Accessibility of chromatin to DNA polymerase I and location of the Fl histone

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ABSTRACT

The effect of prebound poly-L-lysine upon the template activity of DNA, chromatin and Fl histone-depleted chromatin for E.coli DNA polymerase I has been investigated. Measurements have been made in the absence and presence of 0.1M NaCl. From the results we conclude that only ca 60% of the polylysine-accessible DNA, i.e. 22% of the total DNA of the chromatin, is accessible to the DNA polymerase I and that ca. 30% of the polylysine-accessible DNA, i.e. 11% of the total DNA, is associated with the Fl histone.

INTRODUCTION

Chromatin is a poor template for E.coli DNA polymerase I. In a previous paper¹ we have shown that this low template activity arises from the limited accessibility of the chromatin DNA to the polymerase and not from a significantly lower rate of synthesis per enzyme molecule along the DNA, nor from the insolubility of the chromatin in the polymerase assay mixture. The DNA that is accessible to the polymerase is found within those zones that are accessible to poly-L-lysine; these comprise ca. 40% of the total DNA^{2,3}. Since DNA polymerase I has been used to study single-strand breaks in chromatin DNA^{1,4} it is important to know exactly how much of the DNA is accessible to the enzyme. This value is given by the present study, which deals with the changes in template activity of chromatin on addition of varying amounts of polylysine, both before and after removal of the Fl histone; also, information has been obtained about the location of the Fl histone.

MATERIALS AND METHODS**Materials**

DNA-dependent RNA polymerase I was prepared from E.coli B (obtained as a frozen cell paste from M.R.E., Porton) by the method of Jovin et al⁵. The four deoxyribonucleoside triphosphates were purchased from the

Boehringer Corporation (London) Ltd. [^3H]-dATP was purchased from the Radiochemical Centre, Amersham. Poly-L-lysine hydrobromide, mol.wt. 5,000 was obtained from Miles-Seravac Ltd, Maidenhead, Berks. Calf thymus DNA was purchased from Sigma London Chemical Co., Ltd and was activated by incubation with deoxyribonuclease I. Rat thymus chromatin was prepared as described previously⁶. Fl histone-depleted chromatin was prepared from the rat thymus chromatin by treatment with 0.6M NaCl according to the method of Bradbury et al⁷.

Prebinding of Polylysine to DNA and to Chromatin

10 μg activated DNA or DNA as chromatin was mixed with varying amounts of polylysine in a total volume of 100 μl water. The mixture was kept in ice for 30 minutes with shaking at regular intervals.

Assay of Template Activity

Assay mixture was added to the preformed DNA-polylysine or chromatin-polylysine complex so that the total volume was 300 μl and contained 10nmoles of dATP, dCTP and dGTP and 2nmoles of dTTP (1.0×10^6 cpm), 0.05 units DNA polymerase I, 0.07M Tris/HCl pH 7.4, 7mM MgCl₂, and 1mM mercaptoethanol. After mixing, the mixture was incubated at 37^o for 30 minutes. DNA synthesis was measured by precipitation with 5% trichloroacetic acid containing 1% Na₄P₂O₇·10 H₂O and counting the acid-insoluble radioactivity as described elsewhere¹. Under the conditions of these assays there is always an excess of enzyme over enzyme-binding sites on the template so that changes in the number of initiation sites on the template can be detected⁸.

RESULTS AND DISCUSSION

Addition of poly-L-lysine to DNA and to chromatin

In a previous paper we have shown that the addition of polylysine to chromatin in an amount sufficient to neutralise all accessible DNA-phosphates (equivalent to 40% of the total DNA phosphates) reduced the template activity to zero¹. To check that this was in fact due to the blockage of otherwise accessible initiation sites rather than to an effect on the enzyme or to the insolubility of the complex, varying amounts of polylysine were mixed with pure DNA and the template activity assayed. Fig.1. shows that the template activity decreases linearly with increasing ratio of lysyl residues to DNA-phosphates and becomes zero at ca. 100% neutralisation. In contrast, the solubility of DNA does not decrease linearly with increasing amounts of added polylysine but shows an initial lag³. The present result is therefore consistent with our previous finding that

insolubility of the template in the assay is not a major factor determining template activity¹. The action of polylysine must therefore be to reduce progressively the accessibility of the template to the enzyme.

