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Thermal perturbation differential spectra of ribonucleic acids. II. Nearest neighbour interactions

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ABSTRACT

Dinucleoside monophosphates are used here as models for studying sequence dependence of the hypochromic effect correlated with base stacking. It was shown that once the contribution due to the temperature dependent hydration change of the bases is substracted from the thermal perturbation difference spectra of dinucleoside monophosphates, the absorbance change of the dimer only due to unstacking of the bases could be obtained. In order to be able to use these corrected thermal perturbation difference spectra as models for studying nearest neighbour interactions in nucleic acids, it was necessary to normalize them to 100% unstacking of the bases.
To perform this normalization, apparent thermodynamic parameters were extracted from the corrected transition curves by means of the two-state model.

INTRODUCTION

The analysis of the dispersion of the hypochromic effect has often been used to evaluate the percentage of AU and GC rich regions in nucleic acids (1-10). These methods are based on the assumption that the contribution of AU and GC base pairs to the hypochromic spectrum is independent of their environment. However, the nearest neighbour contribution cannot be neglected in the analysis even of long nucleic acids of quasi random sequence.
It has to be taken into account, in the analysis of the spectral dispersion of sharp thermal transitions such as the ones observed in tRNA and 5S rRNA (12,13).

Dinucleoside monophosphates are the simplest molecules for studying sequence dependent properties in nucleic acids. Here they are used as models to get more insight into

the influence of nearest neighbour interactions upon thermal perturbation difference spectra of nucleic acids. It is shown that their temperature perturbation difference spectra can be decomposed into two contributions : one due to the temperature dependent hydration change of the bases and the other to the variation in optical density accompanying unstacking of bases.

A thermodynamic analysis of the later contribution was performed by means of the two-state model. Apparent ΔH and ΔS values thus obtained were used in order to normalize it to 100% stacking of the bases.

It is shown that the corrected temperature perturbation difference spectra thus obtained are all linearly independent.

MATERIALS

ApA, ApG, ApU, GpA, GpC, UpA and CpG were purchased from Miles (Indiana). ApC, CpA, CpU, GpU, UpC, GpC, UpG, UpU, GpU and CpC were purchased from PL Biochemicals (Milwaukee). In all cases, the buffer used was 0.02 M Potassium Phosphate, pH 7.2.

METHODS

Thermal perturbation difference spectra

Thermal perturbation difference spectra of dinucleoside monophosphates were recorded as described in the preceeding paper (1).

Melting curves

Melting experiments were performed on a DMR 10 double beam spectrophotometer (C. Zeiss, Oberkochen). Optical densities of the solutions were recorded every 0.2°C between 3° and 90°, at 260, 270 and 280 nm by means of a papertape punch (TALLY).

Correction for hydration change

Thermal perturbation difference spectra of dinucleo-

side monophosphates were corrected for temperature dependent hydration change as described in the preceeding paper (1) by substracting the thermal perturbation difference spectra of their component mononucleoside phosphates at the same concentration. The corrected melting curves were obtained in a similar way, by substracting the change of the optical density of the corresponding mononucleotides from the change in optical density of a solution of dinucleoside monophosphates, all recorded at the same concentration and temperature.

Determination of ΔH and ΔS for the stacking interaction of dinucleoside monophosphates

Corrected thermal perturbation difference spectra (10 temperatures, 46 wavelengths) were analysed according to a two-state model :

$$NpN_{unstacked} \rightleftarrows NpN\ stacked$$

for this reaction :

$$1) \qquad \ln K = \ln \frac{(\varepsilon u_{(\lambda)} - \varepsilon_{(\lambda,T)})}{(\varepsilon_{(\lambda,T)} - \varepsilon s_{(\lambda)})} = \frac{-\Delta H}{RT} + \frac{\Delta S}{R}$$

where $\varepsilon s_{(\lambda)}$ and $\varepsilon u_{(\lambda)}$ are the mean residue absorbances at wavelength λ of the totally stacked and totally unstacked dimer respectively, while $\varepsilon_{(\lambda,T)}$ is the corresponding mean residue absorbance of the dimer at temperature T.
K, ΔH and ΔS are the equilibrium constant, enthalpy change and entropy change for the stacking equilibrium.

If we set :

$$2) \qquad X_{(T)} = e^{-\frac{\Delta H}{RT} + \frac{\Delta S}{R}}$$

we obtain :

$$3a) \qquad \frac{\varepsilon u_{(\lambda)} - \varepsilon_{(\lambda,T)}}{\varepsilon_{(\lambda,T)} - \varepsilon s_{(\lambda)}} = X_{(T)}$$

and

3b) $$\varepsilon_{(\lambda,T)} = \frac{1}{1 + X_{(T)}} \varepsilon u_{(\lambda)} + \frac{X_{(T)}}{1 + X_{(T)}} \varepsilon s_{(\lambda)}$$

On the other hand, the absorbance $\varepsilon_{(\lambda,T)}$ can be expressed as the sum of the absorbances of the two states :

4a) $$\varepsilon_{(\lambda,T)} = fu_{(T)}\ \varepsilon u_{(\lambda)} + fs_{(T)}\ \varepsilon s_{(\lambda)}$$

or

4b) $$\varepsilon_{(\lambda,T)} = (1 - fs_{(T)})\ \varepsilon u_{(\lambda)} + fs_{(T)}\ \varepsilon s_{(\lambda)}$$

since

4c) $$fu_{(T)} + fs_{(T)} = 1$$

where $fu_{(T)}$ and $fs_{(T)}$ are the mole fraction of unstacked and stacked dimer at temperature T.

