STUDIES ON REDUCED WOOL

III. STARCH-GEL ELECTROPHORESIS OF EXTRACTED WOOL PROTEINS

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[*Manuscript received August* 16, 1963]

Summary

Starch-gel electrophoresis in 8M urea has been used to demonstrate the presence of many components in wool protein fractions extracted from reduced and alkylated wool. All preparations of low-sulphur wool proteins gave multiple bands on starch gel in 8M urea even though some of these had previously been fractionated to give a single peak using moving-boundary electrophoresis in the absence of 8M urea. The heterogeneity suggested by these results is in accord with that found by chromatography of the proteins on DEAE-cellulose in buffers containing 8M urea. With stepwise elution from DEAE-cellulose it is possible to obtain fractions responsible for various sections of the starch-gel electrophoretic pattern.

I. INTRODUCTION

In previous studies on the fractionation of extracted wool proteins (O'Donnell and Thompson 1961, 1962; Gillespie 1962; Thompson and O'Donnell 1962*b*), fractions isolated by chromatographic procedures on DEAE-cellulose using buffers containing 8M urea were shown by amino acid analysis to have differing chemical compositions. This technique thus demonstrated chemical heterogeneity in the purified low-sulphur structural proteins of wool even though these give predominantly single peaks in moving boundary electrophoresis under a variety of conditions (cf. Gillespie and Lennox 1955; Gillespie 1956, 1957; O'Donnell and Woods 1956; Woods 1959).

The present paper reports studies on extracted wool proteins using the technique of electrophoresis in starch gel developed by Smithies (1955). The combined effect of molecular sieving and electrophoretic separation in this procedure coupled with the disaggregating effect of 8M urea has revealed the presence of many components even in purified preparations.

II. MATERIALS AND METHODS

Horizontal starch-gel electrophoresis (Smithies 1955) was used with the discontinuous Tris–borate buffer system of Poulik (1957). Urea (8M) was used in the buffer as a disaggregating agent (Poulik 1960). Hydrolysed starch (20 g) (Connaught Laboratories, Toronto) was heated with 100 ml buffer (pH 8·6) in the usual way and then 80 g urea added before degassing at the water-pump. The starch gels were poured in vessels (15 by 5 by 0·5 cm) such as those described by Smith (1960).

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They were covered with thin polythene sheeting and allowed to set overnight. Proteins were applied as 0·5–0·75% solutions on rectangular pieces of Whatman No. 3MM paper. At concentrations of protein greater than about 1% the patterns were less well-defined. Electrophoresis was carried out at constant voltage in a cold room at 2°C, using a voltage gradient of approximately 16 V/cm. After slicing, the gels were stained with nigrosine in a solution of methanol–water–acetic acid (45:45:10 v/v). For re-running, slices of gel 5 mm wide were cut along the direction of migration of the proteins and inserted in a second gel.

The alkylated low-sulphur wool protein fraction SCMKA and the high-sulphur wool protein fraction SCMKB were generally prepared by the method described previously (O'Donnell, Thompson, and Inglis 1962). The preparations of SCMKA2 (Nos. Z115 and Z34, kindly supplied by Dr. J. M. Gillespie) had been fractionated with ammonium sulphate (Gillespie 1957), the latter preparation (Z34) having been previously used by Thompson for fractionation of its tryptic digests (see Gillespie *et al.* 1960) and by Bradbury (1958) for carboxyl end-group studies. The corresponding freeze-dried preparations of SCMKA and SCMKB from wool roots were kindly supplied by Dr. G. E. Rogers.

Chromatography on DEAE-cellulose was carried out, as described previously, with buffers containing 8M urea–0·01M Tris–0·001M Versene, pH 7·4 (O'Donnell and Thompson 1961; Thompson and O'Donnell 1962*b*), using stepwise elution (15 ml per step) with salt concentrations from 0·01M to 1M. The fractions (1·25 ml) were diluted with 6 ml of water before measurement of their optical density at 276 mμ. Each peak was concentrated by freeze-drying, dialysed versus water, freeze-dried, and made up finally to approximately 1% concentration in the chromatography starting buffer. They were then each subjected to starch-gel electrophoresis.

