Volume 1 number 2 February 1974 Nucleic Acids Research

Thermal denaturation of calf thymus DNA: existence of a GC-richer fraction

Hsueh Jei Li, Benjamin Brand and Arnold Rotter

Department of Chemistry, Brooklyn College of the City University of New York, Brooklyn, New York 11210, USA

Received 10 December 1973

ABSTRACT

In 2.5×10^{-4}M EDTA buffer, the derivative melting curve of calf thymus DNA shows a major band at 47° with a shoulder at about 54°. The fraction of melting area of this shoulder is about 13%. For reconstituted polylysine-calf thymus DNA complexes, in addition to the melting of free DNA regions at about 50° (T_m) there is another melting at about 106° (T_m') of polylysine-bound regions. The melting band of the complex at T_m is not symmetrical. As more polylysine is bound to DNA the melting amplitude is diminished greatly on the major band at 47° but only slightly on the shoulder at 54°. The insensitivity of this shoulder appears to result from the existence of a 13% fraction of calf thymus DNA containing 55% GC. It is not favorably bound by polylysine. It remains in the supernatant after centrifugation and melts at about 54-56°. This conclusion is further supported by two facts: the reconstitution method provides a condition for selective binding of polylysine to AT-rich DNA, and it yields a fully symmetric melting band at T_m for complexes of polylysine with homogeneous bacterial DNA such as the one from M. luteus.

INTRODUCTION

Derivative plots of melting curves are more informative than melting curves themselves, particularly in protein-DNA complexes (1-7). We recently showed a non-symmetrical melting band for pure calf thymus DNA with a major band at 47° and a shoulder at about 54° (7). The asymmetry of this melting band indicates that perhaps calf thymus DNA molecules are heterogeneous with respect to base composition. In other words, though the average base composition of the total calf thymus DNA population is 42% in GC (guanine + cytosine), it is possible that this is only a statistical average of DNA molecules with varied GC contents. The shoulder at about 54° of the melting band might imply the existence of a minor fraction of calf thymus DNA with higher GC content. In order to test this hypothesis the melting properties of polylysine-DNA complexes prepared by salt gradient dialysis (reconstitution method) were carefully studied. It has been shown before (8) that polylysine binding to DNA by this method

is cooperative. The complex prepared can be separated into two fractions by centrifugation. The pellet is the DNA almost fully covered by polylysine while the supernatant is essentially pure DNA. Further, it has also been shown (9) that polylysine preferentially binds AT-rich DNA at high salt. Since by salt gradient dialysis the mixture of polylysine and DNA is exposed to high salt, it is possible that AT-rich DNA molecules may be bound preferentially. Combining these two considerations it may be expected that, if calf thymus DNA is really heterogeneous with respect to its GC content, the AT-richer molecules will be bound more favorably and remain in the pellet after centrifugation, while the GC-richer molecules will be free of polylysine binding and remain in the supernatant.

In this report it is shown that the previous hypothesis of heterogeneous population in calf thymus DNA is correct. Indeed the reconstitution method provides conditions for cooperative binding of polylysine to DNA and its selective binding to AT-rich DNA in a heterogeneous population. It is concluded that calf thymus DNA has a minor fraction (about 13%) with a GC content about 55%, compared with an average of 42% GC for whole calf thymus DNA (10). This report provides another example of ways in which the knowledge of helix-coil transition in DNA and nucleoprotein, when used very carefully, can be a very useful tool for the investigation of physical properties in DNA and nucleoprotein.

MATERIALS AND METHODS

Calf thymus DNA was purchased from Sigma Chemical Co. and was purified by phenol extraction. M. luteus DNA was purchased from Miles Laboratories, E. coli (strain B) and Cl. perfringens DNA from Sigma Chemical Co.; These were used directly without further purification. GC (guanine + cytosine) content of DNA is considered to be 31% for Cl. perfringens, 42% for calf thymus, 53% for E. coli and 70% for M. luteus (10). The following molar extinction coefficients of DNA at 260 nm used is 7400$M^{-1}cm^{-1}$ for Cl. perfringens, 7000 for M. luteus (11) and 6500 for calf thymus. Poly- (L-lysine) hydrochloride (mol wt 170,000) was purchased from Schwarz/Mann.