From (3b) and (4b) :

5a) $$fu_{(T)} = \frac{1}{1 + X_{(T)}}$$

and

5b) $$fs_{(T)} = \frac{X_{(T)}}{1 + X_{(T)}}$$

which are therefore only functions of ΔH and ΔS.
In order to express $\varepsilon u_{(\lambda)}$ and $\varepsilon s_{(\lambda)}$ as a function of the same unknowns, we use equation 4b at two temperatures, T_i and T_j, preferentially at extreme temperatures, e.g. 3°C and 90°C.
From the difference :

6a) $$\varepsilon_{(\lambda,T_i)} - \varepsilon_{(\lambda,T_j)} = (fs_{(T_j)} - fs_{(T_i)}) \times (\varepsilon u - \varepsilon s)$$

we obtain :

6b) $$(\varepsilon u_{(\lambda)} - \varepsilon s_{(\lambda)}) = \frac{\varepsilon_{(\lambda,T_i)} - \varepsilon_{(\lambda,T_j)}}{fs_{(T_j)} - fs_{(T_i)}}$$

Substituting into equation (4b), one obtains :

7a) $$\varepsilon s_{(\lambda)} = \varepsilon_{(\lambda,T_j)} - fu_{(T_j)} \frac{(\varepsilon_{(\lambda,T_i)} - \varepsilon_{(\lambda,T_j)})}{(fs_{(T_j)} - fs_{(T_i)})}$$

7b) $$\varepsilon u_{(\lambda)} = \varepsilon_{(\lambda,T_j)} + fs_{(T_j)} \frac{(\varepsilon_{(\lambda,T_i)} - \varepsilon_{(\lambda,T_j)})}{(fs_{(T_j)} - fs_{(T_i)})}$$

Substituting the expressions for εu and εs given in equations (7a) and (7b) into equation (4b), $\varepsilon_{(\lambda,T)}$ can be calculated for several wavelengths at any other temperature T.

8) $$\varepsilon_{\substack{\text{calculated} \\ (\lambda,T)}} = \varepsilon_{\substack{\text{obs} \\ (\lambda,T_j)}} + (fs_{(T_j)} - fs_{(T)}) \frac{(\varepsilon_{\substack{\text{obs} \\ (\lambda,T_i)}} - \varepsilon_{\substack{\text{obs} \\ (\lambda,T_j)}})}{(fs_{(T_j)} - fs_{(T_i)})}$$

It is compared with the observed value of ε, $\varepsilon_{\substack{\text{obs} \\ (\lambda,T)}}$

Since $fu_{(T)}$ and $fs_{(T)}$ are functions of ΔH and ΔS only (equations (2) to (4)), the values of these two parameters are determined by minimization of the function

9) $$\chi^2 = \int_{\lambda^0}^{\lambda^f} \int_{T^0}^{T^f} (\varepsilon_{\substack{\text{obs} \\ (\lambda,T)}} - \varepsilon_{\substack{\text{calc} \\ (\lambda,T)}})^2$$

The minimization is performed by the consecutive use of a simplex method (14,15) and a gradient method. The latter algorithm is Fletcher's switching variation of the original Davidon Fletcher Powell algorithm (16,17).

Error calculation

Let σ be the experimental error on the measured values of $\varepsilon_{(\lambda,T)}$ and χ^2 min, be the minimum value of the function χ^2 defined in (9). A contour line connecting all points where the function χ^2 takes a value of $\chi^2_{min} \pm \sigma^2$ was drawn in the space

of the two variables ΔH and ΔS (fig. 1).
The values of ΔH_{ext} and ΔS_{ext} on this contour line being furthest apart from the value of ΔH_{min} and ΔS_{min}, for which χ^2 is a minimum, are determined.
The error on these two parameters is defined as the difference between ΔH_{ext} and ΔH_{min} and ΔS_{ext} and ΔS_{min} respectively.
The subroutines used for the minimization and error calculation were taken from the CERN Computer Center program library. All calculations were performed on a UNIVAC 1110 Computer (Orsay).

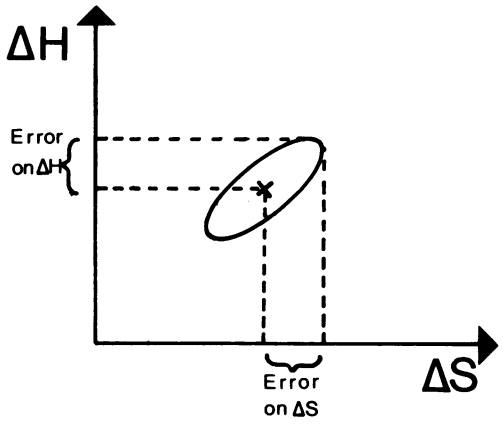


Figure 1 - Example of error calculation on the two parameters ΔH and ΔS. X is the point where χ^2 is minimum. The ellips is the line connecting all the points for which $\chi^2 = \chi^2_{min} \pm \sigma^2$.

Normalization of the temperature perturbation difference spectra to 100% unstacking

The values of $\varepsilon u_{(\lambda)}$ and $\varepsilon s_{(\lambda)}$ were calculated by replacing ΔH and ΔS by their values determined as described above in equations (2) to (7) and $(\varepsilon u_{(\lambda)} - \varepsilon s_{(\lambda)})$ was plotted as a function of wavelength. These plots are defined as "normalized temperature perturbation difference spectra". On the other hand, the % hypochromicity as a function of wavelength is defined as :

$$\% H_{(\lambda)} = \frac{(\varepsilon u_{(\lambda)} - \varepsilon s_{(\lambda)})}{\varepsilon u_{(\lambda)}} \times 100$$

Calculation of the angle between the normalized temperature perturbation difference spectra

In order to get a quantitative estimate of the degree of similarity between the normalized temperature perturbation

difference spectra, these normalized difference spectra digitalized every two nanometers between 220 and 310 nm, were treated as vectors and the angle between each pair of vectors was calculated :

$$\theta = \text{Arc.cos.}(\alpha)$$

where α is the scalar product between the two normalized vectors :

$$\alpha = \frac{\vec{v}_1 \cdot \vec{v}_2}{||v_1|| \; ||v_2||}$$

and the norm : $||v|| = \sqrt{\vec{v} \cdot \vec{v}}$

The larger the angle between two spectra is, the more different they are (18).

