Interactions between the Lysine-Rich Histone F1 and Deoxyribonucleic Acid

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1. The interactions of the lysine-rich histone F1 with DNA have been studied at various histone to DNA ratios, in water and in the presence of uni- and bi-valent cations. In water only, histone F1, even in fourfold excess, is unable to precipitate all the DNA. In 0.14 M-sodium chloride, 0.8 mg. of histone F1 is required to precipitate 1 mg. of DNA, whereas in 0.07 M-magnesium chloride only 0.4 mg. is required. 2. Bivalent cations are also shown to be more effective in dissociating the DNA-histone complex. Histone F1 can be selectively removed from deoxyribonucleoprotein with 0.1 M-magnesium chloride. 3. The precipitation of DNA by histone F1 is a reversible process and the complex can be taken in and out of solution by changing the ionic environment. 4. The bearing of these results on the observed ability of various DNA-histone complexes to act as templates for RNA synthesis is discussed.

It is now well established that the histones are capable of inhibiting *in vitro* the synthesis of RNA formed on a DNA template in the presence of RNA polymerase (Huang & Bonner, 1962; Bonner & Huang, 1963; Allfrey, Littau & Mirsky, 1963; Barr & Butler, 1963; Hindley, 1963; Huang, Bonner & Murray, 1964). These authors have also noted a variation in the extent to which the various histone fractions can inhibit this reaction. Allfrey *et al.* (1963) and Hindley (1963) concluded that the arginine-rich histones were the best suppressors of DNA template activity, whereas Barr & Butler (1963) and Huang *et al.* (1964) reported that the lysine-rich histone was the most efficient. Since these authors have carried out their experiments under very different conditions, with different histone to DNA ratios, different ionic environments and histones prepared by different methods, it is not surprising that the results are at variance. Indeed, it is likely that these problems will not be resolved until much more is known about the interactions of the various histone fractions with DNA under a variety of conditions.

Previous work from this Laboratory has shown that the histones of calf thymus can be separated into five main fractions, designated F1, F2(a)1, F2(a)2, F2(b) and F3 (Johns, 1964, 1967; Phillips & Johns, 1965). The interactions of some of these fractions, and whole histone, with DNA have been studied previously (Bjornesjo & Teorell, 1945; Butler & Johns, 1964; Johns & Butler, 1964), and

in the more recent studies it was found that in 0.14 M-sodium chloride the lysine-rich histone, F1, was the most efficient at precipitating DNA, but that it was the least strongly bound. These investigations have now been extended and the interactions of the lysine-rich histone F1 with DNA have been studied under conditions of varying ionic strength, with uni- and bi-valent cations, and also varying histone to DNA ratios. The bearing of the results on the observed ability of various DNA-histone complexes to act as templates for RNA synthesis is discussed.

EXPERIMENTAL AND RESULTS

All experiments were carried out at room temperature except for the dialysis experiment, which was at 4°. Histone and DNA solutions were always mixed together rapidly with a Vortex mixer (Scientific Industries Inc., Queen's Village, N.Y., U.S.A.). DNA was prepared according to Kay, Simmons & Dounce (1952) and histone F1 was prepared according to Johns (1964), method 1.

Precipitation of DNA from solution with varying amounts of the lysine-rich histone F1. A solution of the lysine-rich histone F1 (1 mg./ml. adjusted to pH 7.0 with 0.1 N-NaOH) was added in varying amounts to 5 ml. portions of a solution of DNA (0.1 mg./ml. adjusted to pH 7.0 with 0.1 N-NaOH). The mixtures were then centrifuged at 2500g for 1 hr. to sediment the insoluble complex of deoxyribonucleohistone and the extinctions of the clear supernatants were measured at 260 m μ . The DNA precipitated was then calculated, after allowing for volume differences, by assuming that the extinction at 260 m μ of a solution to which no histone had

Fig. 1. Precipitation of DNA from solution (0.5 mg./ml.) by the addition of solutions (1 mg./ml.) of the lysine-rich histone F1, under conditions of varying ionic environment. For details see the text. ○, 0.07 M-MgCl₂; □, 0.14 M-NaCl; △, double-distilled water.

