DEHYDRATION OF CYTOSINE MONOHYDRATE AT PHYSIOLOGICAL TEMPERATURES

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ABSTRACT Neutron diffraction, thermogravimetric, and mass spectrographic measurements have been used to show that cytosine monohydrate loses its water of hydration at physiological temperatures (≃37°C) and converts to cytosine. The "activation energy" for the dehydration process has been determined from isothermal weight curves and is 27.1 ± 0.6 kcal · mol^{-1}. It is suggested that pyrimidine dehydration may be involved in structural changes in DNA.

The possibility that cytosine can bind water at its amino site was pointed out in early structural studies on DNA (Langridge et al., 1960). These same studies also indicated that the bases of DNA assume different orientations as a consequence of humidity changes. Although it is generally accepted that the planar surfaces of DNA bases are hydrophobic, there are experimental (Martel, 1979) and theoretical (Port and Pullman, 1973; Scordamaglia et al., 1977) studies that indicate that water can be bound to the edges of these molecules. The strongest forces binding the water molecule to the bases are assumed to be hydrogen bonds. Because the hydrogen bonds in pure water cannot maintain the solid phase at temperatures above 0°C, one may speculate that bound water molecules associated with bases may also be readily freed at relatively low temperatures. In this communication, we report neutron diffraction, thermogravimetric, and mass spectrographic measurements that show conclusively that cytosine monohydrate (CMH) loses its water of hydration at physiological temperatures near 37°C and is converted into cytosine.

Crystals of CMH were grown by slow evaporation of aqueous solutions at room temperature. Their integrity was verified by neutron diffraction measurements on ground polycrystalline samples. These samples were subsequently heated in an oven in which a relative humidity of 57 ± 3% was maintained at every temperature. The lower profile of Fig. 1 shows the results of a typical neutron diffraction run on a polycrystalline sample of CMH that had been heated for 41 h at 35.5 ± 1°C. The background has been subtracted from the raw data. Both solid and dashed lines are drawn through the experimental points. The solid line shows a fit to the CMH portion of the profile for which lattice parameters given by McClure and Craven (1973) were used. The dashed lines correspond to a similar calculation for which the known lattice parameters for cytosine were used (McClure and Craven, 1973). Clearly, the sum of the two calculated profiles is a good description of the experimental data. This result conclusively shows that the heat treatment of CMH at this temperature results in dehydration with the formation of cytosine. In order to facilitate location of the cytosine peaks produced by dehydration, the calculated cytosine profile is reproduced by the middle (dashed) curve. The uppermost dashed profile is a calculation of the diffraction from uracil. This profile clearly does not form any significant fraction of the experimental powder profile. Consequently, the heat treatment of CMH does not result in the formation of uracil by deamination.

A sample of CMH was then heated for equal time periods of 22 ± 2 h at successively increasing temperatures. The sample was kept in an open vessel in the oven described above, and its weight was measured after each time period. The results are shown in Fig. 2, which shows that the weight of the sample began to decrease at ≃35°C and became constant for temperatures ≳55°C. The measurements of Fig. 2 show that the percentage loss of weight for CMH at the highest temperature is ≃14%. This value agrees well with the weight loss expected (13.95%) from the known stoichiometry of CMH, if complete dehydration is assumed. Mass spectrographic measurements on CMH show that a species with mass 18 is emitted at ≃35°C. This result further suggests that water is the molecular species being lost.

Two samples of CMH were then maintained at constant temperatures of 42° and 46°C, respectively, and their weights were continuously monitored as a function of time for periods up to 100 h. At both temperatures, the sample weights decreased to constant values with weight losses of 13.05 and 13.95%, respectively. These results again agree

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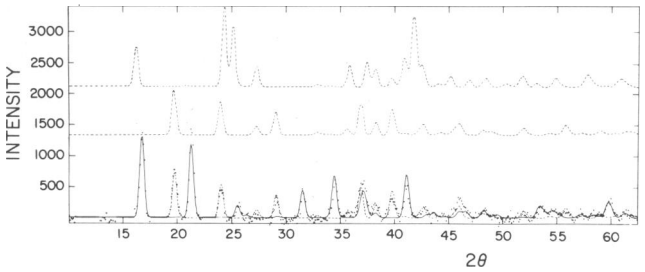

FIGURE 1 Bottom of the figure: neutron diffraction profile observed after polycrystalline cytosine monohydrate had been heated for 41 h at 35.5°C. The scattering angle, 2Θ, is in degrees, and the neutron wavelength was 0.224 nm. The solid-line portion represents scattering from cytosine monohydrate. The dashed curve through a portion of the data points in the lowest profile defines peaks from pure cytosine that evolved upon dehydration. The calculated cytosine profile is reproduced in the middle (dashed) profile. The uppermost (dashed) profile represents a calculation for uracil.

well with the assumption of complete dehydration. Both samples were studied by neutron diffraction after being weighed, and both were found to be pure cytosine with no detectable traces of CMH.