RESULTS AND DISCUSSION

In the preceeding paper two major phenomena were shown to be responsible for the U.V. absorption change observed upon elevation of temperature of a solution of dinucleoside monophosphate. The first is the temperature dependent dehydration of the bases. Its contribution was shown to be identical to the thermal perturbation spectra of the component mononucleotides at the same concentration and temperature. The spectral change only due to the second phenomenon, namely the unstacking of the bases, can therefore be obtained from the dinucleoside monophosphate's thermal perturbation spectra, after substraction of the monomeric contributions.

Fig. 2 shows the thermal perturbation spectra of ApC and CpA before and after substraction of those of the component monomers. The resulting corrected thermal perturbation difference spectra are apparently homothetic and show neat isosbestic points as was shown first for homodinucleoside-monophosphates (1). Corrected thermal perturbation difference spectra of all other heterodinucleosides were obtained in a similar fashion and are shown in Fig. 3.
The hydration change and the stacking interactions are appa-

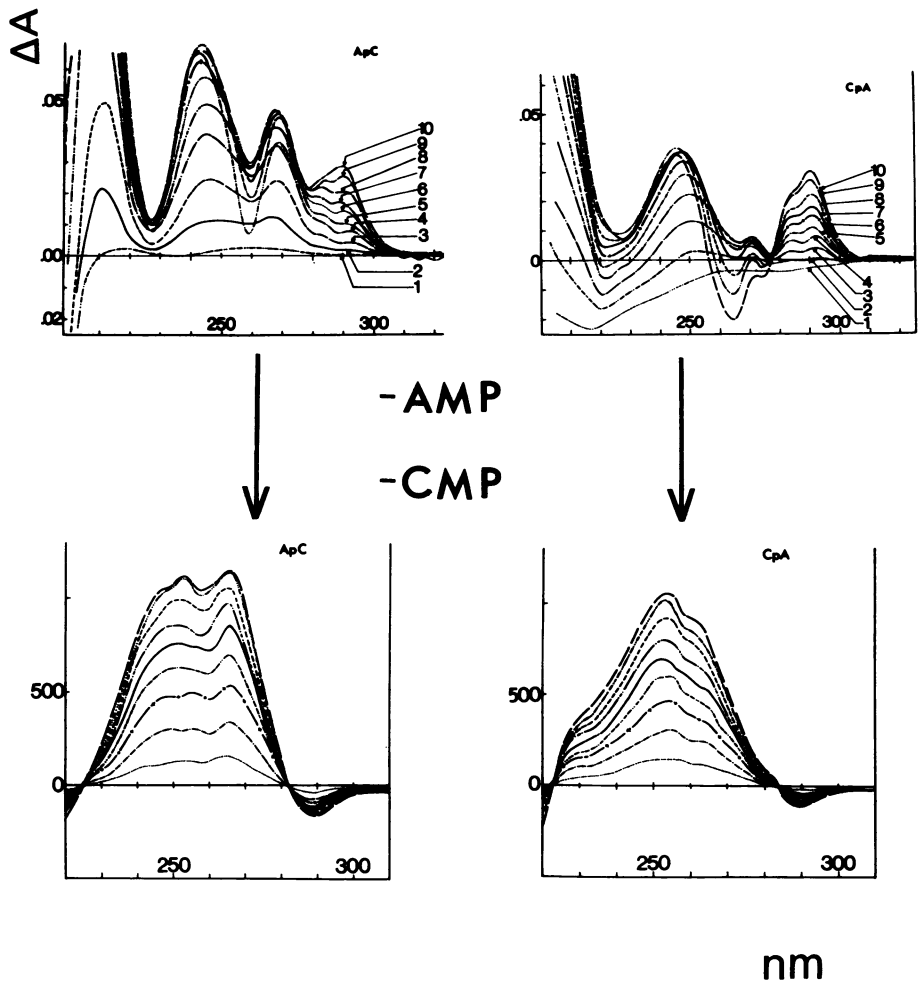


Figure 2 - Influence of hydration change on the thermal perturbation difference spectra of dinucleoside monophosphates. ApC before and after correction for hydration change. CpA before and after correction for hydration change. The correction for hydration change was performed as described in the preceeding paper.
Reference cuvette's temperature : 3°
Sample cuvette's temperature : 1 = 3° ; 2 = 10° ; 3 = 20° ; 4 = 30° ; 5 = 40° ; 6 = 50° ; 7 = 60° ; 8 = 70° ; 9 = 80° : 10 = 90°.

