Journal of Thermal Analysis, Vol. 11 (1977) 377–385

A DSC-TLC ANALYSIS OF THERMOLYSIS REACTIONS INVOLVING 2'-DEOXYNUCLEOSIDES

P. G. OLAFSSON, A. M. BRYAN and K. LAU*

Department of Chemistry, State University of New York at Albany Albany, New York 12222 U.S.A.

(Received September 16, 1976)

Differential scanning calorimetry (DSC) has provided a universal tool for ascertaining the effects of preparation and thermal history on the physical characteristics of polymers. When applied to the study of nonpolymeric compounds, however, the interpretation of thermal analysis data may be complicated by the possible occurrence of chemical as well as physical transformations under thermal stress. In the present study involving 2'-deoxynucleosides, this difficulty has been resolved by utilizing a DSC-TLC combination to correlate thermal phenomena with corresponding chemical and physical changes. This combination shows great promise in extending the application of DSC to the investigation of thermal reactions.

Thermal analysis techniques have received wide acceptance over the past several years in the area of materials characterization such as the identification and characterization of phase transitions in organic and polymeric materials. Consequently differential scanning calorimetry (DSC) has provided a universal tool for ascertaining and predicting the effect of preparation and thermal history on the physical properties of polymers. When DSC is applied to the study of nonpolymeric compounds, however, the situation may become more complex due to the possible occurrence of chemical as well as physical transformations under thermal stress. Such changes would require the utilization of auxiliary techniques to differentiate between them. The establishment of correlations between thermal phenomena and chemical and physical changes can be affected by the utilization of thin layer chromatography.

We have employed this DSC-TLC combination to analyze the response of deoxynucleosides, which are of particular interest in biochemistry, to programmed thermal stress.

The integrity of the N-glycosyl bonds that bind the bases of DNA (deoxyribonucleic acid) to deoxyribose is important to the functioning of DNA. The cleavage of these bonds in vivo may lead to mutations [1] or to inactivation of the DNA via chain breakage [2]. Consequently it is of importance to determine the conditions which result in glycosidic cleavage and the chemical transforma-

* N.S.F. Undergraduate Research Participant from SUNY College at Plattsburg, Summer 1975.

tions associated with this process. Thus the hydrolysis of the bases has been studied via a variety of methods [3–8]. Recently the thermolysis of this bond in the fused state has been investigated [9–11].

Tamm et al. [12] demonstrated a slightly greater initial release of guanine than adenine when DNA was treated with mild acid at 23°. Similarly depurination was found to predominate over depyrimidination in melts of the deoxynucleosides [10], even though glycosidic cleavage in this case would involve a different mechanism. The latter specificity suggests that the N-glycosidic bond involving purines is weaker than that of pyrimidines.

It is the objective of the present investigation to carry out a study of the thermal characteristics of a series of 2'-deoxyribosides and to determine the process associated with each of the enthalpic stages along the thermal cycle.

Materials and methods

The nucleosides and their corresponding bases, utilized in this experiment, were of the highest purity commercially available from the Nutritional Biochemical Corporation and the Mann Research Laboratories Inc. Thermal curves of these compounds were obtained on a Perkin-Elmer DSC-1B, differential scanning calorimeter which was calibrated against standards of known melting points at a scan speed of 20°/min under a dry nitrogen atmosphere. Experiments were conducted with samples in the size range of 0.3 to 1.0 mg.

Isolation of the thermal products was accomplished via thin layer chromatography using two solvent systems [solvent system 1: chloroform – methanol–water (V/V 4:2:1) and solvent system 2: ethyl acetate – 2-propanol – water (V/V 75:16:9)] [13]. The TLC plates utilized in this experiment were commercially available (MN Silica Gel S-HR/UV_{254}). The developed spots were visualized under a short-wave ultraviolet light (Mineralight, Ultraviolet Products, San Gabriel, California).

Samples of the nucleosides were heated in a DSC under a dry nitrogen atmosphere with a flow rate of 30 ml/min at a scan speed of 20°/min and a range of 2mcal/sec full scale. The heating cycle was stopped immediately after the first endotherm was recorded, and the sample quickly removed and cooled to its original temperature. Using a fresh sample, the temperature was programmed until a second peak was recorded. Once again the sample was removed. This procedure was repeated until samples were obtained to cover each stage of the progressive thermal cycle.

Each of the aluminum pans was carefully opened, placed in a small vial and then treated with a drop or two of the same solvent as would be used the development of the TLC plates. A small amount of each sample (unheated) was dissolved in the same solvent to serve as a TLC reference.

