The effect of urea on histones in polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis (PAGE) has been used for the resolution of histones and histone fractions[1,2]. Although the method of McALLISTER *et al.*[1] does appear to be one of the better methods for the separation of the apparently heterogeneous histones, the method does suffer some drawbacks and appears to lead to the formation of some artifacts. This communication reports that the incorporation of urea into the previously used buffer system eliminates the holdback at the point of application of the sample, decreases the number of bands observed in a histone sample and allows for the use of spacer gels if desired. These observed alterations may be due to the lack of aggregation of some of the histone fractions in the presence of urea[3] and thus a decrease in the number of bands in the PAGE system. The previously used PAGE systems have yielded ten or more rather diffuse bands for unfractionated calf thymus histones compared to a smaller number of more discrete bands upon urea incorporation into the buffer system.

Methods and materials

Whole calf thymus histone, as supplied by Worthington Biochemical Corporation, Freehold, New Jersey, was used for this study.

A concentrated solution of buffered Temed (N,N,N',N',-tetramethylethylenediamine) was prepared by adding 2.4 ml of 5 *N* KOH and 1.15 ml Temed to 53 ml of glacial acetic acid. Immediately before use 5.7 ml of this solution is added to 70 mmoles of urea and diluted to a final volume of 10 ml. The gel mixture is composed of two parts of this solution with one part of 2.8 % ammonium persulfate–7 *M* urea and one part of 60 % acrylamide–0.4 % bis (N,N'-methylenebisacrylamide). The spacer gel solution, when used, included 0.09 *M* glycinium acetate buffer (pH 4.0), 2.5 % acrylamide, 0.31 % bis, 0.5 mg % riboflavin, and 5.25 *M* urea. Sample gels were not used. The samples were dissolved in 0.09 *M* glycinium acetate buffer (pH 4.0)–1 *M* sucrose, and one ml of this solution was added to seven mmoles urea (final volume 1.3 ml, final urea concentration 5.4 *M*). This solution was placed directly on the gel and electrophoresis buffer (0.37 *M* glycinium acetate, pH 4.0, with 5.25 *M* urea) was carefully layered over it. The tubes used were 8 mm I.D. (destaining tubes as supplied by Canal Industrial Corporation, Bethesda, Maryland) with a current of 7 mA/tube for 4 h.

Staining and destaining were carried out as recommended by McALLISTER *et al.*[1]

Results and discussion

Fig. 1 compares the electrophoregrams of calf thymus histone in the presence and absence of urea. All of the bands appear more distinct in the urea containing system. It should be noted especially that in the presence of 5.4 *M* urea there is an absence of the band which appears at the point of application of the protein sample. In urea it appears that there is no hold up of the sample at this point which may indicate the lack of aggregation to form high molecular weight complexes which may form in the absence of this denaturing agent. Also, it should be pointed out that the urea containing system allows for the use of spacer gels if desired. The PAGE system run in the presence of urea appears identical with or without the spacer gel.

Another significant difference is the absence of several of the slower moving bands in the urea containing electrophoregrams. Although the molecular sieving effect is not the only factor responsible for the resolution of histones in the PAGE system, it may account for the lack of the high molecular weight species or aggregates which appear as less mobile bands in the urea free system. The difference found in the presence of urea employed here was not reported by DREIDGET *et al.*[2] in gels containing 4 *M* urea. While these authors reported no differences with 4 *M* urea, they did report

