DO HISTONES BIND TO A SPECIFIC GROUP OF DNA SEQUENCES IN CHROMATIN?

A TEST BASED ON DNA LIGASE ACTION ON RECONSTITUTED CHROMATIN

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SUMMARY: In the reconstitution of chromatin, the proteins are dissociated from the DNA by exposure to high ionic strength. If such a procedure is carried out in the presence of DNA containing single-strand breaks, the chromatin proteins will redistribute over this nicked DNA as well as over the DNA of the chromatin. When such reconstituted chromatin is treated with an excess of DNA ligase, about half of the nicks are sealed. If this partially sealed preparation is taken through a second cycle of reconstitution, a large fraction of the previously unsealed nicks are now sealed upon exposure to the DNA ligase. The changes in the fraction of accessible nicks are in quantitative agreement with a random binding model in which all DNA sequences are equally suitable for binding of histones and rule out the binding of histones to a specific subset of DNA sequences in these model chromatin preparations.

A purified DNA ligase from rat liver nuclei (1) has been found to have limited access to single-strand interruptions ("nicks") in the DNA of reconstituted chromatin. About half of the nicks are inaccessible due specifically to histone binding. This result, which will be presented in detail elsewhere (2), suggested the use of the ligase to test whether histones bind to a specific subset of DNA sequences or whether they bind to random sequences of DNA. If the binding is specific, there should be no increase in the fraction of sealed groups upon multiple rounds of reconstitution followed by ligase action, since the histones will return to protect the same DNA sequences. If the binding is random, the fraction sealed should rise with each round of reconstitution followed by ligase action because at each reconstitution there will be a random redistribution of the histones over all of the DNA sequences. This results in some previously covered nicks now being uncovered and able to be sealed by the ligase.

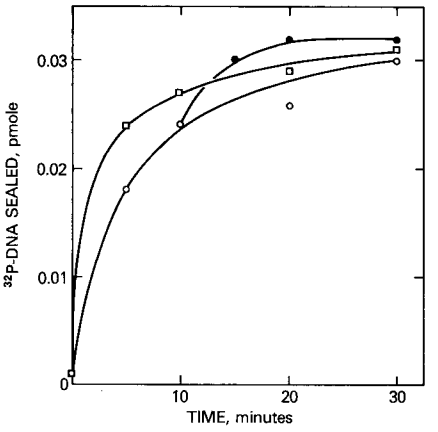

Figure 1. DNA ligase sealing of nicks in the DNA of reconstituted chromatin as a function of enzyme level or time of incubation. DNA ligase added: ○ , 2 units at 0 min; ● , 2 units at 0 min and again at 10 min; □ , 4 units at 0 min. The reconstituted chromatin was produced by a single application of the reconstitution protocol and contained 0.065 pmole of labeled nicks in the aliquot used here.

MATERIALS AND METHODS:

DNA ligase: DNA ligase was purified from rat liver nuclei; enzyme fraction IV stabilized with plasma albumin was used for all measurements. The extent of sealing of the nicked rat liver DNA was estimated by conversion of 5'-$^{32}PO_4$-labeled nicks (one nick per 2000 base pairs) into a form resistant to phosphatase. The ligase has been shown (1) to seal nicks bounded by any of the four usual bases on either the 3'- or 5'- sides in this substrate. Details of these procedures have been described (1). The fraction of labeled nicks in the various reconstituted chromatins which could be sealed by final treatment with an excess of ligase was determined upon 2 µg samples of the reconstituted chromatins under standard ligase assay conditions (1). An illustration of the lack of dependence of this sealable fraction upon the amounts of ligase used or the length of the ligase incubation is shown in Fig. 1. The maximum amount of sealing shown corresponds to about half of the total sealable groups in the labeled nicked DNA present in the reconstitution mixture. The remaining half of the groups could be sealed if the DNA were reisolated from the reconstituted chromatin by phenol extraction. The ligase could seal a maximum of 69% of the acid-precipitable ^{32}P in the nicked DNA; all of the extents of sealing are expressed as fractions of this limit of sealing on the uncomplexed DNA.

Isolation of chromatin: Nuclei were purified from rat liver as before (1) and chromatin isolated essentially by the procedure of Axel et al. (3) except that the detergent treatment of the nuclei was omitted. The chromatin was sheared in a Virtis homogenizer (2 min, 90 v); the final preparation contained 2.0 mg protein and 0.1 mg RNA per mg DNA.

Reconstitution of chromatin: For reconstitution experiments, 1.4 µmoles of chromatin were mixed with 0.18 µmole of ^{32}P-labeled nicked DNA (amounts based on total P contents) in 2.0 ml of 0.02 M β-mercaptoethanol-0.1 mM EDTA. The procedure for reconstitution of this mixture is slightly modified from the protocol of Axel et al. (4). NaCl (350 mg) and urea (900 mg of Schwarz/Mann "ultrapure" grade) were dissolved at 0° in the mixture of chromatin and DNA.