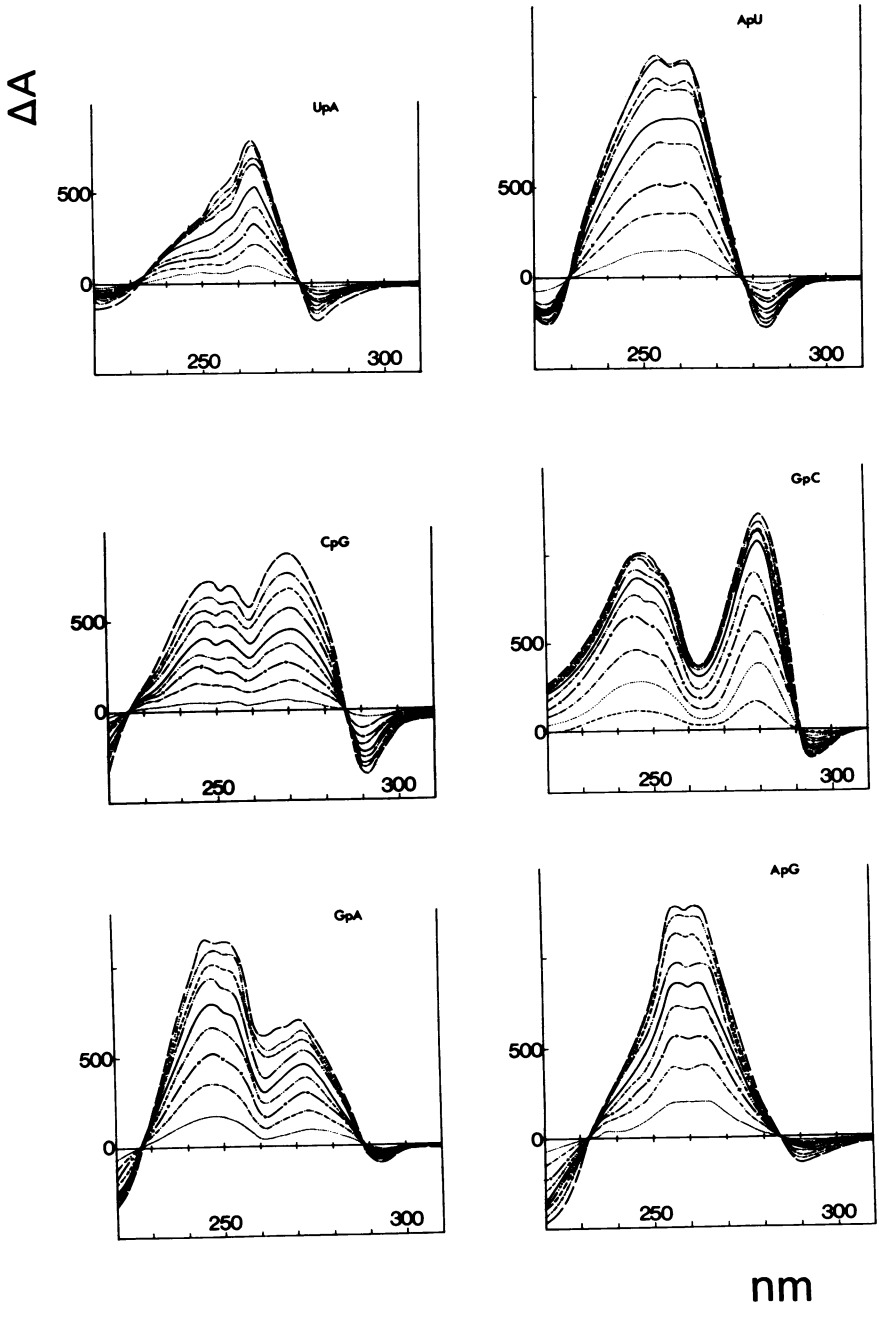
rently independent.

The apparent homotheticity of the corrected thermal perturbation difference spectra suggests that unstacking may be con-

sidered as a two-state phenomenon. If several stacked states of dinucleoside monophosphates coexist in solution, they probably have very similar optical and thermodynamic properties. This implies that the exchange between the different stacked states must be rapid. The hypochromic effect is, however, sensitive to sequence differences. Such sequence dependence is usually observed for optical properties involving the interaction between chromophores such as circular dichroism and optical rotatory dispersion (19-21).
The sequence dependence of hypochromism was also predicted by theoretical calculations (22-25).

Besides the well known hypochromism of the B_{1U} and B_{2U} transitions (26), several features common to all corrected thermal perturbation difference spectra of dinucleoside monophosphates should be noticed.
A small hyperchromic effect at high wavelength, for example, probably arises from the interaction between n - π^* transitions. These transitions being perpendicular to the plane of the bases should be hyperchromic upon stacking. Their presence in the low energy region of the spectrum was predicted by theoretical calculations (27-28). It was also assessed by polarized absorption spectroscopy (29) and U.V. photoelectron spectroscopy (30-31). Moreover, gaussian decomposition of the mononucleotide U.V. spectra at different temperatures shows that the shape of their thermal perturbation difference spectra cannot be accounted for if only shifts and intensity change of the $\pi\pi^*$ transitions are considered (32).

An other interesting feature of the corrected thermal perturbation difference spectra of dinucleoside monophosphates is the appearance of a hyperchromic region below 230 nm, as predicted by the Kuhn - Thomas sum rule (33).
The magnitude of the corrected thermal perturbation difference spectra of dinucleoside phosphates is directly proportional to their unstacking percentage. These compounds, in contrast to double stranded ribonucleic acids are not completely stacked in the temperature range accessible to experiment. In order to be able to use their thermal perturbation difference spectra as models for nearest neighbour interaction in the


ΔA
UpA
ApU
CpG
GpC
GpA
ApG
500
0
250
300
nm

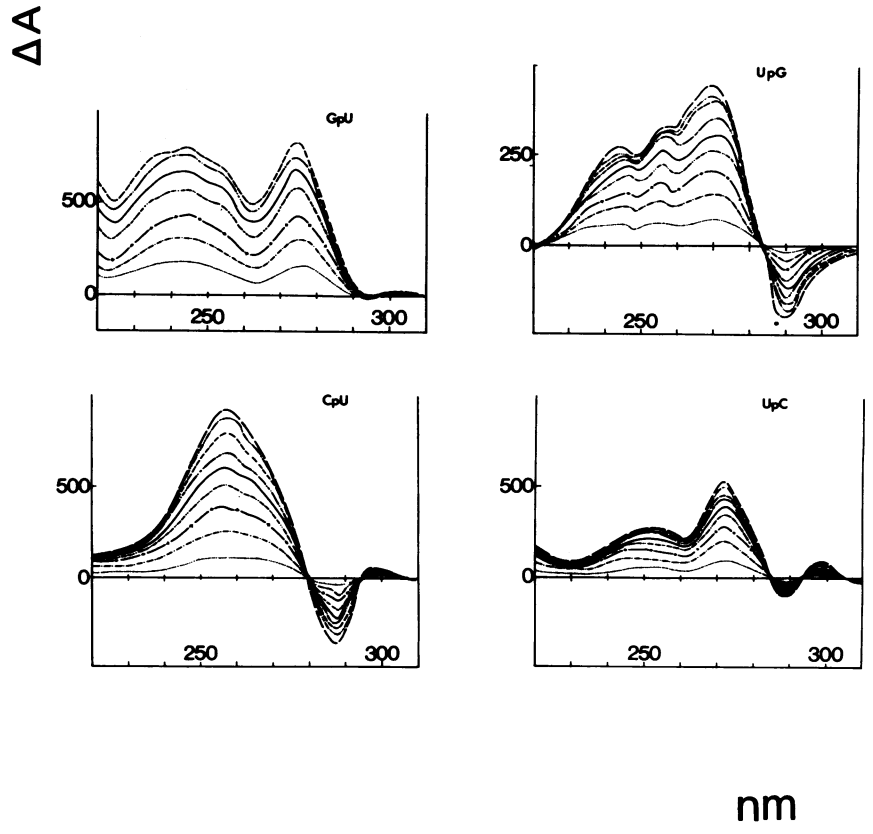


Figure 3 - TPDS of 10 heterodinucleoside monophosphates corrected for temperature dependent change in hydration (see legend to figure 2).

spectral analysis of nucleic acids, it is therefore necessary to normalize them to 100% stacking of the bases.

