POLYLYSINE BINDING TO HISTONE-BOUND REGIONS IN CHROMATIN

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Received April 18, 1972

SUMMARY

Thermal denaturation of calf thymus nucleohistone, polylysine-nucleohistone and polylysine-pure DNA were studied. In calf thymus nucleohistone polylysine binds preferentially to the DNA regions originally bound by non-histone proteins or the gaps between adjacent histone-bound regions and regions covered by less basic half-molecules of histones. Polylysine binds to chromatin with only one half efficiency when compared to polylysine binding to pure DNA. Using polylysine titration to determine the fraction of free DNA regions in chromatin is questionable.

One of the major questions concerning the structure of chromatin is the fraction of DNA free of protein binding. According to the experiments of template activity of chromatin and DNA-RNA hybridization, it was generally accepted that only about 5 to 20% of DNA in chromatin is free of histone binding (1-6). Recently, Clark and Felsenfeld (7) concluded from their studies that about 50% DNA in chromatin is free of protein binding. This conclusion was based upon two experiments showing that in chromatin about 50% of DNA is digestible by nuclease and also about 50% of DNA is titratable by polylysine. Mirsky (8) recently did extensive work on nuclease digestion of chromatin and showed that the amount of DNA digestible by nuclease depends upon the amount of enzyme added and time of enzyme action. In this communication we show that polylysine can also bind histone-bound regions in chromatin so that the binding sites on chromatin for polylysine can not be used as a method for measuring the fraction of free DNA regions in a chromatin.

Materials and Methods

Calf thymus chromatin was prepared according to the procedure of Shih and Bonner (9). It was sheared in a Waring blender for 1 min. and centrifuged for 20 min. at 10,000 rpm using a Sorvall SS-34 rotor. The supernatant was the soluble nucleohistone and was dialyzed against 2.5×10^{-4}M EDTA, pH 8.0.

Poly-L-lysine hydrochloride with molecular weight 170,000 was purchased from Schwarz/Mann. It was dissolved in 2.5×10^{-4}M EDTA and further dialyzed against the same buffer to remove chloride. Calf thymus DNA was purchased from Sigma Company and was purified by phenol extraction. It was also dialyzed into 2.5×10^{-4}M EDTA, pH 8.0 buffer. DNA concentration in both nucleohistone and pure DNA were determined spectrophotometrically by taking $\epsilon_{260_{nm}} = 6{,}500 M^{-1}\ cm^{-1}$ in nucleotide.

To a DNA or nucleohistone solution with a concentration about 1×10^{-4}M in nucleotide was added dropwise a poly-L-lysine solution which was 5×10^{-4}M in lysine residue. The resulting solutions were centrifuged for 10 min. at 10,000 rpm in a Sorvall SS-34 rotor at 4°C. The supernatant was collected and melted. The input ratio of lysine to nucleotide was 0.60. Thermal denaturation of nucleohistone, polylysine-DNA and polylysine-nucleohistone was performed at 260 nm using a Gilford spectrophotometer model 2400-S. The derivative of the melting profile was calculated according to the equation of Li and Bonner (10).

$$\frac{dh_{260}(T)}{dT} = \frac{h_{260}(T + 1) - h_{260}(T - 1)}{2}$$

where $h_{260}(T)$ is the hyperchromicity at 260nm and at temperature T.

Results and Discussion

The results are shown in Fig. 1. Adding poly-L-lysine to pure DNA shifts the hyperchromicity from 42°C for pure DNA to 98°C for the polylysine-DNA complexes. The fraction of the area of the melting band at 98°C is 0.60 of the total and is equal to the input ratio of lysine to nucleotide, indicating the ratio of one lysine per nucleotide in the complexed region (11).

The derivative curve of the melting profile of calf thymus nucleohistone shows

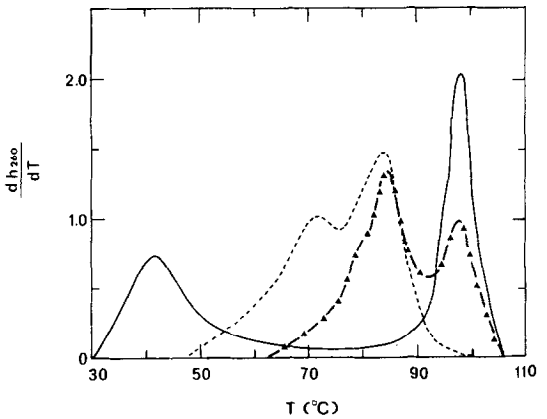

Fig. 1 Derivatives of melting profiles of poly-L-lysine-DNA complex with input lysine/nucleotide = 0.60 (—), of calf thymus nucleohistone (- - -) and poly-L-lysine-nucleohistone complex with input lysine/nucleotide = 0.60 (- △ -).

