

The Conductivity of Single Crystals of 9-Methyladenine

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The conductivity of single crystals of 9-methyladenine(6-amino-9-methyl-purine) has been determined using proton-injecting solution electrodes. The conductivities parallel and perpendicular to the (100) plane are $2.5 \pm 1.9 \times 10^{-13}$ /ohm-cm and $7.0 \pm 2.9 \times 10^{-13}$ /ohm-cm, respectively, at 295°K. The H-bonded pathways lie in the (100) planes. The conductivity is ohmic over the range of applied fields. The temperature dependence of the conductivity was determined parallel and perpendicular to the (100) planes.

Introduction

Although solid-state intrinsic protonic conduction in biological systems has been postulated to occur, the lack of detailed data makes its importance difficult to assess. The biological implications of protonic conduction are far-reaching and thus, the phenomenon deserves further study.

Investigations of the electrical conductivity in biological compounds such as proteins, DNA, or purines and pyrimidines, have revealed that they are predominantly electronic conductors in the dry state but will, after the adsorption of a certain amount of water, become predominantly protonic conductors. The conductivity displayed in the latter state is very much greater than in the former. An adsorbed monolayer of water has been found to raise the conductivity of a protein by $\sim 10^8$ (1). The major effect of the adsorbed water is to lower the true, or dry-state, energy of activation for the conduction process. The reason for the changeover is not known for certain but judging from available experimental data, it appears that there may be several different causes whose importance depends on the structure and properties of the biological material. In hemoglobin, enough water has to be ad-

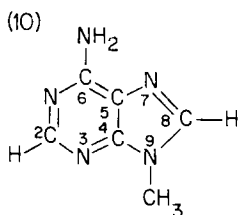
sorbed to create a continuous path of linked water molecules for protonic transport (2). In crystalline DNA, the adsorbed water must cause a change in conformation to a more disordered state in order for protonic conduction to predominate (3). The exact role of water in these processes is not known. It may be possible that the water molecules act (a) as the sole pathway for conduction, (b) as links in the already-present H-bond system facilitating rapid proton transport, (c) to create charge transfer complexes with the host molecule (4), or (d) to function as proton injectors into the biological system.

Although the biological implications have prompted many investigations of the conduction properties of biologically relevant compounds, a major difficulty has been the growth of single crystals suitable for electrical measurements. Consequently, nearly all the investigations have utilized either compressed powders or thin films. Neither of these two forms is particularly advantageous since the measured properties may not be a true reflection of the material itself. Grain boundaries in the powders or films, trapped water or other impurities, and the lack of long-range order in the solid can all interfere with measurements and produce erroneous data. For example, the conductivity of isocytosine was

found to be three orders of magnitude lower for single crystals than for compressed powder samples (5).

Single crystals of several purines and pyrimidines have been grown and X-ray determination of the crystal structures of these compounds have been accomplished. Since these compounds comprise a major part of the polynucleic acids, such as DNA and RNA, and exhibit the H-bonded pairs typical of the macromolecular state, they are of particular interest. The compound chosen for investigation from this group was a purine: 9-methyladenine, MA, (6-amino-9-methyl-purine).

MA is representative of the nucleotide bases that comprise DNA. It closely resembles the nucleoside, thymidine, except that the 2-deoxyribose ring of the latter is replaced by the methyl group. The chemical structure of the compound is shown below.



MA is extensively H-bonded in the solid state and in aqueous solutions to form "base pairs" in the pure state or with other purines or pyrimidines in mixtures. The crystal

structures of MA consists of layers of MA molecules stacked parallel to the *a*-axis (6). Within the layers, the purine molecules are H-bonded to each other as shown in Fig. 1, where a simplified structure is projected onto the (100) plane (not to scale).

As is apparent from Fig. 1, the H-bonding occurring between the MA molecules creates ribbons of interconnected MA molecules, approximately parallel to the *c*-axis. No H-bonds occur connecting the ribbons (parallel to the *b*-axis); this must be due to the presence of the methyl group substituents. Furthermore, no linkages exist between the layers of MA molecules. The interplanar separation, 3.38 Å, is typical of stacking of molecular planes within a crystal, where only van der Waals forces act between the layers.

