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ARTICLE

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Singular value decomposition of 3-D DNA melting curves reveals complexity in the melting process

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Abstract The thermal denaturation of synthetic deoxypolynucleotides of defined sequence was studied by a three dimensional melting technique in which complete UV absorbance spectra were recorded as a function of temperature. The results of such an experiment defined a surface bounded by absorbance, wavelength, and temperature. A matrix of the experimental data was built, and analyzed by the method of singular value decomposition (SVD). SVD provides a rigorous, model-free analytical tool for evaluating the number of significant spectral species required to account for the changes in UV absorbance accompanying the duplex – to – single strand transition. For all of the polynucleotides studied (Poly dA – Poly dT; [Poly (dAdT)]$_2$; Poly dG – Poly dC; [Poly(dGdC)]$_2$), SVD indicated the existence of at least 4–5 significant spectral species. The DNA melting transition for even these simple repeating sequences cannot, therefore, be a simple two-state process. The basis spectra obtained by SVD analysis were found to be unique for each polynucleotide studied. Differential scanning calorimetry was used to obtain model free estimates for the enthalpy of melting for the polynucleotides studied, with results in good agreement with previously published values.

Key words DNA · Thermodynamics · Calorimetry · Statistical analysis · UV spectroscopy

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Introduction

The thermal denaturation of DNA is a topic of fundamental interest and practical importance. An understanding of the thermodynamics of DNA melting provides a basis for the quantitative prediction of the stability of specific DNA sequences. Such a capability is of fundamental use in evaluating the stability of particular regions of the genome, and of practical use in the design of hybridization probes and PCR primers. Several reviews of the extensive literature on DNA melting have appeared (Wartell and Benight 1985; Klump 1988).

While the use of calorimetry to measure the enthalpy of DNA melting is increasing, spectroscopic methods have been most commonly used to study the thermodynamics of the helix to single-strand melting transition. Typically, absorbance at a single wavelength has been monitored as a function of temperature, and the data used to construct melting transition curves of the fraction of helix remaining at each temperature (Marky and Breslauer 1987). The suggested procedure for transforming the primary data in this manner (Marky and Breslauer 1987; Breslauer 1995) tacitly assumes that the absorbance at any temperature is a simple linear combination of only two spectral components, one for the duplex and one for the single strands. Once the transition curve is derived from the primary data, a number of analytical methods may be used to extract thermodynamic parameters describing the duplex melting reaction (Marky and Breslauer 1987; Breslauer 1995). Application of these methods generally rests on the simplifying assumption that the melting transition is a two-state process.

Both of the assumptions typically invoked to analyze DNA melting data require rigorous verification. Are there only two spectral components that contribute to the absorbance changes that accompany the melting of the DNA duplex? It seems unlikely that this should be the case, given what is known about the melting process. In polynucleotides, melting is likely to proceed along pathways that include mixed regions of helix and single strands, giving rise

to loops of various sizes and to junctions between the melted and unmelted regions (Crothers 1969; Wartell 1972; Wartell and Benight 1985). Such transient structures may have distinctive optical properties. Premelting transitions with distinctive optical changes have been identified (Palecek 1976), which might contribute additional complexity into the melting process. The assumption that the fraction of helix can be determined by a simple linear combination of two spectral species is likely to be an oversimplification, but has been perhaps necessary because of a lack of appropriate analytical tools for unraveling a greater number of spectral components. Is the melting of DNA a strict two-state process? If so, there should in fact be only two significant spectral species that contribute to the absorbance changes that accompany melting. A method that can enumerate the number of spectral species can offer a critical test of the two-state assumption.

We report here the first (to our knowledge) application of a powerful analytical tool, singular value decomposition, to the study of DNA melting. We exploit the capabilities of computer controlled spectrophotometers to obtain what we term 3-D melting profiles, in which complete UV spectra are obtained as a function of temperature over the course of the melting transition. The primary data are then used to construct a matrix of absorbance values, with each row of the matrix corresponding to a single temperature, and each column corresponding to a single wavelength. The matrix may then be subjected to singular value decomposition (SVD). SVD is a powerful, model-free, analytical tool for enumerating the number of significant spectral species that contribute to a family of spectra (Johnson 1985, 1992; Henry and Hofrichter 1992; Hendler and Shrager 1994; Vandegriff and Shrager 1994). If the two-state assumption for DNA melting is correct, there ought to be but two significant spectral species, corresponding to absorbance by the DNA helix and the melted single-strands. Larger numbers of spectral species enumerated by SVD would constitute rigorous proof that the two-state assumption is incorrect.