Results and discussion

The curve for the parent deoxyriboside, 2'-deoxyuridine, reveals a fusion endotherm at 438 K, a small sharp exotherm at 506 K followed by a second endotherm at 604 K (Fig. 1). The latter peak results from the fusion of uracil as confirmed by the endothermic peak at 604 K in the curve obtained with pure uracil (Fig. 2). Thus the small exothermic peak in the 2'-deoxyuridine nerve at 506 K was attributed to cleavage of the N-glycosidic bond [10] and verified by TLC (Table 1). It had previously been established by NMR studies [10] that the products of thermal decomposition of deoxynucleosides included the base, furfuryl alcohol and water. TLC data obtained from the sample heated through the weak exotherm did not, however, indicate the presence of furfuryl

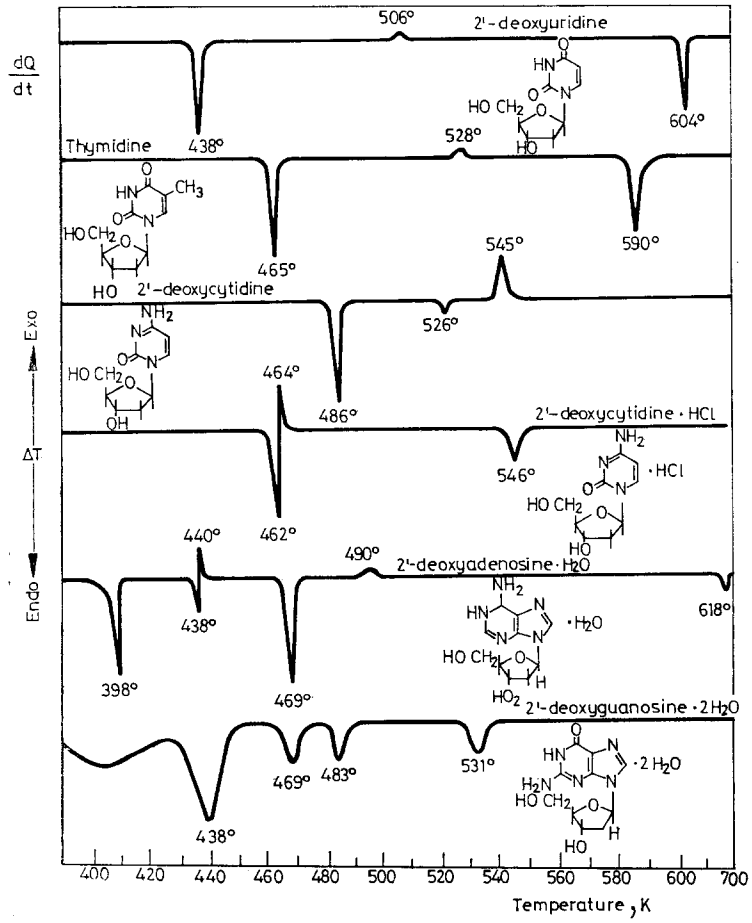


Fig. 1. DSC curves of 2'-deoxyuridines over the temperature range 390—620 K

alcohol. This may be attributed to the fact that the boiling point of furfuryl alcohol (444 K) [14] is reached prior to the separation of the base. Consequently the alcohol vapor escapes from the system.

