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THERMAL STUDIES ON THE POLYMERIZATION OF A URIDINE–URIDINE-3′-(2′)-PHOSPHATE MIXTURE

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The heats of fusion for crystalline uridine and its phosphate derivative, uridine-3′-(2′)-phosphate, as well as the enthalpic and entropic values for the polymerization of a mixture of these compounds, have been determined by Differential Scanning Calorimetry. An analog computer analysis of the exotherm associated with heating of the mixture indicated a minimum of five processes, which could be rationalized on the basis of previously published experimental findings.

The conformational transformations of nucleic acids and their constituents upon being heated in solution have attracted great attention since it is evident that the study of this process can give definite information about the forces that stabilize these structures [1–3].

Less attention, however, has been focussed on the interaction of the individual constituents and the structural changes – physical and chemical – they undergo on heating in the solid or crystalline state. Accordingly the information that can be ascertained from such an investigation – the heats and entropies of fusion – of the different nucleic acid units may prove valuable in the application of theories of melting of ordered polynucleotides [4] to the interpretation of RNA and DNA structures [4–6].

Experimental

The temperature range of a Perkin Elmer DSC-1B differential scanning calorimeter (DSC) was calibrated against standards of known melting points at various scan speeds (5, 10 and 20°/min) under an atmosphere of nitrogen.

A calibration of the DSC in terms of energy (cal/g) was obtained in terms of the known heats of fusion of indium and tin at scans of 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 with literature values [7].

The samples of uridine and uridine-3′-(2′)-phosphate utilized were of the highest purity commercially available (Schwartz Bio Research, and Nutritional Biochemicals Corp.). Each of the transitions or melting points of the compounds under investigation was obtained at three or more scan rates. All readings for a given transition were in agreement within the limits of instrument error and were reproducible to within $\pm 1°$ for sharp changes in transition and $\pm 1.5°$ for broader ones. Minimum samples (0.5 – 1.0 mg) were weighed on a Cahn Balance and encapsulated in aluminium pans. The areas of the curves were measured several times with a planimeter accurate to 0.1 cm^2. The DSC curves were also subjected to curve analysis on a DuPont 310 Curve Resolver [8].

Calculations of enthalpy were based on the measured peak areas and corresponding sample weights. The errors involved in each of these measurements 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% for uridine. For the broader melting point transitions of uridine phosphate and the mixture of uridine—uridine-3′-(2′)-phosphate an error of 8% was estimated. The error in the calculated entropy value was obtained from the square root of the sum of the squares of the errors in enthalpy and temperature. This value was approximately 4% for sharp transitions and 8% for the broad endotherms.

In this investigation, changes in the heat content of crystalline uridine (U), uridine-3′-(2′)-phosphate (U_p) and their mixture, uridine–uridine-3′-(2′)-phosphate ($U - U_p$) when heated at a constant rate, were followed by DSC and were correlated with chemical changes already characterized by Moravek [9].

The appropriate measurements and calculations were made for ΔH and ΔS on the basis that the endothermic processes of (U) and (U_p) 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, $T_f = \dfrac{\Delta H_f}{\Delta S}$ is obtained which relates the transition temperature directly with enthalpy and entropy of transition for the structural change [10].

Results and discussion

The energy absorbed during melting of the monomer (U) is shown in Fig. 1. The curve taken at 20°/min, shows no differential absorption of energy up to 442°K, at which point melting commences, resulting in a sharp transition with a mid-melting point T_m of 447°K. The transition ends with no further absorption of energy, resulting in a level post transition trace above 452°K.

