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A DIFFERENTIAL SCANNING CALORIMETRY–THIN-LAYER CHROMATOGRAPHY STUDY OF RIBONUCLEOSIDES

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ABSTRACT

Thermal mutagenesis has been associated with cleavage of the glycosidic bond and modification of the base structure. Using deoxynucleosides as models, thermal analysis has been utilized to determine the influence of structure on relative ease of these thermolysis reactions. Differential enthalpic analyses of a series of crystalline ribonucleosides indicate that they are more resistent to glycosidic cleavage than are the corresponding 2′-deoxyribonucleosides. This difference in thermal stability is attributed to the retarding influence of the 2′-hydroxyl group on the initiation of ion pair formation in the proposed cyclic transition state. Cytosine exhibits a unique behavior insofar as partial deamination of the base occurs, subsequent to cleavage from the sugar, to yield uracil as indicated by a combined DSC–TLC analysis.

INTRODUCTION

Recently, it has been suggested that heat may be a mutagenic agent which could be more important to evolution than are the ordinary errors in DNA replication[1]. Consequently, it is desirable to know the stability of heredity determinants to thermal energy. Accordingly a program of studies involving thermal analysis, via differential scanning calorimetry (DSC), has been undertaken using deoxynucleosides, the subcomponents of DNA, as model compounds.

The DNA code molecule is a long chain defined by the sequence in which four nucleotide bases appear along a backbone of ribose and phosphate. The key to both the use and duplication of the code, contained in the sequence of the DNA molecule, lies in the concept of complimentary pairing of nucleotide bases by hydrogen bonding. In view of the importance of base pairing to the functioning of DNA, the integrity of the N-glycosyl bonds that bind the individual bases to the DNA polymer chain as well as the maintenance of the character of the base itself are essential to continued identity of the code.

Our previous studies[2] generally indicate, that for pure crystalline deoxynucleo-

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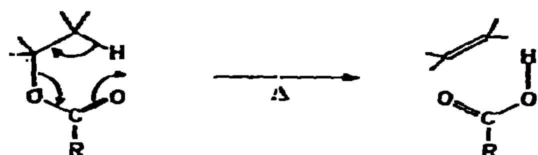
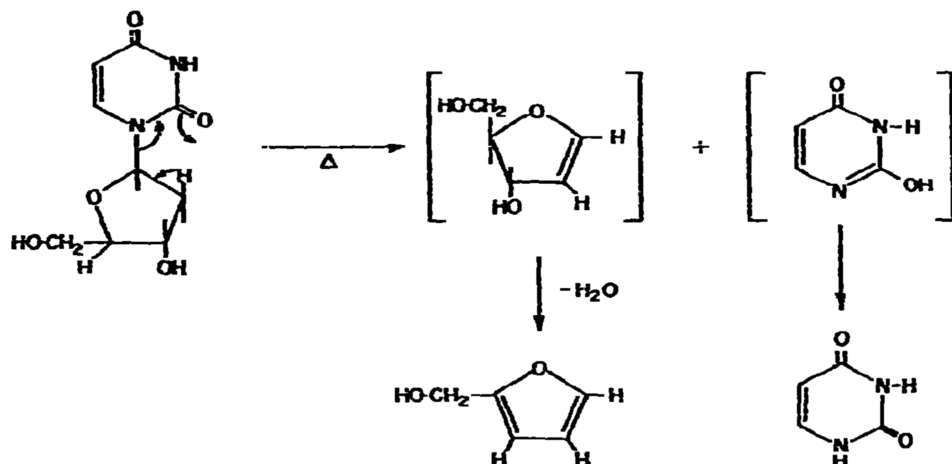
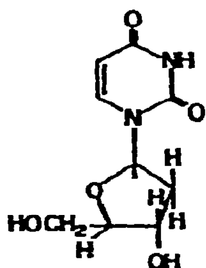
sides, a weak exothermic or endothermic process following fusion is indicative of base cleavage, a process which is accompanied by liberation of furfuryl alcohol and water. The presence of these products suggests the possibility that, in the fused state and in the absence of specific reagents, glycosidic cleavage may be occurring via a pyrolytic E_i elimination resembling that of the ester pyrolysis or Chugaev reactions.



