Localization of low-melting regions in phage T7 DNA

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ABSTRACT

Specific fragmentation of T7 DNA at glyoxal-fixed denatured regions by the S1 endonuclease followed by restriction analysis made it possible to localize four low-melting regions in phage T7 DNA. These regions have the following coordinates: $0.5-1.2$; 14.8 ± 0.3 ; 46.3 ± 0.5 ; 98.4 ± 0.3 (in T7 DNA length units). The location of the low-melting regions was refined by means of electron-microscopic denaturation mapping and gel electrophoresis of partially denatured DNA. The obtained localization of the low-melting regions is consistent with the available data on the sequence of T7 DNA. The map of low-melting regions was compared with the genetic map of T7 DNA.

INTRODUCTION

Bacteriophage T7 is among those best-known both genetically and biochemically (1). Detailed restriction maps, for a great number of endonucleases, are available for T7 DNA (1,2,3). Recently the sequence was determined of almost one third of the T7 genome starting from its left end and the functional properties and structural features of that part of the genome were thoroughly analysed (4,5). Specifically, two AT-enriched regions (79% AT pairs) 61 and 81 bp long were detected, respectively, in the primary replication area and between promoters A0 and A1 at approximately 14.8 and 1.0 units on the T7 map.

We had earlier detected and localized two low-melting regions in T7 DNA (6). Such regions have a much higher than average AT content (72% as against 52% AT pairs) an aggregate length of ca. 400 bp and correspond to a clear-cut peak at the beginning of the differential melting curve for T7 DNA (6-8). A specially elaborated technique was used involving the fixation of melted DNA regions with glyoxal and a subsequent fragmentation by the S1 endonuclease at the regions thus fixed; as a result two low-melting regions were localized in T7 DNA (6). However, the data obtained were not sufficient for orienting the map of those regions relative to the functional and physical maps of T7 DNA or for a direct comparison with the sequence. Besides, as

remarked in (6), possibly not all the low-melting regions were detected. The present paper deals with both these questions.

The map of low-melting regions was oriented relative to the genetic map through a comparison of the fragmentation at the low-melting regions for wild-type T7 DNA and its deletion mutant D111. Restriction analysis of the S1 fragments of T7 DNA supplemented with electron-microscopic denaturation mapping of the promoter fragment and gel electrophoresis of the partially denatured DNA revealed two more low-melting regions and refined the location of those detected earlier.

MATERIALS AND METHODS

DNA. T7 DNA was isolated in the same way as in (6) and made available to us by Dr. L.V. Neumyvakin. T7 D111 DNA was obtained from Dr. L.I. Patrushev. A 1461-bp-long BspI fragment of T7 DNA containing promoters for E.coli RNA polymerase (9) (HaeIII C1 fragment (1)) was given us by Dr. E.F. Zaichikov from the Novosibirsk Institute of Organic Chemistry, USSR Academy of Sciences (Siberian Branch).

Enzymes and DNA cleavage. The BspI endonuclease (isoschizomer of HaeIII) was given us by Dr. M.A. Grachev from the Novosibirsk Institute of Organic Chemistry. The Sau 3A endonuclease (isoschizomer of DpnII) was supplied by Dr. V.E. Alatortsev from the Institute of Molecular Genetics, USSR Academy of Sciences. The S1 endonuclease preparation (Sigma, USA) was used without additional purification. The fixed DNA was cleaved by S1 nuclease in a buffer containing 20 mM sodium acetate (pH 4.6) and 0.1 mM ZnSO₄ as described in (6).

Fixation of DNA. The low-melting regions of T7 DNA were fixed with glyoxal (Sigma, USA) in 0.1SSC at 69°C as described in (6). DNA melting was followed by means of a Cary 219 spectrophotometer.

Electrophoresis and electroelution of DNA. Analytical electrophoresis of DNA fragments was carried out in 3 mm thick agarose or polyacrylamide gel slabs. The preparative separation of DNA fragments was performed by means of electrophoresis in a horizontal 160x130x8 mm 0.8% agarose gel slab. The electrophoresis buffer contained 40 mM Tris-acetate (pH 7.8), 5 mM sodium acetate and 1 mM EDTA. DNA was electroeluted from the gel as described in (10). Ethidium bromide staining and photographing of the gels was performed as in (6). The negatives were traced by means of an IFO-451 microphotometer (USSR).

