IMMUNOLOGICAL STUDIES ON WOOL PROTEINS

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Summary

Immunological methods were used to study the heterogeneity of a major low-sulphur protein isolated from wool. Rabbit antiserum to the purified protein was prepared and by the use of double diffusion in agarose gels, considerable heterogeneity of the protein was demonstrated.

Similar heterogeneity of the protein was also observed in immunoelectrophoretic experiments using soluble extracts of wool which were reduced and alkylated with iodoacetic acid.

It was shown that the *S*-carboxymethyl group is a major antigenic site on these proteins and that precipitation of proteins containing a large percentage of this group could be prevented by the addition of thioglycollate to the gels. The results also show that the heterogeneity of a major low-sulphur protein may be due in part to the presence of contaminating high-sulphur proteins.

I. Introduction

The first objective in studies on the structure of wool proteins is the isolation and purification of a single component. Thompson and O'Donnell (1965) evolved methods for isolating single components from wool after reduction and alkylation of the solubilized proteins with iodoacetic acid. The whole mixture of alkylated proteins is termed SCMK (*S*-carboxymethylkerateine), and if the alkylation is done with acrylonitrile, the mixture is termed SCEK (*S*-cyanoethylkerateine). A component which gives a single band on starch-gel electrophoresis has been isolated from both derivatives (Thompson and O'Donnell 1964; Frater 1966). The protein (component 8), being of lower sulphur content than the original wool, is termed a low-sulphur protein and is one of the major constituents of soluble protein from wool. Both the SCM and SCE derivatives have similar amino acid compositions and have molecular weights of approximately 45,000 as determined by gel filtration on Sephadex G-200 in the presence of 8M urea.

Recent studies on component 8 from SCMK have shown that it is not a pure protein and may well consist of a family of closely related proteins (Thompson and O'Donnell 1967). To provide further demonstration of this heterogeneity, immunological studies of component 8 and other wool proteins were undertaken. The present work was initiated with the SCE derivative of component 8 as this is easier to obtain in a pure form.

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Previous workers have demonstrated that keratins and their derivatives can evoke immunological responses in rabbits (Pillemer, Ecker, and Wells 1939; Yenson and Arat 1961), but these studies were limited to observations of precipitin reactions in test tubes.

II. Materials and Methods

The preparation of SCMK and component 8 from SCMK was as described previously (Thompson and O'Donnell 1965), and the SCE derivative was prepared as described by Frater (1966). The wool used in both cases was from a single Merino fleece and was cleaned in the usual manner (Thompson and O'Donnell 1965).

For the production of antisera, the proteins were dissolved in water and blended with an equal volume of Freund's complete adjuvant (obtained from Commonwealth Serum Laboratories, Parkville, Vic.) so as to form a thick emulsion, and then injected intramuscularly into rabbits. After several injections, spaced a few weeks apart, the rabbits were bled and the serum collected.

Agarose was a sample from Seravac, and a trace of thymol was added to its solutions as a preservative. Acrylamide gels were prepared from acrylamide and N,N-methylene-bis-acrylamide (Eastman Kodak). Antibody–antigen reactions were demonstrated by the double diffusion technique of Elek (1948) in $0 \cdot 5\%$ agarose gels containing $0 \cdot 9\%$ NaCl and $0 \cdot 025$M Tris buffer (pH $7 \cdot 4$).

In the immunoelectrophoresis experiments, a sample of SCMK was separated by acrylamide-gel electrophoresis in 8M urea buffer. The gel contained $7 \cdot 5\%$ polyacrylamide and the other conditions were as described for use with starch-gel electrophoresis (Thompson and O'Donnell 1965). After electrophoresis for 6 hr, a thin slice of the gel (2 mm thick and 1 cm wide) was washed in running water for 30 min to remove urea and then embedded in $0 \cdot 5\%$ agarose solution containing $0 \cdot 9\%$ NaCl and $0 \cdot 025$M Tris buffer (pH $7 \cdot 4$). After the gel had set, troughs were cut parallel to and 5 mm away from the acrylamide slice. Antiserum to SCMK was poured into the troughs and precipitin lines allowed to develop over several days. Another slice of the acrylamide gel was stained with $0 \cdot 5\%$ amido black in 25% acetic acid. After development of precipitin lines for several days in a humid atmosphere, the gels were photographed using dark-field illumination.

