scission of modified DNA's by restriction enzymes.*

Considerable evidence suggests that N^6-methyladenine (6-MA) has profound effects on the susceptibility of double-helical DNA to cleavage by restriction endonucleases. When produced by modification enzymes at those specific DNA sites that can be cut by restriction enzymes, 6-MA protects the DNA from scission (*1*). Little is known about the role of 6-MA, but it is possible that this modified base blocks restriction processes by altering the secondary structure of DNA at restriction sites.

In support of this possibility, we found that, in the crystal structure of 6-MA, the base assumes a conformation which would prevent normal Watson-Crick pairing with thymine in double-helical DNA (*2*). However, this conformation has not been unequivocally established as the most stable for 6-MA, since it may have been simply a consequence of the particular solid state forces in the 6-MA crystal structure.

We now describe the crystal structures of N^6,N^9-dimethyladenine (6,9-DMA) and N^6-methyladenine hydrochloride (6-MA · HCl), both of which display the same conformation as 6-MA, despite considerable difference in their crystalline environments. Our findings provide additional evidence that the preferred conformation of 6-MA is one that would affect the secondary structure of modified DNA.

Monoclinic crystals of 6-MA · HCl were obtained by diffusing ethanol into an aqueous solution. The space group is $P2_1/m$, with $a = 9.345(2)$, $b = 6.584(1)$, $c = 7.314(1)$ Å, $\beta = 114.84(1)°$; the unit cell contains two 6-MA · HCl moieties, and all atoms, except two hydrogen atoms of the methyl group, lie in the mirror planes at $y = \frac{1}{4}$ and $y = \frac{3}{4}$. Monoclinic crystals of 6,9-DMA were obtained from a methanol-chlorobenzene solution by slow evaporation. The space group is $P2_1/c$ with $a = 12.045(3)$, $b = 6.135(2)$, $c = 23.271(10)$ Å, and $\beta = 111.79(3)°$; the unit cell contains eight molecules with two crystallographically independent molecules in the asymmetric unit. Three-dimensional intensity data were collected on an automated diffractometer by use of nickel-filtered copper radiation, a scintillation detector, and a θ–2θ scan technique. All independent reflections with $2\theta \leq 128°$ were measured, including 2615 reflections for 6,9-DMA and 734 reflections for 6-MA · HCl. A suitable trial structure for 6,9-DMA was obtained by direct methods, with the use of the computer program MULTAN (*3*). The trial structure for 6-MA · HCl was obtained by the heavy-atom method. The structures were refined by least squares. Hydrogen atoms were located in difference Fourier maps, calculated during the latter stages of refinement. Final cycles of refinement included all positional parameters, anisotropic temperature parameters for the nonhydrogen atoms, isotropic temperature factors for hydrogen atoms, and a secondary extinction parameter. The final R index ($\Sigma||F_o| - |F_c||/\Sigma|F_o|$), based on all reflections, is 0.053 for 6-MA · HCl and 0.071 for 6,9-DMA (F_o is the observed structure factor and F_c is the calculated structure factor).

Figure 1, a and b, shows the conformations of the two crystallographically independent molecules of 6,9-DMA, and Fig. 1c depicts the conformation of 6-MA · HCl. Like the crystal structure of 6-MA, all three of these molecules display a conformation in which the amino group is nearly coplanar with the purine ring, and the methyl group is directed away from the imidazole moiety of the base [the N(6)-C(MET) bond is *trans* to the C(5)-C(6) bond]. No apparent relation exists between the crystal-packing and hydrogen-bonding schemes in the crystal structures of 6-MA, 6,9-DMA, and 6-MA · HCl. Even in the crystal structure of 6,9-DMA, the two crystallographically independent molecules are in different environments and participate in different hydrogen-bonding and crystal-packing interactions. Thus, we now have four examples of 6-MA derivatives that assume closely related conformations, in spite of the fact that the molecules are subjected to different solid state forces.

