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Note

Formation of artifacts in electrofocussing on acrylamide gel with ampholines

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The electrofocussing technique has been widely used for the separation of protein mixtures. High resolving power and the ability to handle small amounts of proteins are its major advantages. Of the several techniques adopted by recent workers, electrofocussing in a sucrose density gradient or on acrylamide gel have been the commonest choices. Both these methods involve the use of ampholines, a mixture of aliphatic polyaminocarboxylic acids, supplied by LKB (Stockholm, Sweden).

Although the value of these ampholines is not in question, their behaviour needs to be understood. For instance, ampholines have been located as sharply defined bands by Frater[1] during isoelectric focussing of wool proteins on acrylamide gel. This note reports some similar difficulties experienced during the isoelectric focussing of relatively insoluble plant proteins on acrylamide gel.

EXPERIMENTAL AND RESULTS

Isoelectric focussing was performed on 5% acrylamide gel plates by the method of Leaback and Rutter[2]. The gel was prepared by photopolymerization with riboflavin. Ampholine carriers (pH 3–10) and urea (when used) were added to the gel mixture prior to polymerization. The gels, after electrofocussing for 26 h were fixed in 10% trichloroacetic acid (TCA) and then stained either with 0.2% fast green[3] or 0.05% Coomassie brilliant blue[4] by immersing the plate in the stain solution. De-staining was carried out in the same way with stain-free solvent.

Several proteins of different origin, such as microtubular protein extracted from pig brain, fibrillar protein from phloem tissue of *Heracleum* and phloem exudates of several plant species, were investigated and they all showed a satisfactory separation. However, some fine parallel bands extending from one side of the plate to the other were consistently observed. They showed a similarity to the sharply defined bands reported by Frater[1] and it is surprising that there seem to be no other reports on such bands, although this technique has been in use for a considerable number of years.

These sharp bands appeared on the gel only after staining, most of the protein bands being visible as whitish opaque regions immediately after fixation with TCA. In the vicinity of the genuine protein bands the ampholine bands showed a break,

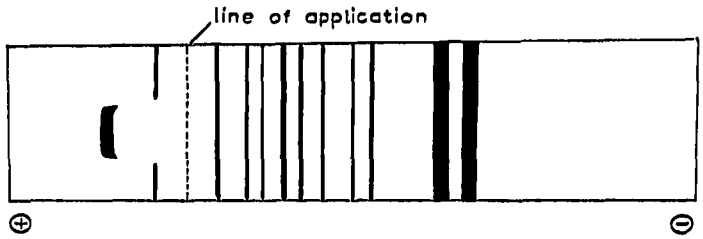

Fig. 1. Isolectric focussing of proteins in phloem exudate of *Heracleum*. Note the protein band and the sharply defined non-protein bands.

as shown in Fig. 1. These were located more frequently in the acidic region of the gel (pH 4–6) than in the neutral and alkaline regions. However, the bands in the neutral and alkaline regions were more prominent, being wider and more intense. A thinning of the gel, which presumably has an effect on the pH gradient (Fig. 2), was noticed, a phenomenon already reported by several workers[5,6]. Most of the faint lines were located in the region between the anode and this thin area.

These sharp bands do not appear to be formed from anything in the protein sample, as they were present on control plates run without a sample (Fig. 3). Similarity in results obtained from several different batches of acrylamide do not

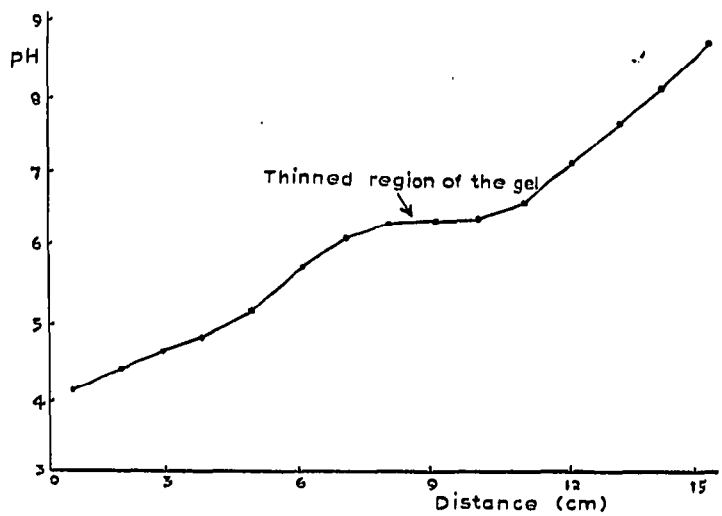

Fig. 2. The pH gradient obtained after isoelectric focussing in acrylamide gel using ampholytes of nominal pH 3–10. Note the plateau region around pH 6.5.

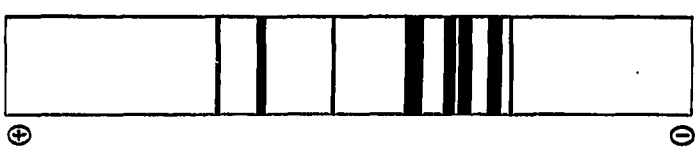

Fig. 3. Bands obtained after electrofocussing without any application of protein to the acrylamide gel, using ampholine carriers of pH 3–10.

seem to indicate that they are formed from any impurity in the acrylamide at these sharp bands. Frater[1] has attributed their formation to non-uniform conductivity. Even though they do not seem to interfere with the separation of proteins it is possible that the identification of genuine protein bands may be difficult, especially where the protein sample spreads over the entire current path. Therefore it is necessary to interpret the band pattern with care.

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