Singular value decomposition has been used to enumerate the number of significant spectral species contributing to changes in circular dichroic spectra arising from conformational transitions in deoxyoligonucleotides. Sheardy et al. (1993) found that the NaCl-induced transition of a 16 bp oligonucleotide from the right-handed B form to a form containing both left- and right-handed DNA (and therefore a B-Z junction) resulted in contributions of at least three significant spectral species to CD spectra. Plum and Breslauer (1995) applied SVD to the analysis of pH-induced changes in the CD spectra of an oligonucleotide containing a triple helix to show that the triplex to duplex transition was two-state, and involved only two significant spectral species.

Our goal in the present report is to apply singular value decomposition to enumerate the number of significant spectral species involved in the DNA melting transition. Utilization of the results of SVD to compute the fraction of intermediate species and to reconstruct their UV absorbance spectra is a more complex computational problem that is beyond the scope of this initial exploration of the application of SVD to DNA melting.

We find, by SVD, that the melting of all deoxypolynucleotides studied results in substantial spectral complexity. Poly dA : poly dT, $[poly(dAdT)]_2$, poly dG : poly dC, and $[poly(dGdC)]_2$ were all found to have more than two significant spectral species contributing to their 3-D melting profiles. While SVD cannot tell us the origin of the complexity in DNA melting, it unambiguously identifies that it is there. Such non-two-state behavior could result from premelting transitions, or from intermediates in the helix to single-strand melting transition.

Materials and methods

Deoxypolynucleotides. Synthetic polynucleotides were purchased as their sodium salts from Pharmacia Biotech (Piscataway, N. J.) and were used without further purification. The size distribution of each polynucleotide was characterized by the manufacturer, and ranged from 600 to 1500 bp, depending on the particular polynucleotide. Polynucleotide samples were dissolved in BPE buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM Na_2EDTA, pH 7.00± 0.01), and then dialyzed against the same buffer for 48 hr before further use using SpectroPor dialysis tubing (MWCO 12,000–14,000). Concentrations of polynucleotides were determined by UV absorbance, using the following molar extinction coefficients (M^{-1} cm^{-1}): poly dA : poly dT, $\varepsilon_{260} = 12{,}000$; $[poly(dAdT)]_2$, $\varepsilon_{262} = 13{,}200$; poly dG : poly dC, $\varepsilon_{253} = 14{,}800$; $[poly(dGdC)]_2$, $\varepsilon_{254} = 16{,}800$. All concentrations are expressed in terms of base pairs (bp).

Differential scanning calorimetry. Differential scanning calorimetry (DSC) experiments were carried out using a MicroCal MC-2 calorimeter (MicroCal, Inc., Northampton, MA). Data collection and analysis were done using an IBM PC and DA2 software (Microcal, Inc., Northampton, MA, v. 2.1). Baselines were obtained over the range 10–98 °C using BPE in both sample and reference cells, and were subtracted from subsequent polynucleotide melting transition profiles. All DSC determinations used a scan rate of 1 °C min^{-1}. Polynucleotide concentrations between 0.46–0.48 mM bp were used for all DSC experiments.

Spectrophotometric-melting studies. Spectrophotometric melting experiments were conducted using a Varian Cary 3E UV-Vis spectrophotometer (Palo Alta, CA), equipped with a Peltier temperature control accessory, and interfaced to a Gateway 386 PC for data collection and analysis. All melting studies used BPE buffer, and 1 cm pathlength cells with Teflon stoppers. Polynucleotide solutions were adjusted to an initial absorbance of 1.0 at 260 nm by appropriate dilution with BPE. Initial experiments were conducted by monitoring absorbance changes at 260 nm while heating at a rate of 1 °C min^{-1}, over the range 10–100 °C. These data were used to set the range of three dimension-

al melting experiments, in which samples were heated at a rate of 0.5 °C min^{-1}, and data were collected at temperatures selected to provide approximately 10 °C of pre- and post-transition baselines. In these experiments, UV spectra were recorded over the range 220 – 300 nm every 0.1 °C, using a scan rate of 320 nm min^{-1}. Heating and spectra collection were controlled by a program included in the ADL software package provided by the manufacturer.

Digital data obtained in the 3-D melting experiment were transferred to the Origin graphics software program (MicroCal, Inc., Northampton, MA), and viewed as a 3-D plot with absorbance on the z axis, wavelength on the x axis, and temperature on the y axis. Origin was used to create a matrix of absorbance values which was transferred as an ASCII file for singular value decomposition.

