Short Communications

Effect of Heating and Cooling on Interaction of Lysine-Rich Histones with Deoxyribonucleic Acid

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DNA and histones are linked to each other in definite proportions. One complete turn of the dyad helix in DNA with 20 nucleotides (ten pairs) would require 7000–9000 mol.wt. of histone for neutralization of the phosphate groups (Phillips, 1962). For the study of the interaction of histones with DNA the precipitation method (Butler & Johns, 1964) is suitable. This showed that the lysine-rich fraction F1 has the greatest ability to combine with and precipitate DNA (Johns & Butler, 1964); this may be due to the greater steric availability of the basic amino acid side chains to the phosphate groups in DNA than is the case with other histone fractions (Johns, 1966a). This appears to be of greater importance than the fact that histone F1 has more basic amino acids and fewer acidic amino acids than the other fractions. According to this idea it is possible that histone F1 would be stretched around the DNA, resulting in a more effective precipitation than is the case with other fractions (Johns, 1966b).

We decided to study the precipitation of the DNA–histone F1 complex when the DNA molecule form was altered. It is known that the DNA form changes with heating, during which process it is denatured by helix–coil transition, with strand separation and breakdown of the helical structure (Doty, 1961). These DNA changes are of particular interest as they are connected with the role of DNA *in vivo*, i.e. they precede the replication of DNA.

For precipitation we used DNA obtained from calf thymus. We prepared the fraction F1 (lysine-rich histones) from the calf thymus by the Johns (1964) procedure, method 1. Precipitation was effected by following the Butler & Johns (1964) method, in which we used the histone F1/DNA ratio 0.8 mg./mg. Our controls were unheated solutions of DNA (in 0.14 M-NaCl, pH 5), which we then added to the histone F1 solutions (in 0.14 M-NaCl, pH 5), whereas the other DNA samples were heated for 10 min. in a boiling-water bath. One group of samples was cooled rapidly after heating by immersion in an ice bath, and the other was cooled slowly by standing at 25°. These DNA solutions were added to histone F1 solutions (before mixing with histone solutions all the samples were

adjusted to 20°). After removing the precipitate we determined the percentage of precipitated DNA by measuring E_{260} spectrophotometrically. In view of the hyperchromic effect produced during denaturation of DNA, we used special controls for each heating and cooling without addition of histone F1. We carried out a series of supplementary experiments in which we added histone F1 heated for 10, 30 or 60 min. to unheated DNA.

When mixing unheated DNA and histone F1 we obtained 94.86% precipitation of DNA. With heated and rapidly cooled DNA we obtained 68.49%, and with slow cooling the percentage of precipitated DNA was only 54.00%. Heating of histones alone resulted in a minimum decrease in the percentage precipitation of DNA: 92.0%, 89.6% and 91.7% after 10 min., 30 min. and 60 min. heating respectively. The results of these tests are presented in Fig. 1.

The results of our experiments show that the deformation of the DNA molecule obstructs the precipitation of the DNA–histone F1 complex. This is certainly due to the strand separation and the helix–coil transition, which results in the DNA phosphate group not remaining in the places corresponding to the structure of the molecule of histone F1, nor the localization of their basic amino acids, so that the lysine-rich histones are not able to

Fig. 1. Percentage of DNA precipitated by mixing with histone F1. (a) Control; (b) DNA heated and rapidly cooled; (c) DNA heated and slowly cooled; (d) histone F1 heated for 10 min.; (e) histone F1 heated for 30 min.; (f) histone F1 heated for 60 min.

stretch around the strand of denaturated DNA as is the case with native DNA molecules. This gives us a picture, although simplified and incomplete, of the relationship of histones and DNA in the process of strand separation in DNA that takes place before replication. It is noteworthy that the precipitation of slowly cooled DNA is more obstructed than that of the rapidly cooled DNA. Doty (1961) claims that the slow cooling results in a renaturation of DNA (previously denaturated by heating) in bacteria and bacteriophages, but this reversible process was not noted in calf thymus DNA because of its heterogeneous structure. We consider that the slowly cooled DNA produces a lower percentage of precipitated DNA than did the rapidly cooled samples because the longer exposure to higher temperatures enhances denaturation (E_{260} was in

this case higher). Heating of histone F1 insignificantly decreases its ability for precipitation of DNA (even for a duration of 1 hr.).

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