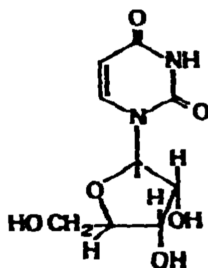
A comparable E_i elimination involving 2'-deoxyuridine might proceed as follows:



It is important to note at this point, that a strong base is not required in the Chugaev or ester pyrolysis[3] and similarly would not be required for the proposed (vide infra) mechanism for glycosidic cleavage of deoxyribonucleosides. Since the above mechanism involves a *cis* elimination it was anticipated that thermolytic cleavage of ribonucleosides (e.g., uridine) could occur in a similar manner to that for 2'-deoxynucleosides, e.g., 2'-deoxyuridine.



2'- DEOXYURIDINE



URIDINE

Therefore a study of the thermal properties of ribonucleosides was undertaken with a view to establishing the validity of this premise.

EXPERIMENTAL

The ribonucleosides used in this experiment were of the highest purity commercially available from ICN, Nutritional Biochemicals and Calbiochem. All calorimetric measurements were obtained on a Perkin-Elmer DSC-1B differential scanning calorimeter. With an instrument of this type, both the transition temperatures and the transition energies are obtained simultaneously with the aid of an Infotronix

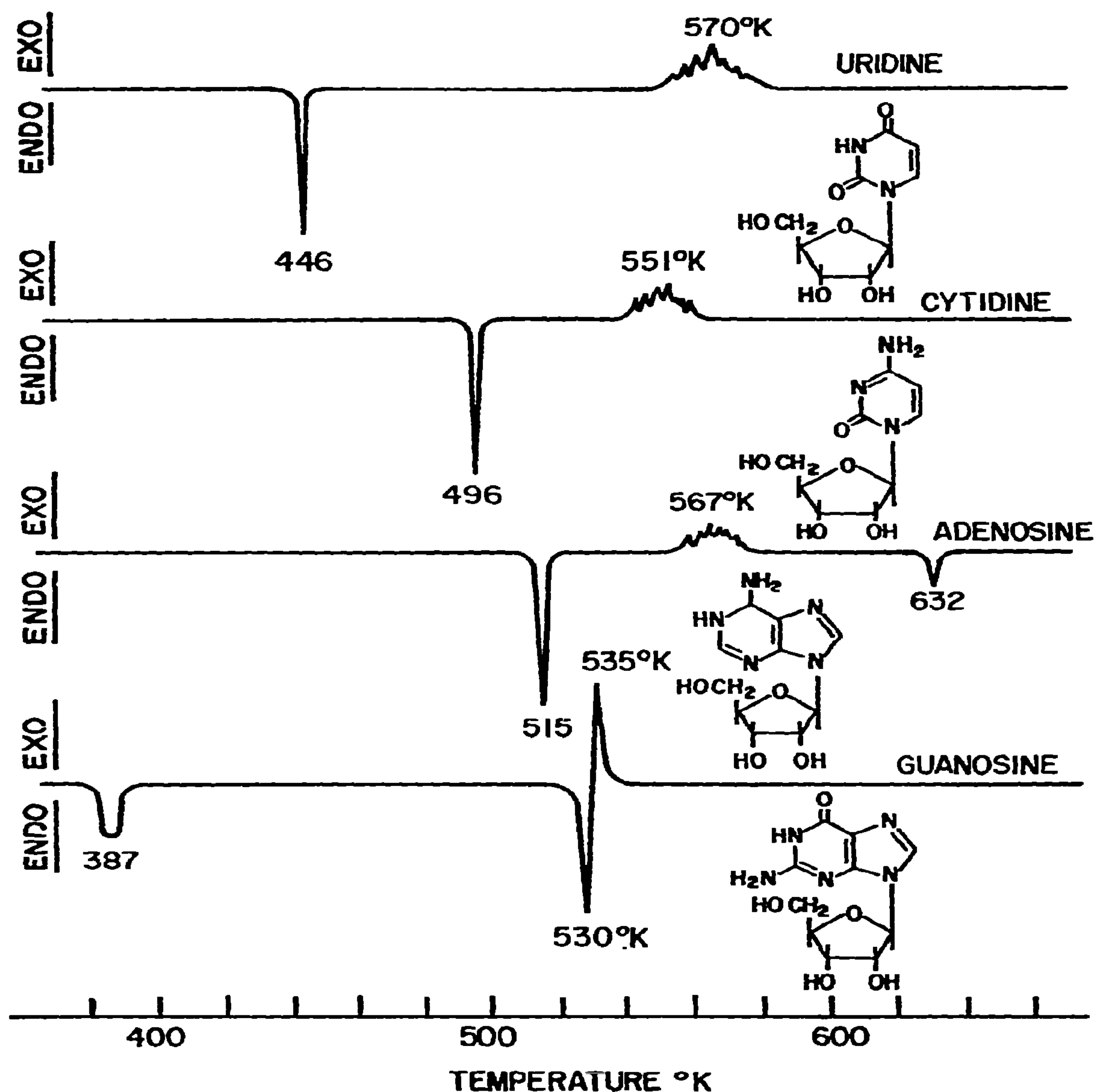


Fig. 1. Thermograms of ribonucleosides over the temperature range 360–640 K.

TABLE I

THIN-LAYER DATA

Compound	Temp. (K)	R_F	
		Solvent system	
		I	II
Uridine		0.91	0.35
Uridine (endo)		0.91	0.35
Uridine (exo)		0.83	0.50
Uracil		0.83	0.50
Cytidine		0.87	0.08
Cytidine (endo)		0.87	0.08
Cytidine (exo)		0.89	0.08
Cytidine (heated)		0.89	0.50
Cytosine		0.81	0.08
Uracil		0.83	0.50
Guanosine		0.75	0.13
Guanosine (endo)		0.75	0.13
Guanosine (2nd endo)		0.75	0.13
		0.01	0.01
Guanosine (exo)		0.01	0.01
Guanine		0.01	0.01