In the case of U_p (Fig. 2) each curve extends over a broad range of temperatures indicating a considerable pre-melting phenomenon, resulting in an inability to obtain a sharp melting point. Consequently it appeared preferable to report the reproducible temperature T_{max} rather than the variable onset temperature at which the pre-melting phenomenon commences. Characteristic curves (Fig. 3) of U_p were obtained which when superimposed on each other, surprisingly show an identical reproducibility of their melting patterns regardless of the heating rates (5, 10 and 20°/min) employed. Respective T_{max} values of 476, 481 and 489°K were obtained. Such a constancy of thermal patterns is in contrast to what might be expected if the compounds were undergoing decomposition and implies that transitions of the melting type were occurring. The complexity of the U_p melting process was further studied by analyzing the nature of the symmetry of the curves obtained at various heating rates. An analog computer analysis of these curves indicated that a minimum of four Gaussians was required to fit each endotherm (Fig. 2). Thus the characteristic endothermic peaks displayed by all the curves represent a summation of many thermal changes. Therefore the resolved curves represent a composite of several of these individual processes which may be pertinent to the structural changes undergone during the solid state thermal treatment. The various possibilities that may account for these changes would involve conformational transformation, alterations in parallel stacking of the bases and breaking of inter- and intra-molecular hydrogen bonds which maintain the crystal lattice of these macromolecules [11, 12].

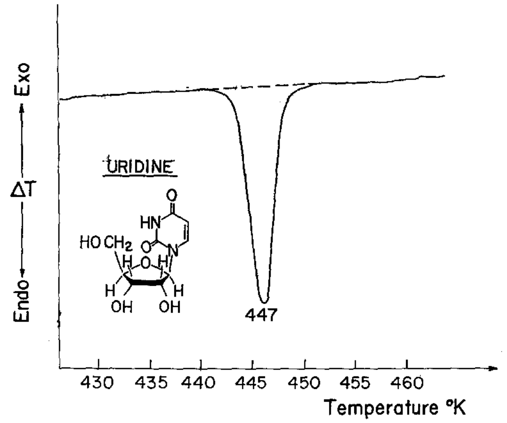

Fig. 1. DSC curve of uridine obtained over the temperature range of 430—460° K at a heating rate of 20°/min and showing a T_{max} of 447° K

The curve obtained on heating a 1 : 1 mixture of U and U_p (Fig. 4) differs from those of the individual components in that it exhibits a strong exotherm in addition to an endotherm. The endothermic portion of the thermogram, as expected, occurs over a lower temperature range (425 – 450°K) than for either of the components but does display the general characteristics noted for U_p. The exotherm, on the other hand, occurs over a much wider temperature range (450 – 670°K) and is somewhat similar to the exothermic processes obtained by DTA for RNA, DNA, and adenosine-3′-monophosphate [13, 14]. The greater complexity of the exotherm was in agreement with what might be postulated for a heterogeneous mixture containing a number of multiple or sequentially formed products. Computer analysis of the exotherm indicated that the minimum of five Gaussian curves was required and suggests that these may well correspond to the number of individual components isolatable from the reaction product. This result is consistent with the findings of J. Moravek [9] who obtained a series of products (eg. U_p, U_pU, U_pU_p, $U_pU_pU_pU_p$ . . .) on heating a $U-U_p$ mixture isothermally at 435°K. It may therefore be postulated that the polymerization proceeds via the formation of the phosphodiester bonds which are predominantly 3′ → 5′ linkage.