Fig. 2. Precipitation and dissociation of the histone F1-DNA complex (histone to DNA ratio 1.6:1) with varying concentrations of uni- and bi-valent cations. For details see the text. ○, MgCl₂; □, NaCl.

been added was equivalent to 100% DNA in solution. The contribution of the added histone to the extinction of DNA at 260 mμ would be very small since $E_{1\text{cm}}^{1\%}$ for DNA is approx. 190 (Kay *et al.* 1952) whereas $E_{1\text{cm}}^{1\%}$ for the lysine-rich histone F1 at this wavelength is about 2.0. For this type of experiment double-distilled water, 0.14 M-NaCl and 0.07 M-MgCl₂ were used for dissolving the histone and DNA. The results are given in Fig. 1.

It can be seen that, when DNA and histone F1 are recombined in water only, it is not possible to precipitate all the DNA. A maximum of about 65% is precipitated at a histone to DNA ratio of 1.6:1.0, after which the addition of more histone F1 apparently causes the complex to become more soluble. This does not occur in the presence of salts,

as can be seen from the other curves. All the DNA is precipitated from 0.14 M-NaCl at a histone to DNA ratio of about 0.8:1.0, whereas in 0.07 M-MgCl₂ the precipitation is complete at a ratio of about 0.4:1.0.

Precipitation of DNA from solutions of varying ionic strength and cation valency, at a constant histone to DNA ratio. Since the precipitation of DNA with histone F1 takes place more readily in the presence of bivalent cations it was of interest to investigate the effect of varying ionic strength with uni- and bi-valent cations at a given histone to DNA ratio. A ratio of histone F1 to DNA of 1.6:1.0 was chosen since this apparently gives maximum insolubility to the complex in water (see Fig. 1). Solutions of DNA and histone F1 were made up as described above. To 5 ml. portions of the DNA solution various volumes of 4 M-NaCl were added, followed by 0.8 ml. of histone solution. The solutions were then centrifuged for 1 hr. at 2500g and the extinctions of the clear supernatants measured at 260 mμ. DNA precipitated was calculated as described above after allowing for differences in volume. A similar experiment was carried out with MgCl₂ in place of the NaCl. The results are given in Fig. 2.

It can be seen that at a ratio of 1.6:1.0 (histone F1 to DNA) complete precipitation is obtained at very low concentrations of NaCl or MgCl₂ (0.02 M). The complex appears to dissociate, however, under these conditions at about 0.4–0.5 M-NaCl, which is precisely the range over which the lysine-rich histone can be selectively removed from the native deoxyribonucleoprotein (Hindley, 1964; Georgiev, Ananieva & Kozlov, 1966). Magnesium chloride appears to be much more effective than NaCl in dissociating the complex, which it does between 0.08 and 0.2 M. Accordingly native deoxyribonucleoprotein, prepared as described previously (Butler, Davison, James & Shooter, 1954), was extracted with a range of concentrations of MgCl₂ and it was confirmed that the lysine-rich histone F1 can be removed from deoxyribonucleoprotein with 0.1 M-MgCl₂.

Effect of changing ionic environment on the complex between histone F1 and DNA. From Fig. 1 it is clear that about 0.8 mg. of the lysine-rich histone F1 is required to precipitate 1 mg. of DNA from solution in 0.14 M-NaCl. However, at this same ratio of histone to DNA in double-distilled water, only about 30% of the DNA is precipitated. It was therefore of interest to see if this was a reversible process, i.e. can the histone-DNA complex be made to go in and out of solution by changing the ionic environment?

Histone F1 (8 mg.) was dissolved in 10 ml. of double-distilled water and the pH adjusted to 7.0 with NaOH. This was then added to 100 ml. of DNA solution (0.1 mg./ml., pH 7.0). A 5 ml. portion was removed, centrifuged at 2500g for 1 hr. and the DNA that had been precipitated determined as described above. The remaining 105 ml. was then dialysed against 5 l. of 0.01 N-NaCl, pH 7.0, for 3.5 hr. at 4°. After the dialysis the bag was opened and a 5 ml. portion removed and the percentage of DNA precipitated determined. The bag was then resealed and the solution dialysed in the same manner against 0.05 M-NaCl. The experiment was continued in this manner, the DNA-histone complex being dialysed against the various solutions shown in Fig. 3. The experiment was carried out over a period of 5 days: two dialyses of 3.5 hr. each during the day, followed by one dialysis of 17 hr. overnight. The results are given in Fig. 3. It can be seen that initially it appeared that the process was not reversible since the dialysis against