III. Results

(a) Immunodiffusion Experiments with Component 8

Component 8 was isolated from Merino wool as the S-cyanoethyl derivative. A solution of this protein in emulsified Freund's complete adjuvant (1 mg/ml final concn.) was injected into a series of rabbits (1 ml every 7 days for 3 weeks), and after several weeks the rabbits were bled and the serum collected.

The double diffusion technique was used to demonstrate the presence of antibodies. The results of such an experiment are seen in Figure 1. Antigen solution (i.e. component 8, SCEK derivative) was placed in the outside wells at the concentrations indicated, and the centre well contained the antiserum (Ab). It can be seen that there are at least three major components in the derivative, and in addition, the precipitin bands are diffuse, indicating that there may be considerable heterogeneity within each component (Lapresle 1959).

The same results were obtained when component 8, isolated from the wool root of the same animal, was used in the antigen wells in this experiment. In control experiments using normal rabbit serum in the centre well, no precipitation was observed.

(b) *Immunoelectrophoretic Experiments*

Even though component 8 as used in these experiments travels as a single band on starch-gel electrophoresis, there is always the possibility that minor or non-staining proteins are present in the preparation. To overcome this problem, the technique of immunoelectrophoresis was used, and to make a more complete study of wool proteins, a total extract of wool was used. Because of the very limited solubility of SCEK derivatives (particularly in the presence of high-sulphur protein), this study was made using the SCMK derivative. A solution of the SCMK in emulsified Freund's complete adjuvant (1 mg/ml final concn.) was injected into rabbits and the antiserum obtained as described before.
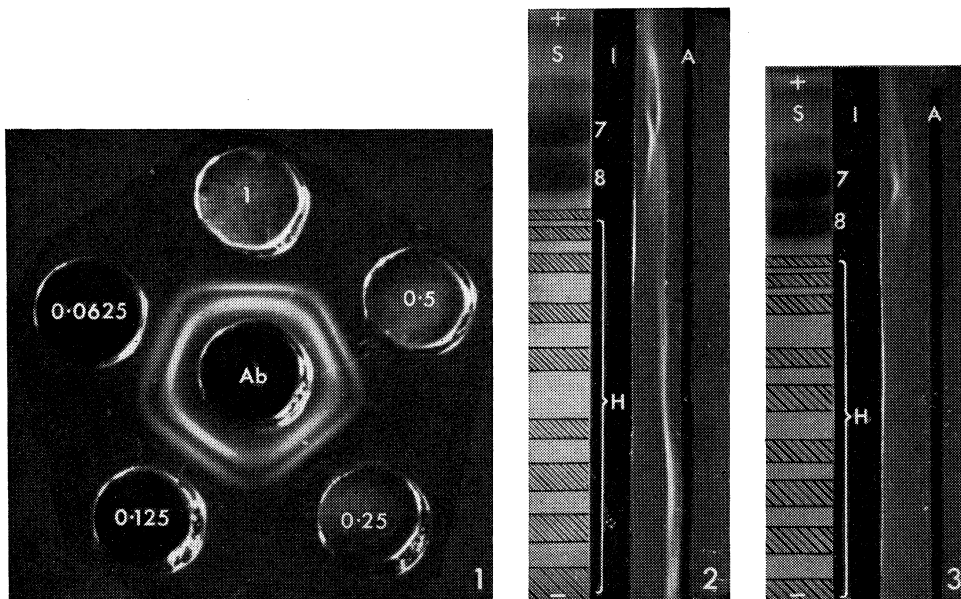

Fig. 1.—Double diffusion gel pattern of component 8 (SCE derivative). The component was placed in the outside wells at the concentrations indicated (all expressed as mg/ml), and the centre well contained antiserum (*Ab*) to the component.

