ARE THE BONDS BETWEEN HISTONE FRACTIONS AND DNA OF DIFFERENT STRENGTHS?

V. D. Paponov, P. S. Gromov, and V. V. Rupasov

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The view that the bonds between histone fractions and DNA differ in strength was formulated some time ago and has been generally accepted for a long time [7, 10, 12]. For that reason active searches have been undertaken for differences in the ability of histones to induce configurational changes in DNA [12], to increase the compactness of DNA [12, 15], and to influence the role of DNA in transcription [7, 15] and replication [6]. Models of nucleohistone have been suggested in which the histones were arranged differently either relative to the base sequence of DNA [13] or relative to the sugar-phosphate skeleton: H3 and H4 in the large gutter, H2A and H2B in the small gutter, and H1 as a cross-linkage between DNA molecules [8]. Such differences in the structural relations between DNA and histone proteins could explain differences in the affinity of histone for DNA.

However, more recently in connection with the nucleosomal model of chromatin, the view has become established that there exists an octamer $(H3\text{-}H4\text{-}H2A\text{-}H2B)_2$ too, on which DNA is wound [2]. In that way the four histone fractions acquired a common structural load and, consequently, a functional significance. The previous evidence in support of differences in the affinity of histones for DNA, derived from the order of dissociation of histones from DNA during modification of the solvent, nowadays does not appear convincing, for by modifying the medium it is possible to break up the histone octamer and to study dissociation of histones whose relations with DNA have already undergone a change.

In a recent publication the authors stated that if the heparin polyanion is used as dissociating agent, histones H3, H4, H2A, and H2B dissociate simultaneously, without exhibiting any differences in their affinity for DNA [9]. These workers associate this result with the transfer of these histones from DNA to heparin in the form of octamers such as exist in chromatin.

The object of the present investigation was to determine whether differences are found in the strength of the bonds between histones and DNA under approximately physiological conditions.

EXPERIMENTAL METHOD

Calf thymus DNA, depolymerized in an ultrasonic disintegrator to a molecular weight of 0.8×10^6, was mixed with total chromatin histones in a medium of near-physiological ionic strength (0.15 M NaCl + 0.7 mM Na-phosphate buffer, pH 7.0). The DNA to histone ratio in the mixture was 1:1. The methods of preparing the DNA and histone samples were described previously [5]. To the nucleohistone thus obtained, total histone was also added and the protein composition of the nucleoprotein complexes was analyzed after separating them from histone not found in DNA by centrifugation of the mixture (6 h, 114,000g, L2-65B centrifuge). Analysis of histones bound with DNA in the mixtures was carried out by subjecting the residue to electrophoresis [11].

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that the initial complexes of DNA with total chromatin histone were transformed on the addition of histone, initially into complexes containing only histone H3, H4, H2A, and H2B, and then into complexes containing DNA + H3 + H4. This order of displacement of the histones from the nucleo-

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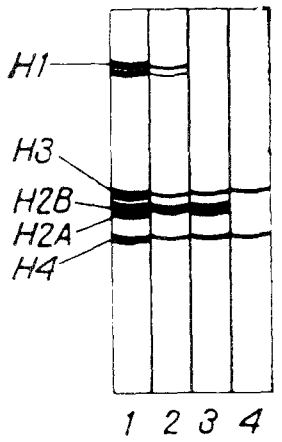

Fig. 1. Electrophoretic analysis of histones contained in nucleohistones obtained in nucleohistones obtained by addition of total chromatin histone to DNA–histone complex. Original DNA–histone complex obtained by direct mixing of DNA with total histone in medium of physiological ionic strength: 0.15 M NaCl + 0.7 mM Na-phosphate buffer, pH 7.0 (ratio of DNA : histone in the mixture 1 : 1). Ratio of DNA to histone in mixtures (w/w): 1) total histone (control); 2) 1 :1 (original nucleohistone); 3) 1 : 5; 4) 1 : 20.

protein complex with an increase in intensity of their competition for DNA shows that as regards strength of the bond with DNA in a medium of physiological ionic strength the histones can be divided into three groups: H1 < H2A + H2B < H3 + H4.

In the medium of low ionic strength (0.7 mM Na-phosphate buffer, pH 7.0) the order of displacement of the histones from the nucleoprotein complex was the same, i.e., histone H1 was the first to be displaced, followed by histone H2A and H2B. The relative strength of the bonds between histones and DNA thus is independent of the ionic strength of the medium, just as is the order of binding of histones with DNA, as the writers showed previously [4]. Using polyanions tRNA and DNA as agents dissociating histones, Varshavskii et al. [1] showed that in a medium of low ionic strength, histones H1, H2B, and H2A dissociate much more readily than H3 and H4. In a medium of physiological ionic strength, only histone H1 could be dissociated with the aid of tRNA and DNA. No difference in the affinity of the remaining histones for DNA could be detected [1]. Presumably the previous attempts [1, 3] to detect differences in the strength of the bonds between histone and DNA in a medium of physiological ionic strength were unsuccessful (at least as regards histones of the nucleosomal nucleus) evidently because the biochemical approaches used were ineffective. Dissociation of histones from DNA with the aid of polyanions probably occurs in a medium of physiological ionic strength also, but the increased power of aggregation of the polyanion–histone complexes under these conditions prevents their fractionation from the residual DNA–histone complexes, so that a false idea of the absence of dissociation is created.

The method of competitive displacement of histone fractions from the nucleohistone complex which we used enabled dissociation of histones H1, H2B, and H2A from DNA to be demonstrated for the first time in a medium of physiological ionic strength, and it thus provides a firm foundation for the many hypotheses on the role of histone dissociation in the functional behavior of the genetic apparatus of eukaryote cells. The mechanisms responsible for differences in the strength of the bond between histones and DNA are not yet clear.

One possible explanation could be the difference in the structural relations of the histone fractions with DNA. However, evidence has been obtained of the absence of specificity in the arrangement of histones relative to the composition of the DNA bases [14]. By using polyphosphate as a model of DNA from which all functional groups other than phosphate have been removed, we showed previously that the relative affinity of histones for DNA is determined by interaction between histones and the phosphate groups of DNA [4]. Relations between histones and the interior of the gutters of DNA thus do not determine differences in the strength of the bonds between histones and DNA. Meanwhile histone H1, which has the highest charge of molecules of all histone fractions, has been found to be least firmly bound with DNA. Possibly the strength of the bond between histones and DNA is influenced by interaction between the histone molecules themselves. That is why destruction of histone complexes in chromatin with the aid of urea makes the relative strength of the bond between histones and DNA correspond to the relative magnitude of the charges on the histones [5].

The results now obtained, indicating differences in the strength of the bonds between histones and DNA in a medium of physiological ionic strength, suggest that the histone octamer of the nucleosomes is not a conservative formation and that the composition of histones in nucleosomes may change under physiological conditions, depending on the degree of competition between the chromatin components for DNA (histones and nonhistone proteins) or for histones (RNA).

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