Fig. 3. Solubility of the histone F1-DNA complex in solutions of changing ionic environment. The histone F1-DNA complex was initially made (ratio 0.8:1) in double-distilled water and subsequently dialysed in sequence against 5l. each of the solutions indicated. For details see the text. 1, 0.01M-NaCl; 2, 0.05M-NaCl; 3, 0.10M-NaCl; 4, 0.15M-NaCl; 5, 0.10M-NaCl; 6, 0.05M-NaCl; 7, 0.01M-NaCl; 8, water; 9, 0.001M-EDTA; 10, 0.001M-EDTA; 11, 0.15M-NaCl, 0.001M-EDTA; 12, 0.001M-EDTA; 13, 0.001M-EDTA; 14, 0.15M-NaCl, 0.001M-EDTA; 15, 0.001M-EDTA.

water, after the first complete precipitation, resulted in only about 20% of the DNA going back into solution. However, the addition of 0.001M-EDTA to all solutions used for subsequent dialyses caused the precipitation to be reversible. Since the dialyses were against 5l. of single-distilled water only, this was probably due to traces of bivalent cations, which from Fig. 1 are seen to be much more effective in bringing about the precipitation of the deoxyribonucleohistone complex. It is noteworthy that the preparation of soluble deoxyribonucleoprotein (Zubay & Doty, 1959) is dependent on the presence of EDTA.

DISCUSSION

From the results given above it can be seen that, in water only, it is not possible to precipitate all the DNA from solution with lysine-rich histone, and that, after a histone to DNA ratio of 1.6:1.0 has been reached, further addition of histone renders the complex more soluble. The reason for this is not known, but the explanation may be the same as for the similar phenomenon in antigen-antibody interactions (Peacocke, 1960). Similarly, at a given ratio of histone F1 to DNA, reversible changes in the solubility of the complex can also be brought about by relatively small changes in the ionic environment. It is known that native deoxyribonucleoprotein becomes more soluble in solutions of low ionic strength (Shooter, Davison & Butler, 1954), particularly in the presence of EDTA (Zubay & Doty, 1959), and, since it appears from the experiments with histone F1 and DNA that this process is quite reversible, changes similar to these may be taking place during the condensation of the chromatin before mitosis.

If the template activity of deoxyribonucleoprotein is dependent on its physical state, as suggested by the experiments of Sonnenberg & Zubay (1965) and Roy & Zubay (1966), then it is not surprising that the various authors using different ratios of histone to DNA, and different ionic environments for assaying for template activity of DNA, have found a variation in the effectiveness of the various histone fractions in inhibiting this activity. For example, it has been shown (Butler & Johns, 1964; Johns & Butler, 1964) that at histone to DNA ratios under 1.5:1.0 only the lysine-rich histone will fully precipitate DNA from 0.14M-sodium chloride solution; it is therefore not surprising that Barr & Butler (1963) and Huang *et al.* (1964), using solutions of lower ionic strength, found the lysine-rich histone to be the most efficient since all their histone to DNA ratios were less than 1.5:1.0. On the other hand Hindley (1963) used a ratio of histone to DNA of 2.5:1.0 and found the arginine-rich fraction to be the best suppressor. Since the assays for template activity are carried out in solutions of relatively low ionic strengths it is quite possible that in this case the large excess of lysine-rich histone over that required to precipitate the DNA was beginning to cause the complex to re-dissolve and thus become available for transcription. It is of interest to discover whether or not the suppression of template activity by the various fractions follows their different DNA precipitation curves and whether or not the histone to DNA ratio is important regardless of the fact that the complex may become more soluble. The work of Allfrey *et al.* (1963), which also indicated that the arginine-rich histones were the best suppressors, was carried out with histone-depleted nuclei and thus the ratio of histone to DNA is difficult to assess, but from the figures given (2-4mg. of histone fraction/40mg. of nuclei) it seems likely that in this case also the histone would be in excess. These problems, together with the effect of the other histone fractions on DNA under conditions similar to those described above, are currently under investigation.

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