Fig. 2.—Immunoelectrophoresis experiment with SCMK. The numbers 7 and 8 show the positions of these components on the gel strip (*I*). This is a composite picture, and the photograph of the stained acrylamide gel strip (*S*) has been added only to indicate the position of the various components as they occur on the unstained gel used in the diffusion part of the experiment. Because the high-sulphur proteins (*H*) do not do not stain well enough on acrylamide gels for photographic reproduction, the location of the protein bands has been indicated by light hatching of the stained areas. *A*, antiserum trough.

Fig. 3.—Immunoelectrophoresis experiment with SCMK in the presence of 0·02M sodium thio-
glycollate. Other details are as described for Figure 2.

Figure 2 shows the results obtained by the immunoelectrophoretic experiment. The heterogeneity of component 8 is still apparent, and in addition, component 7 also shares some of the antigenicity of component 8. The high-sulphur proteins have prominent overlapping antigenic similarities, as signified by the almost con-

tinuous bands of precipitate formed, even though individual bands can be seen on the stained strip after electrophoresis.

An explanation of this may be that the antigenic sites on these proteins were predominantly due to the SCM group, thus, particularly with the high-sulphur proteins, swamping out relatively minor antigenic differences. In support of this conclusion, it was found that unrelated SCM proteins (e.g. SCM pepsin, SCM albumin, SCM ovalbumin), also gave precipitin lines with antiserum to SCMK in the double diffusion test. Pillemer, Ecker, and Martiensen (1939) have reported that thio-glycollic acid acts as a specific inhibitor in the reaction of SCM kerateines with their appropriate antisera. When 0·02M sodium thioglycollate at pH 7·4 was added to the gel in double diffusion tests, the reaction with non-related proteins was eliminated, but a positive reaction (although less intense) was still obtained with SCMK. Assuming then that thioglycollate is acting as an inhibitor of the interaction due to SCM groups, the immunoelectrophoretic experiments were repeated in the presence of this inhibitor.

(c) *Immunoelectrophoretic Experiments in the Presence of Thioglycollate*

SCMK was separated by acrylamide electrophoresis as described previously, and in this case a gel strip was washed in water containing 0·02M sodium thio-glycollate (pH 7·4). The inhibitor was also incorporated into the agarose gel at the same concentration, and the troughs filled with antiserum to SCMK. The results of the experiments are seen in Figure 3. In this case it can be seen that precipitin lines are still formed to the low-sulphur proteins (components 7 and 8) although the number of bands has been lowered. Precipitation against the high-sulphur proteins has been completely inhibited.

The above immunoelectrophoretic experiments were initially attempted with starch gel as in the method reported by Poulik (1959). However, it was found that non-specific precipitates to some component in the gel were formed; both to normal rabbit serum, and to the rabbit antiserum. Thus, in experiments using starch-gel electrophoresis the same results were obtained except that a broad diffuse band due to non-specific precipitation was present along the whole length of the gel.

IV. DISCUSSION

The above results demonstrate that component 8 (either the SCE or SCM derivative) is indeed heterogeneous and that overlapping similarities are apparent between components 7 and 8. It may also be noted that component 8 (SCEK) from the wool root is antigenically identical with component 8 from the wool as far as can be determined in the experiments described.

The results obtained with the use of a specific inhibitor (thioglycollate) support the conclusions advanced by Pillemer, Ecker, and Martiensen (1939) that the SCM group is a major antigenic site on the SCMK protein. The disappearance of several of the precipitin lines against component 8 in the presence of thioglycollic acid (Fig. 3) indicates that this derivative may contain several tightly bound high-sulphur proteins which are not removed in the preparative procedure. It is apparent that

the high-sulphur proteins are very weak in their ability to form precipitates against specific antibodies (if indeed these are present in the antisera) or that their natural antigenic sites are swamped by the presence of a large excess of SCM groups.

Ideally, it would be preferable to repeat the above experiments with a non-substituted kerateine (i.e. in the –SH form), and perhaps this would enable a more precise immunological differentiation to be made among the high-sulphur proteins. However, the problems of maintaining –SH proteins in the reduced state during the multiple manipulations involved in the above immunological experiments make this difficult.

V. ACKNOWLEDGMENTS

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