Singular value decomposition. Singular value decomposition of the 3-D melting data matrix was done using the program MATLAB (The Mathworks, Inc., Natick, MA). The data matrix M is decomposed by the SVD method into the product of three matrices:

$$M = USV^t$$

The matrix U contains the so-called basis spectra, S is a diagonal matrix containing the singular values, and V is a matrix containing the amplitude vectors. The challenge in the use of SVD to enumerate the number of significant spectral species is to rationally decide on the number of statistically significant singular values (Johnson 1985; Henry and Hofrichter 1992; Hendler and Shrager 1994; Vandegriff and Shrager 1994). Our procedure for this will be described in detail in the next section. Briefly, we used several criteria, including the magnitudes of the singular values, values for the first order autocorrelation of the columns of the U and V matrices, and, finally, the randomness of residual plots produced by subtraction of data matrices computed with differing number of singular values from the original data matrix M.

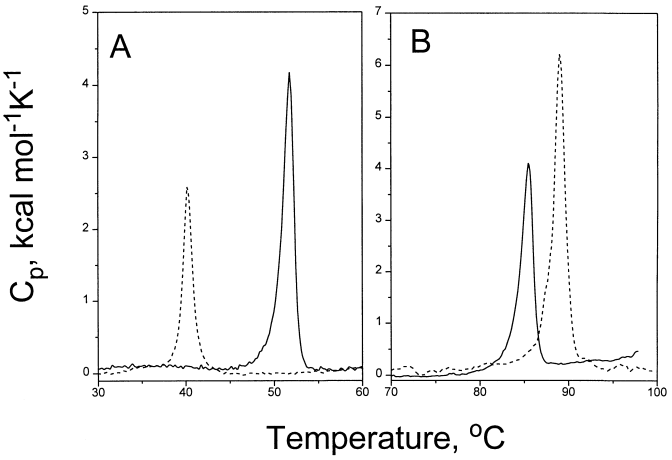

Fig. 1 A, B Results of differential scanning calorimetry experiments on synthetic deoxypolynucleotides. (**A**). Results obtained for AT containing polynucleotides. The helix to coil transitions for Poly dA-Poly dT (*solid line*) and $[Poly (dAdT)]_2$ are shown. (**B**) Results obtained for GC containing polynucleotides. The duplex to coil transitions for Poly dG-Poly dC (*solid line*) and $[Poly (dGdC)]_2$ are shown

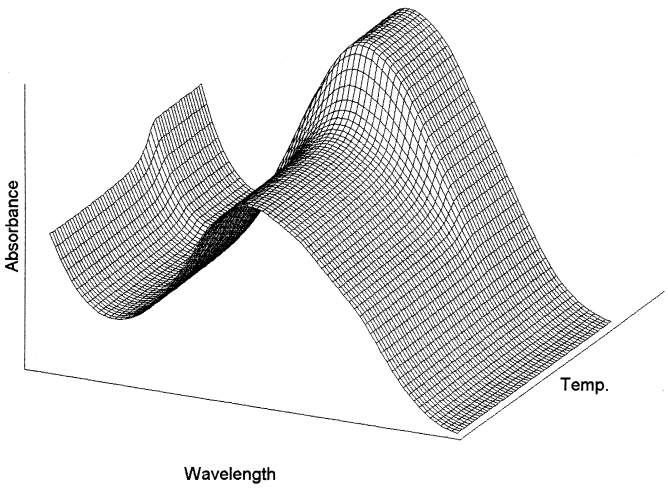

Fig. 2 Results of three-dimensional melting experiments using Poly dA-Poly dT. Complete UV spectra were recorded as a function of temperature, at each 0.1 °C increment. The resulting three-dimensional surface plot of the data is shown, with wavelength plotted on the x axis, temperature plotted on the y axis, and absorbance plotted on the z axis. The range in wavelength is from 220 to 300 nm. The range in temperature is 30 to 70 °C. The T_m of poly dA – poly dT is 51.7 °C

Results and analysis

Figure 1 shows the results of differential scanning calorimetric experiments of the four deoxypolynucleotides used in this study. DSC provides a model-free estimate of the melting enthalpy, ΔH_{cal}, shown in Table 1. An estimate of the van't Hoff enthalpy of melting, ΔH_{vh}, is obtained from the primary data assuming that the melting transition is two-state. These values are listed in Table 1, along with values for the ratio $\Delta H_{vh}/\Delta H_{cal}$, which provides an estimate of the length of the cooperative unit for the melting transition. All polynucleotides showed fully reversible melting transitions, with rescans of the sample after cooling being identical, within error, to the original DSC thermogram.