It is also apparent from Fig. 1 that protonic conduction could occur along the H-bonded chains which lie parallel to the (100) planes. A possible mechanism for protonic transport is illustrated in Fig. 2, where it is proposed that an NH₂ group is protonated, possibly by injection, so that a proton is subsequently transferred to the opposite N, across the H bond. This proton, in turn, could be transferred to the adjacent NH₂ group to the lone pair of electrons on the N forcing further transport of protons.

A transfer of H⁺ from a ring N to the NH₂ group on a neighboring carbon atom is a common occurrence in purines since the transfer creates tautomers of the molecule.

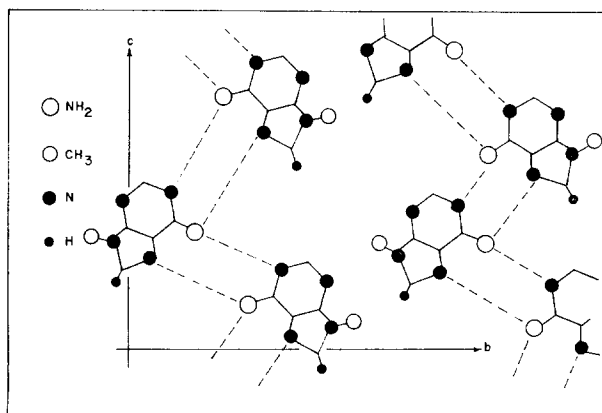


FIG. 1. A schematic illustration of the structure of MA projected onto the (100) plane (6).

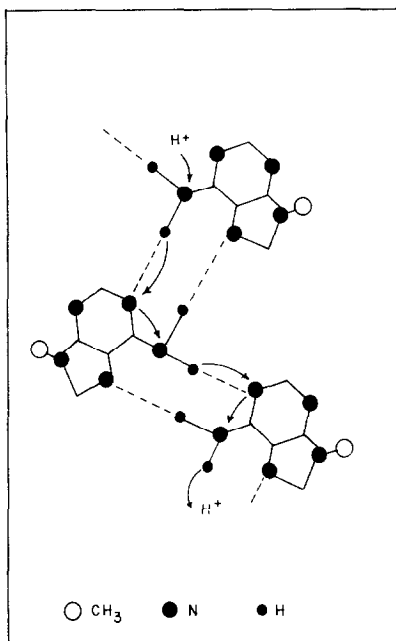
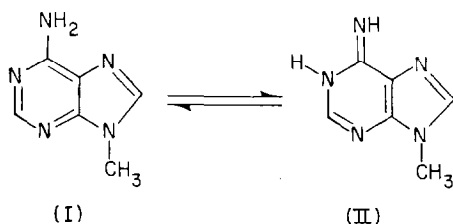


FIG. 2. A possible mechanism for protonic conduction in MA parallel to the (100) planes.

Two structural isomers, which are tautomers of MA, are shown below.



The shift of the H is thought to actually be a transport of a H^+ through an intramolecular H-bond and the subsequent movement of an electron (7). All purines exist in tautomeric forms, so that MA would also exhibit this behavior.

Thus, a proton could be transferred in MA by this process and furthermore, sustained protonic conduction could occur by the NH_2 group rotation reestablishing the initial H-bonded network.

Tautomers of MA are, undoubtedly, already present in MA crystals giving rise to defects within the H-bonded network. For

example, the second tautomer, (II), shown above, would not be able to "pair" in a like manner with (I) as (I) could with itself. If (II) pairs with (I) in the crystal this could give rise to two defects analogous to DL defects in ice. These defects may not, however, play a role in the conduction process.

The crystal structure of MA suggests that it has potential for protonic conduction. Investigation of the conductivity both parallel and perpendicular to the H-bonded planes, (100), should provide information about its ability to conduct protons.

