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Note

Calorimetric investigations of crystalline 2′-deoxynucleosides

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The biological activity of nucleic acids is closely dependent on characteristic short range forces, hydrogen bonding, π-complexing and dipole–induced dipole interactions, which stabilize the helical structure of DNA. These forces are reflected in the stacking and bond-free energies between various purine and pyrimidine base pairs in DNA, and determine in part the melting behavior of that polymer[1,2]. Consequently a quantitative and qualitative understanding of these forces may be obtained through an examination of the melting behavior and thermal properties of the nucleosides themselves in relation to their known structural characteristics, which govern molecular packing and thereby determine the architecture of the crystals.

Differential enthalpic analysis provides an especially suitable method for directly measuring the magnitude of the forces maintaining the crystal lattice and also the energy differences associated with thermal processes. We present here the results of a calorimetric investigation of physical and chemical changes which a series of 2′-deoxyribonucleosides undergo when subjected to a programmed input of thermal energy.

Enthalpimetric measurements were made with a Perkin–Elmer DSC-1B differential scanning calorimeter which was standardized in terms of the known heats of fusion of indium and tin under an atmosphere of nitrogen, scan rate pf 20°/min and a sensitivity setting of 2. The accuracy of this value was checked by determining the heat of fusion of high purity samples of benzoic acid and comparing it with the literature value[3].

The samples of the 2′-deoxynucleosides used in this study (Table 1) were of the highest purity commercially available (Schwartz Bio Research and Nutrional Biochemicals Corp.) and were utilized as obtained. At least three thermal runs were made on each compound. All readings for a given transition were in agreement to within the limits of instrumental error and were reproducible to within ±1 °K for sharp transitional changes and ±1.5 °K for broader ones. Minimum samples (0.5–1.0 mg) were weighed on a Cahn Balance and encapsulated in aluminum pans. The areas of the curves were measured[2] several times with a planimeter accurate to 0.1 cm.

Calculations of enthalpy were based on the measured peak areas and corresponding sample weights. The errors involved in each of these measurements

TABLE I

TEMPERATURES CORRESPONDING TO ENDOTHERMIC MINIMA AND EXOTHERMIC MAXIMA, HEATS OF FUSION AND ENTHALPIES OBTAINED FOR NUCLEOSIDES OBTAINED AT A HEATING RATE OF 20 °K/min

Nucleoside	*Dehydration*		T_{M_1} *Endo*	T_M *Exo*	T_{M_2} *Endo*	ΔH *kcal/mole*
	°K	°K				
2′-deoxyuridine	—	—	438	506	604	6.7
2′-deoxycytidine–HCl	—	—	457	459	542	5.6
2′-deoxyadenosine (H_2O)	398	438	462	496	dec.	8.3
2′-deoxyguanosine·$2H_2O$	400	438	469	—	483	5.8
thymidine	—	—	464	526	588	7.0
2′-deoxyinosine	—	—	483	491	524	7.6
2′-deoxycytidine	—	—	486	—	dec.	5.0

combined with the agreement between the experimental and literature values of ΔH for lead and benzoic acid indicated that ΔH was accurate to within 4% with a precision of 2%. The error in the calculated entropy value was obtained from the square root of the sum of the squares of the error in enthalpy and temperature. This value was approximately 4% for sharp transitions.

The appropriate measurements and calculations were made for ΔH and ΔS on the basis that the initial endothermic processes (following elimination of water) for the series of compounds examined were phase changes of the melting type. If it is assumed that the free energy at the transition point is equal to zero ($\Delta G = \Delta H - T\Delta S = 0$), then a general dependence, $T_t = \Delta H_t/\Delta S_t$ is obtained which relates the transition temperature directly to the enthalpy and entropy of the transitions for the structural change[4].

The response of molecules to thermal treatment has been utilized as a probe or indicator of structural integrity for each of the compounds studied in this investigation Thus the crystalline 2′-deoxynucleosides, when examined by DSC generally exhibit a series of endothermic peaks (Fig. 1) which corresponds to a number of processes related to different structural and compositional properties.

