Vol. 82, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 30,1978 Pages 674-679

THE NATURE OF INTERACTIONS RESPONSIBLE FOR THE DIFFERENCES IN THE AFFINITIES OF HISTONES FOR DNA

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Received July 28,1977

SUMMARY: Electrophoretic studies on the sequential binding of histones to DNA and to polyphosphate in low ionic strength solution have shown that the affinities of histones for both the polyanions decreases in the same order: H4 ~ H3 > H2A > H2B>H1. This permits to suggest that hydrophobic DNA-histone interactions do not determine the relative affinity of histones for DNA. Non-ionic interactions within and between histone molecules participate in determining the histone affinity for DNA affecting electrostatic DNA-histone interactions.

The numerous studies on the sequence of histone dissociation from DNA in various media (1-9), the measurements of binding constants for separate histone fractions to DNA (10,11), and the analyses of the sequential binding of histones to DNA (4,12,13) showed the different affinities of histone fractions for DNA, that may be important for the structural and functional organization of chromosomes. The functional importance of the mentioned fact is still uncertain. It, thus, becomes important that physico-chemical mechanisms responsible for the differences in the affinities of histone repressor molecules for DNA be elucidated.

It is now accepted that electrostatic and hydrophobic interactions as well as hydrogen bonds participate in binding of histones to DNA (14-16). The question arises as to the primary role of one of the three types of interactions in the formation of DNA-histone complexes. It was shown that urea (2-4,8) and detergents (5-7,9) differentially influenced the

0006-291X/78/0822-0674$01.00/0

relations between histones and DNA. These phenomena are often attributed to the influence of the mentioned agents on the hydrophobic interactions between DNA and histones. In this view, hydrophobic DNA-histone interactions are considered to be the decisive factor in establishing the relative affinity of histones for DNA in water-salt solutions.

In fact, in the presence of agents, destroying hydrophobic interactions, histone H1, possessing the highest positive charge, turns out to be the most firmly bound to DNA (2-9), though it is the first to dissociate from DNA at an increase in concentration of salt in aqueous solution (1-9). Nevertheless the mentioned phenomena cannot serve as a direct evidence in favour of the view point that the relative affinity of histones for DNA in water-salt media is determined by hydrophobic DNA-histone interactions, because of the presence of alternative interpretations (4).

This work is an attempt to study the sequential binding of histones to DNA and to polyphosphate in order to elucidate if the interaction between histones and phosphate groups of DNA is sufficient for determination of relative affinity of histone fractions for DNA, or if the character of affinity is under the influence of non-ionic interactions of DNA sugars and nitrogen bases with histones.

MATERIALS AND METHODS.

DNA was isolated from calf thymus as described previously (17) and sonicated with the MSE ultrasonic disintegrator. Its mol wt was 0.88×10^6 daltons determined by viscometry (18). Polyphosphate (mol wt 6610) was kindly provided by Dr M.Skalka. Histones were extracted into 0.4 N HCl from the calf thymus chromatin and dialyzed against 0.9 N acetic acid and lyophilized. Histone solution was prepared in 0.7 mM Na-phosphate buffer (pH 7.0). DNA or polyphosphate solutions at different concentrations in the same solvent was added slowly to equal volumes of the histone solution at concentration of 1 mg/ml. The obtained mixtures, in different histone-polyanion

ratios, were spun for 6 h at 4° in 40.3 rotor of Beckman model L2-65B centrifuge at 114,000 g (10,000 rpm). Histones of the supernatants were analysed in 15% acrylamide gels at pH 2.8 by the method of Panyim and Chalkley (19).

RESULTS AND DISCUSSION.

Electrophoretic analysis of histones in supernatants of DNA-histone mixtures shows that at small DNA-histone ratios, histones H3 and H4 combine preferentially with DNA (Fig.1). Histone H2A is next in order of preference. Histone H1 combine with DNA at the highest DNA-histone ratio in the mixtures, i.e. H1 possesses the smallest affinity for DNA. As seen in Fig.1, histone fractions may be arranged according to the degree of affinity for DNA in the following sequence: H4~H3 > H2A > H2B>H1.

These data are in agreement with the conclusion about the relative affinity of histones for DNA in 0.14 M NaCl (11,12) and with the measurements of binding constants for histone fractions to DNA in higher ionic strength media (10,11), i.e., the relative affinity of histones for DNA is independent of the ionic strength of a medium.