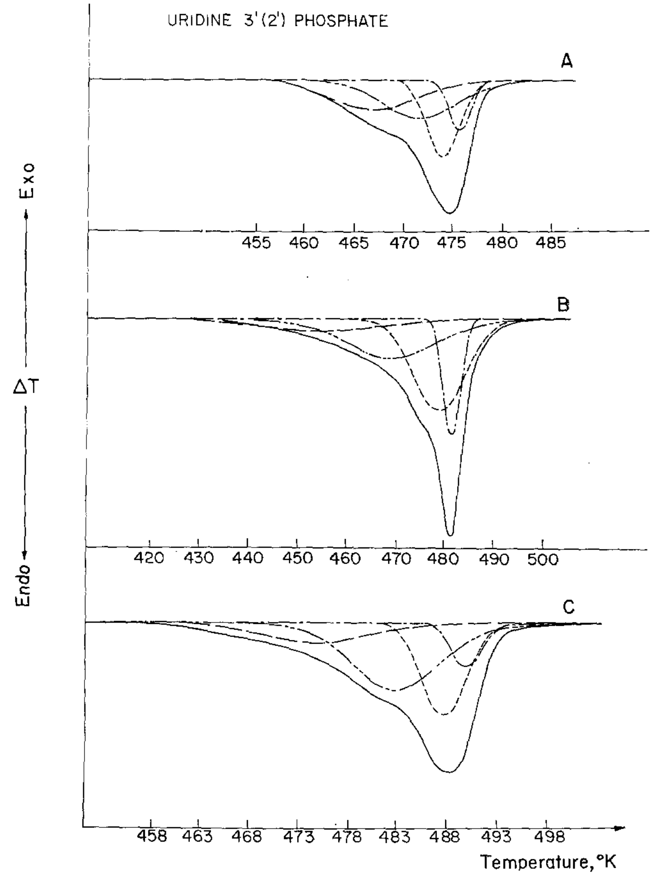

Fig. 2. DSC curves of uridine-3′-(2′)-phosphate obtained over the temperature range of 410—510° K at a heating rate of 5, 10 and 20°/min

The thermodynamic data for the thermal process (Table 1) appear to be the first of their kind reported and are unique in so far as they are free from solvent effects. The heats of fusion for U and U_p are 8.25 and 24 kcal/mole respectively, the higher value for U_p reflecting the strong influence of the phosphate group.

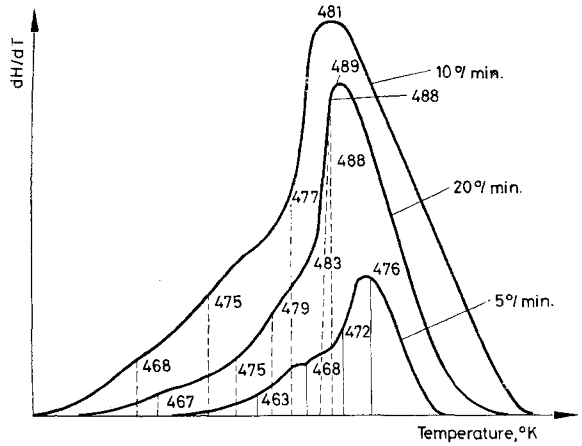

Fig. 3. Comparative DSC curves obtained for uridine-3′-(2′)-phosphate at heating rates of 5, 10 and 20° K per minute showing the similarity of contours

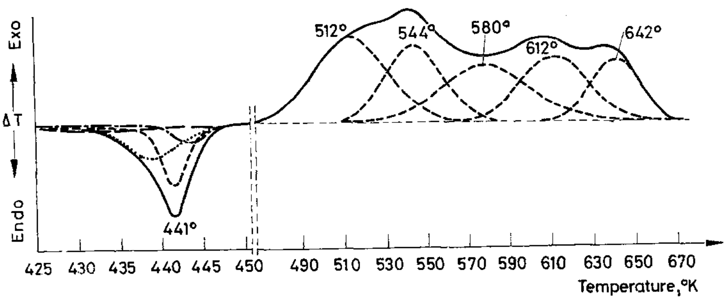

Fig. 4. Curve resolved thermogram obtained for a 1 : 1 mixture of uridine and uridine phosphate over the temperature range 425—670° K and at a scanning rate of 20°/min. The endotherm was recorded at a chart speed of 6″/min, while the exotherm was obtained at a chart speed of 1″/min

Table 1

Summary of thermodynamic data determined for the melting and polymerization of uridine and uridine-3′-(2′)-phosphate by thermal analysis at a heating rate of 20°/min

Compound	T_m, °K at 20°/min	Q cal/g	ΔH kcal/mole	ΔS cal/mole/degree
1. Uridine	(ENDO) 447	33.6	8.25	18
2. Uridine-3′-(2′)-phosphate	(ENDO) 489	77.6	24.0	49.0
3. Uridine + Uridine-3′-(2′)-phosphate mixture	(ENDO) 441 (EXO)	11.4 352	3.2	7.2