The thermodynamic parameters needed to perform this normalization were extracted from the corrected melting profiles by least square fitting to the two-state model, and are listed in table I. They are compared to values published in the literature. The values of ΔH and ΔS determined in this work of course do not agree with values previously determined by applying the two-state model to U.V. melting curves, since in these studies the contribution of temperature

TABLE I - THERMODYNAMIC PARAMETERS FOR THE STACKING INTERACTION OF DINUCLEOSIDE MONOPHOSPHATES

	Determined in this work			From the literature											
				UV absorption			CD - ORD			NMR					
	ΔH Kcal/mole	ΔS e.u./mole	fs 20°	ΔH Kcal/mole	ΔS e.u./mole	Ref.	ΔH Kcal/mole	ΔS e.u./mole	Ref.	ΔH Kcal/mole	ΔS e.u./mole	fs 20°	Réf.
ApA	-5.4±0.4	-17.3±1.4	0.67	-8.5		19	-5.3	-20	18			0.38	43
				-10		34	-6.5	-23	38				
				-9.4		35	-5.3	-19	39	-3	-13		38
				-8.5	-30	36	-6.5	-21.4	40	-3	-10		45
				-5.5	-18	37	-7.9	-25	41	-41	-15		"
				-7.0	-20	32	-8.5	-29.8	42				
ApU	-7.0±0.2	-24.4±0.3	0.48				-8.4	-32	39			0.34	44
							-6.7	-24	46	-6	-21		45
										-4	-17		"
ApG	-6.3±0.2	-21.3±0.3	0.52				-4.8	-18	39			0.25	43
										-3	-12		45
										-9	-32		"
ApC	-5.6±0.2	-19.3±0.3	0.45				-6.1	-21	46			0.38	43
							-6.2	-22	39				
UpA	-9.3±0.2	-31.4±1.4	0.53	-2.0	-8	38	-5.1	-21	39			0.15	44
							-7.0	-29.2	47	-4	-17		45
										-4	-13		45
UpU	-3.8±0.9	-18±1.5	0.08				-7.8	-29	39			0.08	43
							-6.0	-21	46				
							-6.1	-22.2	47				
UpG	-7.0±0.9	-24.8±3	0.38				-6.8	-25	39			0.10	44
							-6.7	-23.3	47				

	Determined in this work			From the literature										
				UV absorption			CD - ORD			NMR				
	ΔH Kcal/mole	ΔS e.u./mole	fs 20°	ΔH Kcal/mole	ΔS e.u./mole	Ref.	ΔH Kcal/mole	ΔS e.u./mole	Ref.	ΔH Kcal/mole	ΔS e.u./mole	fs 20°	ref.
UpC	-4.9±0.3	-18.2±1.7	0.32				-6.2	-22	39			0.18	43
							-5.4	-18.9	47				
GpA	-9.5±0.8	-32±0.3	0.57				-5.6	-20	39			0.3	43
							-6.1	-22	46	-9	-30		45
										-5	-17		"
GpU	-6.5±0.4	-23.1±1	0.39				-4 to 6.4	-15.3 to -24	48			0.27	44
							-6.8	-25	39				
GpG	-4.9±0.3	-16.4±0.9	0.31 (60°)	-4.5	-17	37						0.13 (60°C)	Calculated from data in 49
GpC	-12.1±2	-40.7±0.4	0.58				-7.8	-28	39			0.45	44
CpA	-3.9±0.9	-13.9±2.6	0.42	-6	-18	38	-7.3	-27	39			0.24	44
							-7.0	-24	46				
CpU	-4.7±0.4	-16.4±1.1	0.46				-7.8	-28	39			0.32	43
							-6.8	-24	52				
CpG	-9.2±0.2	-30.6±0.4	0.59				-4.8	-17	39			0.25	44
CpC	-4.8±0.3	-17.4±2.5	0.39	-5.5	-16	37	-8.6	-30	36			0.35	43
							-6.9	-25	39				
							-7.5	-25	46				
							-5.5	-19.2	50				

dependent hydration change to absorption was most of the time ignored (19, 34-36). However, in two more recent publications (37,38), the contribution of the monomers was substracted from the dimers ("in order to eliminate errors due to base line shifts")(37-38). Ogasawara and Inoue (37) analysed the temperature dependence of U.V. difference spectra of ApA, CpC and GpG by the two-state model and their results are in good agreement with ours. Watts and Tinoco (38), on the other hand, analysed melting data of ApA, UpA and CpA. The agreement of their results with ours is not as good. This discrepancy may be explained by the fact that these authors considered the absorption of the completely unstacked dimer equal to that of the monomers.
We observed that neglecting the residual hypochromism of dimers leads to systematic discrepancy between the measured and calculated melting curves.

On the other hand, comparison of our results with circular dichroism and optical rotatory dispersion data (18,38-42,46-48,50) is difficult because these data show a wide range of variation among themselves. This situation can be partly explained by the intrinsic difficulty of applying the two-state model to dinucleoside monophosphates melting curves, which are very smooth and show no neat plateau in the temperature range accessible to experiment. Therefore a reliable determination of the apparent ΔH and ΔS requires a great number of experimental points. The discrepancy may also be explained by the fact that circular dichroism and absorption show different geometry dependence. The value of the fraction of bases stacked at 20° calculated from the absorption data is also usually higher than that calculated by Lee and al. and Ezra and al. from PMR data using the ribose coupling constants (43-45). Again, the temperature dependence of chromophore interactions in absorbance spectra will be different from the conformational variations of the sugar puckering during unstacking.

