AN IMPROVED CHROMATOGRAPHIC SEPARATION OF WHEAT
GLUTEN PROTEINS*

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Gel electrophoresis is at present the best procedure for the analytical fractionation of wheat gluten proteins. Procedures more suitable for preparative studies, such as gel filtration and column chromatography, have provided only partial fractionation on the basis of gel electrophoresis (Graham 1963; Lee et al. 1963; Wright, Brown, and Bell 1964). Gel electrophoretic patterns of fractions resulting from the carboxymethyl cellulose (CMC) fractionation of Simmonds and Winzor (1961) have been published by Graham (1963) and by Lee et al. (1963). These results showed that each fraction was heterogeneous, with considerable overlapping of electrophoretic bands from neighbouring fractions, and that fractions eluted late in the chromatogram were contaminated because of tailing of earlier fractions. The modification of the Simmonds and Winzor procedure described below results in an improved fractionation of gluten proteins as judged by starch-gel electrophoresis.

Gluten proteins were extracted from Gabo flour as described previously (Coates and Simmonds 1961; Lee et al. 1963). Columns (1·5 by 25 cm) containing about 3 g CMC (Whatman CM70 powder) were maintained at 40°C and were eluted at 1 ml/min; fractions were collected at 10-min intervals. Gluten proteins (21 mg nitrogen) were applied to the column in sodium lactate buffer (0·01 M sodium hydroxide adjusted to pH 4·1 with lactic acid) containing 1 M dimethyl formamide (DMF). Protein elution by the solvents described in Figure 1 was followed continuously by directing part of the effluent to a Technicon AutoAnalyser for analysis by the Lowry colorimetric method.

For starch-gel electrophoresis samples were dialysed against 0·01 N acetic acid and freeze-dried. The freeze-dried material was suspended in 2·5 M urea at a concentration of between 0·5 and 1·0%. Vertical starch-gel electrophoresis was carried out as described by Graham (1963) in 0·017 M aluminium lactate–2 M urea, pH 3·1.

Figure 1 shows the protein elution profile obtained by the above procedure. Starch-gel electrophoresis (Plate 1) of fractions selected from this chromatogram showed that the procedure resulted in considerably less tailing and better resolution than the original method of Simmonds and Winzor (1961). Complete separation of the slowest-moving electrophoretic bands was obtained as these were not retained by the column (Fig. 1, tubes 5 and 6). The 0·5 M salt fraction contained the proteins with high electrophoretic mobility generally regarded as albumins and globulins. Gel electrophoresis of the contents of each tube from salt-gradient elution showed that the starch-gel pattern changed progressively from tube to tube as each electrophoretic component appeared in the column effluent at its characteristic salt concentration. It seemed that the appearance of a peak in the elution profile did not necessarily indicate the appearance of a single electrophoretic component at that point.

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A fraction appearing late during gradient elution (Fig. 1, tubes 56-59) produced a diffusely stained region on electrophoresis. Gel filtration of the gluten proteins on Sephadex G-100 separated this material from the sharp bands and showed its molecular weight to be in excess of 100,000.

The main reason for the improvement in resolution appeared to be the increase in temperature of chromatography from room temperature (Simmonds and Winzor 1961) to 40°C. When chromatography was performed at 5, 25, and 40°C, respectively, there was a progressive improvement in resolution and a decrease in tailing. An increase in temperature would tend to weaken hydrogen bonding and so allow ion exchange to make a larger contribution to chromatography with a resultant improvement in resolution and recovery and a reduction in tailing.

![Chromatographic elution pattern of gluten proteins.](image)

The importance of DMF to the separation was shown by excluding DMF from the chromatographic procedure. Almost all resolution into peaks was lost and there was gross tailing on the column. However, only slightly better resolution was obtained by increasing the DMF concentration from 1M to 4M. Probably DMF, like increased temperature, served to decrease non-ionic binding. Because of its higher dissociation constant, lactic acid proved better than acetic acid for the control of pH in presence of DMF. The lower protein loading used in these experiments also contributed to the overall improvement in resolution.

The amount of protein eluted in the NaOH fraction was minimal when chromatography was performed at 40°C or at higher temperatures. At 5°C the NaOH fraction contained 30% of the applied protein and at 40°C it accounted for 13%. The NaOH fraction obtained at 40°C was identified with the material that remained at the origin on gel electrophoresis. This material appears to be similar to the α1 fraction of Jones, Taylor, and Senti (1959) and Woychik, Boundy, and Dimler (1961) who regard it as glutenin. The additional material appearing in the NaOH fraction at lower temperatures presumably represented material remaining after incomplete elution of earlier fractions.
Starch-gel electrophoresis of gluten protein fractions (see chromatogram, Fig. 1). Tube numbers are indicated at the origin of each electrophoretic pattern.

Gluten fractions from the procedure described above contain fewer components (as judged by gel electrophoresis) than fractions from any other method hitherto described. It is hoped that these fractions will provide suitable material for a more critical examination of the gluten protein complex.

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References