III. Results and Discussion

Plate 1, Figures 1–4, shows the starch-gel patterns obtained with the low- and high-sulphur wool protein preparations. Generally it is seen that the bands in the SCMKB preparations have run faster than those of the SCMKA preparations. This is a consequence of their higher net charge and lower molecular weight. The multiplicity of components of SCMKB is in accord with the heterogeneous nature of this material as revealed by moving boundary electrophoresis (Gillespie 1963). We have as yet made no attempt to find the optimum conditions for electrophoretic separation of these high-sulphur proteins on starch gels. The SCMKA preparations revealed a number of distinct bands, usually two major and one minor leading bands, and two or three diffuse slower-moving bands (see Plate 1, Figs. 1–4). There are also several faint faster-moving bands, some of which correspond in position to the bands obtained with SCMKB and some of which have a rate in between those of the high- and low-sulphur wool proteins.

Several SCMKA preparations, including the purified preparations Z34 and Z115 gave essentially identical patterns except that in these purified preparations the traces of faster-moving bands appeared to be less intense than in the less-fractionated preparations.

One possible explanation for the multiple bands in the SCMKA pattern was that the reduction of the disulphide bonds in the original wool had not been complete and that the residual disulphide bonds were cross-linking some molecules to give larger molecules. However, re-reduction with mercaptoethanol in 8M urea followed by carboxymethylation and re-fractionation at pH 4·4 (Gillespie, O'Donnell, and Thompson 1962) did not change the starch-gel pattern. Likewise, running the starch-gel electrophoresis in the presence of approximately 0·1M mercaptoethanol or 0·01M Versene did not simplify the pattern, although the intensities and sharpness of some of the bands did alter. Increasing the ionic strength of the starch gel by the addition of potassium chloride to the buffer (up to 0·05M) resulted in a better separation of the two major leading and the three minor diffuse bands from SCMKA, but the leading bands became more diffuse and no advantage was to be gained from increasing the ionic strength in this way.

The presence of the fast trace components (some of which correspond to high-sulphur proteins) is not surprising in view of the protein–protein interactions which have been demonstrated by O'Donnell and Thompson (1962) to occur between high- and low-sulphur proteins from oxidized wool due to secondary valence bonding. Trichloroacetic acid precipitation, which is effective in removing some high-sulphur contaminants from the low-sulphur proteins from oxidized wool, i.e. α-keratose, could not be used with the proteins from reduced and carboxymethylated wool since these are all precipitated by trichloroacetic acid.

Starch-gel patterns (Plate 1, Fig. 4) of the SCMKA proteins obtained with wool root preparations (Rogers 1959) showed fewer components and a marked intensification and broadening of the major leading band. This band had a slightly higher mobility than the corresponding band from SCMKA from wool.*

Starch-gel electrophoresis of (α—X) keratose preparations (O'Donnell and Thompson 1962) was unsatisfactory because of the difficulties in staining these oxidized proteins. The patterns which were obtained were indistinct but showed a similarity to the patterns from SCMKA preparations.

It might be contended that the various strong bands in the starch-gel pattern of SCMKA are due to different states of aggregation, even in the presence of 8M urea. However, their discreteness would not favour this idea, and re-running these bands in the second dimension in starch gel gave an identical pattern in the second dimension with the bands lying on a diagonal straight line.

The simplest and most obvious explanation of the presence of several strong bands in the starch-gel pattern of the low-sulphur proteins is that they each represent different chemical species. In order to investigate the proteins corresponding to the bands in the starch-gel pattern of the SCMKA fraction each will have to be obtained in sufficient quantity for amino acid analysis and "finger-printing" of

* In contrast when a fresh 8M urea–0·01M Tris (pH 8·8) extract of wool roots was prepared, it was found that after reduction with mercaptoethanol and alkylation with iodoacetate, followed by dialysis against 8M urea–Tris buffer to remove excess salts, the starch-gel pattern showed the same two major bands present in a corresponding wool extract and there was an exact correspondence of mobilities. The other bands were mostly still present but appeared relatively less intense.

their enzymic digests (Thompson and O'Donnell 1962*a*). Preparative starch-gel electrophoresis to isolate the protein bands does not appear practical at present due to the relatively small amount of wool protein tolerated by the gel without streaking on subsequent electrophoresis.