Studies of the electrical properties of the purines and pyrimidines have been mainly concerned with their electronic conductivity. The electronic conductivities of compounds structurally similar to MA in the solid state are given in Table I. The conductivities range from 10^{-17} to 10^{-15} $\text{ohm}^{-1} \text{cm}^{-1}$, with the exception of thymidine which has a somewhat higher value. The activation energies for conduction lie between 2 and 4 eV molecule^{-1} . It is, therefore, not surprising that purines and pyrimidines are generally considered to be effective insulators. Solid-state measurements of the electrical conductivity of polynucleotides also yield very low values, for example the sodium salt of DNA has a conductivity of $\sim 10^{-16}$ $\text{ohm}^{-1} \text{cm}^{-1}$ (8), (when measured as loose fibers or lightly compressed powder).

These values have been obtained almost exclusively from compressed powders so they may not represent the true conductivities but they give an indication of the general behavior of these solids.

The energies of activation for the compounds in Table I have been calculated from the standard equation for electronic semiconductors or insulators which is very similar to the Arrhenius expression for ionic conductors, i.e., $\sigma = \sigma_0 \exp -E_a/RT$, except that E_a is replaced by $E_G/2$. E_G corresponds to the difference in energy between the valence and conduction band, the "band gap," and $E_G/2$ represents one-half of this difference.

The possibility for protonic conduction in these and similar solids has not been investigated with single crystals, except for isocytosine, IC (5, 11), which resembles MA

TABLE I

ELECTRONIC CONDUCTIVITIES OF PURINE AND PYRIMIDINE COMPOUNDS AND THE ASSOCIATED ENERGIES OF ACTIVATION^a

Compound	σ (ohm ⁻¹ cm ⁻¹)	E_a (ev molecule ⁻¹)	(kj-mole ⁻¹)	T (°K)	Reference
Adenine (CP)	5.9×10^{-17}	2.4	229	393	(9)
Adenosine (CP)	10^{-15}	4.5	429	298	(10)
Cytosine (CP)	2.9×10^{-15}	2.4	229	393	(9)
Quanine (CP)	8.3×10^{-17}	2.6	248	393	(9)
Thymine (CP)	1.1×10^{-15}	1.96	187	393	(10)
Thymidine (CP)	10^{-11} - 10^{-13}	4.7	448	298	(10)
Isocytosine (CP)	$\sim 10^{-11}$	2.8	267	303	(11)
Isocytosine (SC)	7×10^{-14}	2.0	190	473	(11)
Isocytosine (SC)	$\sim 10^{-20}$	2.0	190	298	(11)
Thymine (SC)	2.6×10^{-14}	—	—	298	(12)
Thymidine (SC)	2.3×10^{-13}	—	—	298	(12)

^a (CP) = compressed powder, (SC) = single crystal.

in its H-bonded structure, and thymine and thymidine (12). Although Thomas *et al.* (11) make no statements as to whether or not IC is a protonic conductor in the ultra-dry state, it does not show a greater conductivity with proton-injecting electrodes, Pd/H, than with non-injecting electrodes, gold, which may indicate that conduction is electronic.

It is concluded that the potential for protonic conduction in purines and pyrimidines is still nearly unknown and the study of MA, in the form of single crystals, may provide useful information as to whether or not these compounds can conduct *via* protons.

Experimental

Single crystals of MA were grown by slow evaporation from a saturated solution of MA in distilled and deionized water at 331°K. The MA used was of very high purity (Cyclo Chemical Company).

The typical crystalline habit of MA is shown in Fig. 3. It belongs to the monoclinic class of crystals and the $P2_1/C$ space group. MA crystallizes predominantly in the form of needles, elongated along the a axis and exhibits perfect cleavage parallel to the (100) plane.

Crystals of suitable size and free of visible defects are not easily grown and the typical

sizes of the crystals obtained were $3.0 \times 0.8 \times 0.5$ mm. The crystals were examined microscopically and only those free from visible defects were selected for conductivity measurements.