The final volume was 2.7 ml corresponding to 2.2 M NaCl and 5.6 M urea. After 1 hr at 0°, the mixture was dialyzed at 5° for 1 hr periods against 200-volumes of 5 M urea-0.02 M β-mercaptoethanol containing 1.2 M, 1.0 M, 0.6 M and finally 0 M NaCl. The samples were then dialyzed against two changes of 0.1 mM EDTA for 1 hr and 16 hours, respectively. Chromatin reconstituted in this manner is indistinguishable from the original chromatin in a number of respects (2,4,5).

Double reconstitutions: The product of a single round of reconstitution was subjected to a second round of reconstitution (i.e., the exposure to concentrated NaCl and urea followed by the stepwise dialyses, as outlined above) in several cases. Between the rounds of reconstitution, some samples were treated with an excess of ligase (10 or 20 units of ligase with 0.14 μmole of DNA-P in a total volume = 0.5 ml; medium as for ligase assays (1); incubation for 1 hr, 37°).

Calculation of expected DNA ligase sealing: The results of the double reconstitution experiments have been interpreted in terms of two extreme models for the binding of histones to DNA:

Random binding model. Chromatin components which block DNA ligase action bind with equal frequency on all sequences of the DNA.

Specific binding model. Chromatin components which block DNA ligase action bind to a specific subset of sequences on the DNA.

Let α = fraction of DNA inaccessible to the ligase at the in vivo ratio of histones to DNA, and x = amount of histones per unit DNA relative to the in vivo amount of histones per unit DNA. The value of x was obtained from the relative amounts of chromatin and labeled nicked DNA present during the reconstitution,

$$x = \frac{P_{\text{chromatin}}}{P_{\text{chromatin}} + P_{[^{32}P]DNA}}.$$

After one or more rounds of reconstitution without exposure to ligase, the fraction of nicks which can be sealed, s, by an excess of ligase under either model should be, $s = 1 - \alpha x$. (Eq. 1). If a reconstituted chromatin is sealed with an excess of DNA ligase and then subjected to a second reconstitution, the fraction of sealed groups, t, which this "reconstituted-sealed-reconstituted" preparation will show after a final treatment with DNA ligase will depend upon the model assumed.

Random binding model: $t = 1 - \alpha^2 x^2$ (Eq. 2)

Specific binding model: $t = 1 - \alpha x^2$ (Eq. 3)

We used the experimental value of s from samples reconstituted two times without intervening ligase treatment (s = 0.55, Table I) in conjunction with the known value of x (x = 0.89) to yield a value of $\alpha = 0.51$ by Eq. 1. Then x and α were used in equations 2 and 3 to predict the values for t under the two models.

RESULTS AND DISCUSSION

The extent of sealing with ligase offers an estimate of the fraction of the DNA which is not accessible to the enzyme and, hence, presumed to be bound by chromatin proteins. If the reconstitution proceeds to an equilibrium redistribution of proteins, then this limit should be the same after one cycle

TABLE I

Do histones bind to a specific group of DNA sequences? A test based on DNA ligase limits on reconstituted chromatin.

Sequence of Manipulations	Fraction of labeled nicks sealed in the final reconstituted chromatin		
	Observed	Theory*	
		Random binding	Specific binding
Reconstitution	0.00		
Reconstitution-ligase	0.49		
Reconstitution-reconstitution-ligase	0.55		
Reconstitution-ligase-reconstitution-ligase	0.77, 0.79‡	0.80	0.60

* See Methods.

‡ The two values were obtained from samples exposed to 10 or 20 units of ligase, respectively, between the two reconstitutions.

of reconstitution as after two cycles. This was essentially the case for the salt-urea reconstitution procedure used here (Table I).

If two rounds of reconstitution are each followed by sealing with an excess of DNA ligase, the total amount of sealed nicks is greater than after two reconstitutions followed by a single exposure to an excess of DNA ligase. Indeed, the limit after two rounds of reconstitution, each followed by ligase treatment, agrees well with that predicted for the random binding model (Table I). It seems justifiable to conclude that the bulk of the histones are not restricted to binding at a particular set of DNA sequences; however, a small proportion of sequence specific binding would not be detected in this type of experiment. Also, one must note that we are extrapolating from histone binding behavior in the vicinity of nicks to binding in the continuous unnicked stretches of DNA. The quantitative agreement with prediction for random binding supports this extrapolation, for the second reconstitution is done on chromatin in which half of the nicks have been removed.

Several properties of reconstituted chromatins have been found to be relatively unaffected by substituting a heterologous DNA into the reconstituted mixture in place of the homologous DNA. For example, the "supercoiling" of chromatin is relatively unaffected (6) as is the pattern of sizes of DNA pieces produced by digestion of the reconstituted chromatin with staphylococcal nuclease (4). These results as well as the present experiment all indicate from quite different points of view that the interaction of histones with DNA does not employ a particular group of DNA sequences.

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