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Differential Scanning Calorimetry of Various Mutant DNAs of Plasmid

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ABSTRACT

Differential scanning calorimetry of a linear double-stranded plasmid DNA showed a multimodal profile characteristic of the plasmid. This demonstrates that the helix-coil transition of DNA occurs blockwise along the DNA chain. From the results of the analysis of plasmid derivatives having deletions and duplications, the peaks of the DSC curves are assigned at regional sequences including DNA insertion element IS1 present in the plasmid. The small change in a plasmid DNA sequence caused by cleavage of a plasmid with different restriction enzyme was also clearly reflected on their DSC curves.

INTRODUCTION

There are a number of studies on helix-coil transition, or melting, of double-stranded DNA to provide physico-chemical information for the genetic material. The melting process of DNA during temperature rising is monitored most commonly by the hyperchromicity of UV light near 260 nm of wave length (1-7). It has been hypothesized that the process occurs cooperatively over a few tens or hundreds of base pairs along the DNA chain. Based on statistical mechanics, a theoretical model predicting the stepwise melting of cooperative regions of DNA molecule has been constructed (8). Note, however, that the theory could not always account for the experimental result, for example, dependence of the multimodal melting profile of DNA on ionic strength(9).

A number of thermal studies on the helix-coil transition of DNA have been also carried out (10-16). Most of the DSC curves reported so far using large chromosomal DNA have shown broad and monotonical heat absorption profiles. Most recently, however, we have shown that the multimodal transition profiles of DNA can be obtained by the differential scanning calorimetry (DSC), using the small DNA of the chloramphenicol resistance plasmid pJL3-TB5 whose entire base sequence has been determined (17). The DSC curves of the linear form of this plasmid DNA have shown at least six peaks over the temperature range of 82 to 98°C. The analysis of GC distribution along the DNA chain has predicted that each peak is attributed to a particular GC content region (17).

In this paper we describe the results of DSC performed using pJL3 derivatives with mutations, such as deletions and duplications, to obtain experi-

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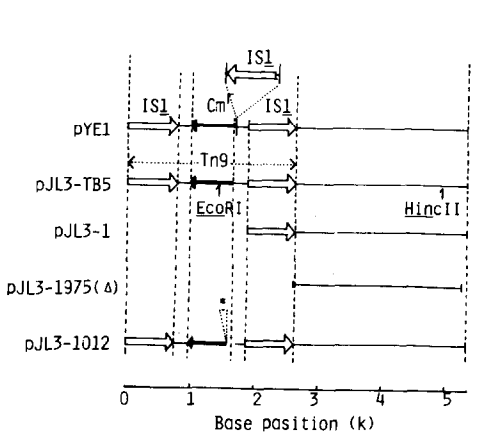
mental evidence that each peak previously observed is due to the helix-coil transition of a particular sequence. This experimental approach to the attribution of transition peak of double-stranded DNA could be useful for the theoretical understanding of DNA helix-coil transition. We also describe the effect of a small variation caused in the DNA sequence on their DSC curves using linear DNAs cleaved at the different recognition site with restriction endonuclease. The results show that the DSC curves of plasmid DNA reflect remarkably in detail structure of DNA sequence.

MATERIALS AND METHODS

Plasmids.

Plasmids used were pJL3-1975(Δ), pJL3-1, pJL3-TB5, pYE1 and pJL3-1012. Their structures are schematically shown in Fig.1. These plasmids are derivatives of pJL3 [5265 base pairs (bp) in length], which contains the entire sequence of the chloramphenicol resistance (Cm^r) transposon Tn9 (2638 bp) flanked by two copies of IS1 (768 bp) (18). Plasmid pJL3-TB5 (5277 bp) has an insertion of a 12 bp sequence containing a BamHI recognition sequence within the Cm^r gene of pJL3 (18). Plasmid pYE1 (6096 bp) has an insertion of the 819 bp sequence containing the entire sequence of IS1 at the BamHI site of pJL3-TB5 (Y.Endo, unpublished results). Thus, pYE1 carries three copies of IS1. Plasmid pJL3-1 deletes the sequence containing the Cm^r gene and has only a single copy of IS1



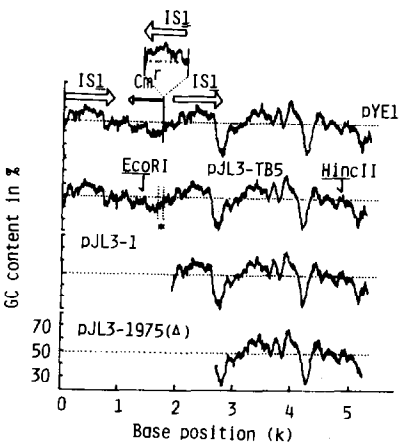


Fig.1 (left). Structures of derivatives from plasmid pJL3. The molecules are actually circular duplexes, but are displayed in a linear representation. * indicates 10 bp BamHI linker sequence inserted.