Finally, it should be noticed that values of ΔH and ΔS for ApA calculated here show a good agreement with those determined from calorimetric measurements by Breslauer et al.

(51). Indeed, these authors measured the ΔH corresponding to the pH induced disruption of that degree of base stacking that exists in ApA at 28° and found a value of ΔH of 2.65 Kcal ± 0.23. The value of ΔH we find for the overall reaction is 5.4 ± 0.3 and the fraction of bases stacked at 28° is fs = 0.61 ± 0.13 ; therefore, the corresponding value of ΔH for this fraction of stacked bases at 28°C will be 3.3 Kcal/mol ± 0.9. The stability of the stacking interaction between nucleic acid bases is in the order RpR > $\frac{YpR}{RpY}$ > YpY.
It is roughly proportional to the polarizabilities of the bases, outlining the importance of polarization forces in the stacking process (52).

Plots of the calculated hypochromicity as a function of wavelength for the hypothetical 100% stacked state of dinucleoside phosphates are presented in figure 4.

Homodinucleoside phosphates are usually more hypochromic than heterodinucleoside phosphates. Among the later, pyrimidine 3'-purine 5' stacks are the ones which display the smallest hypochromic effect (except in the case of CpA). These observations can probably be correlated to the geometry of the base - base overlap.

Use of thermal perturbation difference spectra of dinucleoside phosphates as models in the analysis of longer polynucleotide thermal perturbation difference spectra also requires that these difference spectra are linearly independent. In order to evaluate quantitatively their degree of similitude, we calculated the angle between each pair of difference spectra (table II).
From this evaluation no clear relation between the similarity of the difference spectra and their content in AU and GC appears. Therefore, analysis of nucleic acids thermal perturbation difference spectra using only poly A . poly U and poly G . poly C as models in order to evaluate their content in AU and GC are probably oversimplified.