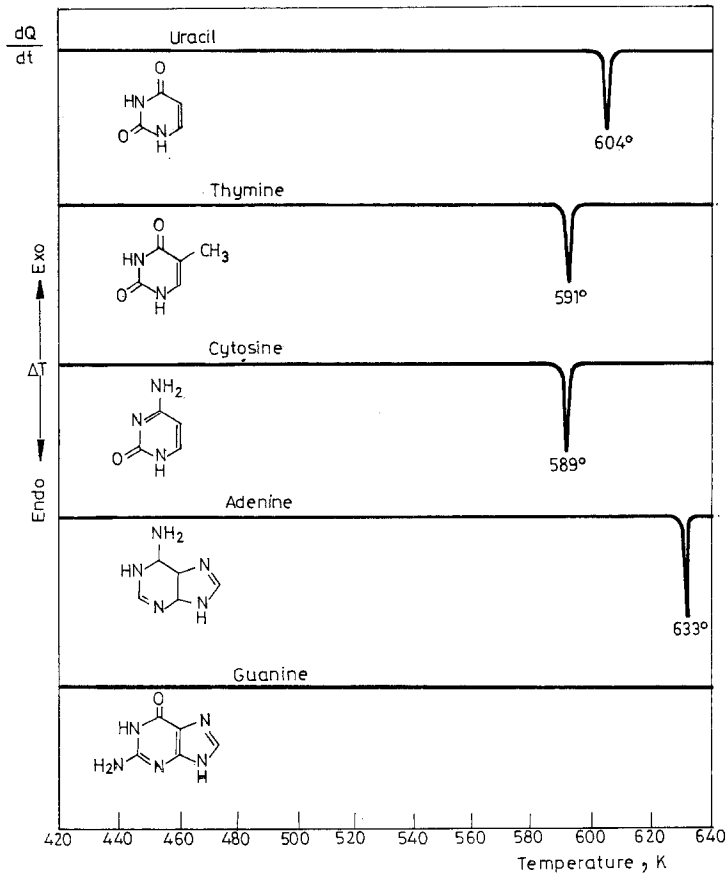


Fig. 2. DSC curves of purine and pyrimidine bases over the temperature range 420—640 K

The curve for thymidine (5-methyl-2'-deoxyuridine) reveals a similar pattern to that of the parent structure – a fusion endotherm at 465 K, an exotherm at 528 K followed by a second endotherm at 590 K. TLC data confirms the following assignments: fusion endotherm of thymidine at 465 K, glycosidic cleavage 528° associated with formation of thymine and the fusion endotherm of thymine at 604 K (Figs 1 and 2).

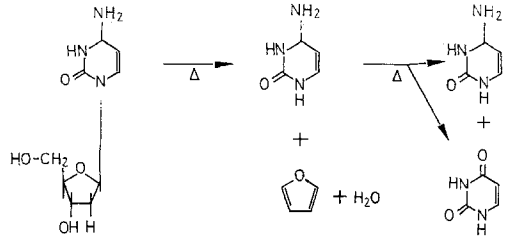
The curve for 2'-deoxycytidine (Fig. 1) is very different from that of 2'-deoxyuridine. The first endotherm is associated with fusion at 486 K and agrees

with the literature value [14]. The second endotherm at 526 K was immediately followed by an exotherm at 545 K but no endotherm was obtained at 589 K as noted in the curve for cytosine. TLC data indicates that fusion occurs at 486 K and undergoes glycosidic cleavage at the second weak endotherm at 526 K with the liberation of cytosine. The exotherm at 545 K is associated with partial

Table 1

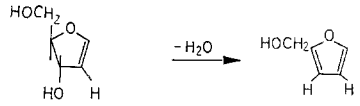
	Temp. K	R_FI	R_FII
2'-Deoxyuridine	unheated	0.87	0.42
	445	0.87	0.42
	515	0.82	0.51
	610	0.82	0.50
uracil	RT	0.82	0.50
Thymidine	unheated	0.79	0.58
	475	0.79	0.58
	535	0.71	0.68
	605	0.71	0.68
thymine	unheated	0.71	0.68
2'-Deoxycytidine	unheated	0.68	0.06
	495°	0.68	0.06
	535°	0.59	0.09
	555°	0.59	0.09
		0.80	0.50
cytosine	unheated	0.59	0.09
uracil	unheated	0.81	0.50
2'-Deoxycytidine · HCl	unheated	0.74	0.07
	470°K	0.68	
		0.74	0.07
	480°K	0.68	0.07
	555°K	0.68	0.07
cyctosine	unheated	0.68	0.08
uracil	unheated	0.79	
2'-Deoxyadenosine	unheated	0.70	0.39
	405	0.70	0.39
	438	0.70	0.39
	445	0.70	0.39
	470	0.70	0.39
	505	0.63	0.41
	620	0.63	0.41
adenine	unheated	0.63	0.41
2'-Deoxyguanosine	unheated	0.71	0.16
	430	0.71	0.16
	450	0.71	0.16
	475	0.71	0.16
	500	0.71	0.16
			0.00
	540	0.00	0.00
guanosine	unheated	0.00	0.00

deamination of the cytosine as indicated by the presence of two spots on TLC plate for sample heated to 10° above the exotherm (Table 1). One of these spots is indistinguishable from the R_F of commercial cytosine and the other with that of uracil.

