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Note

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A method for differentiating between arginine-rich histones and others in polyacrylamide gel

The histones of somatic cell nuclei have been separated into five main fractions by a variety of techniques[1]. These fractions have been designated F1 and F2B, lysine-rich histones; F3 and F2A1, arginine-rich histones; and F2A2 which is an intermediate type having a molar ratio of lysine to arginine of about one[2,3]. There is also a unique fraction, designated F2C, found only in nucleated erythrocytes, which is also lysine-rich[4].

These fractions have all been well characterised by amino acid analyses and by polyacrylamide gel electrophoresis[1]. A number of methods are available for their separation in polyacrylamide gel[5,6], and techniques for running two samples on one gel have been described[7], so that an accurate comparison of mobilities can be obtained. With such methods, any histone fraction, or mixture of fractions, can be compared with the well characterised mammalian fractions. However, recent work has demonstrated that a species specificity does exist in certain histone fractions[8,9] resulting in small changes in the amino acid sequence, or in modifications to some amino acids. These small differences result in corresponding small changes in the mobilities of some of the fractions when they are separated in polyacrylamide gel[9]. Thus, the identification of fractions from different species on the basis of mobility alone becomes more uncertain. As an additional test to aid in the identification of the various fractions in polyacrylamide gel a method is described which enables a mixture of histone fractions to be judged as arginine-rich or not, on the basis of staining with bromophenol blue, followed by the differential elution of the stained bands.

Experimental

Polyacrylamide gel electrophoresis was carried out essentially as described previously[5,10] but with the following modifications. The gels contained 1 *M* urea and before polymerisation they were overlayered with isobutyl alcohol, instead of distilled water[11]. All proteins were dissolved in sample solvent (1 *M* sucrose, 2 m*M* acetic acid) at a concentration of 1 mg/ml. 40 μl of whole histone solution (40 μg of protein) or 20 μl of the individual fractions (20 μg of protein) were applied to the gels. The samples were run for 6.5 h at 175 V (approximately 3 mA/tube). After removal from the tubes the gels were stained by immersion in 40 ml of 0.01 % aqueous bromophenol blue at pH 3 for 16 h. Control gels were stained in a similar manner using naphthalene black (0.5 % in 30 % ethanol, 7 % acetic acid) or alizarin black (0.5 % in 30 % ethanol). All gels were destained in tubes containing 40 ml of 40 % *n*-propanol at 55° for a total time of 48 h. The destaining solution was changed five times during this period.

The results obtained are shown in Fig. 1. It can be seen that using naphthalene black and alizarin black all bands are fully stained and remain stained during the

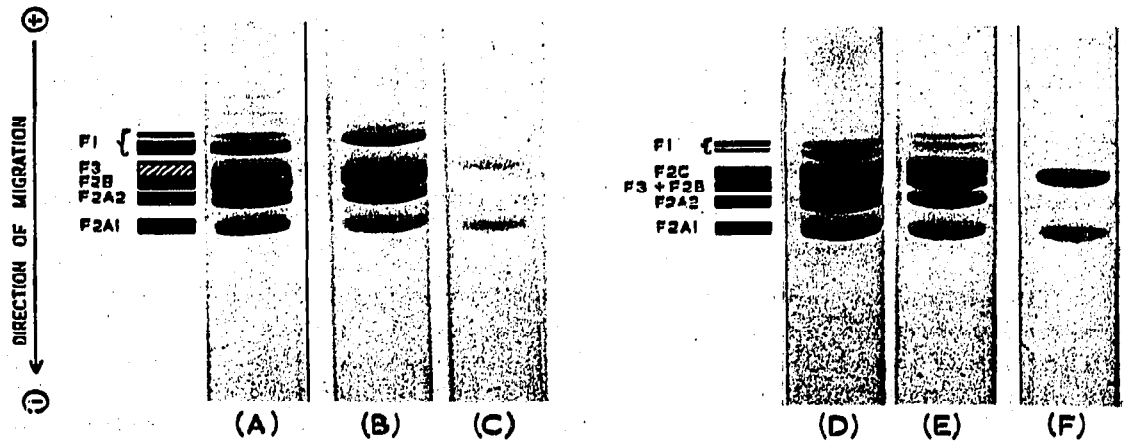

Fig. 1. Polyacrylamide gel electrophoretic patterns of calf thymus and chicken erythrocyte whole histones stained by conventional techniques and by the differential method described here. (A) Calf thymus whole histone stained with naphthalene black. (B) Calf thymus whole histone stained with Alizarin black. (C) Calf thymus whole histone stained with bromophenol blue. (D) Chicken erythrocyte whole histone stained with naphthalene black. (E) Chicken erythrocyte whole histone stained with alizarin black. (F) Chicken erythrocyte whole histone stained with bromophenol blue. All gels destained in 40% *n*-propanol.

the extended washing in 40% *n*-propanol. With bromophenol blue, however, only the arginine-rich histone fractions remain visible after the washing.