DNA melting maps. The BspI C1 fragment of T7 DNA with fixed melted regions

was prepared and the electron-microscopic denaturation maps were constructed in the same way as in (11) with the exception that the glyoxal concentration was somewhat higher in the first and second steps of fixation: 1 and 10 mM respectively. This change in the fixation procedure allows a reliable visualization of short (100 to 200 bp) regions, which is close to the maximum resolution of the electron-microscopic method used. The unwinding of the denatured regions from the ends in the course of fixation did not exceed 20 to 30 bp.

RESULTS

Orientation of the map of low-melting regions.

Figure 1 shows the electrophoretic separation of S1 fragments after cleavage at the low-melting regions for wild-type phage DNA (Fig.1b) and its deletion mutant D111 (Fig.1a). The deletion is seen to alter the mobility of the shortest C fragment (Fig.1c). Quantitative analysis of the electrophoretic data shows that the difference in the mobility of C and C' fragments corresponds to a ca. 1200 bp difference in size. This value is consistent with the size of the D111 deletion (1120 bp). The deletion has the coordinates 1.30 to 4.10 (in per cent of the total DNA length) and covers the region of early genes to the right of the A2 promoter up to gene 0.6 (1). Hence S1 C fragment contains early genes of phage T7.

It has been shown earlier that A,B and C fragments produced by S1 nuclease are situated on T7 DNA in the order A-B-C (6). Under the accepted orientation for T7 DNA the early genes are disposed from the left end of the molecule. Therefore, since C fragment includes the early part of the genome, the correct orientation of the fragments is C-B-A.

Identification and localization of low-melting regions.

The procedure described above for the detection of low-melting regions cannot reveal DNA fragments of a few hundred bp, whereas such fragments might form if there were low-melting regions at a distance of a few hundred bp from the regions detected or from the ends of the DNA molecule. To check this we made use of the restriction analysis of the S1 fragments.

A,B and C fragments produced by S1 nuclease were separated through preparative electrophoresis, then individual fragments of T7 DNA were electroeluted from the gel. Figure 2 shows the electrophoretic separation of the BspI fragments of S1 C fragment (Fig.2a) and a total T7 DNA (Fig.2b). The notation of the BspI fragments for the early region of T7 DNA is taken from (1). One can see that the BspI cleavage of S1 C fragment yields D1 and E1

FIGURE 1. Electrophoresis in 0.8% agarose of DNA fragments resulting from S1 cleavage at low-melting regions: a) deletion mutant D111; b) wild type T7 DNA; c) mixed fragments of both types.

fragments, while 1461-bp-long C1 and 561-bp-long H5 fragments are absent. At the same time a ca. 950-bp-long fragment was detected, which we denoted C1*. It follows from Figure 3 that C1* fragment could only have formed upon the cleavage of C1, the longest of the BspI fragments making up the S1 C fragment.

A special study was carried out in order to localize the low-melting region in the C1 fragment. Figure 4 shows the differential melting curve of

FIGURE 2. Electrophoresis in a 2% agarose gel of BspI fragments of a) S1 C fragment of T7 DNA; b) total T7 DNA.

FIGURE 3. Map of low-melting regions in the early part of T7 DNA. Arrows indicate low-melting regions. BspI restriction maps of the early region of T7 DNA and S1 C fragment are shown. The notation of fragments is taken from (1).

the C1 fragment of T7 DNA. Up to 69°C (the temperature at which the low-melting regions were fixed with glyoxal in the whole T7 DNA) the regions melting in the C1 fragment give rise to peaks 1 and 2 (see also (10,12)). The regions giving rise to either peak were localized by means of electron-microscopic mapping in the way described in (11). The melted regions were fixed with glyoxal at 67°C and 69°C then the fixed DNA preparations were subjected to electron microscopy. Figure 5 shows the denaturation maps corresponding to either temperature of fixation. One can see that the first peak on the differential melting curve of the C1 fragment is associated with

FIGURE 4. Differential melting curve of the BspI C1 fragment of T7 DNA in 0.1SSC. Arrows indicate the fixation temperatures of low-melting regions in the C1 fragment.

