HETEROGENEITY IN A HIGH-SULPHUR PROTEIN FROM WOOL*

By R. Frater†

Introduction

In a previous paper (Frater 1968) a study was made of the immunological properties of a protein mixture isolated from reduced and carboxymethylated wool (SCMK). In addition, the heterogeneity of a low-sulphur component isolated from SCMK was demonstrated.

Recently, Moschetto and Lesage (1967) have stated that the high-sulphur proteins derived from keratins give very weak immunological responses when injected into rabbits, and further that any positive reaction may be due to the presence of contaminating low-sulphur proteins.

In the present paper, experiments to illustrate the immunological behaviour of a purified high-sulphur protein will be described, and it will be shown that in fact the immunological response to a typical high-sulphur protein is approximately equal to that shown by SCMK itself.

Materials and Methods

The high-sulphur protein used in these experiments, SCMKB2, was prepared as described by Gillespie (1963). Amino acid analysis, performed by the method of Crestfield, Moore, and Stein (1963), showed that the protein was free of contaminating low-sulphur proteins, as these contain substantial amounts of lysine and histidine, whereas SCMKB2 contained neither of these amino acids. Methionine was also absent in SCMKB2, but present in the low-sulphur proteins.

Comparisons of the amino acid composition of SCMKB2 and SCMKA are seen in Table 1.

S-Carboxymethylkerateine (SCMK) was prepared as described by Thompson and O'Donnell (1965).

For production of antiserum to SCMKB2, the protein was dissolved in water and emulsified with an equal volume of Freund's complete adjuvant (from the Commonwealth Serum Laboratories, Melbourne). A sample of the emulsion (1 ml) containing 1 mg of the protein was injected intramuscularly into the hind leg of a rabbit. The injection was repeated twice at intervals of 2 weeks, and, following a delay of 3 weeks after the last injection, blood was collected from an ear vein and the serum prepared.

Samples of antisera to SCMK were prepared in a similar way.

Antigen–antibody reactions were demonstrated by double diffusion in gels containing 0·5% Agarose, 0·9% NaCl, and 0·02M Tris (pH 7·7).

Starch-gel electrophoresis of SCMKB2 in 8M urea was carried out for 6 hr as described by Thompson and O'Donnell (1964), the only modification being that the gel contained 30% starch instead of the usual 22%. With this higher concentration, the proteins from SCMKB2 were more retarded on the gel and consequently better separated.

After electrophoresis, a slice of the gel was stained in a solution containing 10% acetic acid, 5% trichloroacetic acid, and 0·25% each of nigrosin and amido black, and destained in 10% acetic acid.

* Manuscript received November 18, 1968.

† Division of Protein Chemistry, CSIRO Wool Research Laboratories, Parkville, Vic. 3052.

For immunoelectrophoresis, a slice of the gel was washed in running water for 90 min and then embedded in an Agarose gel contained within a Petri dish. A trough was cut parallel to and 4 mm away from the starch gel and filled with antiserum to SCMKB2 containing 0·005% bacterial amylase (Mycoform α-amylase). The addition of amylase prevents the non-specific precipitation previously encountered in the use of starch gels (Frater 1968).

TABLE 1
AMINO ACID COMPOSITION OF SCMKA AND SCMKB2
Values expressed as percentage of total nitrogen

Amino Acid	SCMKA*	SCMKB2†	Amino Acid	SCMKA*	SCMKB2†
Lysine	5.44	0	Proline	2·85	10·13
Histidine	1·33	0	Glycine	5·91	7·44
Arginine	19·53	14·35	Alanine	4·32	3·05
S-Carboxymethyl-cysteine	4·55	19·86	Valine	3·98	3·60
			Isoleucine	2·46	3·95
Aspartic acid	5·46	0·94	Leucine	6·89	1·75
Threonine	2·95	9·42	Tyrosine	2·88	2·20
Serine	4·90	12·15	Phenylalanine	2·02	0·85
Glutamic acid	9·49	10·31			

* From Thompson and O'Donnell (1962).
† From Lindley, Gillespie, and Haylett (1968).

Results

(i) *Immunoelectrophoresis of SCMKB2.*—The results of this experiment are seen in Figure 1. It is apparent that the SCMKB2 preparation is not homogeneous, there being at least two major components travelling near the front, and several minor components spread out behind these. Precipitin lines are well developed to all of these components and the continuous nature of the lines indicate that there is present a whole range of antigenically closely related high-sulphur proteins spread out over the whole length of the starch gel.

(ii) *Comparison of Precipitin Lines to SCMK and SCMKB2.*—In this experiment, the object was to compare the precipitin line densities of antigen–antibody complexes for SCMK and SCMKB2 proteins. The results of a double-diffusion experiment are shown in Figure 2 and demonstrate that SCMKB2 [Fig. 2(*b*)] is as efficient antigenically as SCMK [Fig. 2(*a*)], which contains both high- and low-sulphur proteins. During the first 3 hr of precipitin line development, two zones developed (Fig. 3) and later merged together. This provides further evidence for the heterogeneity of the protein.

Discussion

Two facts emerge from these experiments; firstly, that the high-sulphur protein SCMKB2 is not homogeneous (similar heterogeneity was shown for several different samples of the protein) and secondly, that the high-sulphur proteins as typified by SCMKB2 are similar to other wool proteins in their power to elicit immunological response in rabbits.