Of the purines and pyrimidines examined, the lowest enthalpy value (1.8 kcal/mole) was obtained for 2′-deoxyguanosine hydrate. Haschmeyer and Sobell[5] have described this compound as having the unique characteristic of existing in the syn form and having little restriction on rotation about the glycosidic bond. The syn form could only be stabilized by a possible intramolecular hydrogen bond between the N(3) of guanosine and the O(5′) of the sugar. None of the other possible hydrogen bond donors or acceptors appear to contribute to the overall stabilization of the lattice, a factor which accounts for the low enthalpic value obtained. Thus it appears that most of the hydrogen bonding sites are involved in complexation of water molecules within the crystal lattice, necessitating that the lattice be held together by different forces (*e.g.* dipole–induced dipole and π-complexation).

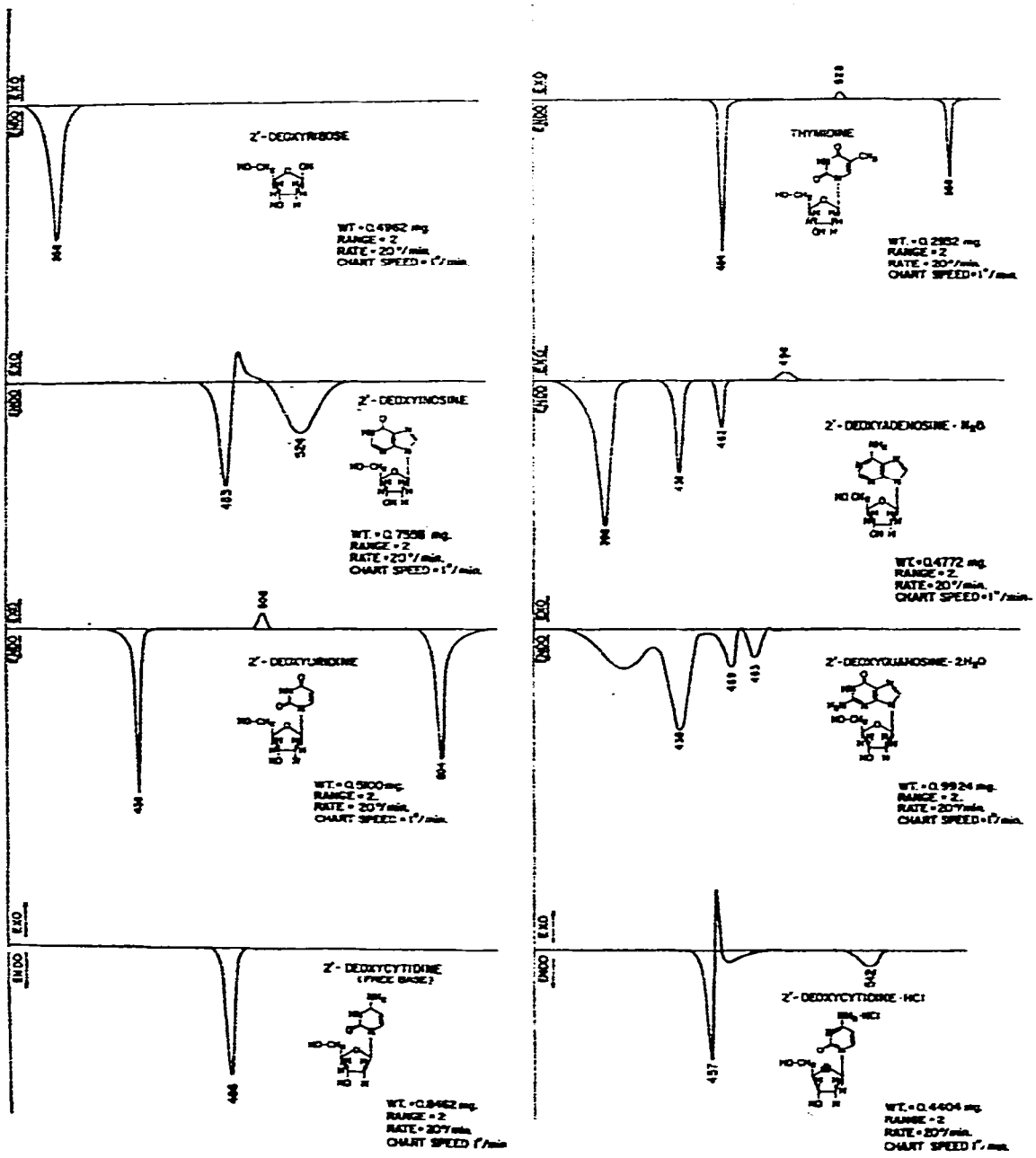

Fig. 1. Thermograms of a series of 2′-deoxyribonucleosides obtained over the temperature range of 300–600 °K.

A low enthalpic value cannot be attributed to all hydrated forms of the 2′-deoxynucleosides however. While the dihydrate of 2′-deoxyguanosine provides the lowest value for the series examined, the monohydrate of 2′-deoxyadenosine shows the highest (8.3 kcal/mole) indicating an alteration in the intermolecular forces. Indeed

an outstanding feature, of the deoxyadenosine monohydrate system, is that packing of the molecules in the crystal is determined by hydrogen bonds in which all available groups participate and the resulting infinite chains of HN···H bonds between bases are related by a screw axis and a distorted trigonal arrangement of HO···H bonds formed by water molecules[6].

A further difference between the thermograms of hydrated and non-hydrated nucleosides is the presence, as anticipated, of additional endothermic peaks. For 2′-deoxyadenosine, the values of T_M for these peaks are 398°K and 438°K, whereas the dehydrated compound melted at 465°K (T_M), a value similar to the reported melting point[7]. The response of deoxyguanosine on heating, on the other hand, is represented by four endothermic peaks, the first two of which are similar to those of 2′-deoxyadenosine and occur at temperatures, 398°K and 438°K, indicating the removal of water of crystallization, which is retained by the complex in possibly lattice and coordinated forms. Of the two additional endotherms, the one at 467°K is associated with melting while the higher one at 483°K corresponds to a process generally characteristic of 2′-deoxynucleosides.