FIGURE 5. Denaturation maps of the BspI C1 fragment of T7 DNA corresponding to two fixation temperatures. The ordinate is the probability of a given section being unwound. The shaded areas are the difference between the bottom and the top maps. This method demonstrates the DNA regions melted upon the transition from one fixation temperature to the other.

the melting of a ca. 160-bp-long region with the coordinates 0.7 and 1.1 (as referred to the total length of T7 DNA in accordance with the notation in Fig.3). The second peak in Figure 4 is mainly due to the melting of a ca. 100-bp-long region to the left of the first one. As a result a melted region appears with the coordinates 0.5–1.2; its cleavage by the S1 nuclease yields the C1* fragment.

Thus there are two low-melting areas in the early part of T7 DNA, one of them including two regions that are only slightly different in thermal stability and are located close to each other (0.5–1.2). The other area is in the BspI H5 fragment of T7 DNA. Since we have not detected any residue of the fragment after the low-melting part splits out, we have for the time being defined the coordinates of the low-melting region by those of the entire H5 fragment: 14.6–15.1.

The BspI experiments only allow the localization of those low-melting regions that are located in the early part of the T7 genome. To refine the mapping of the low-melting regions detected and identify more such regions

FIGURE 6. Electrophoresis in 0.8% agarose of Sau3A restriction fragments of a) native T7 DNA; b) S1 fragments of T7 DNA. Also shown is the electrophoresis of S1 fragments of T7 DNA - c). The notation of fragments is explained in the text. The left column corresponds to line (a), the right column corresponds to line (b).

we cleaved the S1 fragments of T7 DNA with the Sau 3A endonuclease.

Figure 6 presents the electrophoresis of DNA fragments obtained after the Sau3A treatment of native T7 DNA (Fig.6a) and S1 fragments of T7 DNA (Fig.6b). These results can be conveniently analysed with the help of the Sau3A restriction map for T7 DNA from (2) (Figure 7). To identify the bands in Figure 6a we determined fragment sizes from their electrophoretic mobility. The sizes of fragments produced by Sau3A cleavage of S1 fragments of fixed T7 DNA are shown in the left column of Table I. Intensity measurements based on densitometry of the negatives have demonstrated that band D* in Figure 6b has more than double the intensity of band E* (data not shown). The right column of Table I shows the sizes some of the Sau3A fragments of T7 DNA reported in (2).

In view of the known location of the low-melting regions in the early part of the T7 genome we can expect the cleavage of the Sau3A B fragment at these regions to yield subfragments sized 13.4, 5.6 and 0.7 (in T7 DNA length units). Table I shows that these are the sizes of B* and F* fragments in Figure 6b (the shortest leftmost fragment goes out of the gel under the chosen conditions of electrophoresis). The cleavage of the Sau3A A fragment at the low-melting region (whose approximate position can be

FIGURE 7. Map of low-melting regions. Arrows indicate low-melting regions. Shown below are the Sau3A restriction maps of T7 DNA and S1 fragments of fixed T7 DNA. The genetic map of phage T7 with a number of functionally important regions specified is indicated.

inferred from the data in (6)) must yield subfragments sized 43.4 and 10.1. These are respectively A* and C* fragments in Figure 6b. D and E fragments (Fig.6a) do not contain low-melting regions, hence they correspond to bands D* and E* in Figure 6b. Figure 6b does not show a band corresponding to Sau3A C fragment. It means that the Sau3A C fragment contains a low-melting region whose splitting yields a fragment close in size to Sau3A D fragment, which explains the double intensity of band D* compared to E*.

Thus the Sau3A-based analysis of fragment lengths has made it possible to refine the location of the three low-melting regions detected previously and to detect another region near the right end of T7 DNA.

Table 1. The sizes of fragments obtained by Sau 3A cleavage of S1 fragments of T7 DNA and native T7 DNA. Fragment sizes are expressed in T7 DNA length units. The sizes of the Sau 3A fragments of T7 DNA are taken from (2).

S1/Sau 3A fragments	Length	Sau 3A fragments	Length
A*	44.5 $\pm$ 3	A	53.57
B*	13.4 $\pm$ 0.3	B	20.67
C*	10.3 $\pm$ 0.2	C	9.55
D*	7.8 $\pm$ 0.1	D	7.82
E*	7.1 $\pm$ 0.1	E	7.10
F*	5.9 $\pm$ 0.1

FIGURE 8. Electrophoresis in a 6% polyacrylamide gel of BspI restriction fragments of a) native T7 DNA; b) glyoxal-fixed partially denatured T7 DNA. Arrows indicate fragments with reduced mobility. C) - electrophoresis of the BspI C1 fragment.