Fractionation of the SCMKA preparation by stepwise-elution chromatography on DEAE-cellulose in buffers containing 8M urea (Thompson and O'Donnell 1962*b*) has been repeated, and the fractions obtained examined by starch-gel electrophoresis.

It is seen (Fig. 1; Plate 1, Figs. 5 and 6) that it is possible to concentrate proteins responsible for a particular band in the starch-gel pattern. Experiments now in progress are aimed at isolating each protein in as pure a condition as possible for its characterization.

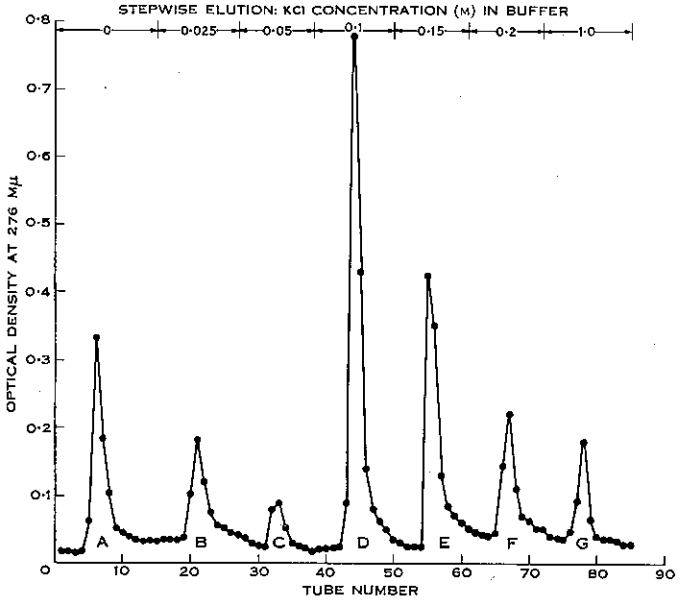

Fig. 1.—Chromatographic behaviour of SCMKA on DEAE-cellulose at 25°C in 8M urea–Tris buffer, pH 7·4. Fraction size was approximately 1·25 ml. Stepwise elution with increasing concentrations of potassium chloride in the buffer (15 ml for each step). Hold-up volume of the 0·9 by 15 cm column was approximately 7·5 ml. The starch-gel patterns corresponding to the original mixture and isolated peaks A–F are shown in Plate 1, Figures 5 and 6.

The complexity previously found with tryptic digests of SCMKA2 (Gillespie *et al.* 1960) could well be due to the presence of several different proteins, in accord with the present observations, and until such components are separated and identified attempts to determine amino acid sequence seem unwarranted.

IV. Acknowledgments

We wish to thank Dr. J. Williams, Molecular Biology Laboratory, Cambridge University, and Dr. M. A. Jermyn, Division of Protein Chemistry, for technical advice.

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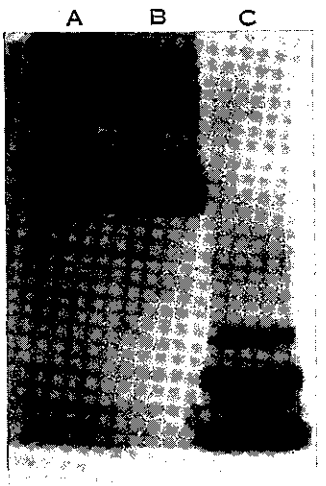