Fig.2 (right). The GC distribution maps of the pJL3 derivatives at the intervals of 101 bp. See ref.17 for construction of a GC distribution map in detail. * indicates the portion deleted in pJL3-1012.

(19). Plasmid pJL3-1975(Δ) (2521 bp) deletes most of Tn9. The deleted region is substituted by a BamHI linker (10 bp in length). Plasmid pJL3-1012 (5168 bp) deletes a 119 bp sequence in Tn9. It has the BamHI recognition sequence of 10 bp at the deleted region. All these plasmids have commonly one cleavage site for restriction endonuclease HincII. pJL3-TB5, pYE1 and pJL3-1012 have also one cleavage site for restriction endonuclease EcoRI.

Fig.2 shows the distribution maps of GC content at the moving intervals of 101 bp along the DNA chain (see ref.17).

All these plasmid DNAs were prepared and purified, as described previously (20). The covalently closed circular DNA samples prepared were converted to linear DNAs by digesting with restriction endonucrease HincII or EcoRI, purchased from Takara Shuzo Co. Ltd., Kyoto, using the conditions as recommended by the enzyme supplier. DNA concentrations were determined at 260 nm of wave length by use of a spectrophotometer, UV-3000, from Shimazu Seisakusho Ltd., Tokyo.

Differential Scanning Calorimetry (DSC).

The calorimetric measurements were performed by means of an adiabatic differential scanning calorimeter, MC-1, available from MicroCal Inc., Amherst, MA. The amount of 0.7 cm^3 of the linear plasmid DNA solution dissolved in SSC buffer was put in the sample vessel of the calorimeter and the equal amount of buffer solution without DNA in the reference vessel. DSC runs were carried out at the heating rate of 20 K/h. The DNA concentration of the solutions used in the calorimetric measurements was 0.05-0.06%.

A high sensitivity and heat flux type of differential scanning calorimeter, SSC 560U, available from Seiko Instruments & Electronics Ltd., Tokyo, was also used as previously described (17). The processing of DSC data was conducted using a personal computer system (17).

RESULTS AND DISCUSSION

Effect of Mutations with Deletions and Duplications on Melting of Plasmid DNA

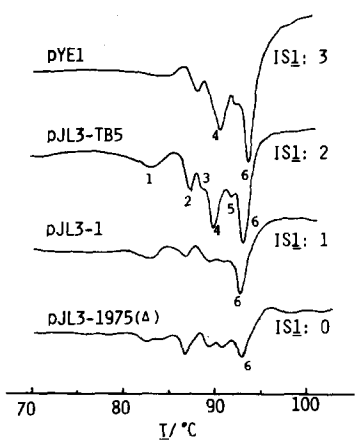
DSC runs of the linear double-stranded pJL3-TB5 DNA digested with HincII were performed. At least six endothermic peaks are seen in the temperature range over 81 to 96 °C on the DSC curve as shown in Fig.3. This demonstrates that these peaks which are numbered 1 to 6 in the order of increasing temperature are due to blockwise melting along the DNA chain. Two peaks 4 and 6 constituted main peaks with a peak top at 90°C and 93°C. As shown in Fig.4, the histograms of GC content were constructed from the GC distribution maps shown in Fig.2 for pJL3-TB5 and the plasmids to be analyzed below. The two large peaks seen in pJL3-TB5 correspond to the two main peaks of the DSC curve for

the plasmid. This analysis suggests that the 90°C-peak is attributed to 46-50 % GC content regions including the Cm^r gene in the plasmid used and 93°C-peak to the 54-58 % GC region containing an insertion element IS1 (768 bp) (Figs.1 and 2).

DSC runs of the other pJL3 derivatives were performed (Fig.3). The most drastic change is observed in peak-6. The change depends on the number of copies of IS1 which has the highest GC content in the plasmids employed in this study (Fig.2), so that the height of peak-6 increases approximately in proportion to the number of IS1 copies. This result experimentally confirms the suggestion mentioned above. However, peak-6 considerably remains still in plasmid pJL3-1975 (Δ) possessing no IS1 sequence. This must be due to the high GC content region in the non-IS1 portion (Fig.2).

