

## Brief Note Water Soluble Protein Fraction of Stratum Corneum

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**Key words :** Stratum corneum — Water soluble protein fraction —  
Fibrous keratin

Stratum corneum which is the terminal differentiation of keratinocytes mainly consists of insoluble protein such as keratin fiber and membranous components. An extremely small amount of water soluble protein in the stratum corneum is known to exist,<sup>1-3)</sup> while the significance of its existence is not explained. Yoshiike *et al.*<sup>4)</sup> extracted normal water soluble protein to compare with that of patients with keratinization disorders. In this time, we studied the antigenicity of the water soluble protein fraction against antibody to fibrous keratin, and compared with that of patients with psoriasis vulgaris.

Stratum corneum were obtained from plantar skin of healthy persons and patients with psoriasis vulgaris by scraping. They were chopped to sizes smaller than 1.0 mm<sup>3</sup> to be placed in a buffer solution which contained 8 M urea and 0.05 M tris-HCl at pH 9.0 with 0.025 M 2-mercaptoethanol (2ME), and were stirred for 4 hours at 37°C. Cellular debris was removed by centrifugation at 30,000×g for 30 minutes, and then at 250,000×g for 2 hours. The supernatants were then dialyzed against 5 mM tris-HCl at pH 8.0 and 0.025 M 2ME for 48 hours at 4°C, and centrifuged at 250,000×g for 2 hours at 4°C. The supernatant resulting from centrifugation at 250,000×g was dialyzed against distilled water for 48 hours at 4°C and then centrifuged at 250,000×g for 2 hours. This procedure was performed twice on the resultant supernatant until the pellet was no longer visible. Then, afterward, the resultant supernatant lyophilized was designated the water soluble protein fraction. This water soluble protein fraction was analyzed by SDS-polyacrylamide gel electrophoresis as described by Laemmli<sup>5)</sup> using the 15% acrylamide gels. The antigenicity of the water soluble protein fraction against antibody to fibrous keratin of stratum corneum was examined by Ouchterlony gel diffusion technique. The extraction and purification of fibrous keratin was carried out by the method of Ogawa *et al.*,<sup>6)</sup> and the antibody was taken by the following method : One ml of purified fibrous keratin (5 mg/ml) in 8 M urea-0.05 M tris-HCl buffer, pH 7.0 containing 3 mM dithiothreitol (DTT) was mixed with an equal volume of Freund's incomplete adjuvant and injected into the skin of Japanese white rabbits. Three weeks later, a booster injection was administered subcutaneously into the neck of each animal, and 7 days after the injection, sera were obtained from the sensitized animals. Moreover, the fibrous keratin and water soluble protein fraction were subjected to electrophoresis and the proteins were transferred to nitrocellulose sheets. The blotting was performed in 25 mM tris-129 mM glycine/15% methanol/0.1% SDS at 4°C, a voltage of 10 V/cm was applied

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for 4 hours. Then, immunological detection against antibody to fibrous keratin on nitrocellulose was carried out by immunoperoxidase method. Normal water soluble protein fraction weighed  $0.062 \pm 0.01\%$  of whole stratum corneum (dry weight/fresh stratum corneum), and formed 11 bands from 14,000 to 69,000

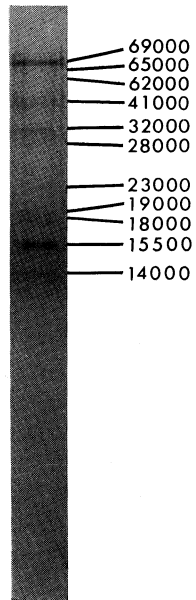


Fig. 1. SDS gel electrophoresis of water soluble protein fraction.

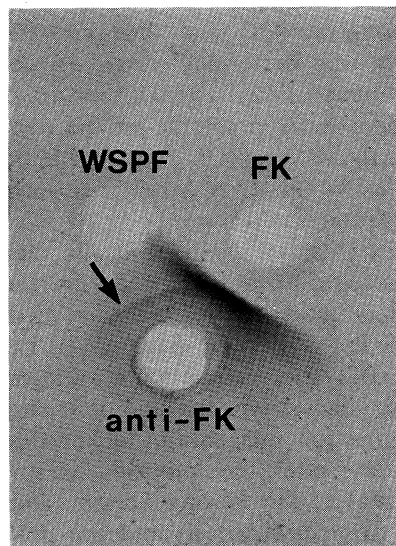


Fig. 2. Double diffusion of antibody to fibrous keratin and fibrous keratin and water soluble protein fraction.  
 WSPF : water soluble protein fraction.  
 FK : fibrous keratin.  
 anti-FK : antibody to fibrous keratin.

