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IMMUNOLOGIC PROPERTIES OF THE α-FIBROUS PROTEIN OF HUMAN EPIDERMIS

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Abstract. Rabbits could be immunized to the S-carboxymethyl derivative of the α-fibrous protein of human epidermis, but not to the unmodified protein. It was shown that antibodies were formed not only to the S-carboxymethyl groups, but to other sites as well. The data indicated that the α-fibrous protein was heterogeneous rather than homogeneous as earlier chemical studies suggested. Several possibilities are offered to explain these results. The immunologic techniques described offer an additional approach to studying the structural proteins of epidermis.

The structural proteins of epidermis differ from those of hair in a variety of ways, but particularly with regard to their cystine content (2, 5). It has not been possible to identify separate filamentous and matrix proteins in epidermis (2), although electron microscopic studies have suggested they are present (4). The structural proteins that can be isolated from epidermis by reducing agents appear to exist as large homogeneous aggregates which have been dissociated only by enzymatic digestion (1). Frater (6) recently described the use of immunologic techniques for investigating the structural proteins of wool and has indicated that heterogeneity exists in certain components that appeared to be homogeneous by chemical studies. The purpose of this report is to describe the immunologic behavior of the fibrous proteins isolated from human epidermis and to show that there is more molecular heterogeneity than has previously been recognized (2).

MATERIAL AND METHODS

Human epidermis obtained by stretching and scraping surgically excised skin was extracted with 20 volumes of 6 M urea in 0.1 M tris, pH 9.0, for two 24 hour periods at room temperature. The washed tissue was then extracted in the same buffer with 0.1 M mercaptoethanol for 24 hours at room temperature to obtain the α-fibrous protein (2). The S-carboxymethyl (SCM) and aminoethylated derivatives were prepared as previously described (2).

Antisera were prepared by dissolving or suspending the protein in 0.1 M tris, pH 8.0, blending with Freund's complete adjuvant (Difco) and then injecting rabbits intramuscularly. Booster intravenous injections of protein were given at 3 week intervals and the animals bled from the ear 7 days after each injection.

Antigen-antibody reactions were studied by three techniques. The double diffusion method was done in 0.5% agar with 0.1 M sodium chloride and 0.02 M tris, pH 7.4 (7). Immunoelectrophoresis was carried out as previously described but the buffer used contained 0.05 M tris, pH 7.5 (3). Microcomplement fixation was done by the method of Wasserman & Levine (9).

RESULTS

Antisera to α-fibrous protein

Since the α-fibrous protein is insoluble in neutral buffer, diffusion techniques could not be used for immunologic studies. This difficulty could be overcome by carrying out complement fixation with suspensions of protein. Although some complement was bound, the results were the same for immunized and control animals, thus indicating that there was no specific immunologic reactivity. The antisera were also tested against the SCM derivative of the protein by the double diffusion and complement fixation methods and no evidence of a specific reaction could be observed. The sera of 8 animals, given from 1 to 25 mg of antigen, were found to be unreactive by these tests.

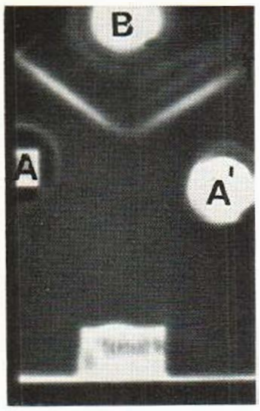

Fig. 1. Double diffusion gel pattern of the SCM α-fibrous protein. The antigen was placed in wells A^1 (1%) and A (0.25%) and the antibody in B ($^1/_2$ dilution). Bands 2 and 3 were visible in the wet plate at the lower concentration of antigen.

Antisera to the SCM α-fibrous protein

The SCM derivative of the α-fibrous protein, because of its solubility at neutral pH, can be studied by gel diffusion techniques. By using varying concentrations of antigen and antibody it was found that at least three bands could be observed by double diffusion (Fig. 1). The third band appeared at times to consist of two components. The same qualitative results were obtained with serum from different rabbits, but there was some variation in the intensity of the bands. No reactivity of the antibody with unmodified α-fibrous protein could be demonstrated when using complement fixation or the double diffusion method. The antibody was also tested with a number of soluble proteins and derivatives (Table I). No reaction was seen with bovine and human serum albumin, but the SCM derivatives gave a sharp single line. The antibody reacted with the SCM α-fibrous protein obtained from individuals whose epidermal tissue has not been used to immunize the rabbits. The aminoethylated derivative of the α-fibrous protein also showed a reaction to the antibody. The results of immunoelectrophoresis can be seen in Fig. 2. Three bands were seen with the SCM derivative and only two with the aminoethylated protein.