Polylysine-DNA complexes were prepared by salt gradient dialysis as described by Olins et al. (8), except that 0.01M Tris, pH8.0, was used rather than cacodylate buffer. This method of reconstitution is essentially similar to the one first used by Huang et al. (13). The complexes were finally dialyzed against 2.5 x 10^{-4}M EDTA, pH8.0 (EDTA

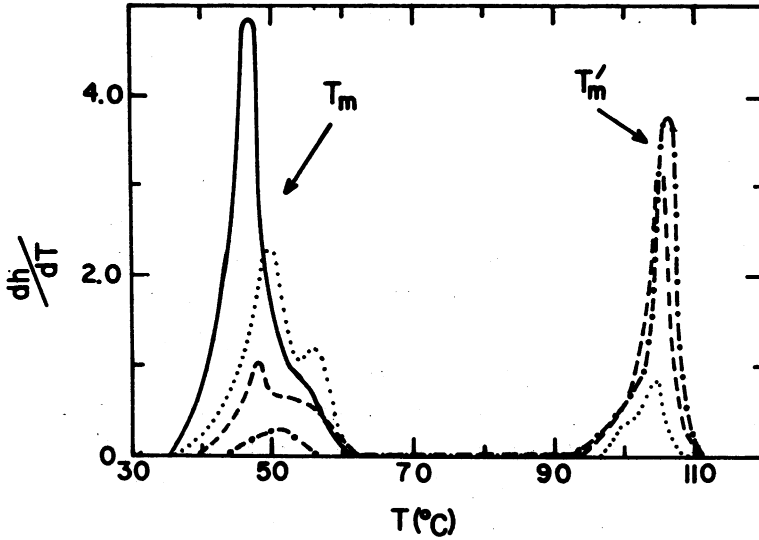
buffer). The input ratio of polylysine to DNA in each complex is reported in terms of lysine/nucleotide. Thermal denaturation experiments were performed on a Gilford Spectrophotometer model 2400-S and the results were analyzed as described earlier (2).

RESULTS

The melting band of pure calf thymus DNA is not symmetrical (7). There is a major band at 47° and a shoulder at 54°. The fraction of melting area under this higher melting band is 13% (Fig. 1). It indicates that possibly calf thymus DNA molecules are heterogeneous with respect to base composition. In other words, though the average base composition of the total calf thymus DNA population is 42% in GC, it is possible that this represents a statistical average of different DNA molecules of varied GC contents. In order to test this hypothesis results of the following experiments were analyzed.

Fig. 1 shows the derivative plots of melting profiles of polylysine-DNA complex with input ratio of 0.6 lysine residue per nucleotide. The results of the pellet and the supernatant of this complex after centrifugation at 10,000 rpm for 30 min in Sovall SS-34 rotor are also included. The complex itself shows a biphasic melting with 57% of the total hyperchromicity occurring at 106.5° (T_m') and 43% at 50° (T_m). The striking feature is that the melting band at T_m is not symmetrical. Compared with the melting band of pure DNA, the main peak at 47° is greatly reduced while the shoulder at about 54° is reduced only slightly.

The results in Fig. 1 show that the binding of polylysine to DNA is cooperative. For instance, in the pellet, 87% of the total melting area occurs at T_m' while only 13% occurs at T_m whereas for the original complex 57% is at T_m' and 43% at T_m. The supernatant, on the other hand, has only 15% of the total melting area at T_m' and 85% at T_m. The failure of attempts to separate the complex into two fractions with monophasic melting (at T_m with the supernatant and at T_m' with the pellet for instance) may indicate a non-perfect cooperative binding. The residual melting either at T_m for the pellet or at T_m' for the supernatant also appears in the report of Olins et al (8), but it is more clearly shown by the derivative plot in Fig. 1 than by a melting profile of Olins et al (8). The use of low ionic strength in this report also gives a better separation of T_m and T_m' and shows the residual melting as a distinguishable band (Fig. 1).