two bands with peaks at about 71 and 83°C, which are equivalent to the 66 and 81°C bands observed in pea bud nucleohistone (10). These bands are attributed to the melting of DNA covered by the less basic half- and the more basic half-molecules of histones. The derivative curve (Fig. 1) of calf thymus nucleohistone shows a tail between 50 and 60°C, in contrast to a shoulder near 52°C in pea bud nucleohistone (10). It can be due to either non-histone protein binding or to weak coupling of melting at the boundaries between free DNA and histone bound segments or to the small gaps between adjacent histone-bound segments. According to our previous study on thermal denaturation of pea bud nucleohistone, the result here indicates that there are no big stretches of free DNA regions in calf thymus chromatin because there is no melting near 42°C which is the melting temperature of pure DNA.

When polylysine was added to the nucleohistone, most of the hyperchromicity from 50 to 70°C disappears and is shifted to a new melting band near 98°C, corresponding to the melting of polylysine-DNA. However, there is only a slight decrease in hyperchromicity of the melting band near 83°C with a slight shift of the melting peak from 83.5°C to 84.5°C. The area of the melting band at 98°C is only about 30% of the total, indicating only 30% of the DNA base pairs in

calf thymus nucleohistone is complexed with polylysine, though the input ratio of polylysine to DNA is 0.60 lysine/nucleotide (11). The binding of polylysine to calf thymus nucleohistone is therefore not as effective as in pure DNA. This is not surprising because most of the DNA regions are covered by histones and are electrostatically shielded.

The most striking feature in Fig. 1 is that polylysine binds preferentially to those regions originally covered by non-histone proteins or to those gaps between adjacent histone-bound DNA regions (about 50 to 60°C), and then to the DNA regions covered by the less basic half-molecules of histones (near 71°C) and only little to the DNA regions covered by the more basic half-molecules of histones (near 83°C). This is consistent with our expectation and with our previous studies (10) which showed that electrostatic shielding on the phosphate lattice of DNA follows the opposite order. The DNA regions which are electrostatically more shielded will bind polylysine less favorably.

Our results here imply that polylysine does not replace histone when it binds to DNA. If it did, the melting near 83°C of the more basic half-molecule-bound regions would also disappear. This is in agreement with the results of Clark and Felsenfeld (7). However, whether polylysine replaces only the less basic half molecules of histones or simply binds superimposedly on them is still not answered by the present experiments.

Clark and Felsenfeld (7) used the amount of polylysine taken by unit chromatin (input lysine per nucleotide) to measure the fraction of free DNA regions in chromatin. This method can be legitimate only when (A) polylysine binds only free DNA regions and (B) every input lysine residue binds one phosphate or one nucleotide. The results in Fig. 1 show that both assumptions are not true. Polylysine can also bind protein-bound DNA regions which are electrostatically less shielded. Compared to pure DNA, lysine residue in polylysine binds DNA nucleotide in nucleohistone with only about 50% efficiency, namely the area under the melting band at 98°C in nucleohistone is only about 50% that of polylysine-pure DNA though the input lysine per nucleotide is identical in both cases. Therefore,

the conclusion of 50% free DNA regions in chromatin based upon polylysine titration (7) is obviously too high. Recently, a theory has been developed with equations which can be used for measuring the fraction of DNA regions covered by histones in chromatin. Based upon this theory and the results from Li and Bonner (10), it was concluded that 75 ± 8% of base pairs in pea bud nucleohistone are covered by histones (11).

Acknowledgment

This work is in part supported by a Faculty Research Award from the City University of New York. Manuel Weiskopf is a recipient of NSF undergraduate summer research award. The comment on this manuscript from Professor A. Lukton is acknowledged.

References

1. Paul, J., and Gilmour, R. S., J. Mol. Biol., 16, 242 (1966).
2. Marushige, K., and Bonner, J., J. Mol. Biol., 15, 160 (1966).
3. Bonner, J., Dahmus, M. E., Fambrough, D., Huang, R. C. C., Marushige, K., and Tuan, D. Y. H., Science, 159, 47 (1968).
4. Smith, K. D., Church, R. B., and McCarthy, B. J., Biochemistry, 8, 4271 (1969).
5. Kurashina, Y., Ohba, Y., and Mizuno, D., J. Biochemistry, 67, 661 (1970).
6. Shih, T. Y., and Bonner, J., J. Mol. Biol., 48, 469 (1970).
7. Clark, R. J., and Felsenfeld, G., Nature New Biology, 229, 101 (1971).
8. Mirsky, A. E., Proc. Nat. Acad. Sci., 68, 2945 (1971).
9. Shih, T. Y., and Bonner, J., Biochim. Biophys. Acta., 182, 30 (1969).
10. Li, H. J., and Bonner, J., Biochemistry, 10, 1461 (1971).
11. Li, H. J., manuscript to be submitted for publication.