When a similar experiment was carried out with chromatin, a biphasic decrease was observed, the template activity decreasing linearly to ca. 15% of the initial value and then decreasing more slowly with further increase of polylysine (see Fig.2). The template activity became zero at a ratio of lysyl residues to DNA phosphates of ca. 38%. Extrapolation of the initial phase to zero activity corresponded to a lysyl residue to DNA-phosphate ratio of ca. 22%.

The biphasic decrease in template activity of chromatin on addition of increasing polylysine suggests that there are two regions of polylysine-accessible DNA-phosphates, only one of which is accessible to DNA polymerase I. Also, the polylysine binds more strongly to the polymerase-accessible region than to the remainder. The intercepts corresponding to zero template activity show that ca. 38% of the DNA-phosphates are accessible to polylysine and that only ca. 60% of these are accessible to DNA polymerase I. The value of 38% accessible DNA-phosphates agrees favourably with the value of 40% accessible to polylysine obtained by direct titration^{2,7}.

Addition of polylysine to DNA and to chromatin in the presence of 0.1M NaCl

In these experiments polylysine was bound to DNA or to chromatin and also assayed in the presence of 0.1M NaCl. In the absence of pre-bound polylysine the effect of the NaCl is to reduce the template activity of the DNA to 45% and the template activity of the chromatin to 31% (Figs. 1 and 2). In the presence of polylysine the template activity of DNA in 0.1M NaCl decreases linearly and reaches zero at ca. 100% neutralisation of DNA-phosphates (see Fig.1). In the case of chromatin in 0.1M NaCl both the biphasic decrease in template activity and the ratio of lysyl residues to DNA-phosphates at which the activity becomes zero, namely ca. 38%, resemble the results in the absence of NaCl. However, extrapolation of the initial linear phase to zero activity corresponds to a higher ratio of lysyl residues to DNA-phosphates than in the absence of NaCl, namely to ca. 30% (see Fig.2). Thus the accessibility of the DNA to polylysine is unaffected by the presence of 0.1M NaCl whereas the accessibility to DNA polymerase I increases from 22% to 30%.

Fig.1. Effect of prebound polylysine on the template activity of activated calf thymus DNA for *E.coli* DNA polymerase I. O DNA alone. X DNA in the presence of 0.1M NaCl. The activity of activated DNA in the absence of both NaCl and polylysine is taken as 100.

Fig.2. Effect of prebound polylysine on the template activity of rat thymus chromatin for *E.coli* DNA polymerase I. O Chromatin alone. X Chromatin in the presence of 0.1M NaCl. The action of chromatin in the absence of both NaCl and polylysine is taken as 100.

Addition of polylysine to F1 histone-depleted DNA in the presence and absence of 0.1 M NaCl

It seemed very probable that the F1 histone was responsible for blocking part of the polylysine-accessible DNA to DNA polymerase I for it had previously been shown that in dilute NaCl (0.1 - 0.3M) large parts of the F1 histone are freed from the chromatin⁷. To test this possibility, chromatin was depleted of F1 histone and the template activity was assayed in the presence and absence of polylysine. Removal of F1 histone was checked by polyacrylamide gel electrophoresis using the method of Senshu⁹: this showed that the depleted chromatin contained no F1 histone while the fraction removed from the chromatin by 0.6M NaCl had the F1 histone band with a very small amount of the band comprising the F2b plus F3 histones (see Fig.3).

Fig.4. shows that in the absence of pre-bound polylysine the template activity of the F1 histone-depleted chromatin was ca. twice that of the original chromatin but in the presence of 0.1M NaCl was about 1.5 times the latter. With increasing amounts of pre-bound polylysine, both in the absence and presence of 0.1 M NaCl, the template activity of the F1 histone-depleted chromatin decreased linearly until it reached ca. 5% of the original activity of the depleted chromatin: it then remained constant up to a very high level of polylysine (corresponding to a ratio of lysyl residues to DNA-phosphates of over 100%). This constant level was reached at a ratio of lysyl residues to DNA-phosphates of ca. 40%, while extrapolation of the initial linear phase to zero activity corresponded to a ratio of ca. 33%.