Figures 2 and 3 show the 3-D UV melting profiles obtained for poly dA : poly dT and poly dG : poly dC, respectively. Distinct differences between the plot surfaces obtained for the two polynucleotides are evident. The plot surface for poly dG : poly dC is more complicated than that observed for poly dA : poly dT, with a distinct shoulder visible near 290 nm. A similar difference is observed in the 3-D UV melting plots of the alternating polynucleotides, $[poly(dAdT)]_2$ and $[poly(dGdC)]_2$ (data not shown). Primary data such as that shown in Figs. 2 and 3 may be used to construct the data matrix M for use in singular value decomposition. The matrix M consists of rows and columns

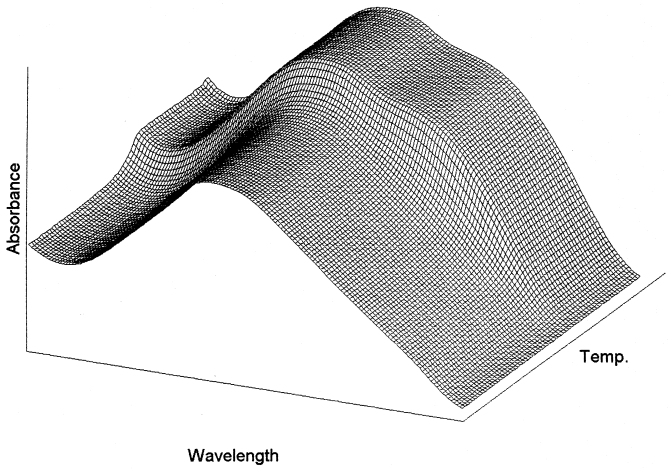

Fig. 3 Results of three-dimensional melting experiments using Poly dG-Poly dC. Complete UV spectra were recorded as a function of temperature, at each 0.1 °C increment. The resulting three-dimensional surface plot of the data is shown, with wavelength plotted on the x axis, temperature plotted on the y axis, and absorbance plotted on the z axis. The range in wavelength is from 220 to 300 nm. The range in temperature is 75 to 95 °C. The T_m of poly dG – poly dC is 85.4 °C

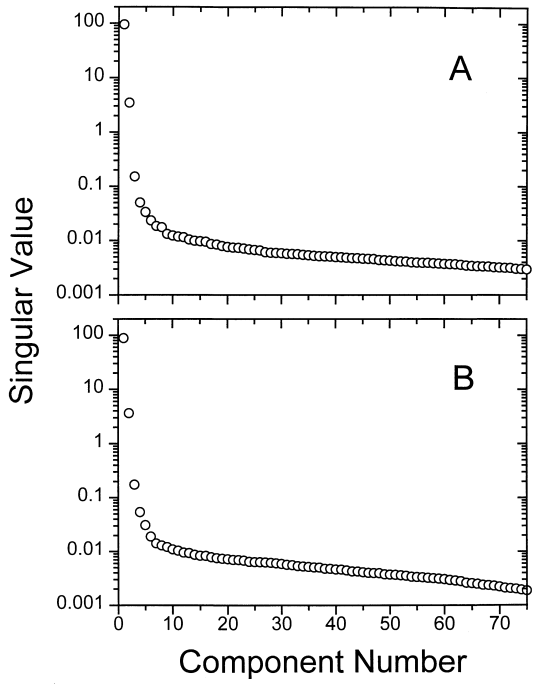

Fig. 4 Plots of the first 75 singular values obtained from the analysis of the melting of Poly dA-Poly dT (**A**) and Poly dG-Poly dC (**B**)

Table 1 Results from differential scanning calorimetry experiments of deoxypolynucleotide melting

Polynucleotide	T_m °C	ΔH_{cal} kcal mol^{-1}	ΔH_{vh} kcal mol^{-1}	$\Delta H_{vh}/\Delta H_{cal}$
Poly dA-Poly dT	51.7±0.05	8.9+0.4	385±10	43.4
$[Poly(dAdT)]_2$	40.5±0.05	8.4±0.4	247±10	29.5
Poly dG-Poly dC	85.4±0.05	7.5±0.4	539±10	72.1
$[Poly(dGdC)]_2$	88.9±0.05	12.5±0.4	507±10	40.7

DSC scans for poly dA-poly dT, $[poly(dAdT)]_2$, and poly dG-poly dC were carried out in BPE buffer, pH 7.00. For $[poly(dGdC)]_2$, a 1 : 4 dilution of BPE was used in order to keep the T_m of the polynucleotide below 100 °C. All polynucleotides were diluted from concentrated stocks with the appropriate dialysate to give final concentrations between 0.46 – 0.48 mM bp. The data shown above were obtained using a scan rate of 1 °C min^{-1}

of absorbance values, with each element of the matrix corresponding to the absorbance observed at a particular pair of temperature and wavelength values.