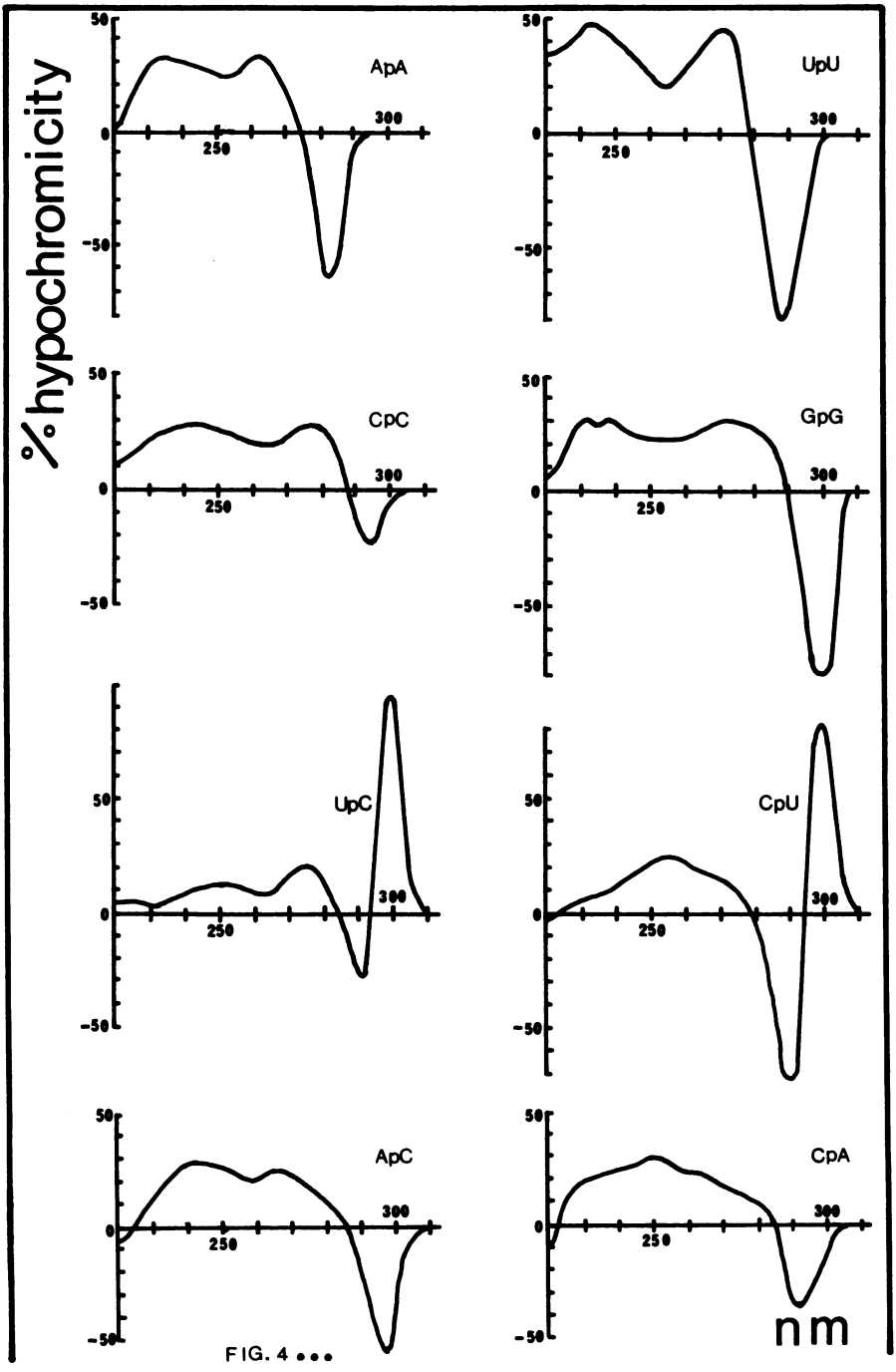

% hypochromicity
ApA
UpU
CpC
GpG
UpC
CpU
ApC
CpA
50
0
-50
250
300
nm

FIG. 4 • • •

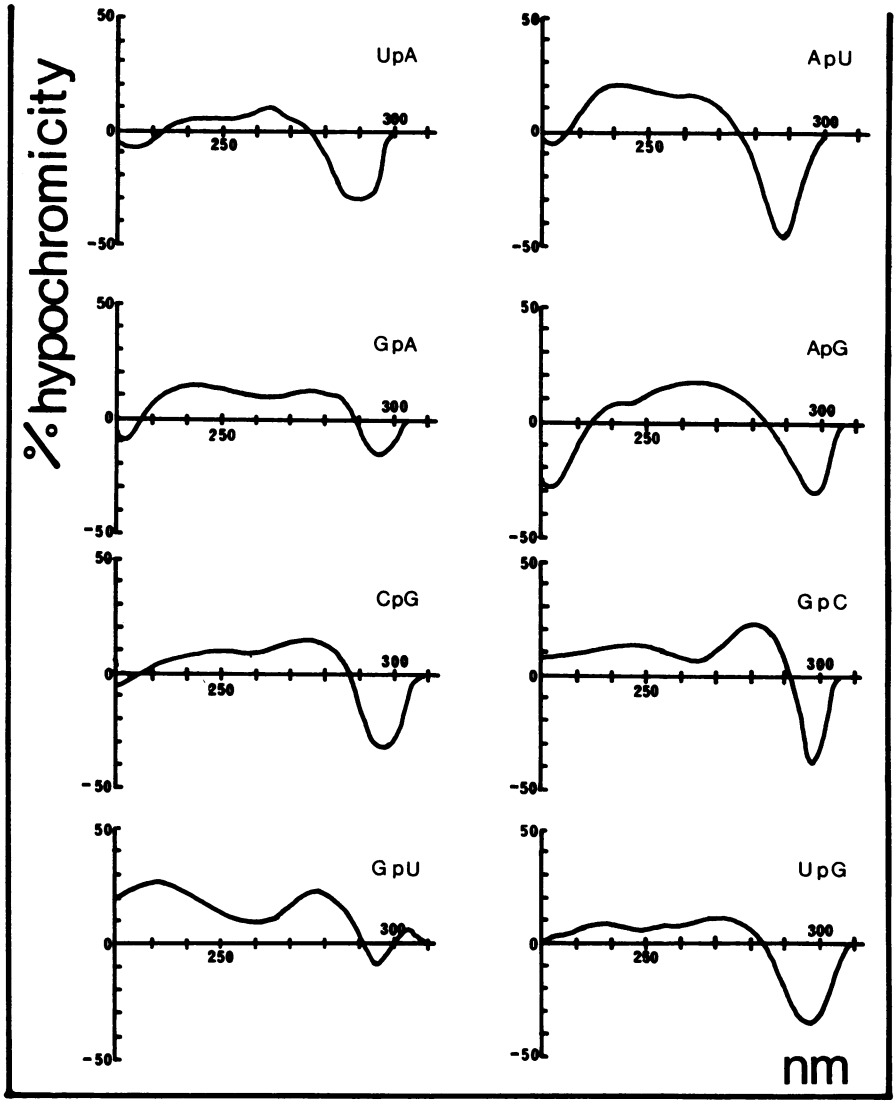


Figure 4 - Plot of the percentage hypochromicity as a function of wavelength for the hypothetical completely stacked state of the 16 common dinucleoside monophosphates.

TABLE II - ANGLE BETWEEN PAIRS OF DINUCLEOSIDE MONOPHOSPHATES CORRECTED THERMAL PERTURBATION DIFFERENCE SPECTRA

	ApA	ApU	ApG	ApC	UpA	UpU	UpG	UpC	GpA	GpU	GpG	GpC	CpA	CpU	CpG	CpC
ApA	0	12.7	28.2	21.6	19.4	30.4	35.2	45.4	32.1	45.3	37.5	50.8	20.3	18.9	39.9	44.0
ApU		0	25.8	16.6	21.1	31.2	31.0	40.5	24.4	42.8	32.8	48.1	15.0	13.8	35.1	41.2
ApG			0	25.0	19.5	39.0	28.5	37.6	29.3	50.9	34.1	50.6	29.3	22.9	29.2	48.6
ApC				0	28.3	24.9	18.9	29.5	14.1	31.1	18.8	35.6	10.8	20.0	20.4	28.6
UpA					0	34.5	35.8	46.0	38.3	56.3	44.0	60.5	31.8	20.0	41.2	54.4
UpU						0	23.8	29.0	35.0	31.8	31.7	42.3	29.6	27.8	32.7	30.6
UpG							0	18.9	22.6	29.6	18.1	33.3	25.2	26.1	12.9	26.6
UpC								0	28.2	27.3	21.9	29.9	33.6	34.2	20.0	26.1
GpA									0	28.8	13.2	29.0	16.2	28.3	18.1	27.0
GpU										0	19.8	14.9	31.2	43.4	28.8	6.3
GpG											0	18.9	22.2	33.7	12.5	18.0
GpC												0	36.0	49.3	27.6	17.0
CpA													0	19.4	26.3	29.0
CpU														0	32.8	41.3
CpG															0	25.7
CpC																0

CONCLUSION

Two major phenomena were shown to be responsible for the change in optical density observed in dinucleoside monophosphates upon temperature elevation : the temperature dependent hydration change of the bases and the unstacking of the residues.

The optical change due only to this last phenomenon could be analysed by means of the two-state model. Thermodynamic parameters needed to normalize these corrected thermal perturbation spectra to 100% base unstacking were thus extracted.

A quantitative evaluation of the degree of similarity between these normalized difference spectra revealed that these could not be classified according to their base composition only, but were very sensitive to sequence differences. Whether the difference spectra obtained in this work can be used as model for the analysis of longer nucleic acids or not, depends on the similarity of the geometry of the base stack in the dinucleosides and in the polynucleotides. This will be the object of a forthcoming paper.