This result shows that removal of the F1 histone from the chromatin leads to a large increase in the amount of DNA accessible to the DNA polymerase I and that the amount of DNA accessible to the enzyme is unaltered by the presence of 0.1M NaCl. Estimation of this amount from the increase in template activity (ca. two times) in the absence of polylysine is vitiated by the possibility that the preparation of the F1 histone-depleted chromatin introduces extra single-strand breaks (i.e. new initiation sites which would further increase the template activity) in the DNA. However, the extrapolation to zero template activity, which would be unaffected by any such extra breaks, shows that the amount of DNA polymerase I-accessible DNA increases on removal of the F1 histone from 22% to 33%. Thus the F1 histone is associated in the polylysine-accessible zones with ca. 11% of the total chromatin DNA. Table 1 summarises the results.

DNA-PHOSPHATES (% of total)		
	Accessible to Polymerase	Accessible to Polylysine
Normal chromatin	22%	38%
Normal chromatin in 0.1M NaCl	30%	38%
Chromatin minus F1	33%	40%
Chromatin minus F1 in 0.1M NaCl	33%	40%

Table 1. Accessibility of DNA in chromatin under a variety of conditions to DNA polymerase I and to polylysine, as measured by the template activity.

Titration of the F1 histone-depleted chromatin with poly-L-lysine, using methyl orange to detect unbound lysyl residues^{2,3} showed that the percentage of accessible DNA phosphates was 39% and 43% in two separate preparations: i.e. as might be expected, the value was similar to that of the original chromatin and to the value obtained in Fig.4. corresponding to the constant minimum template activity of the F1 histone-depleted chromatin. This means either that very few of the basic residues of the F1 histone are bound to the DNA-phosphates or that they are only weakly bound, thus allowing polylysine, but not DNA polymerase I, access to the DNA in this F1 histone-associated region. Even after removal of the F1 histone, however, access is still lower by 7% of the total DNA to the polymerase than to polylysine. Whether this is due to steric hindrance by other proteins or parts of proteins associated with the polylysine-accessible zones is unknown.

The constant, low template activity of the F1 histone-depleted chromatin at ratios of lysyl residues to DNA-phosphates above ca. 40% shows that there are regions of DNA accessible to the polymerase but not to polylysine. The exact nature of these regions is uncertain. Also, the reason for the differences in reduction of template activity of DNA, chromatin and F1-depleted chromatin on addition of NaCl in the absence of polylysine is unknown.

CONCLUSIONS

Several previous studies have indicated that part or all of the F1 histone is only weakly associated with the chromatin DNA compared to other histone fractions^{7,10-15} and that it is more accessible than the latter¹⁶. This is confirmed in the present study.

We conclude that in intact chromatin only ca. 60% of the DNA accessible to polylysine is accessible to DNA polymerase I. Further, from the absence of a detectable increase in polylysine accessibility, together

Fig.3. Polyacrylamide gel electrophoresis of the proteins from (a) intact chromatin (b) chromatin depleted of F1 histone by 0.6M NaCl and (c) the fraction removed by 0.6M NaCl. BPA: bovine plasma albumin.

Fig.4. Effect of prebound polylysine on the template activity of F1 histone depleted rat thymus chromatin for *E.coli* DNA polymerase I. O F1 histone depleted chromatin alone. X F1 histone depleted chromatin in the presence of 0.1M NaCl. The activity of intact chromatin in the absence of both NaCl and polylysine is taken as 100 (see Fig.2).

with the increase in accessibility to DNA polymerase I either on removal of the F1 histone from the chromatin or in intact chromatin in the presence of 0.1M NaCl, we conclude that three quarters of the remaining accessible DNA - 11% of the total DNA - is associated with the F1 histone. This conclusion holds whether or not proteins are redistributed^{14,17,18} and non-histone proteins removed by the 0.6M NaCl treatment for removing F1 histone.

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