Deoxyadenosine and deoxycytidine, both of which have an amino group at the C(4) position of the pyrimidine moiety, differ from other 2′-deoxynucleosides examined, insofar as the melting transitions (462 and 486°K respectively) are followed by decomposition on further heating over the range examined (300–600°K) However, when deoxycytidine is converted to the hydrochloride, the salt is sufficiently stabilized so that the initial melting process which occurs at 457°K, is followed by a second endothermic process characteristic of thymidine, 2′-deoxyuridine, 2′-deoxyinosine and 2′-deoxyguanosine (Table 1).

The enthalpic values obtained for 2′-deoxyinosine, thymidine and 2′-deoxyuridine (7.6, 7.0 and 6.7 kcal/mole respectively) indicate that the base stacking forces decrease in the order:

2′-deoxyinosine > thymidine > 2′-deoxyuridine

The greater stacking force of the purine, 2′-deoxyinosine is attributable to increased π delocalization of the purines in comparison to the pyrimidines and hence has a greater availability of π electrons.

In the case of thymidine, the introduction of a methyl group on to the pyrimidine ring increases the electron density on the ring due to a positive inductive effect, thereby increasing the π bonding between infinite stacks of partially overlapping bases.

Thymidine displays two distinctly separated endotherms with their respective endothermic maxima T_{M_1} and T_{M_2} at 464°K and 588°K and a very weak exotherm at 528°K. The higher temperature endotherm is associated with a reversible process whereas the lower one is not. It is apparent that some chemical change has occurred following melting. The second endotherm is attributed to the melting of a new compound resulting from homolysis of the glycosidic bond[8]. This homolytic process appears to be common to all 2′-deoxyribonucleosides examined, with the exception

of 2′-deoxyadenosine and 2′-deoxycytidine, which undergo decomposition, and accounts for the highest temperature endotherms obtained in these studies. The fact that cytidine hydrochloride shows an exotherm associated with melting of rearranged reagent whereas the free base does not, suggests that the rearrangement occurs at a temperature above the melting point, possibly in the region of the weak exotherm. The exact nature and mode of formation of these compounds is presently under investigation.

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