The uridine curve shows a sharp endothermic peak, characteristic of the normal melting process ($\Delta H_f = 33.6$ cal/g) whereas the broad endothermic curve for uridine-3'-(2')-phosphate ($\Delta H_f = 77.6$ cal/g), is indicative that crystal lattice degradation is being accompanied by additional endothermic processes. A similar broad endotherm is obtained for the $U - U_p$ mixture. The much lower enthalpy value obtained (11.4 cal/g) for this endotherm must result from the onset of the large exothermic reaction – polymerization – during melting. The fact that the endothermic process is followed directly by an exotherm is in agreement with this possibility. Furthermore, if fusion and polymerization are occurring simultaneously, then the entropy for the overall process (7.2 cal/mole/degree) would be anticipated to be lower than that for uridine (18 cal/mole/degree) which gave no indication of polymerization accompanying fusion.

A linear relationship was shown to exist when corresponding enthalpic values and entropic values were plotted. This relationship can only be accounted for qualitatively on the basis that as the potential energy of interaction between the molecular species becomes more negative, the reactants are bound more firmly thus losing more translational and rotational entropy in the process.

Polymerization is associated with a decrease in entropy since the formation of dimer (e.g., $U_p - U_p$) or trimer (e.g., $U_p - U_p - U$) from monomer units results in a decrease in the total number of molecules and a corresponding loss in degrees of freedom. Moreover, since the phosphate groups exert a repulsive force against each other, the introduction of U_p molecules into the polymer leads to a further loss in entropy, as a result of cancellation of charge repulsion.

As the polymer chain length increases, the opportunity for base stacking also increases with a further concomitant decrease in entropy. Thus the value of ΔS provides strong evidence for the importance of specific structural effects in the bases. This factor is important for the stability of macromolecules basic to biological systems.

Conclusions

These calorimetric studies indicate that while U_p does not undergo polymerization, the presence of U in a 1 : 1 mixture leads to a strong exotherm. U has been shown to be thermally stable over the temperature range required to induce polymerization of the $U - U_p$ mixture. Furthermore, polymerization of the $U - U_p$ mixture accompanies melting and as a result the heat of fusion obtained represents a minimal value. The orders of magnitude of the entropy values associated with the condensation can be rationalized on the basis of the polymerization process.

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Résumé — Détermination par analyse enthalpique différentielle de la chaleur de fusion de l'uridine cristallisée et de son dérivé phosphaté, l'uridine-3′(2′) phosphate, ainsi que de l'enthalpie et de l'entropie de polymérisation de leur mélange. A l'aide d'un calculateur analogique, analyse du phénomène exothermique associé au chauffage du mélange et mise en évidence d'au moins 5 processus expliqués en se servant de données expérimentales déjà publiées.

Zusammenfassung — Die Werte der Schmelzwärmen von kristallinem Uridin und seines Derivats, Uridin-3′-(2′)-phosphat sowie Enthalpie- und Entropiewerte der Polymerisation einer Mischung dieser Verbindungen wurden durch Differential Scanning Kalorimetrie bestimmt. Eine Analog-Komputeranalyse der mit dem Erhitzen der Verbindung verbundenen Exotherme wies auf mindestens fünf Prozesse, die aufgrund von früher publizierten experimentellen Resultaten gedeutet werden konnten.

Резюме — На дифференциальном сканнинг-калориметре определены теплоты образования кристаллического уридина и его производного уридин-3'(2')-фосфата, а также величины энтальпии и энтропии для полимеризации смеси этих соединений. Анализ экзотермических процессов, связанных с нагреванием смеси, проведенный на вычислительной машине аналогичного типа, показал минимум пять процессов, природа которых объяснена на основании ранее опубилкованных экспериментальных данных.