The effect of thioglycolate as described by Pillemer et al. (8) was studied by using agar containing 0.02 M sodium thioglycolate in addition to the usual buffer. No reaction was observed by the double diffusion method when the SCM serum albumins were used, but the SCM α-fibrous protein gave two or three lines. Band 1 was sharp, 2 less intense, and 3 either lost or barely discernible. Similar tests with the aminoethylated protein showed a strong and a weak band corresponding closely in position to bands 1 and 2 of the SCM α-fibrous protein.

DISCUSSION

Attempts to immunize rabbits with human epidermal α-protein were unsuccessful as no specific immunologic response could be detected by complement fixation. Furthermore, serum from the treated rabbits did not react with the SCM derivative when using double diffusion in agar. Since antibodies to some insoluble antigens can be prepared, the lack of responsiveness is not necessarily explained by the insolubility of the α-protein. This suggests that either the protein is not species-specific or it is not a good antigen.

The SCM derivative of the α-protein, however, proved to be an effective antigen, and three, or possibly four, bands could be detected by diffusion techniques. The antibodies formed were not entirely specific since they reacted with the SCM derivative of entirely different proteins. When the double diffusion experiments were carried out in the presence of thioglycolate the SCM albumins no longer reacted and one or more bands produced by the SCM α-fibrous protein appeared to be lost. This suggests that not all the antibodies produced against the SCM α-protein were directed

Table I. *Reactivity of various proteins and their derivatives in double diffusion studies to an antibody against the epidermal SCM α-fibrous protein*

Protein	Reactivity (no. of bands)
α-fibrous protein	0
Bovine serum albumin	0
Human serum albumin	0
SCM α-fibrous protein	3[b]
SCM α-fibrous protein[a]	3[b]
SCM bovine serum albumin	1
SCM human serum albumin	1
Aminoethylated α-fibrous protein	2[b]

[a] SCM α-fibrous protein from a different individual than the immunizing SCM α-fibrous protein.
[b] An additional band may be present.

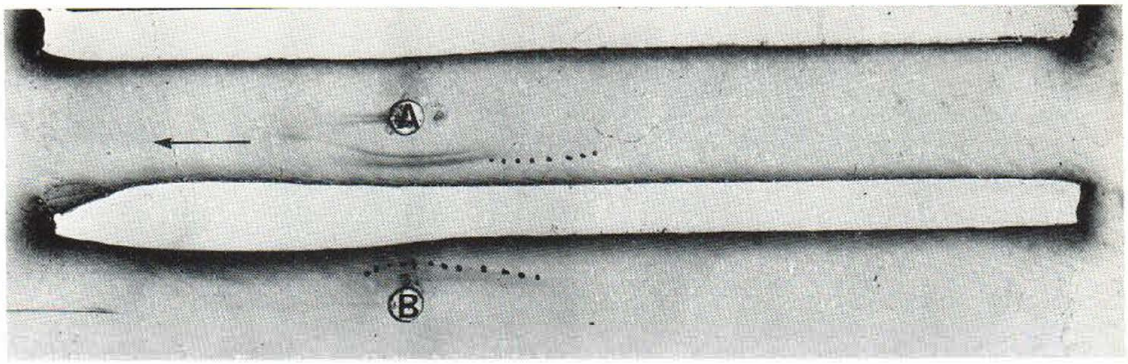

Fig. 2. Immunoelectrophoresis of derivatives of the α-fibrous protein. The SCM protein was placed in well A, and the aminoethylated protein in B. The dotted lines are bands which were clearly visible when the gel was wet, but were too faint to be seen on drying.

to the SCM group. The finding that the aminoethylated α-protein also reacted to the rabbit antibody confirms this hypothesis.

Frater (6) prepared an antibody to SCM wool protein and found many bands by immunodiffusion techniques. He showed that several but not all of the bands were lost when the experiments were conducted in the presence of thioglycolate. His data, coupled with our own, suggest that immunization with an SCM α-fibrous protein produces antibodies not only to the SCM group but to other antigenic sites which must be formed or exposed as a result of permanent rupture of cystine bonds. It is not clear, however, whether these sites are specific for the fibrous protein of keratin or might be found in a variety of unrelated proteins. If it can be shown that only SCM proteins derived from keratin can react with the antibody, these immunologic techniques may prove to be a valuable analytical tool for studying the structural proteins.

Our results show clearly that the α-fibrous protein extracted from human epidermis cannot be homogeneous as was suggested by previous chemical data. There are several possible explanations for the heterogeneity of the α-protein. The α-protein itself may be heterogeneous, as has been shown clearly in the case of wool and hair. The techniques which have been used for separation in the past may not have been suficiently sensitive to distinguish different components. Secondly, minor components which should have been removed by the urea extraction may not be released until the α-fibrous protein is solubilized. Thirdly, nonfilamentous components linked to the α-fibrous protein may be solubilized by the extraction procedure but not separable by the methods used for isolation. As indicated by Frater, immunologic techniques should prove helpful in studies of structural proteins of keratin.

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