A NEW METHOD FOR STUDYING HISTONE-HISTONE INTERACTIONS

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1. Introduction

It is now well established that the histones, the basic proteins associated with the DNA in most animal somatic cell nuclei consist of five main proteins. These have been designated F1 and F2B, lysine-rich histones; F3 and F2A1, arginine-rich histones; and F2A2 which is an intermediate type having an approximately equal molar ration of lysine to arginine [1]. It is thought that the histones are non-specific gene repressors which function by forming higher order structures with the DNA. The way in which the histones combine with each other and with DNA is under active investigation in many laboratories and recent work has indicated that precise histone-histone interactions may be extremely important [2–4]. This paper describes a simple method for the investigation of certain types of histone–histone and histone–DNA interactions.

2. Materials and methods

Histone fractions were prepared and purified as described previously [5].

The diffusion technique used is based on the well-known double diffusion technique of Ouchterlony [6] normally used for the demonstration of antigen–antibody complexes. In the work described here the positively charged proteins are allowed to diffuse towards the central well which contains a negatively charged dye or mixture of dyes. When they meet, a precipitation line is formed. Information is obtained about the complexing of mixtures or self aggregation of proteins by their relative rates of diffusion, i.e. the positions of the precipitation bands.

For basic proteins agarose is used for the gel since agar contains negatively charged polysaccharides which prevent free migration.

25ml of agarose solution (0.5% w/v in 0.14M–NaCl at 100°C) was poured into plastic petri dishes (88mm diam) and allowed to cool. Wells (7mm diam) were then cut in the gel variously spaced as shown in the figures, using a sharpened stainless steel tube. The centre well contained the dye, which in the experiments described here was made by mixing equal volumes of aqueous solutions of 2% (w/v) alizarin black and 0.1% (w/v) Ponceau S. The mixed dye system was used after a number of trials because it gave different coloured bands with the different histone fractions and was therefore useful for their identification in mixtures. This mixed dye system has been used previously for the differential colour staining of lysine-rich and arginine-rich histones after their separation in polyacrylamide gel [7]. Satisfactory results can, however, be obtained using a single dye system.

Histone fractions and various mixtures of histone fractions were then placed in the peripheral wells and allowed to diffuse for from 24 to 48 hr. The precipitation bands formed were photographed approximately 24 hr after their first appearance.

3. Results

In fig.1 various histone fractions have been run against the dye and precipitation bands formed. It can be seen that all fractions with the exception of F2A1 (well 2) diffuse freely into the gel. F2A1, however, has not moved far from its well. This would not be expected on the basis of their molecular weights since histone fraction F2A1 is the smallest of all the mammalian histones having a mol. wt of 11 300, compared

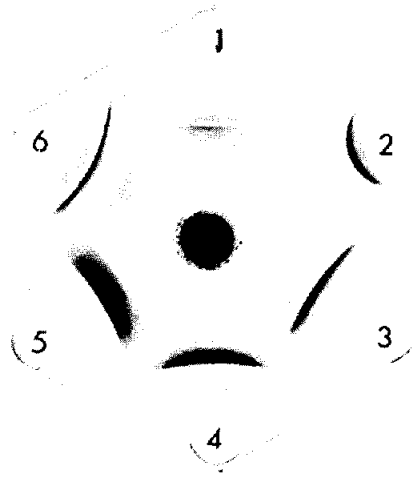

Fig.1. Histone fractions (5 mg/ml) in water, pH 7.0, run against dye. Well 1, histone F1; Well 2, histone F2A1; Well 3, histone F2A2; Well 4, histone F2B; Well 5, histone F2C; Well 6, histone F3.

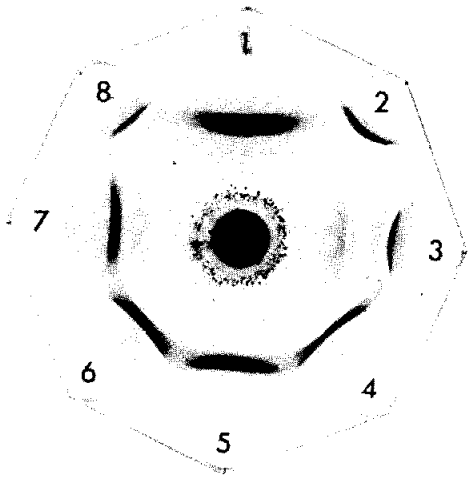

Fig.2. Mixtures of F2A1 with various histone fractions and whole histone, run against dye. All fractions are at a final concentration of 4 mg/ml in water at pH 7.0. Well 1, whole histone, unfractionated (20 mg/ml); Well 2, F2A1 (4 mg/ml); Well 3, F2A1 (8mg/ml) + F1 (8mg/ml) 1:1, v/v: Well 4, F2A1 (8 mg/ml) + F2A2 (8 mg/ml) 1:1, v/v; Well 5, F2A1 (8 mg/ml) + F2B (8 mg/ml), 1:1, v/v; Well 6, F2A1 (8 mg/ml) + F3 (8 mg ml), 1:1, v/v; Well 7, whole histones, reconstituted from individual fractions (20 mg/ml); Well 8, F2A1 (4 mg/ml).

with approx. 21 000 for histone F1. However, it is well known that histone F2A1 self-aggregates under a variety of conditions [8.9] and forms soluble polymers which presumably account for the low rate of diffusion in the agarose gel.

In fig. 2 an equal quantity of histone F2A1 has been added to each of the other histone fractions, and with the exception of histone F1 the other histones have prevented the self-aggregation of histone F2A1. This has presumably occurred because of fairly specific complexes being formed between F2A1 and the other histones since any large non-specific aggregates would not diffuse readily through the gel. The fact that histone F1 does not appear to prevent the self-aggregation of F2A1 is of interest since this is the only histone which has not been implicated previously in complex formation between different histone molecules. These results would indicate that the three histone fractions F3, F2B, and F2A2 can all form simple complexes with histone fraction F2A1. This is in agreement with the recent results of D'Anna and Isenberg [10].

4. Discussion

These results are given because of the relative simplicity of this technique and its applicability to many different problems. It is obvious that the conditions of interaction of any proteins under study can be varied very easily. For example, the agarose gels can be made up at varying pH values, at varying ionic strengths, containing different valency ions or other complexing substances such as ion-exchange resins. The diffusate in the central well need not of course be an interacting dye and in some of our more recent studies other biological macromolecules have been used including DNA of varying molecular sizes. The possibility of determining molecular weights of proteins using DDS agarose gels is also being investigated Other conditions which can be varied are of course the distance between the wells, and the relative concentrations of substances in the wells, both of which influence the concentration gradients of the interacting components.

The method is extremely versatile and we are currently obtaining complex precipitation bands by migrating the various histone fractions against DNA. By the correct spacing of the wells it is also possible to allow

various histone fractions to merge with one another before interacting with a third substance such as DNA and in this way with the interactions between the varying concentration gradients the desirable ratios for interaction of the various components are more easily obtained. By the appropriate radioactive labelling of the various interactants the ratios of the components in a precipitation band can be determined.

The results on the histone-histone interactions indicate that histones F3, F2B and F2A2 can all form fairly simple complexes with histone F2A1. It will be of interest to see how the various fractions compete with one another under these conditions and how the various mixtures of two, three and four histones can complex with one another and combine with DNA.

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