Due to the small size and fragility of the crystals, the apparatus used for earlier conductivity measurements had to be modified (13). The O-rings in the previously described

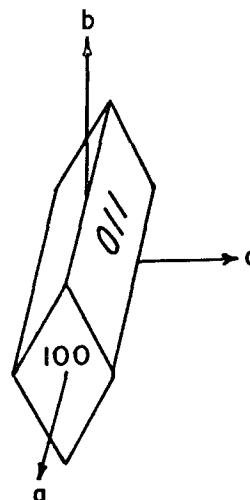


FIG. 3. Crystal habit of MA single crystals employed in this investigation.

apparatus were replaced by Teflon "caps" which were machined to fit the solution chambers exactly. The electrode area could then be reduced to less than $5 \times 10^{-3} \text{ cm}^2$. It was found necessary to keep the "lip" of the Teflon cap at the smallest possible diameter in order to minimize the surface area touching the crystals in order to prevent fracture of the crystals.

The solution chambers were filled with a syringe with the caps in place. Teflon caps appeared to help keep the solution from being trapped between the lip of the cap and the surface of the crystal. Very little excess water would cling to the Teflon after being filled because of its nonwettable nature.

The fragility of the MA crystals necessitated that the technique of keeping the solution chambers tightly clamped to the crystal had to be altered from that used earlier (13). A micrometer head, mounted in a brass plate, was used to apply positive pressure to one side of the apparatus. The micrometer could be adjusted so that pressure could be increased by very slight increments. Coiled springs were placed on the guideposts between nylon spacers to provide counterpressure. With this arrangement, sufficient pressure could be applied to the sides of the crystal to insure proper sealing but damage to the crystals was avoided.

The crystals were mounted between the chambers, after they had been filled, and the apparatus sealed. The apparatus and crystal were allowed to air dry, if the atmospheric humidity was low, for 12–24 hr. If the humidity was high, the apparatus was placed in a dessicated chamber for a like time period. The modified apparatus reproduced the previously measured conductivity of a copper formate tetrahydrate crystal (13).

Measurements of the conductivity (dc) were made with the same equipment as described earlier (13), except that saturated aqueous solutions of MA (in distilled and deionized water) were used as injecting electrodes. The temperature dependence of the conductivity was measured at constant applied field.

To avoid contributions from adsorbed water, the crystals were measured in a dessi-

cated chamber if the atmospheric humidity was greater than 15%. The dessicant used was CaCl_2 which reduces the humidity in a closed vessel to $\sim 7\%$.

Results and Discussion

The conductivity of MA was measured both parallel to the (100) planes ($\parallel(100)$) containing the H-bonded chains, and perpendicular to the (100) planes ($\perp(100)$). The conductivity measured $\parallel(100)$ exhibited ohmic behavior up to the maximum field applied, $\sim 25 \text{ kV-cm}^{-1}$, with no evidence of space-charge-limited behavior. A current density against field plot typical of those measured for MA is shown in Fig. 4. The ohmic conductivity measured $\parallel(100)$ was $2.5 \times 10^{-13} \pm 1.9 \times 10^{-13} / \text{ohm-cm}$ at 295°K , an average of trials on four different crystals. The crystals were too small to incorporate guard rings so that the measurements were made under dry conditions as discussed above.

Under application of the field the current underwent a sharp decrease in magnitude, indicative of polarization within the crystal,

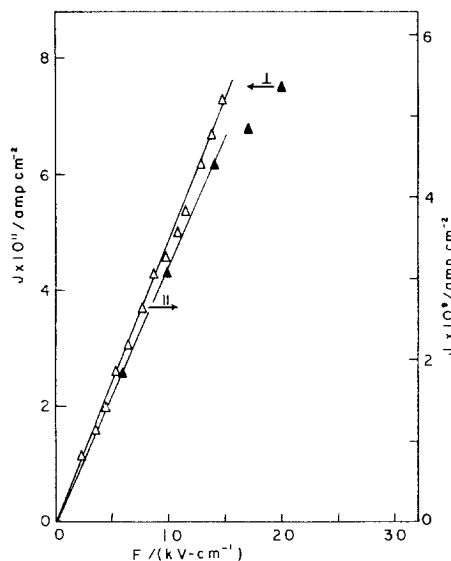


FIG. 4. A plot of current density against field for conduction of MA with proton-injecting electrodes, parallel to (100) planes and perpendicular to (100) planes (295°K).

and then leveled off to a stable value after approximately 20 min, as shown in Fig. 5. This stable value was taken as the conductivity. The current slowly decreased from this value after the field has been applied for ~ 45 min, possibly indicating the presence of another type of time-dependent polarization.