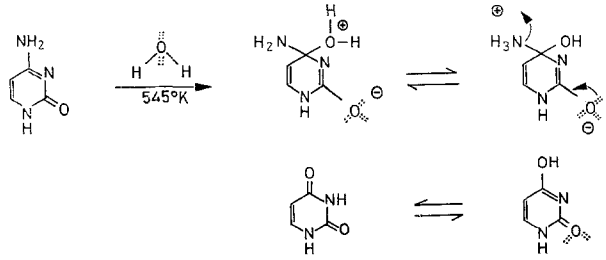


Deamination of cytosine has been noted in other cases. Ulman et al. [15] reported that cytosine residues in DNA are specifically deaminated by exposure to 1*M* NaOH at 70°. Furthermore, Fujishima et al. showed that cytidine or deoxycytidine was deaminated in 0.5 to 2*N* NaOH or KOH at over 70° to yield uridine or deoxyuridine [16].

The formation of uracil might be accounted for in terms of the water released from the sugar portion of molecule following glycosidic cleavage.



Some of the water released by the above process may result in displacement of the amino group in cytosine. A possible route might be as follows:



The curve of 2'-deoxycytidine hydrochloride (Fig. 1) differs from that of 2'-deoxycytidine. The fusion endotherm at 462 K is immediately followed by a sharp exotherm associated with glycosidic cleavage. The lowering of the temperature for glycosidic cleavage of 2'-deoxycytidine (526 K) to 464° for that of 2'-deoxycytidine · HCl is not unexpected since it has previously been established that

electron withdrawing groups on the ring weaken the glycosidic bond. In the case of the hydrochloride, the NH_3^+ group is a strong withdrawing group. The second endotherm for 2'-deoxycytidine · HCl was lower (546 K) than was expected for cytosine (589 K), suggesting that cytosine is present as the hydrochloride salt melting at 536 K. The fact that TLC analysis indicates this thermal product to be cytosine rather than the hydrochloride is understandable since the plate is developed in solvents containing water which would reach equilibrium with the hydrochloride.

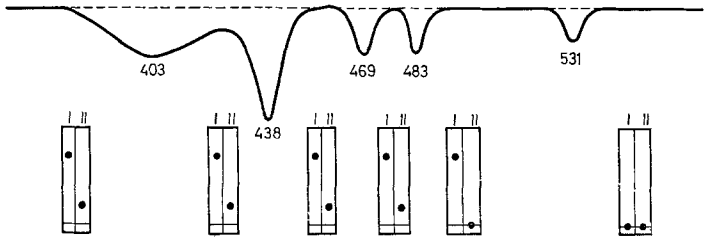


Fig. 3. DSC curves and corresponding thin layer chromatographic separations associated with the heating of 2'-deoxyguanosine dihydrate over the range 390—620 K

The curves of the deoxypurines are very different from those of the deoxypyrimidines. Thus 2'-deoxyadenosine reveals two endotherms at 398 K and 438 K followed immediately by an exotherm at 440 K prior to the fusion endotherm for this compound at 469 K; a second exotherm at 490 K was suspected of being associated with glycosidic cleavage. A fourth endotherm at 618 K completed the curve. A comparison of the latter curve with that of adenine (Fig. 2) indicated that the peak at 618 K was probably the fusion point for impure adenine. The thin layer results (Table 1) indicate that the first four peaks involve 2-deoxyadenosine. The first endotherm results from loss of absorbed water from the monohydrate. The second endotherm is associated with the loss of water of crystallization. At this temperature the anhydrous adenosine undergoes a phase change which is weakly exothermic. The strong endotherm at 469 K is the fusion point for anhydrous 2'-deoxyadenosine. Following heating through the 490 K exotherm, the product is adenine as indicated by TLC. Thus the weak exotherm at 490 K is associated with glycosidic cleavage.

The response of 2'-deoxyguanosine dihydrate on heating results in five endothermic peaks. TLC following each of the first three of these yields a single component with the same R_F value as 2'-deoxyguanosine. Thus each of these peaks is associated with loss of water. The first broad endotherm is coincident with removal of adsorbed water whereas the second (438 K) and third (469°) are the result of loss of a mole of bound water in each case. Fusion of 2'-deoxyguanosine occurs at 483° and is accompanied by glycosidic cleavage and liberation of guanine as apparent from TLC data (Table 1) (Fig. 3). The broad endotherm of guanine is caused by sublimation of the base.

Conclusions

The nature of physical and chemical changes associated with enthalpimetric transitions, resulting from the application of thermal stress to 2'-deoxynucleosides has been established by the combined utilization of DSC and TLC. This study has been particularly effective in demonstrating the power of this system to differentiate thermal transitions associated with physical processes from those of chemical reactions, such as glycosidic cleavage and the anomalous conversion of cytosine to uracil.