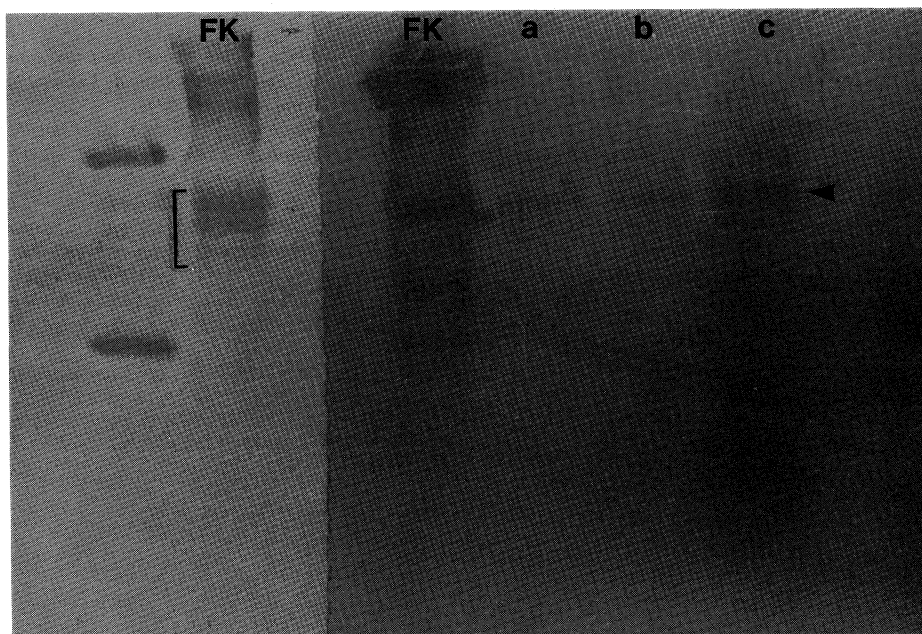


Fig. 3. Immunological detection of transferred protein against antibody to fibrous keratin by immuno-peroxidase method.  
 a, b : WSPF obtained from healthy persons.  
 c : WSPF obtained from the patients with psoriasis vulgaris.  
 left : gel stained with 0.1% amidoblack.

daltons on electrophoresis (Fig. 1). It had no difference in the content and electrophoretic pattern between those obtained from the both subjects mentioned above.

In a double diffusion system, the water soluble protein fraction showed the precipitin line against antibody to fibrous keratin of stratum corneum, and this precipitin line had something common with that caused by fibrous keratin (Fig. 2). The antigenicity against antibody to fibrous keratin was detected in every band of fibrous keratin (Fig. 3) and only in the 69,000 dalton subunit of water soluble protein fraction (Fig. 3). It is indicated that the 69,000 dalton subunit of fibrous keratin has higher solubility to water than other subunit does, and has a part of water soluble protein of stratum corneum. No difference even in these immunological characteristics was shown between those obtained from healthy persons and patients with psoriasis vulgaris.

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## REFERENCES

- 1) Matoltsy, A.G. and Matoltsy, M.N. : A study of the soluble proteins of normal and pathologic horny tissues by a modified disc electrophoresis technic. *J. Invest. Dermatol.* **41** : 255-257, 1963
- 2) Hook, B., Neufahrt, A. and Leonhardi, G. : Separation of water soluble proteins in psoriatic scales with different polyacrylamide gel concentrations and molecular weight estimations of the separated bands by disc-electrophoresis. *Arch. Dermatol. Forsch.* **250** : 245-252, 1974
- 3) Leonhardi, G., Ross, L. and Neufahrt, A. : Disc-electrophoretic separation of water soluble proteins from scales of different forms of ichthyosis. *Arch. Dermatol. Res.* **260** : 227-230, 1977
- 4) Yoshiike, T., Negi, M., Manabe, M., Hattori, M. and Ogawa, H. : Fractionation and characterization of the human epidermal stratum corneum in keratinization disorders. *J. Dermatol.* **9** : 243-251, 1982
- 5) Laemmli, J.K. : Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227** : 680-685, 1970
- 6) Ogawa, H. and Hattori, M. : Immunological characterization of fibrous protein isolated from human stratum corneum. *Arch. Dermatol. Res.* **268** : 71-78, 1980