The fall in initial current to a low stable value after application of the field has been observed in other systems (14). It may arise from polarization of mobile dipoles, dielectric displacement currents. After the field was removed, a negative residual current, comparable in magnitude to the positive value of the current carried prior to the removal of the field, was observed. This current decays

in a nearly exponential manner (Fig. 6). The decay may be associated with the relaxation of the polarization under field-free conditions.

The conductivity measured $\perp(100)$ also displayed ohmic behavior under the same fields used for the parallel measurements, although the conductivity was of lesser magnitude. A typical plot of J versus F for the conductivity measured $\perp(100)$ is given in Fig. 4. The value for the conductivity in this direction, from the data of three different crystals, is $7.0 \times 10^{-15} \pm 2.9 \times 10^{-15} \text{ ohm}^{-1} \text{ cm}^{-1}$ at 295°K. This value of σ is 10^2 times lower than that obtained $\parallel(100)$ which indicates significant anisotropic conduction.

Because of the low currents measured

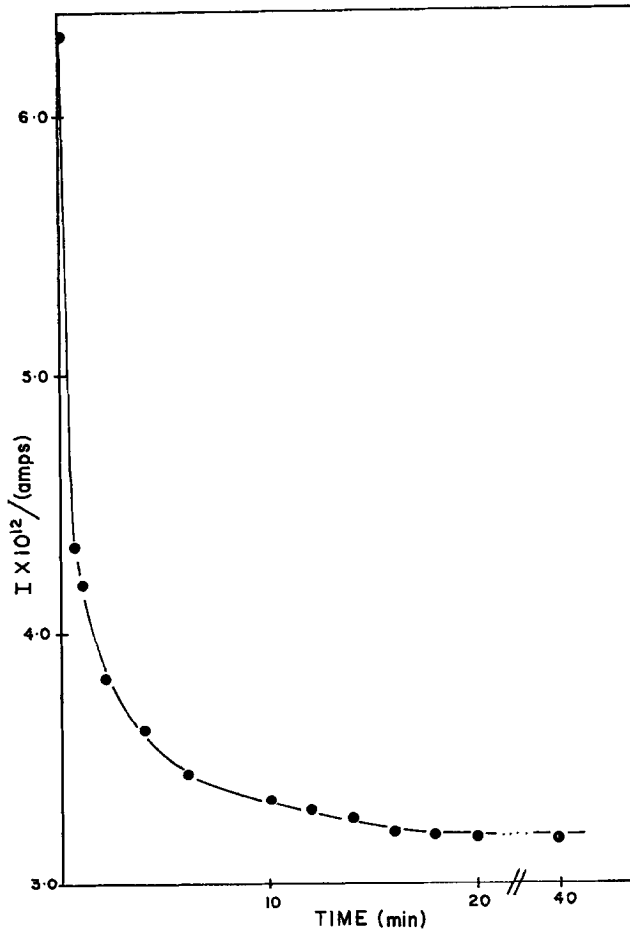


FIG. 5. A typical time dependent plot of current for a MA crystal measured parallel to the (100) planes at a specific applied field (295°K).