Adenosine		0.70	0.22
Adenosine (endo)		0.70	0.22
Adenosine (exo)		0.60	0.24
Adenosine (heated beyond exo)		0.60	0.24
2'-Deoxyuridine	unheated	0.87	0.42
2'-Deoxyuridine	445	0.87	0.42
2'-Deoxyuridine	515	0.82	0.51
2'-Deoxyuridine	610	0.82	0.50
Uracil	unheated	0.82	0.50
Thymidine	unheated	0.79	0.58
Thymidine	475	0.79	0.58
Thymidine	535	0.71	0.68
Thymidine	605	0.71	0.68
Thymine	unheated	0.71	0.68
2'-Deoxycytidine	unheated	0.68	0.06
2'-Deoxycytidine	495	0.68	0.06
2'-Deoxycytidine	535	0.59	0.09
2'-Deoxycytidine	555	{ 0.59 { 0.80	{ 0.09 { 0.50
Cytosine	unheated	0.59	0.09
Uracil	unheated	0.81	0.50
2'-Deoxyadenosine	unheated	0.70	0.39
2'-Deoxyadenosine	405	0.70	0.39
2'-Deoxyadenosine	438	0.70	0.39
2'-Deoxyadenosine	445	0.70	0.39
2'-Deoxyadenosine	470	0.70	0.39

TABLE 1 (continued)

Compound	*Temp. (K)*	R_F	
		Solvent system	
		I	*II*
2'-Deoxyadenosine	505	0.63	0.41
2'-Deoxyadenosine	620	0.63	0.41
Adenine	unheated	0.63	0.41
2'-Deoxyguanosine	unheated	0.71	0.16
2'-Deoxyguanosine	430	0.71	0.16
2'-Deoxyguanosine	450	0.71	0.16
2'-Deoxyguanosine	475	0.71	0.16
2'-Deoxyguanosine	500	0.71	{ 0.16 0.00
2'-Deoxyguanosine	540	0.00	0.00
Guanosine	unheated	0.00	0.00

CRS-110. The calibration of the DSC instrument was carried out in the usual manner. All experiments were performed with samples in the weight range of 0.3 to 0.6 mg at a scan rate of 20 K min^{-1} and range 2.

Thin-layer chromatographic (TLC) separations were carried out on commercially available plates (MN Silica Gel S-HR/U_{254}) using the solvent system I: chloroform–methanol–water; v/v 4:2:1 and system II (ethyl acetate–isopropanol–water; v/v 75:16:19 (ref. 4). The ribonucleosides were examined in this manner and exhibited the behavior of a single component.