References

1. E. FREESE, Molecular Genetics (J. H. Taylor, ed.), Academic Press, New York, NY 1963, Part 1, p. 207.
2. B. S. STRAUSS and R. WAHL, Biochim. Biophys. Acta, 80 (1964) 116.
3. J. R. TITTENSOR and R. T. WALKER, Eur. Poly. J., 3 (1967) 705.
4. H. S. SHAPIRO, Methods of Enzymology (L. Grossman, and K. Mildave, eds), Academic Press, New York, NY 1967, 12, Part. A, p. 18.
5. R. SHAPIRO and S. KANG, Biochemistry, 8 (1969) 1806.
6. E. R. GARRETT, J. K. SEYDEL and A. J. SHARPEN, J. Org. Chem., 31 (1966) 2219.
7. E. R. GARETT, T. SUZUKI and D. J. WEBER, J. Am. Chem. Soc., 86 (1964) 4460.
8. R. SHAPIRO and M. DANZIG, Biochemistry, 11 (1972) 23.
9. P. G. OLAFSSON and A. M. BRYAN, Arch. Biochem. Biophys., 165 (1974) 46.
10. P. G. OLAFSSON, A. M. BRYAN, G. C. DAVIS and S. ANDERSON, Can. J. Biochem., 52 (1974) 997.
11. P. G. OLAFSSON, A. M. BRYAN and W. LAU, J. Chromatogr., 124 (1976) 388.
12. C. TAMM, M. E. HODES and E. CHARGAFF, J. Biol. Chem., 195 (1952) 49.
13. J. CADET and R. TEOULE, J. Chromatogr., 76 (1973) 407.
14. H. A. SOBER, Handbook of Biochemistry, Second Ed., Section 6, The Chemical Rubber Co., Cleveland, Ohio, 1970.
15. J. S. ULLMAN and B. J. MCCARTHY, Biochim. Biophys. Acta, 294 (1973) 396.
16. T. FUJISHIMA, K. UCHIDA and H. YOSHINO, Japan, Kokai, 62 (1973) 772.

ZUSAMMENFASSUNG — Die Differential–Scanning–Kalorimetrie (DSC) ist besonders geeignet zum Nachweis des Einflusses der Herstellungsbedingungen und thermischen Vorgeschichte auf die Beschaffenheit von Polymeren. Beim Einsatz zur Untersuchung nicht-polymerer Verbindungen kann jedoch die Deutung der Daten der Thermoanalyse schwierig werden, da durch thermische Stresswirkung chemische sowie physikalische Unwandlungen stattfinden können. In diesem Beitrag, der sich mit 2'-Desoxynucleosiden befasst, wurde diese Schwierigkeit durch Anwendung einer Kombination von DSC und TLC behoben, welche die Korrelation thermischer Erscheinungen mit den entsprechenden chemischen und physikalischen Änderungen gestattete. Diese Kombination von DSC und TLC ist hinsichtlich der Erweiterung des Anwendungsgebiets der DSC auf die Untersuchung thermischer Reaktionen vielversprechend.

RÉSUMÉ — L'analyse calorimétrique différentielle (DSC) est une méthode universelle pour mettre en évidence les effets de la préparation et du passé thermique sur les caractéristiques physiques des polymères. Cependant, si on l'applique à l'étude de composés non-polymères, l'interprétation des données d'analyse thermique peut être compliquée par l'intervention possible de transformations chimiques ou physiques sous l'effet du traitement thermique. Dans la présente étude sur les désoxy-2'-nucléosides cette difficulté est éliminée en combinant la DSC et la TLC afin d'établir une corrélation entre les phénomènes thermiques qui correspondent aux changements chimiques et physiques. L'emploi combiné de ces deux méthodes semble promis à une grande extension.

Резюме — Дифференциальная сканирующая калориметрия является универсальным методом для установления эффектов получения и термической предыстории полимеров на их физические характеристики. Однако, когда метод используется для изучения не полимерных соединений, интерпретация данных термического анализа может быть осложнена воемożным проявлением как химических, так и физических превращений при термическом напряжении. В настоящем изучении, включающем 2-дезоксинуклеозиды, эта трудность была преодолена использованием комбинированной техники ДСК—ТСХ для корреляции термических явлений с соответствующими химическими и физическими изменениями. Эта комбинированная техника является многообещающей в расширении применения ДСК к исследованию термических реакций.