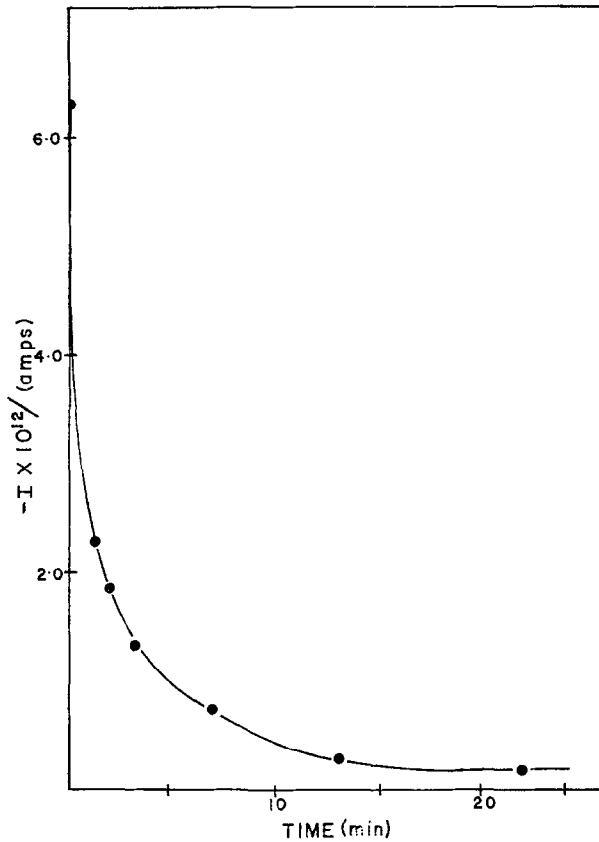


FIG. 6. A typical plot of the time dependent decay of the residual current of an MA crystal measured parallel to the (100) planes (295°K).

$\perp(100)$, considerably more scatter was evident in the J - V characteristic curves and the correlation coefficient was generally only about 0.8, however SCL behavior does not appear to obtain (Fig. 4). At high fields, the current tended toward saturation, which was not observed in the measurements $\parallel(100)$. The behavior of the current after the initial application of the field was similar to that observed $\parallel(100)$.

The effect of humidity on the ohmic conductivities of the MA crystals was small, generally causing a small increase in the conductivity. However, nonohmic behavior was introduced by adsorbed water. Such an effect on the conduction behavior by increased humidity is illustrated in Fig. 7, where A is the behavior of the crystal under dry conditions and B is that under an atmosphere of 12 mm of water vapor pressure,

for a crystal measured $\parallel(100)$. The appearance of nonohmic behavior due to adsorbed water has also been noted in IC (11). Other indications of the presence of adsorbed water were that the current did not drop off as sharply after the initial application of the field as observed under "dry" conditions (Fig. 5) and that instead of stabilizing after about 20 min, the current began to rise slowly with time.

When water is adsorbed on crystals of high resistivity, its presence is usually indicated by a large increase in conductivity. For example, the conductivity of the sodium salt of DNA, as previously mentioned, is 10^{-16} $\text{ohm}^{-1} \text{cm}^{-1}$ in the dry state, but increases to $\sim 10^{-7}$ $\text{ohm}^{-1} \text{cm}^{-1}$ when 27 g of H_2O is adsorbed per 100 g of the salt (8). Even at one-third this amount of adsorbed water, the conductivity was $\sim 10^{-10}$ $\text{ohm}^{-1} \text{cm}^{-1}$.

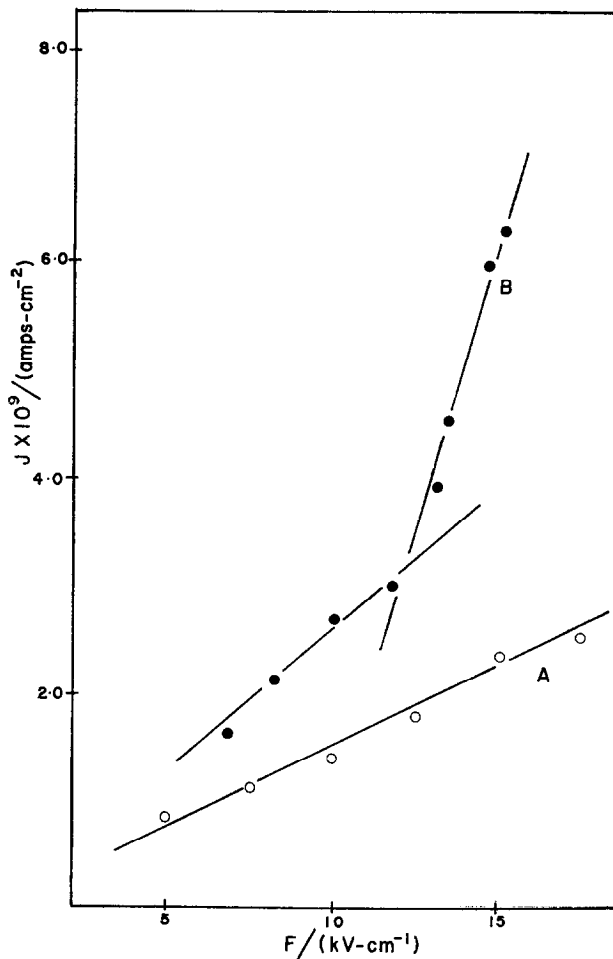


FIG. 7. The effect of water vapor pressure on the current density versus field relationships for MA with proton-injecting electrodes measured parallel to the (100) planes. \circ , dry conditions; \bullet , 11-mm Hg water vapor pressure (295°K).