This has been confirmed by running the individual purified fractions and staining and destaining under the same conditions when only histones F2A1 and F3 remain stained.

It is of interest to note that if immediately after the electrophoresis the gel is placed in 10% glutaraldehyde for 1 h and then stained and destained according

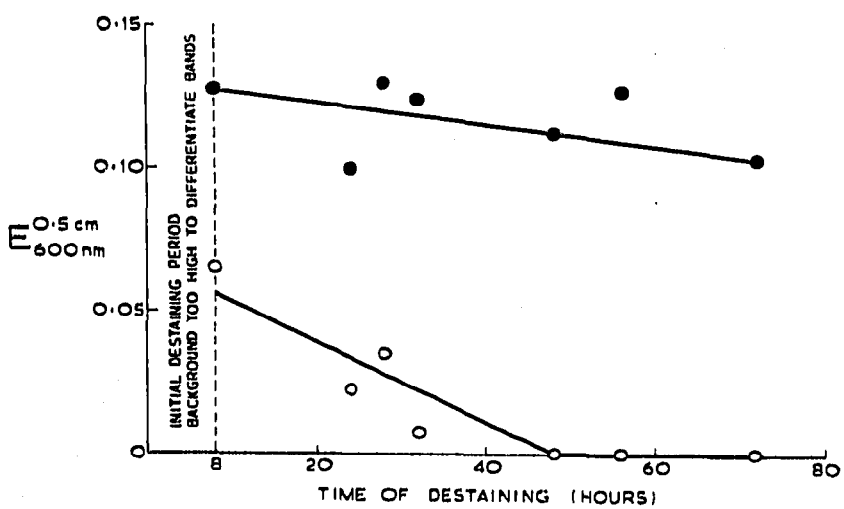

Fig. 2. The differential elution of colour from bromophenol blue stained bands of purified lysine-rich (F1) and arginine-rich (F2A1) histones. ○, Histone fraction F1; ●, histone fraction F2A1.

to this procedure all bands remain visible and the patterns are identical to those obtained with the naphthalene black and the alizarin controls.

As the bands remaining visible after this specific staining and destaining technique (F3 and F2A1) were far less intense than the corresponding bands in the control gels (Fig. 1) a study was undertaken to determine the rate at which colour was lost from both an arginine-rich histone (F2A1) band and a lysine-rich histone (F1) band. This was carried out by running a series of gels containing the two histone fractions, staining as described above with bromophenol blue, and destaining for various times. The colour remaining in the bands was then determined quantitatively as described previously[5] by elution with dimethyl sulphoxide. It can be seen from Fig. 2 that the F1 band is completely removed after 48 h whereas the removal of colour from the F2A1 band appears to plateau after this time and even prolonged destaining does not remove it completely.

Discussion

The method described in this paper for the differentiation of the arginine-rich histones developed out of a study of a method described by RINGERTZ AND ZETTERBERG[12] for the cytochemical demonstration of histones and protamines. Their paper describes a method for the differentiation of basic and acidic proteins using an alkaline bromophenol blue binding reaction. In an attempt to use this method in polyacrylamide gels, in order to differentiate between histone and non-histone proteins we noticed that under the conditions we were using for destaining, some of the histone bands were also eluted. This observation was followed up and various destaining regimes investigated in order to optimise this effect.

The method as developed now allows the individual fractions in a mixture of histones to be identified as arginine-rich or not, after separation in polyacrylamide gels. This appears to be correct for all histone fractions tested so far, but the precise mechanism of the differential elution is not clear. It appears, however, that with the fractions which are no longer visible after the differential destaining in the propanol–water mixture, the dye is being removed from the protein, since a subsequent staining with naphthalene black causes them to reappear. After this treatment, however, the low-molecular-weight F2A2 fraction is much less sharp, presumably due to some diffusion of the temporarily unfixed protein.

The differential elution does not appear to be dependent simply on the isoelectric point of the protein since non-basic proteins of higher molecular weight like bovine serum albumin do not, once stained, destain easily. Only with the lower-molecular-weight proteins does it appear to be dependent on the isoelectric point. As the results indicate, there is probably elution of all stained bands in this system but the arginine-rich histones are much more resistant to the destaining than the other histones. The low rate of elution of the stained arginine-rich fractions may of course not be due to a removal of stain from the protein, but due to a slow elution of the stained complex which occurs with most destaining techniques when carried out for long periods.

The polyacrylamide gels used contained 1 *M* urea and are at pH 2.8 at the end of the electrophoresis. We have also tested this method using the electrophoretic technique of PANYIM AND CHALKLEY[6] which is at a slightly higher pH (3.2) and uses gels containing 6.25 *M* urea. No differences were observed and the differential staining

was equally effective. Gels run at higher pH values may give different results since it is known that the arginine-rich histones aggregate at pH values above 4, but these have not been tested.

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