Molecular orbital calculations indi-

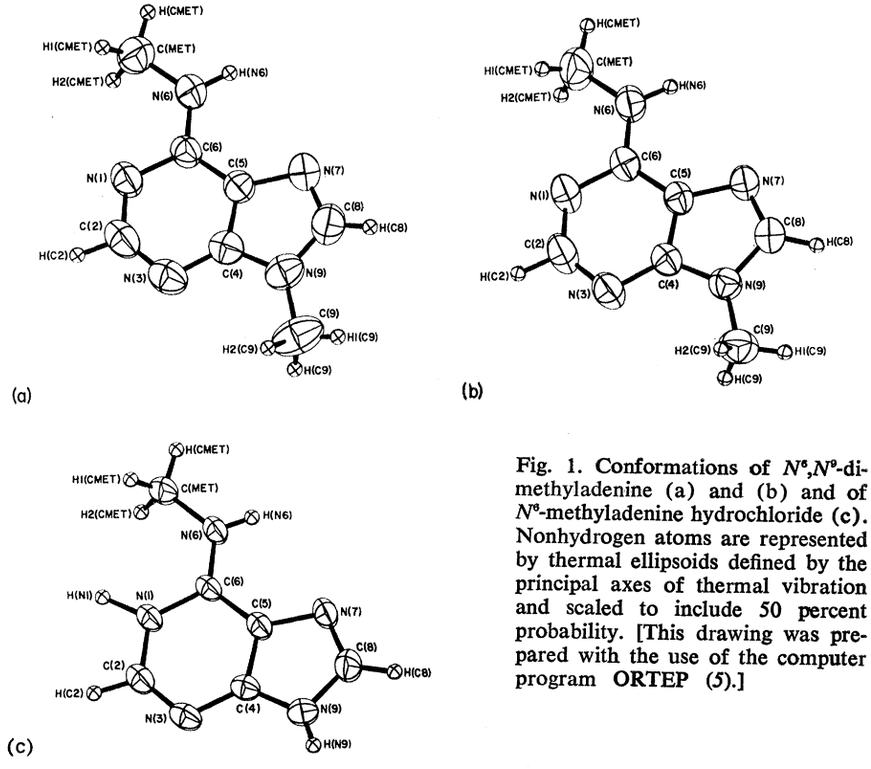

Fig. 1. Conformations of N^6,N^9-dimethyladenine (a) and (b) and of N^9-methyladenine hydrochloride (c). Nonhydrogen atoms are represented by thermal ellipsoids defined by the principal axes of thermal vibration and scaled to include 50 percent probability. [This drawing was prepared with the use of the computer program ORTEP (*5*).]

cate that 6-MA has two minimum energy conformations corresponding to configurations in which the amino group is coplanar with the purine moiety and the methyl substituent is directed either toward, or away from, the imidazole ring of the base (*4*). When directed toward the imidazole moiety, the methyl group would not prevent Watson-Crick pairing between 6-MA and thymine. However, in the alternate conformation, 6-MA would interfere with normal Watson-Crick base pairing, because the methyl group blocks the N(6)-H site that is used for hydrogen bonding between adenine and thymine. In agreement with our crystallographic results, the molecular orbital calculations predict that the most stable conformation for 6-MA is the one that would disrupt normal Watson-Crick pairing. A Corey-Pauling-Koltun space-filling molecular model of 6-MA indicates that there is steric hindrance between the methyl group and atom N(7) when the N(6)-C(MET) bond is *cis* relative to the C(5)-C(6) bond, whereas no analogous interaction occurs when C(6)-C(MET) is *trans* to C(5)-C(6).

Since the conformation we have observed for 6-MA is sufficiently stable to persist through various crystalline environments, it is possible that 6-MA assumes the same conformation within double-helical DNA. If so, this modified base would probably exert appreciable effects on DNA secondary structure. By interfering with Watson-Crick pairing within modified DNA, 6-MA could effectively denature those double-helical regions which in the unmodified state would be recognized and cleaved by site-specific restriction enzymes. It is reasonable to assume that such effects on the secondary structure could interfere with the binding of restriction enzymes to DNA, which might explain the role 6-MA plays in protecting DNA from scission. However, if 6-MA were incapable of participating in Watson-Crick pairing, then it would be difficult to rationalize how modified DNA's can replicate and be transcribed with high fidelity. Possibly a less stable conformation which permits Watson-Crick pairing is imposed on 6-MA by replication and transcription enzymes, thereby permitting modified DNA's to function adequately. However, it is also quite possible that the preferred conformation of 6-MA within double-helical DNA is different from that of the free base, since the stabilization provided by Watson-Crick pairing between 6-MA and thymine may be sufficient to maintain the orientation in which N(6)-C(MET) is *cis* to C(5)-C(6).

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Psychologic Stress and Threshold for Repetitive Ventricular Response

Abstract. *A psychologically stressful environment reduced the threshold of the dog's ventricle for repetitive response. Elicitation of such a response indicates the presence of electrical instability and a predisposition to ventricular fibrillation, the mechanism of sudden death.*

Sudden death claims over 400,000 lives annually in the United States. While in the majority of victims the underlying basis is coronary heart disease, the immediate mechanism is an electrical derangement in heart rhythm believed to be ventricular fibrillation (VF). If the afflicted patient is promptly resuscitated, survival is possible for many years. Thus, this arrhythmia represents a reversible electrical accident. It has been postulated that the susceptible patient has an electrically unstable heart characterized by a reduced threshold for ventricular fibrillation (*1*). The present report indicates that psychologic factors can predispose to electrical instability of the heart.

In the normal as well as the infarcted heart, markedly suprathreshold pulses are required to trigger VF when they are delivered during the brief interval of the ventricular vulnerable period. However, when three early sequential pulses are administered, the current required for VF is markedly reduced; and in the animal with infarction, currents at threshold level for diastolic depolarization suffice to precipitate VF (*2*). This technique, designated as sequential R/T pulsing (*3*), was employed in dogs to measure the threshold for repetitive ventricular response in stressful and nonstressful environments. A repetitive ventricular response rather than VF was selected as the end point. The animal does not perceive a repetitive response and therefore can be retested frequently, whereas VF with the attendant traumatic resuscitative procedures precludes psychologic studies. We have found that repetitive firing evoked by sequential R/T pulsing consistently anticipates the development of VF (*4*); the repetitive response occurs when 66 ± 4 percent of the VF threshold current is administered.

In these experiments two bipolar catheters, with platinum electrodes having an interelectrode distance of 1.5 cm, were placed at the apex of the right ventricle via a jugular vein. Both catheters were exteriorized at the nape of the neck and the dogs were permitted to recover for 1 week. Electrical pulsing of the awake animal was achieved with square wave cathodal pulses of 2-msec duration. The timing of each pulse could be varied with an accuracy of ± 3 msec. The current intensity ranged from 0 to 100 ma (constant current, accuracy ± 3 percent). The amplitude of the first stimulus (S_1) was set at twice the mid-diastolic threshold for a single propagated response. The pulse was discharged progressively earlier in the cycle, in 10-msec steps, until a response no longer occurred. This defined the boundary of the effective refractory period for a stimulus of twice threshold intensity (*5*). The delay of S_1 was