Melting fine structure of filamentous fungus nuclear DNA

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ABSTRACT
Melting fine structure of the nuclear DNA isolated from the filamentous fungus Fusarium graminearum Schwabe is presented. Optical melting profiles of nuclear DNA were analyzed by using a combination of curve fitting and derivative techniques. The "melting components" were obtained from the derivative curve by a simple decomposition technique. Differential optical melting curves of unsheared nuclear DNA indicate the presence of 15 "melting components" in filamentous fungus nuclear genome. It should be emphasized that the "melting components" observed here are different from the "thermalitea" which can be observed in bacteriophage DNA. The "melting components" reported here represent the separately melting of large "blocks" of fungus nuclear DNA.

INTRODUCTION
Spectrophotometrically monitored thermal unfolding of deoxyribonucleic acids/DNA/ may be a way to study their submolecular characteristics. Thermally induced transitions of DNAs could provide an approach to structural studies. During recent years the sensitivity of spectrophotometric methods increased permitting precision measurements of optical density with the high precision of temperature measurement and control, permitting the construction of derivative or differential melting curves with respect to temperature. The derivative of a seemingly uninteresting melting curve frequently reveals much previously unsuspected fine structure of different DNAs. During the last few years several authors have called attention to the fact that differential melting curves of DNA on heating may exhibit a fine structure of simple procaryotic genome such as those of bacteriophages1-5. DNAs from several bacteriophages/T2,T4,
T5,T7 etc. possess quasirandom sequences. Using the differential melting curve technique Mayer et al. were able to detect the fine structure of melting curves for the DNA from Rhizobium phage which possess quasirandom sequences as judged from the small width of melting curves. For small genomes quasirandom base sequences called "thermalites" become visible. For creatures with genomes larger than a few hundred thousand base pairs the detail reflects the existence of "blocks" of DNA. The "blocks" differ from each other by the average \( \%G+C\) content. Large "blocks" of DNA with distinctive base composition occur in both bacteria and higher organisms. The eucaryotes have multiple thermal transitions indicative of large "blocks" of DNA with similar base composition. Higher organisms DNA possess different families and amounts of repetitive sequences. According to Mayfield transitions in the DNA melting of different eucaryotes represent families of repeated DNA. Each family of repeated DNA should in general have a distinctive base composition and behave as a "blocks" in the DNA melting. Thermal denaturation profiles have also been used to characterize reassociated repetitive DNA sequences. One of the most widely used approaches in the investigations of DNA base-pair distribution involves the analysis of DNA melting curves, since the melting temperature of each given region depends on its length and \( \%G+C\) content. The thermal stability of AT-pairs is much lower than that of GC-pairs therefore, the AT and GC clusters in DNA molecule should denature at different temperatures. The low-melting segments with large content of AT-pairs melt out first, followed by the higher-melting segments. In the present work optical melting profiles of the filamentous fungus Fusarium graminearum nuclear DNA have been analyzed by using a combination of curve fitting and derivative techniques. The "melting components" were revealed from the differential profile by a simple decomposition technique.

**MATERIALS AND METHODS**

**DNA preparation.** DNA was prepared from F. graminearum strain F-6. Cultivation of fungus strain has been described elsewhere.
Isolation of nuclei using Nonidet P-40/Sigma, London, to lyse the cells was carried out as previously described. The urea-phosphate method of DNA isolation from nuclei, originally developed by Britten et al., was used as described earlier. Molecular weights of unsheared DNAs were $3.5 \times 10^6$ daltons as presented in previous paper. Purity of DNA preparations was tested by optical spectra: $A_{260}/A_{280}$ = 1.88 to 1.90; $A_{260}/A_{230}$ = 2.28 to 2.30, and by spectrophotometric melting. All DNA preparations had hyperchromicity of 26% or greater in 0.1xSSC/0.015 M NaCl plus 0.0015 M sodium citrate, pH 7.0. The unsheared DNA solutions were dialyzed against five changes of 0.1xSSC solution to avoid any small differences in solvent conditions. They were used as stock solutions.

Optical melting curves and their differentiation. Melting curves were automatically and continuously recorded by the Unicam SP 1750 Ultraviolet Spectrophotometer/Pye Unicam Ltd, England/ equipped with an SP 876 Series 2 Temperature Programme Controller, heated cell block and a Scalamp Thermocouple Galvanometer/W.G. Pye & Co Ltd, England/. The temperature was continuously monitored in the reference cuvette with the galvanometer. The spectrophotometer was interfaced to a Hewlett-Packard 97S calculator programmed for automatic sampling of data. An unsheared DNA sample in 0.1xSSC was first passed through a Metricel GA-6/Gelman/ filter to remove dust particles and then degassed under a partial vacuum for 10 min. Absorbance/A$_{260}$ nm/ of DNA solution was always adjusted to around 1.5 at the beginning of heating. The limit of the resolution of the present analysis was 0.001 absorbance, but each point of the melting curve was computed from the mean of 20 readings. Noise levels were reduced with large slit width/1.0 mm/ together with increased DNA concentrations/75-80 µg/ml/. The cuvettes/UV grade silica cells, 1.3 ml, 5 mm path length; Pye Unicam Ltd/ were filled so that the air space was less than 10% of the fluid volume. Evaporation was prevented with a teflon stopper to less than 0.5% per h. A programmed temperature rise of 0.25°C/min was provided by the Temperature Programme Controller in an electrically heated cell block. The temperature difference between two sampled points was 0.125°C. The heating was
continued until denaturation was complete. Measurements of the melting curves of DNA were repeated 20 times. Absorbancy values were not corrected for changes of the solution volume due to heating. The melting curves were smoothed by fitting to a cubic polynomial including either 13-points at a time according to Ansevin et al.\textsuperscript{22}, or nine points at a time but with two iterations. In the latter method after the first fitting we obtained the points of a "smoothed" melting profile and these points were fitting again to obtain the value of dH/dT at each center point. We found that this "double-smoothing" procedure was better than the 13-point smoothing.