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REFERENCES

1. Fréchet, D., Ehrlich, R., Remy, P., accompanying paper.
2. Guschlbauer, W. (1966) Biophysik 3, 156-164
3. Felsenfeld, G. and Sandeen, G. (1962) J. Mol. Biol. 5, 587-610
4. Felsenfeld, G. and Cantoni, G.L. (1964) Proc. Nat. Acad. Sci. USA 51, 818-826
5. Felsenfeld, G. and Hirschman, S.Z. (1965) J. Mol. Biol. 13, 407-427
6. Hirschman, S.Z. and Felsenfeld, G. (1966) J. Mol. Biol. 16, 347-358

7. Felsenfeld, G. (1967) Proc. Nucl. Acids Res. 2, 233-244
8. Fresco, J.R., Klotz, L.C. and Richards, E.G. (1963) Cold Spring Harbor Quant. Biol. 28, 83-90
9. Mahler, H.R., Kline, B. and Mehrotra, B.D. (1964) J. Mol. Biol. 9, 801-811
10. Cox, R.A. (1970) Biochem. J. 120, 539-547
11. Gould, H.J. and Simpkins, H. (1969) Biopolymers 7, 223-239
12. Ehrlich, R., Remy, P., Fréchet, D., Renaud, M., Reiss, C. and Gabarro-Arpa (1978) Cold Spring Harbor Symp. Quant. Biol. (tRNA Meeting), August 22nd-27th, p. 48
13. Ehrlich, R., Fréchet, D., Renaud, M., Remy, P., Gabarro-Arpa, J. and Reiss, C. (1978) VIth International Biophysics Congres, Kyoto, Workshop on High Resolution Melting Studies of DNA and RNA
14. Nelder, J.A. and Mead, R. (1965) Computer J. 7, 308-313
15. Fletcher, R. (1960) Computer J. 13, 317-322
16. Fletcher, R. and Powell, M.J.D. (1963) Computer J. 6, 163-168
17. Eadie, W.T., Drijard, D., James, F.E., Roos, M. and Sadoulet, B. (1971) in Statistical Methods in Experimental Physics, North Holland Publ. Co., Amsterdam
18. Marck, Ch., Schneider, Ch. and Brehamet, L. (1978) Biopolymers 17, 1065-1074
19. Warshaw, M.M. and Tinoco, I., Jr (1966) J. Mol. Biol. 20, 29-38
20. Warshaw, M.M. and Tinoco, I., Jr (1965) J. Mol. Biol. 13, 54-64
21. Bush, C.H. and Tinoco, I., Jr (1966) J. Mol. Biol. 23, 601-614
22. Danilov, V.I. and Pechenaya, V.I. (1974) Studia Biophys. 44, 33-49
23. Pysh, E.S. and Richards, J.L. (1972) J. Chem. Phys. 57, 3680-3685
24. Balcerski, J.S. and Pysh, E.S. (1974) J. Phys. Chem. 78, 444-446
25. Balcerski, J.S. and Pysh, E.S. (1976) Biopolymers 15, 1873-1875
26. Clarck, L.B. and Tinoco, I., Jr (1965) J. Amer. Chem. Soc. 87, 11-15
27. Hug, H. and Tinoco, I., Jr (1976) J. Amer. Chem. Soc. 96, 665-673
28. Bush, A.C. (1970) J. Chem. Phys. 53, 3522-3530
29. Stewart, R.F. and Davidson, N. (1963) J. Chem. Phys. 39, 255-264
30. Yu.C, Peng,S., Akiyama, I., Lin, J. and Le Breton, P.R. (1978) J. Amer. Chem. Soc. 100, 2303-2307
31. Pavda, A., Le Breton, P.R., Dinerstein, R.J. and Ridyard, J.N.A. (1974) Biochem. Biophys. Res. Comm. 60, 1262-1268
32. Fréchet, D., These d'Etat, Université Louis Pasteur, Strasbourg
33. Thiery, J. (1965) J. Chem. Phys. 43, 553-560
34. Leng, M. and Felsenfeld, G. (1966) J. Mol. Biol. 15, 455-466
35. Applequist, J. and Damle, V. (1966) J. Amer. Chem. Soc. 88, 3895-3900
36. Powell, J.T., Richards, E.G., Gratzer, W.B. (1972) Biopolymers 11, 235-250

37. Ogasawara, N. and Inoue, Y. (1976) J. Amer. Chem. Soc. 98, 7054-7060
38. Watts, M.T., Tinoco, I., Jr (1978) Biochemistry 17, 2455-2463
39. Davis, J. and Tinoco, I., Jr (1968) Biopolymers 6, 223-242
40. Poland, D., Vournakis, J.M. and Scheraga, H.N. (1966) Biopolymers 4, 223-236
41. Van Holde, K.E., Brahms, J. and Michelson, A.M. (1965) J. Mol. Biol. 12, 726-739
42. Johnson, N.P. and Schleich, T. (1974) Biochemistry 13, 981-987
43. Lee, C.H., Ezra, F.S., Kondo, N.S., Sarma, R.H. and Danyluk, S.S. (1976) Biochemistry 15, 3627-3639
44. Ezra, F.S., Lee, C.H., Kondo, N.S., Danyluk, S.S. and Sarma, R.H. (1977) Biochemistry 16, 1977-1987
45. Lee, C.H. and Tinoco, I., Jr (1977) Biochemistry 16, 5403-5414
46. Brahms, J., Maurizot, J.C. and Michelson, A.M. (1967) J. Mol. Biol. 25, 481-495
47. Catlin, J.C. and Guschlbauer, W. (1975) Biopolymers 14, 51-72
48. Guschlbauer, W., Frick, I. and Holy, A. (1972) Eur. J. Biochem. 31, 1-13
49. Ts'o, P.O.P., Kondo, N.S., Schweizer, N.P. and Hollis, P.P. (1969) Biochemistry 8, 997-1029
50. Topal, M.D. and Warshaw, M.M. (1976) Biopolymers 15, 1775-1793
51. Breslauer, K.J., Charko, G., Hrabar, D. and Oken, C. (1978) Biophys. Chem. 8, 393-397
52. Hanlon, S. (1966) Biochem. Biophys. Res. Comm. 23, 861-867