Singular value decomposition produces three matrices, U, S and V, from the data matrix M. The diagonal S matrix contains the singular values, the first 75 of which are shown for poly dA : poly dT and poly dG : poly dC in Fig. 4. The first 10 singular values obtained for all polynucleotides studied are given in Table 2. The magnitudes of the singular values provide the first indication of the number of significant spectral species. In Fig. 4, the first 4 – 6 singular values appear to deviate from the smooth, systematic decline characteristic of singular values 10 through 75. Inspection of the data in Table 2 indicates that, for all polynucleotides, more than two singular values are significant.

A second criterion for determination of the number of significant spectral species is the value of the first-order autocorrelation function for columns of the U and V matrices. The function is:

$$C(X_i) = \Sigma(X_{j,i})(X_{j+1,i})$$

where $X_{j,i}$ and $X_{j+1,i}$ are the *j*th and *j*th + 1 row elements of column *i* from either the U or V matrix. The value of $C(X_i)$ is, in effect, a measure of the smoothness between adjacent row elements. Since the column vector of U and V are normalized to unity, $C(X_i)$ varies between 1 and –1. Values near –1 indicate rapid row to row variations, or "noise", and indicate random behavior. Significant singular values have non-random corresponding columns in the U and V matrices. A $C(X_i)$ value of 0.8 corresponds to a column with a signal to noise ratio of 1.0 (Henry and Hofrichter 1992). A value of $C(X_i) \geq 0.8$ for both the U and the V matrices was therefore selected as a cut off criterion for accepting a significant singular value. By this criterion, the data of Table 2 indicate the following number of significant singular values for each polynucleotide: poly dA : poly dT, 5; $[poly(dAdT)]_2$, 4; poly dG : poly dC, 5; $[poly(dGdC)]_2$ 5.

A final criterion for the number of significant singular values is the randomness of residual plots for the difference between the original data matrix M and a computed data matrix calculated with a truncated number of singular values. The difference matrix D is defined as:

$$D = M - US'V^t$$

Table 2 Results from singular value decomposition of deoxypolynucleotide melting data

Polynucleotide	Singular values	Autocorrelation	
		U Matrix	V Matrix
A. Poly dA : Poly dT	96.605	0.9955	0.9933
	3.436	0.9950	0.9912
	0.151	0.9933	0.9626
	0.050	0.8659	0.9343
	0.033	0.8966	0.8250
	0.023	0.8479	0.5399
	0.018	–0.2268	0.4306
	0.017	0.3109	0.4988
	0.013	0.0748	0.2019
	0.012	0.2722	–0.0583
B. $[Poly(dAdT)]_2$	98.049	0.9959	0.9926
	4.492	0.9926	0.9898
	0.115	0.9862	0.9677
	0.039	0.9052	0.9348
	0.028	0.8774	0.3300
	0.021	–0.0339	0.4676
	0.016	0.6138	0.3748
	0.014	0.3723	0.1739
	0.013	0.0670	0.2173
	0.012	–0.1583	0.1434
C. Poly dG : Poly dC	88.242	0.9978	0.9869
	3.618	0.9975	0.9816
	0.174	0.9905	0.9110
	0.054	0.9170	0.6504
	0.031	0.9078	0.9504
	0.019	0.8015	–0.2685
	0.014	0.3372	0.4517
	0.013	–0.1860	0.0335
	0.012	0.2348	0.1516
	0.011	0.1574	–0.1914
D. $[Poly(dGdC)]_2$	85.345	0.9982	0.9872
	4.466	0.9962	0.9830
	0.102	0.9946	0.9250
	0.081	0.9559	0.9383
	0.017	0.9203	0.8612
	0.012	0.8798	0.2512
	0.008	0.6517	0.1334
	0.007	0.4691	0.0182
	0.007	0.1287	–0.1447
	0.006	–0.0408	0.0262