The isothermal curves of sample weight as a function of time are shown in Fig. 3. The curves are analogous to the isothermal annealing curves of defects in metals, and both can be well described by an exponential decay as a function of time. If the fractional weight of water molecules is n, and the inverse time constant for decay is C, then both curves are represented by $n = n_0 e^{-Ct}$, where n_0 is 0.145 and 0.139 of the total sample weight for 42° and 46°C, respectively. By "least squares" fitting these curves, we find $C(42°C) = 0.0442 \pm 0.0008\ h^{-1}$ and $C(46°C) = 0.076 \pm 0.002\ h^{-1}$. If we assume that the dehydration is an activation process, then its rate equation has the form $dn/dt = -F(n)\ K_0 e^{-E/kT}$, where $F(n)$ is any continuous

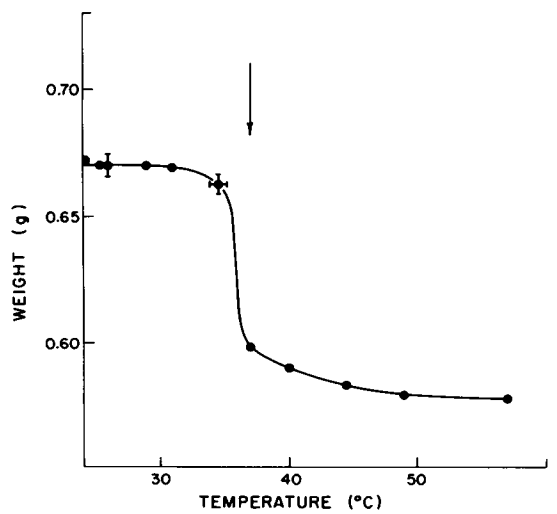

FIGURE 2 Observed weight of a sample of CMH as a function of successive heat treatments each lasting 22 ± 2 h (isochronal curve). The arrow is at 37°C.

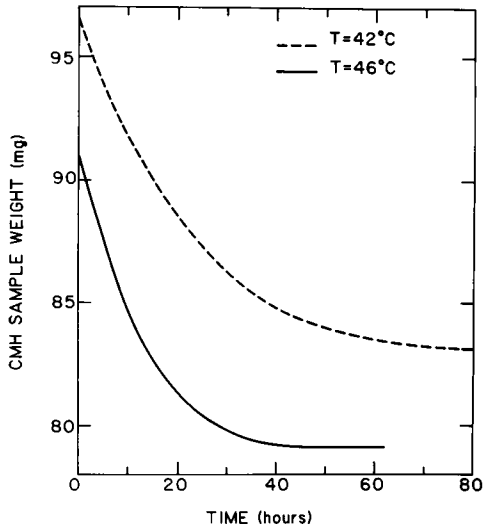

FIGURE 3 Observed weights of samples of CMH held at constant temperatures (42° and 46°C) as a function of time (isothermal curves).

function of n, K_0 is a constant, and the activation energy, E, is assumed to be independent of n. It can be shown that the times, t_1 and t_2 needed to reach the same fractional weight of water molecules, n, at temperatures T_1 and T_2 are related by

$$\ln\left(\frac{t_1}{t_2}\right) = \frac{E}{k}\left(\frac{1}{T_1} - \frac{1}{T_2}\right).$$

But since, within experimental error, n_0 is constant (=13.95%), then $t_1/t_2 = C_2/C_1$. For the curves shown in Fig. 3, we find $E = 27.1 \pm 0.6$ kcal · mol^{-1}. A recent calculation (Powell and Martel, 1981) suggests that the binding energy of a water molecule in the CMH lattice is ≃14 kcal · mol^{-1}. The difference between these two energies may be due to several effects. The experimental activation energy includes a contribution for the energy necessary to restructure the CMH crystal lattice into that of anhydrous cytosine and a further contribution for the energy necessary to promote diffusion to the surface and subsequent evaporation of the water molecule.

In conclusion, our measurements show that water is readily evolved from CMH at temperatures near 37°C with the formation of cytosine. The dehydration appears to satisfy the rate equation for activation processes and has an activation energy of 27.1 ± 0.6 kcal · mol^{-1}. There is also evidence that both thymine monohydrate (Gerdil, 1961) and 5-nitrouracil monohydrate (Craven, 1967) slowly effloresce at room temperature. These results all suggest that water is rather weakly bound to DNA pyrimidines (at least in a monohydrate configuration) and may become mobile at temperatures near 37°C. Such mobility might facilitate conformational changes during replication or recombination. Local heating by biochemical action could, for instance, lead to the dehydration near a recombination tetrad where the bases would be in close contact. CMH

provides a simple, well-defined model system for testing calculations such as those of Scordamaglia et al. (1977) of the binding energy of water in biological systems. It is a significantly simpler system than the coenzyme B_{12} recently studied by Finney (1979).

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