Comparable behavior was observed for isocytosine (11).

The fact that the humidity has little effect on the magnitude of the conductivity of MA indicates that the measured conduction is a bulk property of the crystal. The anisotropic conduction further supports this, since comparable values of σ measured \parallel and \perp (100) would have been expected if adsorbed water were a major contributor to the conductivity.

A comparison of the conductivity of MA, measured \parallel (100) with values in Table I, shows that the observed conduction is greater than the listed values by about 10^2 – 10^4 times. It is

also greater than that measured for isocytosine by a factor of 10^6 .

The conductivity measured \perp (100) is within the range of conductivities given for electronic conduction (Table I). The conductivity may have a protonic contribution, however, as an examination of the results of the energy of activation studies suggests.

The temperature dependence of the conductivity was measured both \parallel (100) and \perp (100) over the temperature range, 292–341°K (Fig. 8). The temperature dependence has been plotted as $\sigma = \sigma_0 \exp - E/RT$ rather than by the more exact expression $\sigma T = \sigma_0' \exp -$

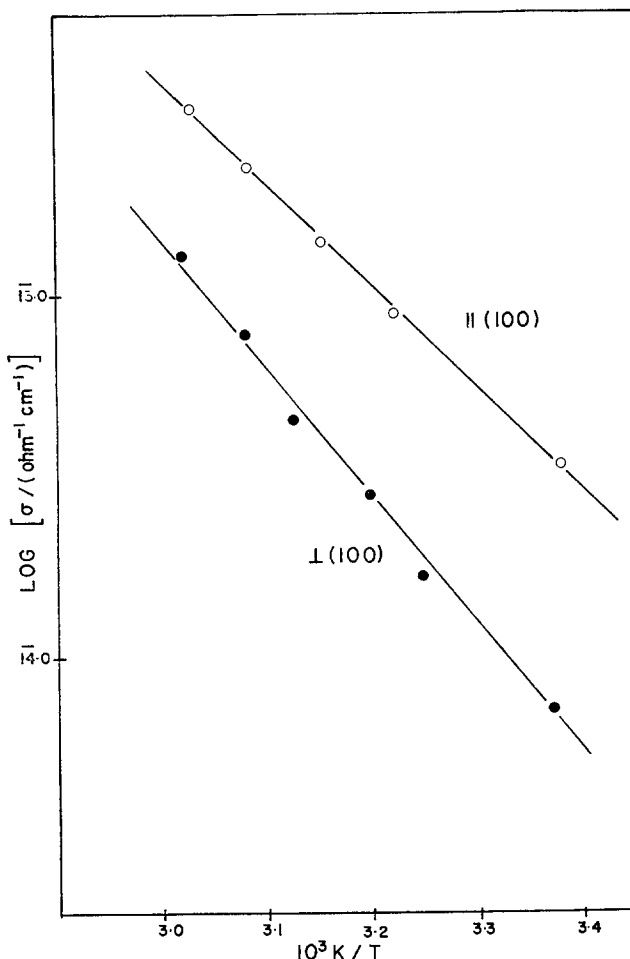


FIG. 8. Arrhenius' plot for conductivity of MA measured with proton-injecting electrodes. o, parallel to (100) planes; ●, perpendicular to (100) planes.

E'/RT because it is believed that the accuracy of the data do not justify the need for the more exact expression. An error of about 5% of E_{act} is introduced according to Glasser (14). The energy of activation for conduction $\parallel(100)$ was determined to be $51.5 \pm 13.4 \text{ kJ/mole}^{-1}$, an average based on one crystal measured several times. By contrast, the energy of activation measured $\perp(100)$ was $75.4 \pm 19.0 \text{ kJ/mole}^{-1}$, based on the data from three different crystals.