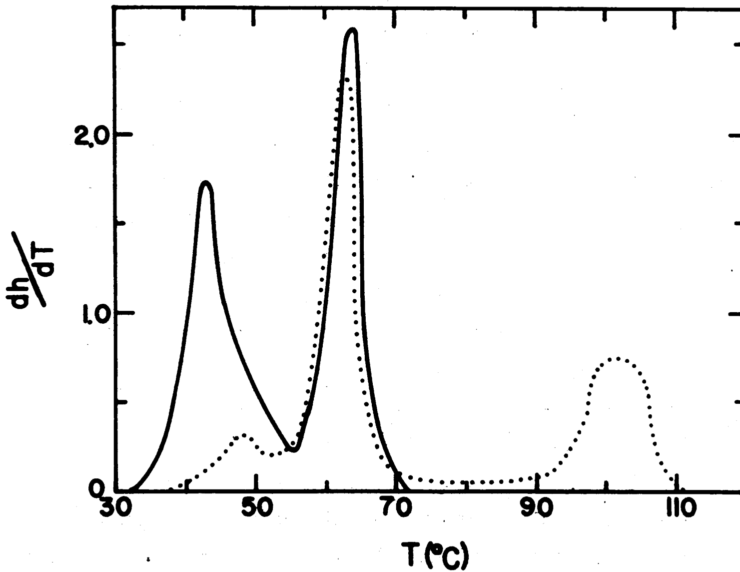


Fig. 1. Derivative melting curves of a reconstituted polylysine-DNA complex with 0.6 lysine/nucleotide. Original complex (– – –), pellet (– . –) and supernatant (. . . .). Also included is DNA (——).

Fig. 2. Derivative melting curves of a mixture of Cl. perfringens and M. luteus DNA and its complex with polylysine prepared by reconstitution. Input ratio of these two DNA is one to one. Input lysine/nucleotide is 0 (——) and 0.22 (. . . . .).

As discussed above the interpretation of the small amount of melting at T_m of the pellet can be due to a non-perfect cooperative binding which leaves a small amount of short free DNA gaps in a complex without 100% coverage on the DNA by polylysine. The pellet is not the key point for this investigation. The key point is in the melting at T_m of the supernatant (Fig. 1). It has a main peak at 49° and a strong second peak near 56°. The shift of T_m and a stronger contribution of the second band at 56° can not be accounted for by small free DNA gaps between polylysine-bound regions, because in the supernatant only 15% of the total melting occurs at T_m'. This fraction, therefore, must be due to DNA molecules with GC content higher than 42% which have lower affinity for polylysine and therefore remain in the supernatant after centrifugation.

The conclusion that heterogeneous DNA molecules of varied GC contents can be separated by reconstituted polylysine-DNA complex formation using salt gradient dialysis is supported by the results shown in Fig. 2. A mixture of equal amount of M. luteus DNA of 70% GC and Cl. perfringens DNA of 31% GC is complexed with polylysine by salt gradient dialysis. In the absence of polylysine, Cl.perfringens DNA melts at 42° and M. luteus DNA at 63°. With an input ratio of 0.22 lysine per nucleotide, nearly all of Cl. perfringens DNA is bound, while almost all of the M. luteus DNA is free. Salt gradient dialysis therefore provides the condition for selective binding of polylysine to AT-richer DNA in a heterogeneous population.

If the conclusion concerning the shoulder at about 54° in the derivative melting curve of calf thymus DNA is correct, it is expected that no shoulder will appear in a complex of polylysine with a homogeneous DNA population such as that from bacteria.

Reconstituted polylysine-M. luteus DNA complexes were prepared by the same method of salt gradient dialysis. Thermal denaturation results are shown in Fig. 3. Just as in the case of calf thymus DNA, the derivative melting curves of the complexes can be separated into two regions, one at 62.5 ± 0.5° (T_m) corresponding to the melting of free DNA base pairs and the other at about 111.5 ± 0.5° corresponding to that of polylysine-bound base pairs. Compared with calf thymus DNA, M. luteus DNA shows a symmetric melting band without any significant shoulder. The derivative melting bands at T_m of the complexes are also symmetric with respect to their peaks and there is no shift in melting temperature within 0.5°. That these results are completely different from those of complexes with calf thymus DNA fully supports our conclusions.

