ELECTROPHORETIC COMPARISON OF ACIDIC PROTEINS OF CHROMATIN FROM DIFFERENT ANIMAL TISSUES

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1. Introduction

The acidic proteins of chromatin have recently been isolated in an undenaturated soluble form thus allowing the fine study of their electrophoretic migration. In the present paper, the electrophoretic patterns of chromatin acidic proteins from different tissues of the same species are compared: calf thymus, liver and kidney as well as chicken's liver and erythrocytes. The electrophoretic pattern of the same organ (liver) in various homeotherms are also compared (rat, calf, chicken). Indeed, if these proteins have a role in gene's control as repressors or derepressors as suggested, important variations may be expected in different tissues.

Our results indicate that the electrophoretic patterns of these proteins are indeed different from one tissue to another within the same species, the nucleated erythrocytes acidic proteins showing the greatest difference. The patterns of chromatin acidic proteins from a single tissue also differ within various species.

2. Material and methods

Liver, thymus and kidneys from calf were obtained at the slaugterhouse immediately after death of the animals: wistar rats and chickens have been killed by decapitation.

Nuclei have been prepared by the Chauveau method and their purity checked by phase contrast microscopy; chicken erythrocytes have been prealably lysed with 0.1% tween 80 (Shaerer and MacCarthy). Acidic proteins of chromatin have been prepared by Wang's technique as modified by Loeb. After washing the nuclei with a solution containing NaCl 0.05 M, F Na 0.05, tris 0.05 M, pH 7.6, the chromatin was extracted by 2 M NaCl. The acidic proteins of chromatin were obtained in solution by lowering the NaCl concentration to 0.15 M at which nucleohistones are precipitated.

Electrophoretic analysis has been done by the technique of Uriel in a mixed gel containing 5% of acrylamide and 0.8% of agarose at pH 8.6. This method allows simultaneous migrations of more than one sample on the same plate. Proteins have been concentrated to 2 mg/ml by swelling biogel P 6 in the protein solution and pressing the gel through a nylon tissue.

3. Results

Fig. 1 indicates the electrophoretic patterns of chromatin acidic proteins of different calf tissues: thymus, liver and kidney. These patterns differ significantly from one tissue to another. The number of bands, their mobility and the relative intensity of staining are different in each organ.

Differences in the mobility of calf liver and thymus acidic proteins are also clearly shown by the mixture of these two fractions. The number of bands thus obtained is 18, this is superior to the number of the bands of each tissue separately, 12 for liver, 11 for thymus. One may conclude that there are at least six bands of different mobilities.

The electrophoretic patterns of two tissues of chicken, liver and erythrocytes are shown in fig. 2. They are obviously very different. The number of

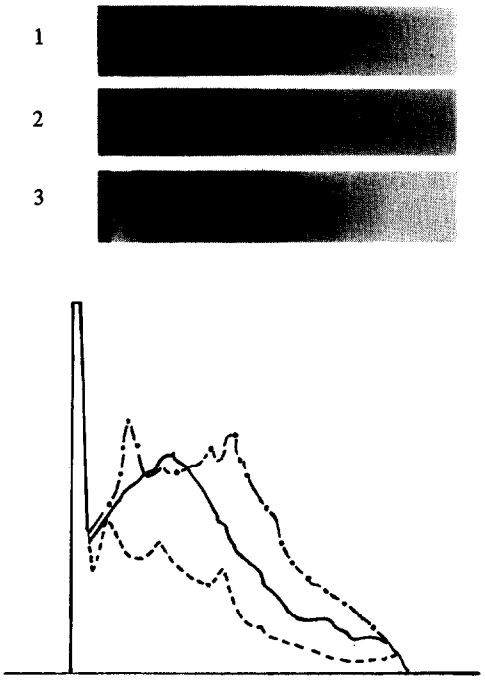

Fig. 1. Electrophoretic patterns and densitometry of acidic proteins of calf thymus (1; – – – –), liver (2; ———) and kidney (3; –·–·–).

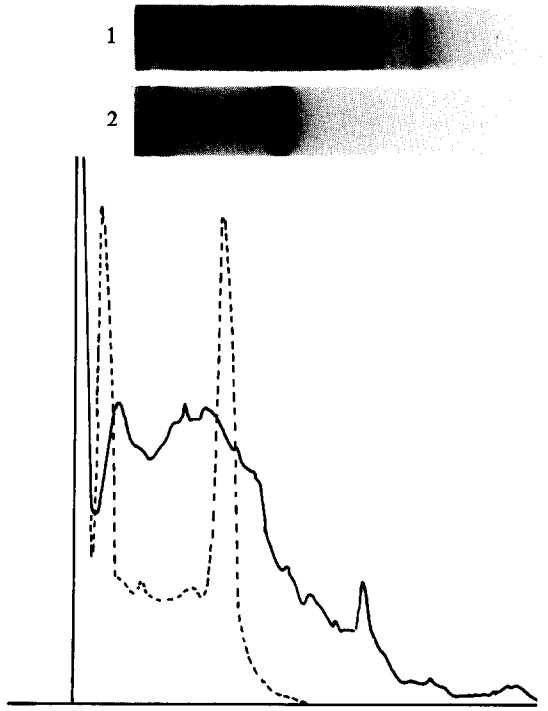

Fig. 2. Electrophoretic patterns and densitometry of acidic proteins of chicken liver (1; ———), erythrocytes (2; – – – –).