We can see in Fig.1 that histones, according to the degree of affinity for polyphosphate, are arranged in a sequence equivalent to one for DNA. It enables us to conclude that hydrophobic DNA-histone interactions, if they exist at all, do not determine the relative affinity of histones for DNA. The influence of urea and detergents upon the affinity of histones for DNA should be interpreted in this connection as a consequence of the modification of intra- and intermolecular protein interactions of non-ionic nature (hydrophobic interactions and hydrogen bonds). This modification leads to the alteration in the ability of histones to interact with

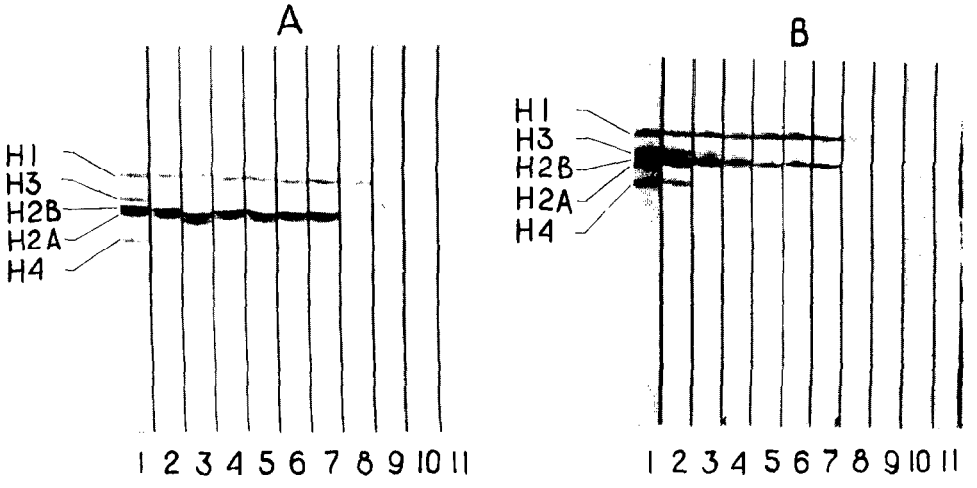

Fig.1. Electrophoretic analysis of histones remaining in supernatants after centrifugation of (A) DNA-histone and (B) polyphosphate-histone mixtures .
A. DNA-histone ratio in mixtures (w/w):
(1) Whole calf thymus histone - control; (2) 1:20; (3) 1:10; (4) 1:8; (5) 1:7; (6) 1:6; (7) 1:5; (8) 1:4; (9) 1:3.5; (10) 1:3; (11) 1:2.5
B. Polyphosphate-histone ratio in mixtures (w/w):
(1) Whole calf thymus histone - control; (2) 1:20; (3) 1:10; (4) 1:8; (5) 1:7; (6) 1:6; (7) 1:5.5; (8) 1:5; (9) 1:4.5; (10) 1:4; (11) 1:3

DNA electrostatically. In fact, the conformational variations in histones induced by urea and detergents change the space distribution of the charged amino acid residues (the density of the charge) and consequently the electric potentials of histone molecules. Destroying of histone complexes by urea and detergents decreases the value of the charge of the unit to be bound to DNA.

The discovery, in histone solutions, of specific complexes sensitive to the action of urea and several other agents (20,21) supports our conclusion. The fact that the histones H3 and H4 do not exhibit essential difference in the affinities for DNA is in agreement with the data (20) showing that these fractions

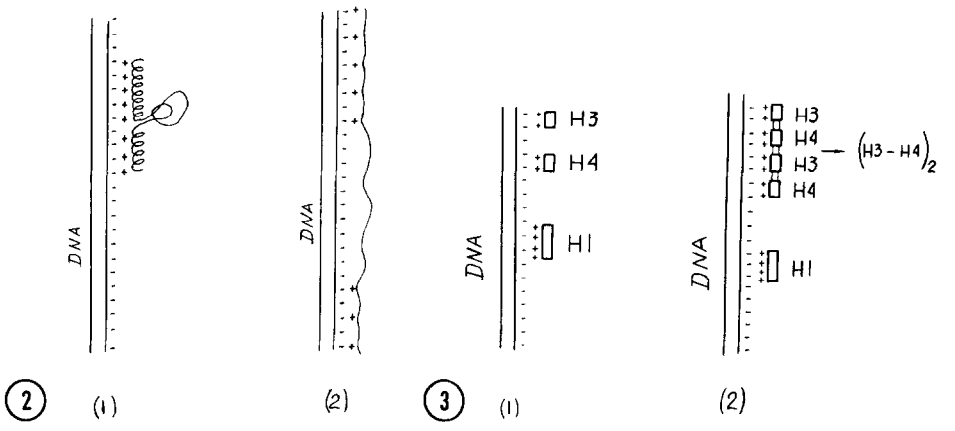

Fig.2. The influence of the charge density of histone molecules on the electrostatical DNA-histone interaction.
1. In water-salt solution.
2. In presence of agents destroying non-ionic interactions.

Fig.3. The influence of mutual histone complexing on the electrostatical DNA-histone interaction.
$E_{H3\text{-}DNA} \sim E_{H4\text{-}DNA} < E_{H1\text{-}DNA} < E_{(H3\text{-}H4)_2\text{-}DNA}$
1. In presence of agents destroying non-ionic interactions.
2. In water-salt solution.

exist in aqueous solutions as complexes $(H3)_2(H4)_2$. This may account for a higher affinity of histones H3 and H4 for DNA in comparison with H1. Histone H1 has a higher charge than single H3 and H4 molecules, but it is unable to aggregate (20,21). Therefore, in addition to electrostatic DNA-histone interactions, non-ionic interactions within and between histone molecules participate in determining the histone affinity for DNA affecting the charge density of histone molecules and inducing the formation of histone complexes, which have higher charges than single histone molecules.

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