The difference in activation energies correlates well with the expected anisotropy if the conduction is protonic and occurs by the postulated mechanism (Fig. 2). The magnitude of the energy of activation is nearly equal to

that observed other protonic conductors. In order to be compared to values given in Table I, the value must be doubled and therefore becomes 103 kJ/mole^{-1} . This value is still significantly less than values which have been attributed to electronic conduction. Therefore, the energy of activation measured $\parallel(100)$ supports the view that conductivity in MA is protonic.

The energy of activation obtained for the conductivity $\perp(100)$, when doubled, is also smaller than observed for other biologically important compounds, and is not inordinately high for protonic conduction. Within the ideal lattice structure of MA, there is no pathway for protonic conduction $\perp(100)$,

but it is possible that water molecules are incorporated between the MA layers. Such water molecules would provide a means of protonic transport $\perp(100)$.

The crystals used were grown from water in the same manner as for the X-ray and neutron diffraction studies, and these studies did not reveal any regular incorporation of water molecules. Therefore, if impurity water molecules are present in the lattice, they must be present in very small amounts.

To determine the amount of water incorporated, if any, in MA crystals, samples of each type of crystal were heated in an oven at 403°K for several days. The MA crystals showed no evidence of dehydration. Their weight loss was negligible.

From the magnitude of the conductivity $\perp(100)$, and an assumed carrier mobility of $\sim 10^{-3} \text{ cm}^2 \text{ V}^{-1} \text{ sec}^{-1}$ (the estimated mobility for protons in ice (14) and lithium sulfate monohydrate (14, 18)), the number of charge carriers necessary would be 10^7 cm^{-3} . If only 10^{-5} of the impurity water molecules present donated protons to the conduction process, the necessary concentration of such water molecules in MA would have to be $\sim 10^{12} \text{ cm}^{-3}$. This concentration would not be detectable using dehydration techniques and would also not be visible in the crystal structure determinations. Therefore, it seems possible that MA could contain enough water to provide for the conduction pathways $\perp(100)$.

It is very likely that the water molecules, if present, would be located between the layers of MA molecules since in the hydrates of analogous compounds, e.g., thymine monohydrate (15) and quanine monohydrate (16), the waters are located between the layers. The water molecules are H-bonded to either an O or N atom that are not already H-bonded. The spacing between the MA layers is large enough to accommodate such inter-layer water.

The conductivity of MA is, therefore, probably protonic, $\perp(100)$, or, at least, has a very significant protonic contribution, and this would account for the comparatively low value of the energy of activation.

The σ_0 values for the MA crystals \parallel and

$\perp(100)$ were 10^{-5} and $10^{-2} \text{ ohm}^{-1} \text{ cm}^{-1}$, respectively. Both values are too low to indicate intrinsic protonic conduction, according to the criteria proposed by Schmidt (17) so that the conduction may be from extrinsic sources, such as injected protons or defect waters. In contrast, the value of σ_0 for the compressed powder measurements of isocytosine is $10^6 \text{ ohm}^{-1} \text{ cm}^{-1}$, while the σ_0 for the single crystal measurements is $10^{-3} \text{ ohm}^{-1} \text{ cm}^{-1}$ (5, 11).

In conclusion, it would appear very likely that MA is capable of protonic conduction. The observed anisotropy of conductivities and measured energies of activation are consistent with protonic conduction and the proposed mechanisms. Furthermore, the values of the energies of activation are of the magnitudes expected for protonic conductors, and are much lower than those expected for electronic conduction.

The absence of nonohmic behavior while indicating, perhaps, a lack of proton injection, also implies a negligible contribution from adsorbed water to the measured conductivities.

These conductivity data represent one of the few examples of successful measurements on single crystals of a purine. As a protonic conductor MA is not as good as ice but is comparable to the inorganic hydrates, $\text{Li}_2\text{SO}_4 \cdot \text{H}_2\text{O}$ (18) and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (19). Therefore, MA's ability to conduct protons is comparatively good and may indeed have a role in biological processes.

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