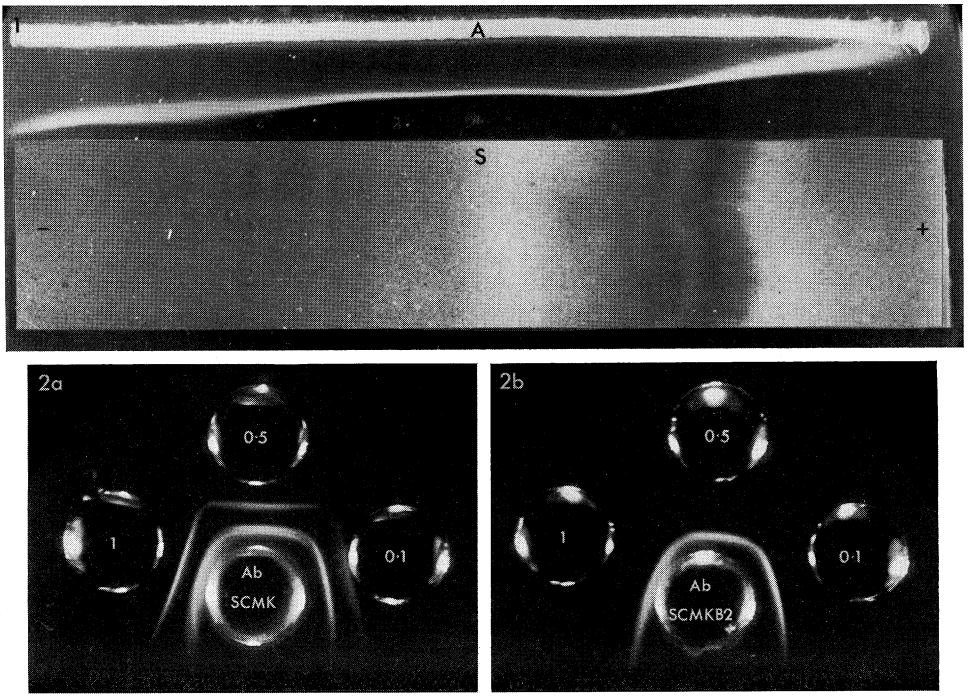

Fig. 1.—Immunoelectrophoresis of SCMKB2. After starch-gel electrophoresis in 8M urea of SCMKB2, a washed slice from the gel was embedded in 0·5% Agarose gel and immunodiffusion allowed to proceed for 24 hr at 25°C. *A*, Antiserum trough. *S*, Stained starch gel strip.

Fig. 2.—Immunodiffusion of SCMK (*a*) and SCMKB2 (*b*) in 0·5% Agarose gels. Protein concentrations (mg/ml) are indicated on the figures.

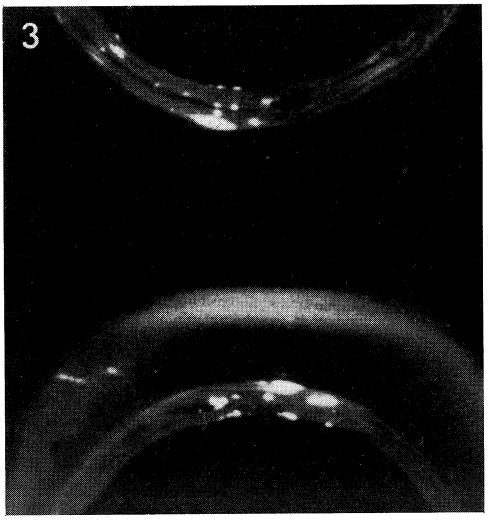

Fig. 3.—Immunodiffusion pattern of SCMKB2 3 hr after start of development. The concentration of SCMKB2 was 1 mg/ml.

The results of Moschetto and Lesage (1967), showing that precipitation of their antiserum by SCMKB could be eliminated by pretreatment with SCMKA is doubtless due to the fact that SCMKB and SCMKA will cross-react with antiserum to SCMK owing to the presence of common antigenic sites on both types of protein—i.e. the *S*-carboxymethyl group (Frater 1968).

It has also been demonstrated (Frater 1968) that the low-sulphur proteins are still capable of reacting with antiserum to SCMK in the presence of the inhibitor potassium thioglycollate, whereas reaction with the high-sulphur protein is eliminated.

Thus if SCMKB were added to antiserum against SCMK, precipitation could still be obtained on the addition of SCMK or SCMKA due to the presence of low-sulphur protein in these preparations.

In summary, inhibition of SCMKA will completely precipitate antibodies to SCMKB, but relatively incomplete inhibition of precipitation occurs when SCMKB is added to antiserum against SCMK.

Acknowledgments

I wish to thank Mr. R. E. Guthrie, Division of Protein Chemistry, CSIRO, for valuable technical assistance and Mr. D. W. T. Piercy, Division of Animal Health, CSIRO, for the preparation of antisera.

References

Crestfield, A. M., Moore, S., and Stein, W. H. (1963).—*J. biol. Chem.* **238**, 622.
Frater, R. (1968).—*Aust. J. biol. Sci.* **21**, 815.
Gillespie, J. M. (1963).—*Aust. J. biol. Sci.* **16**, 241.
Lindley, H., Gillespie, J. M., and Haylett, T. (1968).—Proc. Int. Symp. Fibrous Proteins, Canberra, 1967. p. 353.
Moschetto, Y., and Lesage, R. (1967).—*C.r. hebd. Séanc. Acad. Sci., Paris* **265**, 1004.
Thompson, E. O. P., and O'Donnell, I. J. (1962).—*Aust. J. biol. Sci.* **15**, 757.
Thompson, E. O. P., and O'Donnell, I. J. (1964).—*Aust. J. biol. Sci.* **17**, 277.
Thompson, E. O. P., and O'Donnell, I. J. (1965).—*Aust. J. biol. Sci.* **18**, 1207.