**Fine structure analysis.** Resolution of polyphasic melting profiles into Gaussian-components was carried out according to Medgyessy\textsuperscript{23}. Following parameters were used for characterization of Gaussian-components of differential melting profiles: $T_m$/temperature of a maximal point of the component/, 2 $\sigma$/transition width in °C/, G+C content/percentage molar fraction of guanine plus cytosine/, S/area under the Gaussian curve/, %/percentage of total area of component/. The G+C-content of each Gaussian-component of the DNA was calculated according to equation 6 of Blake and Lefoley\textsuperscript{12}.

**RESULTS AND DISCUSSION**

The differential optical melting profile of Fusarium graminearum nuclear DNA/\textsuperscript{25}/ is clearly multimodal, with a huge peak/No.11/ at about 72.5°C and at least 16 other reproducibly observed components. Some of them always appear as distinct peaks/No.1,2,3,4,5,6,9,16,17/, others as shoulders/No.7,8,10,11,12,14,15/. On the basis of the differential melting profile we can reproducibly distinguish 15 "melting components"/Gaussian-components/ by a simple decomposition technique according to Medgyessy\textsuperscript{23}. Each "Gaussian-component"/\textsuperscript{25}/ was characterized by the temperature of a maximum point of the component/$T_m$/, by the transition width in °C/2 $\sigma$/, by the percentage molar fraction of guanine plus cytosine/G+C content/, by the area under the Gaussian curve/S/, and by the percentage of total area of
Fig. 1. Differential melting profile of Fusarium graminearum nuclear DNA in 0.1xSSC.

Table I gives these parameters of "Gaussian-components" of fungus nuclear DNA. The $T_m$ spectra of fungus nuclear DNA are shown in Fig. 3. Results of the analysis of the differential optical melting profiles indicate that the
Fig. 2. Gaussian components of differential melting profile of Fusarium graminearum nuclear DNA.

fungus nuclear DNA investigated consist of approximately 15 "melting components" representing the molecular "blocks" of DNA differing in their base composition/Tab. I/. This differential melting profile suggests a highly heterogeneous
Table I. Characteristic parameters of "melting components" (Gaussian components) of Fusarium graminearum nuclear DNA

<table>
<thead>
<tr>
<th>No.</th>
<th>T_m/°C</th>
<th>2σ/°C</th>
<th>%G+C</th>
<th>S</th>
<th>%</th>
</tr>
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<tr>
<td>1</td>
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<td>2</td>
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<td>3</td>
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<td>5</td>
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</tr>
<tr>
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<tr>
<td>13</td>
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<td>0.73</td>
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Fig. 3. T_m spectra of Fusarium graminearum nuclear DNA
distribution of base pairs in fungus nuclear DNA. In the present analysis, the "melting components" may be divided into three main groups. The first group includes the biggest component/No.11 in Fig.1,2 and Tab.1/ which represents 68% of the total area of the profile. This "melting component" seen in the profile occurs over 16°C/Fig.1,2/. This medium temperature denaturing fraction represents the main band DNA and the largest portion of the total hyperchromicity. The second group includes the components melting at low temperature/low temperature denaturing fractions/. These are adenine plus thymine /A+T/ enriched components/No.1-10 in Fig.1,2 and Tab.1/. The third group includes the components melting at high temperature/high temperature denaturing fractions/. These are guanine plus cytosine/G+C/ enriched components/No.12-15 in Fig.2 and Tab.1/. It should be emphasized that the multiple melting transitions observed here are quite different from the much narrower "thermalites" which can be observed in bacteriophages1-5. These "thermalites" are less than one-half of a degree in width and represent the cooperative melting of single base sequences a few hundred to a few thousand nucleotide pairs in length. The "melting components" reported here are typically one to five degrees in width/Tab.1/ and represent the separately melting large "blocks" in fungus nuclear DNA, with very similar base composition. These "melting blocks" are interspersed among and covalently linked with other segments of different base composition. We suspect these "melting blocks" represent the magnified effects of repetitive sequences17 superimposed on a broad background of unique sequences. In the present study a more sensitive method was used for measurement of absorption melting curves and a suitable numerical differentiation technique than previously16 when 13 "melting components" were presented in a similar filamentous fungus/F. culmorum/ nuclear DNA. In spite of sensitive methods we could not distinguish more than 15 "melting components" in F. graminearum genome, but their parameters could be determined more exactly.
REFERENCES


2526