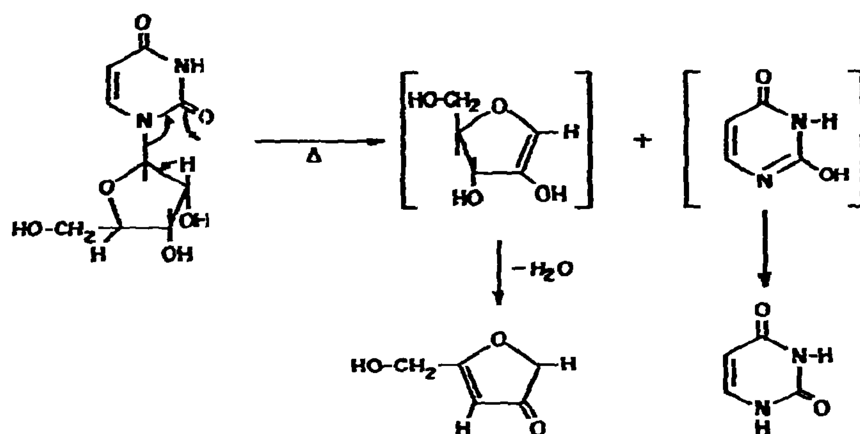
Samples of the ribonucleosides, as obtained commercially, were individually encapsulated and heated under nitrogen at a scan rate of 20 K min^{-1} and a range of 2mcal sec^{-1} full scale. The heating cycle was stopped immediately after the first peak was recorded, and the sample quickly removed and cooled to its original temperature. Using a fresh sample, the temperature was programmed until the 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 then carefully opened, placed in a small vial and then treated with a drop or two of the same solvent as would be used in 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 thermogram for uridine (Fig. 1) reveals an endotherm at 446 K and a serrated exotherm at 570 K. Thin-layer data (Table 1) verify that the initial endotherm results from fusion at 446 K. The exotherm at 570 K results in glycosidic cleavage with liberation of uracil as indicated by TLC (Table 1).

Cytidine responds to heat in a manner similar to that of uridine, exhibiting an endotherm at 496 K and an erratic exotherm at 551 K (Fig. 1). The first endotherm is due to fusion without decomposition (Table 1). Thin-layer data indicate that glycosidic cleavage occurs at 551 K but results in the liberation of uracil along with cytosine. The formation of uracil might be accounted for in terms of a subsequent reaction involving water released from the sugar portion of the molecule following glycosidic cleavage.



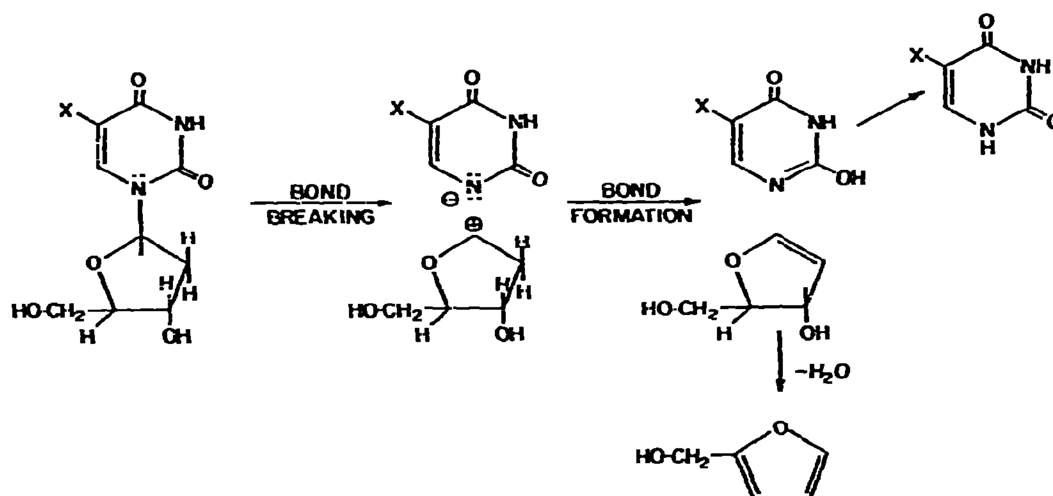
It is interesting to note in connection with this reaction, that deamination of cytosine residues, rather than depurination, has been suggested as the cause of heat mutagenesis, at least in T4 bacteriophage[1]. Calorimetric data indicate, however, that in the fused melt, cytidine is not converted to uridine since glycosidic cleavage occurs prior to deamination.