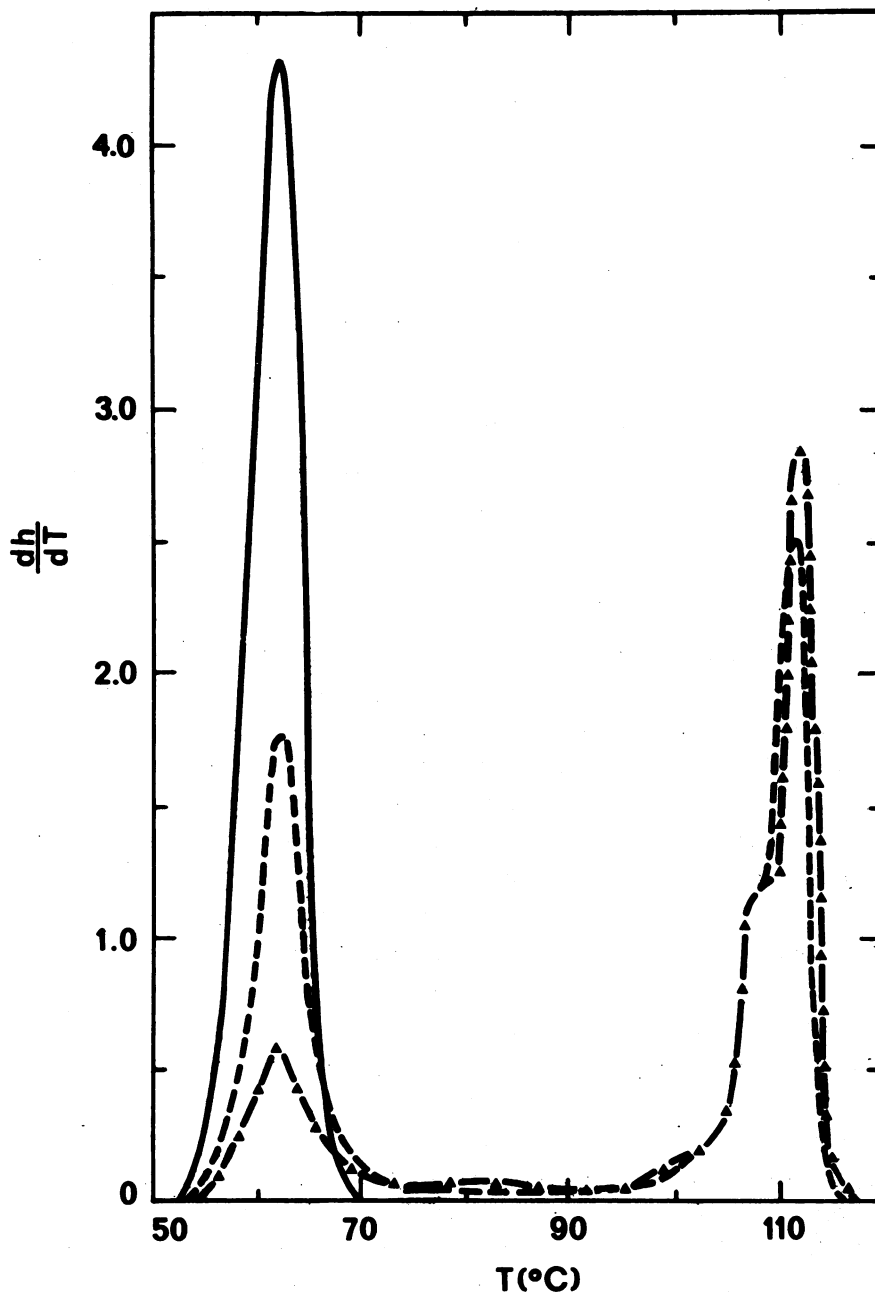


Fig. 3. Derivative melting curves of reconstituted polylysine-M. luteus DNA complexes. Input lysine/nucleotide is 0 (——), 0.4 (- - -) and 0.6 (- ▲ -).

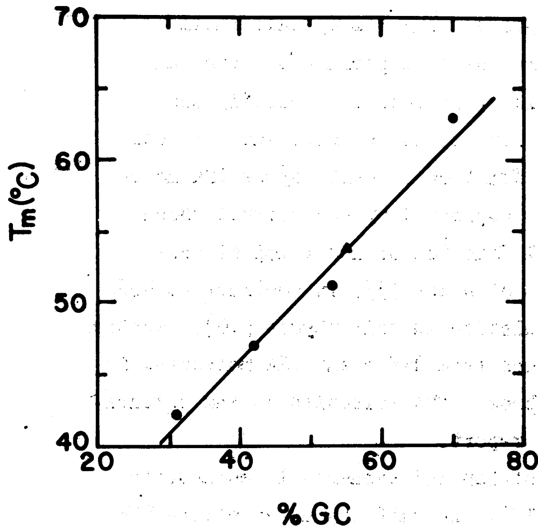


Fig. 4. Linear relation between GC content of DNA and its T_m in EDTA buffer. DNA from M. luteus, E. coli, calf thymus (T_{mI}) and Cl. perfringens (●). T_{mII} (54°) of the minor fraction in calf thymus (▲).

In order to determine the GC content of the minor fraction in calf thymus DNA molecules, the T_m's of four DNA with varied GC contents were determined using the same buffer of 2.5×10^{-4}M EDTA, pH8.0. The results are shown in Fig. 4. As expected (14), there is a linear relation between the T_m and the GC content. From this plot it is determined that there is a minor fraction of about 13% calf thymus DNA molecules which has approximately 55% GC compared with an average of 42% for the whole calf thymus DNA.