The second main peak numbered 4 in plasmids pYE1 and pJL3-TB5 was diminished in the others, pJL3-1 and pJL3-1975(Δ), as seen in Fig.3. The latter two plasmids do not have the intermediate GC content region of Tn9 containing the Cm^r gene (Fig.2), while the former two plasmids have this region, confirming that peak-4 is attributed to this intermediate GC content region.

Plasmid pJL3-1012 deletes only a 119 bp sequence (see Fig.1). The deleted region is relatively low in GC content(42-43%, Fig.2). pJL3-1012 as well as pJL3-TB5 were digested with restriction enzyme EcoRI with which both plasmids are cleaved only at a single site, and used for DSC runs. As shown in Fig.5,



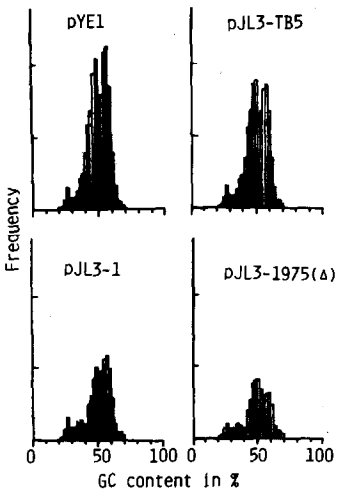


Fig.3 (left). Effect of large deletions and duplications on plasmid DNA melting. The adiabatic calorimeter, MC-1, was used. All the curves were normalized to the equal molar concentration.

Fig.4 (right). Histograms for GC content in moving segments of each plasmid. See ref.17 for construction of a histogram in detail. All the histograms were normalized to an equal molar concentration.

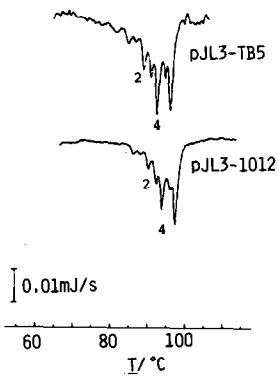
both peak-2 and peak-4 decrease in pJL3-1012 compared with pJL3-TB5. This result must be due to the effect of deletion of the low GC content region, suggesting that the DSC of plasmid DNA is so sensitive that a small change in the sequence affects the profile of DSC curves.

Effect of a Structural Change in DNA Sequence on Melting

DSC runs of a plasmid cleaved with restriction enzymes at different recognition sites were performed. Fig.6 illustrates the DSC curves for the two linear DNA samples prepared by digesting pJL3-TB5 with restriction endonucleases EcoRI and HincII, respectively. The change of profile of DSC curves is significant in the peaks 1-2, in a low temperature region. This suggests that the terminal sequences of linear DNA chain tend to melt at the lower temperature in comparison with the internal region of the same chain. Similar results have been described using a spectrophotometrical method (22).

The results above show that the DSC curves of plasmid DNA reflect remarkably in detail the structure of DNA sequence through blockwise or cooperative melting profile.

We have demonstrated in the present and previous papers that the fine structure of DNA melting can be observed also by use of DSC, when the relatively small DNA such as plasmid DNA is used. Furthermore, in this paper, we have obtained experimental evidence for the attribution of a few peaks predicted from the previous GC distribution analysis of the plasmid DNA chain (17). The results suggest that the relation of Marmur and Doty that the melting tempera-



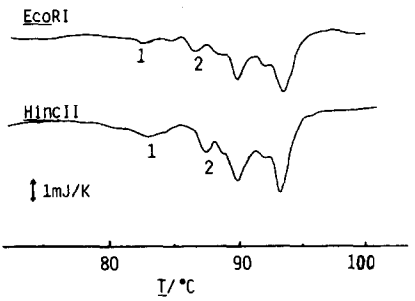


Fig.5 (left). Effect of a small deletion on plasmid DNA melting. The heat flux type of calorimeter, SSC 560U, was used.

Fig.6 (right). Effect of cleavage with different restriction enzyme on pJL3-TB5 DNA melting. The adiabatic calorimeter, MC-1, was used.

ture (Tm) of DNA is proportional to its overall GC content seems to apply approximately in the regional sequence within the molecule of DNA (21).

We believe that thermal methods have some advantage over spectroscopical method, as follows: (i) data can be obtained in a differential form proportional to reaction rate, (ii) enthalpy can be measured directly, and (iii) the measurement of DNA melting is possible in the solvents containing some chemicals which have absorption band at 260 nm. We therefore expect that the DSC method to plasmid DNA is useful for the study of interaction between DNA and various drugs and for the study of the structure of higher order such as supercoil and cruciform which are assumed to be important for expression of genes. Also the detailed determination of enthalpy for each transition along DNA chain probably provides more information for physico-chemical properties of DNA structure.

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