Fig. 1

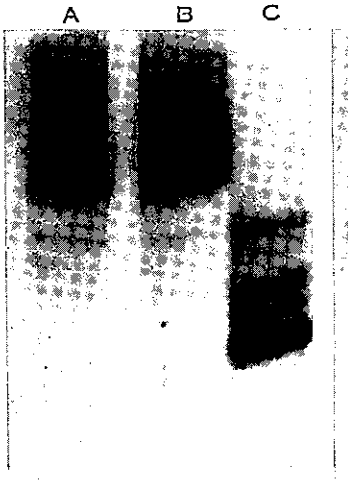


Fig. 2

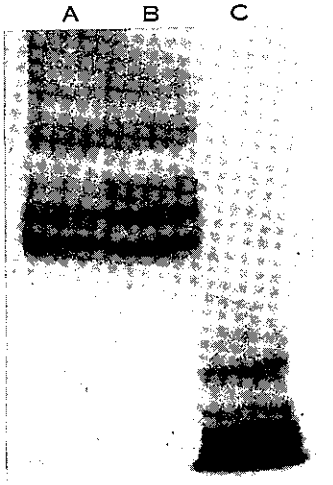


Fig. 3

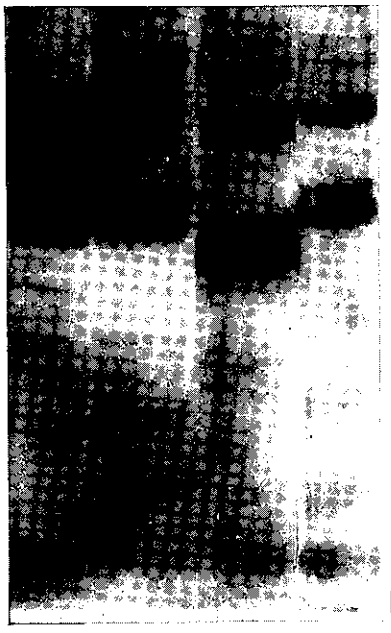


Fig. 4

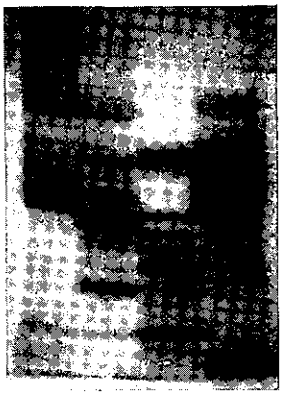


Fig. 5

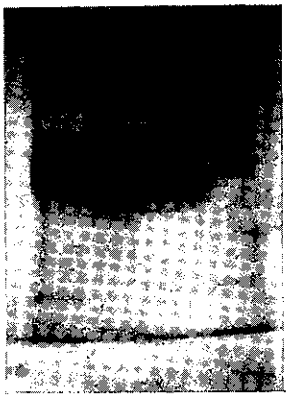
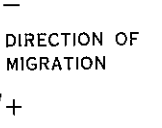


Fig. 6



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Explanation of Plate 1

Starch-gel patterns of wool protein fractions. Origin of each electrophoretic pattern coincides with the top of each figure

Fig. 1.—Normal gel, pH 8·6 (see Section II): *A*, SCMKA; *B*, SCMKA2, preparation No. Z115; *C*, SCMKB.

Fig. 2.—Normal gel containing 0·1M mercaptoethanol: *A*, SCMKA; *B*, re-reduced, carboxymethylated, and re-fractionated SCMKA; *C*, SCMKB.

Fig. 3.—Normal gel containing 0·01M Versene: *A*, SCMKA; *B*, SCMKA2, preparation No. Z115; *C*, SCMKB.

Fig. 4.—Normal gel: *A*, SCMKA; *B*, low-sulphur carboxymethylated hair-root protein from guinea pig; *C*, low-sulphur carboxymethylated wool-root protein; *D*, high-sulphur carboxymethylated wool-root protein.

Figs. 5 and 6.—Starch-gel patterns corresponding to SCMKA and isolated peaks A–F (see Fig. 1, p.280) after stepwise elution from DEAE-cellulose.