DISCUSSION

The dependence of a melting curve of DNA upon its GC content is well documented (14). Its dependence upon the distribution of GC pairs along a DNA molecule has also been well described (15, 16). Melting properties of calf thymus DNA have been studied for some time. Since a symmetrical melting curve with respect to its T_m is expected, where T_m was previously taken as the midpoint of a transition, the small tail of a melting curve at higher temperatures might have not been taken seriously before. However, when a derivative melting curve is plotted, the small tail in a melting profile becomes a non-trivial

shoulder. If it had not been repeated many times, this small shoulder would have been considered as an experimental error and neglected in the plot. Nevertheless, we took it seriously and designed experiments as reported here to investigate whether this shoulder might be due to a small fraction of calf thymus DNA molecules with higher GC content. As supported by experiments shown in Figs. 1 to 4, there is a small fraction of about 13% of calf thymus DNA which has a GC content of about 55%, in contrast to 42%, the average of the whole DNA population in calf thymus (10). Whether this heterogeneity originally comes from different DNA molecules in calf thymus chromosomes or from broken DNA molecules during preparation can not be answered by this report.

Heterogeneity in base composition and sequence in eucaroytic DNA is a well known phenomenon (17). In calf thymus satellite DNA with densities higher than the main DNA had been isolated. These satellite DNA renatured much more rapidly than the main DNA after denaturation, indicating the enrichment of repeated base sequence in the satellite fraction (18). Recently Pakroppa and Müller (19) also showed the existence of and further separated, several minor species sonicated calf thymus DNA with higher GC contents by using a hydroxyapatite column with a GC-specific DNA ligand. The existence of a minor fraction of calf thymus DNA which melts at 54° rather than 47° in EDTA buffer as reported here is in agreement with previous findings by using other techniques.

This report provides another example of the usefulness of thermal denaturation in obtaining information about DNA and nucleoprotein. Our laboratory is currently investigating the mechanism of helix-coil transition in nucleoprotein through the use of thermal denaturation in the belief that this approach can be used as a reliable tool for obtaining information of protein-DNA interaction such as in polypeptide-DNA complexes and chromatin.

ACKNOWLEDGEMENT

This work was supported by Grants GM20596-01 from National Institute of Health, GB35459 from National Science Foundation and Research Foundation of the City University of New York. B.B., and A.R. were receipients of National Science Foundation Undergraduate Summer Research Awards.

REFERENCES

1. Shih, T. Y. and Bonner, J. (1970) J. Mol. Biol. 48, 469-487.
2. Li, H.J. and Bonner, J. (1971) Biochemistry 10, 1461-1470.
3. Ansevin, A.T. and Brown, B.W. (1971) Biochemistry 10, 1133-1142.
4. Ansevin, A.T., Hnilica, L.S., Spelsberg, T.C. and Kehn, S.L. (1971) Biochemistry 10, 4793-4803.
5. Li, H.J. (1972) Biopolymers 11, 835-847.
6. Li, H.J. (1973) Biopolymers 12, 287-296.
7. Li, H.J., Chang, C. and Weiskopf, M. (1973) Biochemistry 12, 1763-1772.
8. Olins, D.C., Olins, A.L. and von Hippel, P.H. (1967) J. Mol. Biol. 24, 157-176.
9. Leng, M. and Felsenfeld, G. (1966) Proc. Nat. Acad. Sci. U.S 56, 1325-1332.
10. Sober, H.A. (1970) Handbook of Biochemistry (The Chemical Rubber Co., Cleveland, Ohio), p. H-84-100.
11. Felsenfeld, G. and Hirschman, S.Z. (1965) J. Mol. Biol. 13, 407-427.
12. Huang, R.C.C., Bonner, J. and Murray, K. (1964) J. Mol. Biol. 8, 54-64.
13. Olins, D.E. (1969) J. Mol. Biol. 43, 439-460.
14. Marmur, J. and Doty, P. (1962) J. Mol. Biol. 5, 109-118.
15. Crothers, D.M. (1968) Biopolymers 6, 1391-1404.
16. Crothers, D.M., Kallenbach, N.R. and Zimm, B.H. (1965) J. Mol. Biol. 11, 802-820.
17. Britten, R., and Kohne, D. (1968) Science 161, 529-540.
18. Polli, E., Ginelli, E., Bianchi, P. and Corneo, G. (1966) J. Mol. Biol. 17, 305-308
19. Pakroppa, W. and Müller, W. (1973) Proc. Nat. Acad. Sci. U.S. (in press).