where S′ is a matrix created to contain diagonal elements only for those singular values believed to be significant, and set to zero everywhere else. The matrix D should contain only random noise when the correct number of singular values is used in S′. An example is shown in Fig. 5 for poly dA : poly dT. The difference matrix D is shown for several numbers of assumed significant singular values. D is shown as a contour plot, looking down the absorbance axis. Several matrices containing random noise were first generated and examined to train the eye to recognize random residual plots in this representation (not shown). The upper left contour plot, computed with only first 2 singular values listed in Table 2, show distinct nonrandomness. This result shows, categorically, that poly dA – poly dT melting cannot be a strictly two-state process. Inclusion of the third singular value (Fig. 5, upper right) leads to increased randomness, but regions of the contour plot near the lower edge are still structured. Data computed with 4 singular values (Fig. 5, lower left) are more random, but the contour plot still shows a subtle structured region near the lower edge. Finally, inclusion of 5 singular values results in an essentially random residual plot (Fig. 5, lower right). Inclusion of more than 5 singular values does not discernibly improve the residual plots. These results confirm for poly dA : poly dT that 5 singular values are significant, as was inferred from the magnitudes of the singular values and from the autocorrelation values calculated from the U and V matrices. Contour plots of the residuals were examined for all of the remaining polynucleotides (not shown). The results confirmed in all cases the number of significant singular values inferred above.

To summarize the results to this point, we have found that none of the polynucleotides studied have only two singular values. Their melting transitions, therefore, cannot be a simple two state process. There are 4 significant singular values for $[poly(dAdT)]_2$; all of the remaining polynucleotides have at least 5 significant singular values.

Figures 6 and 7 show the first three basis spectra for polynucleotides containing AT and GC base pairs, respectively. These basis spectra do not correspond directly to the true spectra of intermediates in the melting process, but rather are mathematical constructs that may be used to reconstruct true spectra. The shapes of these spectra are neither simple nor intuitive. The most significant basis spectra (Figs. 6 A, 7 A) represent the single shape that best describes each of the spectra in the family of spectra obtained as a function of temperature. Remaining basis spectra represent changeable features in the spectral families above the noise in the data. While these basis spectra do not correspond directly to physical spectra of the polynucleotides, they do reveal interesting differences between alternating and nonalternating polynucleotide sequences. Figure 6 shows the basis spectra for poly dA : poly dT and $[poly(dAdT)]_2$. The first basis spectra (Fig. 6, panel A) are virtually identical for the two polynucleotides. The second and third basis spectra (Fig. 6, panels B and C), however, show striking differences. Similarly, the first basis spectra for poly dG : poly dC and $[poly(dGdC)]_2$ are similar, but there are distinctive differences in the second and third basis spectra (Fig. 7). These results indicate that there are clear, but subtle, spectral differences for the melting of alternating and nonalternating sequence polynucleotides.

Discussion

Singular value decomposition of 3-D DNA melting profiles reveals several significant spectral species for all polynucleotides studied. DNA melting is therefore more complex than has been commonly assumed.

Calorimetry offers a model-free analysis of the DNA melting process, and provides the total enthalpy for the helix to single-strand transition directly. The ΔH_{cal} values determined for the polynucleotides used in this study are in