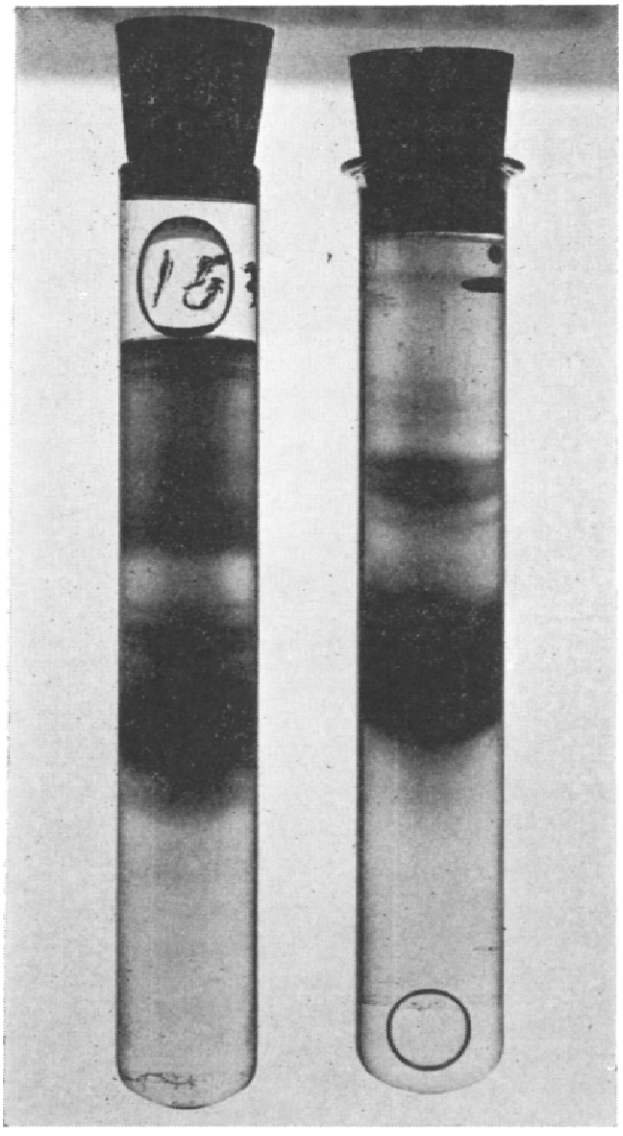

Fig. 1. The electrophoregrams of whole calf thymus histone run in the absence (left) and presence (right) of 5.4 *M* urea.

slight variation in the positions, intensities and number of bands of histones in the presence of some metal ions at a final concentration of 10^{-3} *M*. VANDE WOUDE AND DAVIS have also recently reported a considerable sharpening of histone bands when the PAGE was run in the presence of urea at concentrations up to 8 *M*[4].

These various observations point out the need for a reevaluation of the PAGE

system applied to the resolution of histones. Aggregate formation of some of the histone fractions may be one of the major factors responsible for some of the numerous bands reported in these electrophoresis systems. Employment of urea, as suggested here and in the recently reported preparative electrophoresis of proteins on polyacrylamide gels[5], may be necessary to eliminate the anomalous behavior of histones in PAGE systems.

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Partition chromatography of some organic acids on a cation exchange resin

It is known that air dry 10% divinylbenzene cross-linked sulfonated polystyrene resin, when equilibrated with 70% aqueous acetone, absorbs water preferentially, so that the water content of the liquid absorbed is found to be higher than that of the external solution[1]. The distribution of an organic substrate which is sparingly soluble in water between these two phases will be greatly in favour of the outer solution and thus provides an explanation both for the negative adsorption and for the low catalytic activities of sulfonated polystyrene resin in the hydrolysis of various aliphatic esters in 70% acetone in water[2].

RUCKERT AND SAMUELSON[3] have also shown that strongly polar nonelectrolytes, such as sugars, can be taken up effectively from mixed solvents by means of ion exchange resins. On the basis of this, they were able to separate sugars chromatographically on anion exchange resin. Dowex 1-X8 in the sulfate form was used as the stationary phase and the eluent was 74% aqueous ethanol[4].

Separation of various organic acids using similar chromatographic systems has been attempted, in some cases successfully. In the present work cross-linked sulfonated polystyrene resin, Amberlite CG-120, in the hydrogen ion form was used. Citric acid, malic acid and tartaric acid could be separated using the mixture acetone–dichloromethane–water (160:100:9, v/v) as eluent, while an eluent containing more dichloromethane (acetone–dichloromethane–water (20:15:1, v/v), made possible the separation of fumaric acid, glutaric acid and succinic acid.