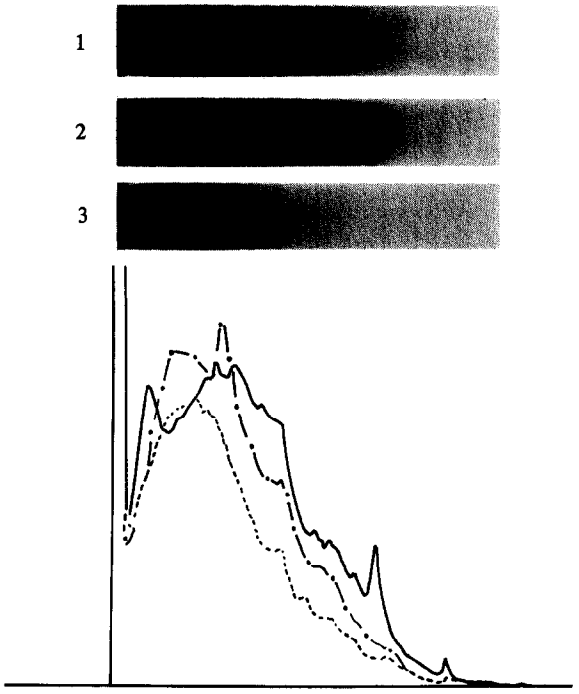

Fig. 3. Electrophoretic patterns and densitometry of acidic proteins of liver from rat (1; –·–·–), chicken (2; ———) and calf (3; ------).

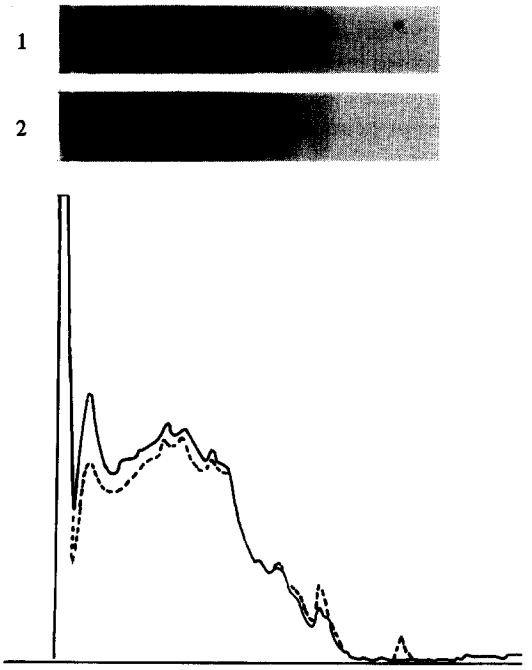

Fig. 4. Electrophoretic patterns and densitometry of acidic proteins from chicken liver, left one night at 4° (1; – – – –), at 20° (2; ———).

bands is more important in the chicken liver, the pattern of which shows some similarities with that of calf liver, whereas in erythrocytes there are fewer bands with only two strongly stained.

The mobilities are different as shown by the pattern obtained by a mixture of equal parts of the proteins of the two tissues. The number of bands is superior to those of each fraction separately. However, for chicken erythrocytes, only part of the acidic proteins is extracted by the technique utilised.

Fig. 3 shows the pattern of the same organ liver in different species: rat, calf, chicken. If the diagrams possess some similarities, there are however differences as shown by the mixture of two tissues. The number of bands in the mixture is each time higher than that in one tissue alone.

Differences between electrophoretic patterns could come from a more or less strong proteolytic action. Presence of cathepsins in cell nuclei has been reported. However, determination of proteolytic activity after electrophoresis by the technique has given negative results at neutral pH. Meanwhile we have compared electrophoretic patterns from one sample kept one night at +4° or at +20°. As shown in fig. 4, no significant difference was observed in relative mobilities, relative intensity, or sharpness of the bands.

It can be concluded that differences encountered in the diagrams correspond to differences in the number and the nature of the proteins belonging to each tissue.

4. Discussion

Our results show that acidic proteins of chromatin differ from one tissue to another in the same species. The differences are particularly strong between chicken liver and erythrocytes where the electrophoretic patterns are very different. These results are in contradiction with the situation found with histones where the electrophoretic diagram and the amino-acid composition are identical in cell tissues from the same animal, the only exception being erythrocytes where a supplementary fraction is present (Neelin).

There are also differences between the acidic proteins of the same tissue in different species of higher animals although the histones of these species are nearly identical.

It is difficult to explain the true signification of these differences. Some enzymes have been described in this fraction, in particular DNA polymerase and RNA polymerase [1] are probably present in all tissues studied but in different quantities. There is a possible exception with chicken erythrocytes.

The presence of hormonal receptors has been described in this nuclear fraction and one may expect that these receptors are present only in target cells. Some authors by analogy with the situation in bacteria (and phages) have postulated the presence of repressors among the acidic proteins of chromatin. One could expect in this respect large differences between tissues. Our results do not contradict this hypothesis. However, these repressors are certainly present in very small amounts which are not detectable by electrophoresis. However, it is not excluded that there are proteins of close electrophoretic mobility which together could give a visible band.

Other authors have stated that this fraction contains derepressors such as the phosphoprotein described by Langan. The nature and distribution of these derepressors may vary among tissues. Future researches on the nature of acidic proteins, their binding with DNA and histones, their metabolism, and their influence on synthesis primed by DNA can help to elucidate their function.

Similar results have been found by Kruh and Tichonicky [12] who have observed differences in electrophoretic patterns and aminoacid composition of acidic proteins of chromatin from different tissues of rabbit.

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