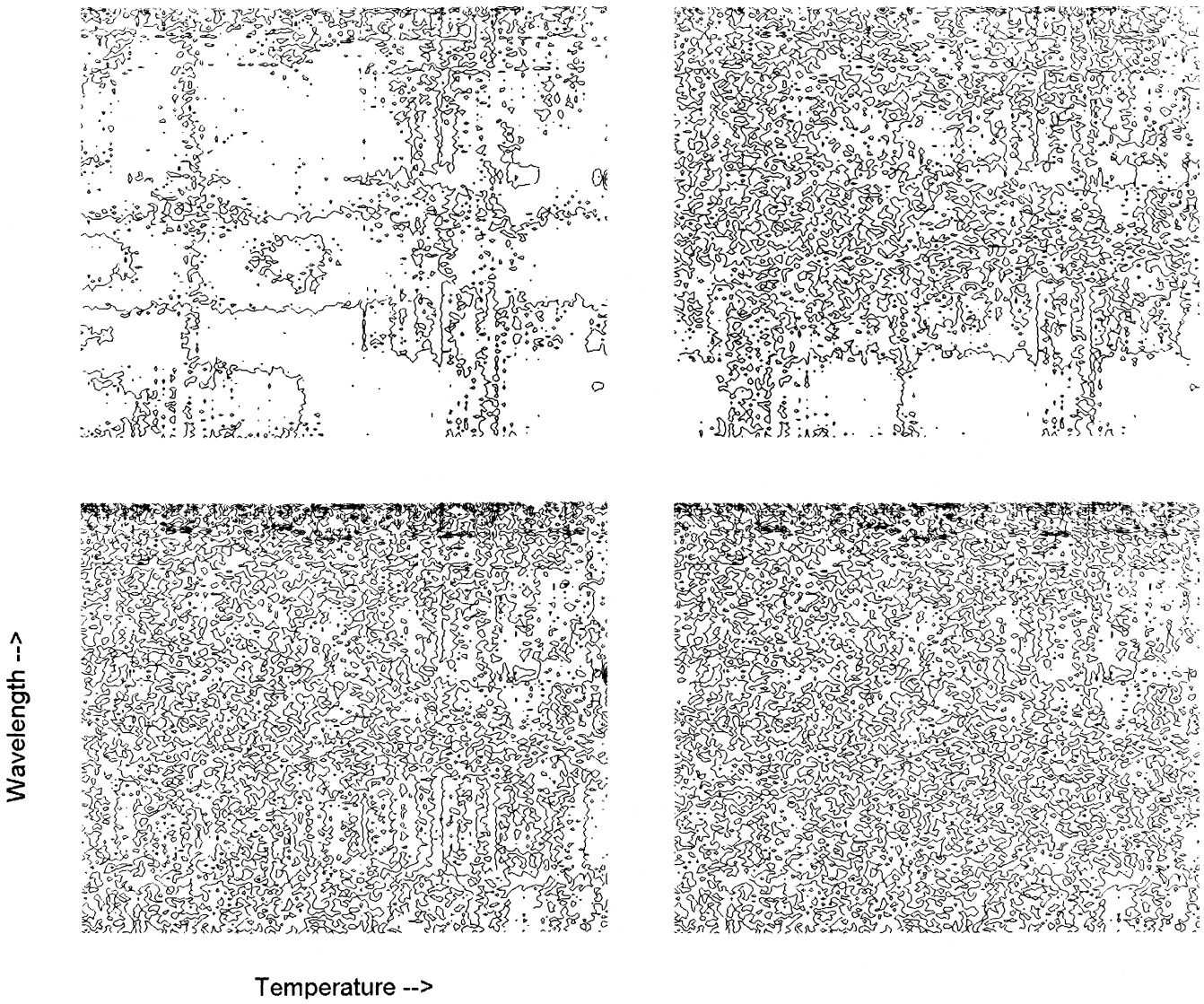

Fig. 5 Contour plots for the difference between the original experimental data matrix and data matrices computed with limited numbers of significant singular values. Data for Poly dA-Poly dT are shown. These residual plots show the view looking down the absorbance difference axis. The temperature and wavelength axes are indicated in the *lower left* figure. The number of singular values used to compute the data matrices were: 2 (*upper left*); 3 (*upper right*); 4 (*lower left*); 5 (*lower right*)

excellent agreement with previously published calorimetrically obtained values under similar ionic conditions (Breslauer 1986; Filmanov 1986; Klump 1988). The complexity uncovered by SVD is therefore not due to peculiarities of the samples. The van't Hoff enthalpy estimates from calorimetric data are typically obtained under a two-state assumption. Such values are, in light of the SVD results, not strictly valid, nor are the estimates of the length of the cooperative unit derived from the ratio of the van't Hoff and calorimetric enthalpy estimates.

While SVD unambiguously and rigorously identifies complexity in the DNA melting process, it does not identify the mechanism of the transition or the nature of the intermediate species. The results of SVD analysis point to the need for more detailed kinetic and equilibrium studies of the melting process aimed toward the identification of the reaction pathway and of possible intermediate species. One likely source of complexity in DNA melting lies in premelting helix-to-helix transitions (Palecek 1976). For poly dA-poly dT, such a premelting transition has been identified and examined in detail (Herrera and Chaires 1989; Chan et al. 1990).

Friere and Biltonen (1978 a, b) have derived a general analytical procedure for the analysis of differential scanning calorimetric data that makes no assumptions about the two-state nature of the thermal transition. A statistical mechanical approach for the deconvolution of transition curves in terms of any number of sequential transitions was developed, and applied to the melting of nucleic acids (Friere and Biltonen 1978 b). The SVD analysis described here indicates the presence of intermediates in the melting