The thermogram for adenosine (Fig. 1) reveals a similar pattern to that of uridine. The fusion endotherm occurs at 515 K while glycosidic cleavage was associated with a serrated exotherm at 567 K as established by TLC. A small endotherm was obtained at 632 K due to fusion of the free base.

Guanosine exhibits an initial broad endotherm at 387 K followed by a sharp endotherm at 530 K continuing abruptly into an exotherm at 535 K. The 530 K endotherm results from fusion, a value corroborated by TLC data. Following disintegration of the crystal lattice, the compound immediately undergoes cleavage yielding guanine. The exothermic portion of this thermogram differs greatly from that associated with glycosidic cleavage in uridine, cytidine and adenosine, insofar as it does not give an erratic exotherm. It is important to note, in connection with this phenomenon, that the thermolysis temperature for guanosine lies anywhere from 15–35 K below that required by other ribonucleosides. It is therefore probable in the case of guanosine and for the sample sizes utilized, that the pressure build-up due to vaporization of the by-products of thermolysis is not quite sufficient to cause leakage from the sealed sample holder.

While the thermolysis temperature for guanosine is much lower than that required by other ribonucleosides, it is much higher than that required to effect cleavage in the case of the 2'-deoxyribonucleosides (Table 1), indicating that the presence of the 2'-hydroxyl group increases thermal stability of ribonucleosides. Therefore if an E_i elimination is involved as previously suggested, the 2'-hydroxyl group must increase the energy required to pass through the cyclic transition state. On the basis of examinations of space-filling models, assuming this cyclic arrangement, it appears probable that the retarding influence of the hydroxyl group is electronic in nature rather than steric.

Like other elimination mechanisms, the E_i elimination may tend to proceed to an ion pair as a result of bond breaking preceding bond making or vice versa. If the thermolysis of ribonucleosides occurs via such a route, then the ease of the reaction would also be influenced by electronic factors at other locations on the molecule. Indeed previous studies have shown a direct relationship between the increased ease of glycosidic cleavage and the inductive effect of a 5-halo substituent on 2'-deoxyuridines[5]. Furthermore, the 5-methyl group in thymidine has been shown to decrease the ease of thermolysis of 2'-deoxyuridines. Consequently, these data indicate the importance of electron withdrawal from the N(1) atom in facilitating the reaction. Thus, if the bond-breaking step involving glycosidic cleavage precedes bond formation with the development of an ion pair, then the original bonding electrons of the glycosidic bond must move towards the N(1) atom and away from the C(1) carbon of the furanose ring. In ribonucleosides, therefore, the 2'-hydroxyl group through its negative inductive effects, would inhibit development of a positive charge on the C(1) atom.



Thus the greater thermal stabilities of ribonucleosides over the corresponding 2'-deoxynucleosides can be accounted for within the framework of the postulated E_i mechanism.

CONCLUSION

The nature of physical and chemical changes associated with enthalpimetric transitions, resulting from the application of thermal stress to crystalline ribonucleosides has been established by the combined utilization of DSC and TLC. These studies indicate that the ribonucleosides, like the corresponding 2'-deoxyribonucleosides, undergo thermolytic cleavage of the glycosidic bond but at a higher temperature. It is postulated that the thermolysis reaction involves a cyclic mechanism similar to that of the Chugaev reaction. The retarding effect of the 2'-hydroxyl group is attributed to its destabilizing influence on ion pair formation in the rate-determining step.

REFERENCES

1 R. H. Baltz, P. M. Burgham and J. W. Drake, *Proc. Natl. Acad. Sci., U.S.A.*, 73 (1976) 1269.
2 P. G. Olafsson, A. M. Bryan, G. C. Davis and S. Anderson, *Can. J. Biochem.*, 52 (1974) 997.
3 R. F. Brown, *Organic Chemistry*, Wadsworth, Belmont, Calif., 1975, p. 388.
4 J. Cadet and R. Teoule, *J. Chromatogr.*, 76 (1973) 407.
5 P. G. Olafsson and A. M. Bryan, *Arch. Biochem. Biophys.*, 165 (1974) 46.