The low-melting regions of T7 DNA have the following coordinates:
 0.5-1.2, 14.8 ± 0.3 , 46.3 ± 0.5 , 98.4 ± 0.3 (see Fig. 7).

Electrophoresis of partially denatured DNA.

We used electrophoresis of partially denatured DNA in polyacrylamide gel for an additional check of the localization of the low-melting regions. The electrophoretic mobility of DNA was found in (13) to be sharply reduced by its partial denaturation. We have shown this to be also true of glyoxal-fixed partially denatured DNA (14). The low-melting regions in T7 DNA were fixed with glyoxal, then the DNA was treated with the BspI endonuclease and the resulting fragments were separated in 6% and 10% acrylamide gels under normal conditions.

Figures 8 and 9 show the results of these experiments. Figure 8b lacks the bands corresponding to C1, F3 and H5 fragments. Figure 9b presents the separation of the shorter BspI fragments of T7 DNA; here the band corresponding to L4 fragment is also absent. Hence C1, F3, H5 and L4 fragments contain denatured low-melting regions which reduced their mobility.

These data confirm the presence of low-melting regions in the C1 and H5 fragments. Although the F3 and L4 fragments have not been localized, the location of the low-melting regions suggests that one of them is in the

FIGURE 9. The same as in Figure 8 a,b,c except that the gel is 10% polyacrylamide.

central part of the molecule and includes the 46.3 ± 0.5 region, while the other is at the right end of the molecule and includes the 98.4 ± 0.3 region.

DISCUSSION

The localization of the low-melting regions in T7 DNA is summarized in Figure 7 which shows a partial functional map of T7 DNA as well as a map of the low-melting regions.

It is interesting to compare our results concerning the location of the low-melting regions, which have an average length of 100 bp and an average AT content of ca.72%, with the known sequence of T7 DNA. The primary structure of about one third of T7 DNA is reported in (4,5). According to (4), the early part of the T7 genome contains two sizable AT-enriched regions of more than 60 bp with an AT content of 79%. One of them is situated between the A0 and A1 promoters and has the coordinates 0.92-1.12. It is here that a low-melting region was detected (see Figure 7). In fact it follows from the melting curve and equilibrium mapping (Figures 4,5) that this part of the T7 genome contains not one but two low-melting regions with a 2°C difference in melting temperature. Analysis of the base sequence shows that there are indeed two AT-enriched regions between the A0 and A1 promoters, one of them 137 bp long (0.89-1.23) with 77% AT pairs, the other 89 bp long

(0.66–0.88) with 64% AT pairs. These results are in good agreement with our data concerning the location and size of the low-melting regions in this part of the molecule and the difference in their melting temperatures. The second AT-enriched region reported in (4) has the coordinates 14.83–14.97. Here we have mapped another low-melting region (14.8 ± 0.3).

Figure 7 makes it clear that the first two low-melting regions are located in functionally important parts of the T7 genome. The first is between the A0 and A1 promoters. The second is behind gene 1 encoding phage RNA polymerase, and it is here that the primary replication site of T7 DNA is situated (15). It is supposed in (4) on the basis of the sequence data that the 1.0–1.2 area, i.e. the region between the A0 and A1 promoters containing the first low-melting region, may be important to the functioning of the secondary replication site. It is surmised in the same paper that this terminal section of T7 DNA may be involved in concatemer formation, packing or maturation of DNA ends. If this is true, a similar functional section may exist at the right end of T7 DNA. It is reported in (4) that the right end of T7 DNA promoted replication of recombinant plasmid during T7 infection. Therefore it may not be at all coincidental that there is a low-melting region at practically the same distance from the right end of T7 DNA as from its left end. The central low-melting region is located on the boundary between the genes encoding late RNA II and late RNA III, which may be its functional significance.

Thus T7 DNA has regions that are different in thermal stability from the rest of the molecule and are located in functionally important parts of the T7 genome.

Electrophoresis in polyacrylamide gel of fixed partially denatured DNA (see Figures 8 and 9) is a fast and convenient technique for identifying the fragments containing low-melting regions. It can also be used for restriction and denaturation mapping (14).

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