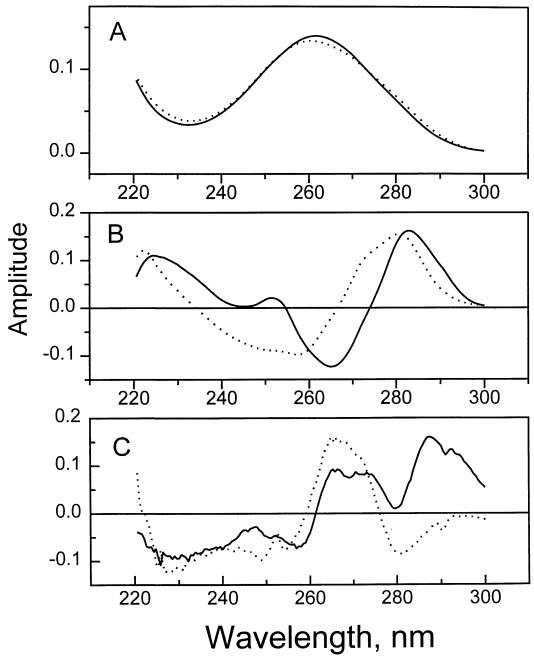

Fig. 6 A–C The first three basis spectra from the U matrix for AT containing polynucleotides. The *solid lines* are those for [Poly (dAdT)]$_2$, while the *dotted lines* are for Poly dA-Poly dT

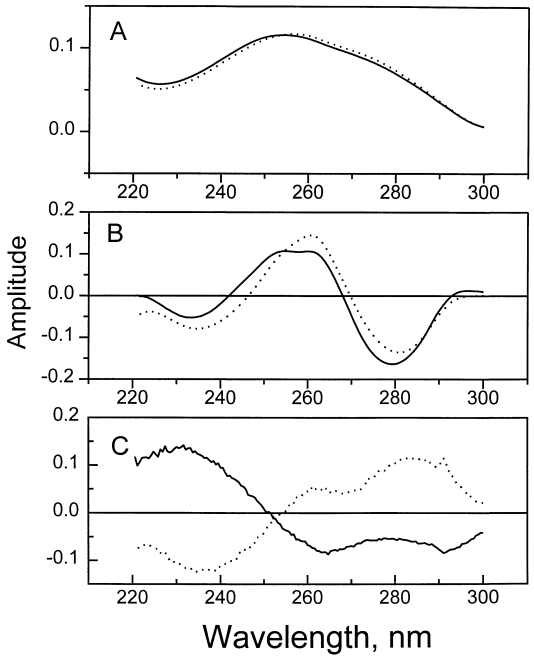

Fig. 7 A–C The first three basis spectra from the U matrix for GC containing polynucleotides. The *solid lines* are those for [Poly (dGdC)]$_2$, while the *dotted lines* are for Poly dG-Poly dC

process, and suggests that the method of Friere and Biltonen might be the preferred procedure for the analysis of calorimetric data obtained for the melting of nucleic acids. We are exploring the application of their method to the melting of a variety of synthetic deoxypolynucleotides and natural DNA samples.

Three-dimensional melting curves have been described and utilized by Jovin and coworkers in studies of the thermal stability of parallel-stranded DNA (Ramsing and Jovin 1988; Ramsing et al. 1989). In their procedure, the hyperchromicity was plotted as a function of wavelength and temperature, describing a surface which showed distinctive features for the melting of parallel stranded duplexes. Interpretation and analysis of such 3D hyperchromicity plots was largely qualitative, and no attempt was made to use the 3D data for an evaluation of the number of significant spectral species. The SVD method described here represents both an extension and an advance over the 3D hyperchromicity plots presented previously by using the 3D data to extract quantitative, model-free, information about the nucleic acid melting transition.

The basis spectra for AT and GC containing polynucleotides show clear differences (Figs. 6, 7), as do the basis spectra for polynucleotides of alternating and nonalternating sequence. That the UV spectral changes accompanying the melting of AT and GC base pairs differ is well known (Bloomfield, et al., 1974). The results of Figs. 6 and 7 show that the spectral changes depend on the exact sequence as well, with alternating and nonalternating AT and GC sequences showing unique spectral signatures upon melting. The origin of these spectral differences is not immediately apparent, but could arise from the subtle differences in base stacking of dinucleotide steps present in each polynucleotide. Poly dA : poly dT, for example, has only the ApA (TpT) dinucleotide step, while [poly(dAdT)]$_2$ has the steps ApT and TpA. Dinucleotide steps differ in stacking geometry, and the disruption of each unique geometry might reasonably be expected to give rise to distinctive, if subtle, spectral changes. SVD appears to be sensitive to such subtle differences. We caution, again, that the basis spectra shown in Figs. 6 and 7 do not represent directly the true spectra of intermediate species, but do indicate unambiguously that there are distinctive spectral differences among the polynucleotides studied.

Summary

Singular value decomposition reveals that the melting of deoxypolynucleotides of defined sequence is more complicated than has previously been assumed. SVD has enumerated 4–5 significant spectral species within the melting transition, a finding that unambiguously shows that melting cannot be a simple two-state process. More complex analyses of optically obtained melting curves that account for intermediate species is therefore required.

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