Inhibition of Keratinases by α_2-Macroglobulin

We studied the effect of sera from patients with dermatophytosis, noninfected adults and newborn babies on the proteolytic activities of the keratinases isolated from *Trichophyton mentagrophytes*[1,2]. Sera from patients inhibited the activities of the enzymes; however, the same inhibitory effect was also obtained with sera from individuals without clinical signs of dermatophytic infection and with sera from newborn babies. Preliminary investigations using immunoelectrophoretic analyses indicated that the inhibitor resided in the α-globulin fraction of the human serum. The inhibitor was finally identified as α_2-macroglobulin and isolated.

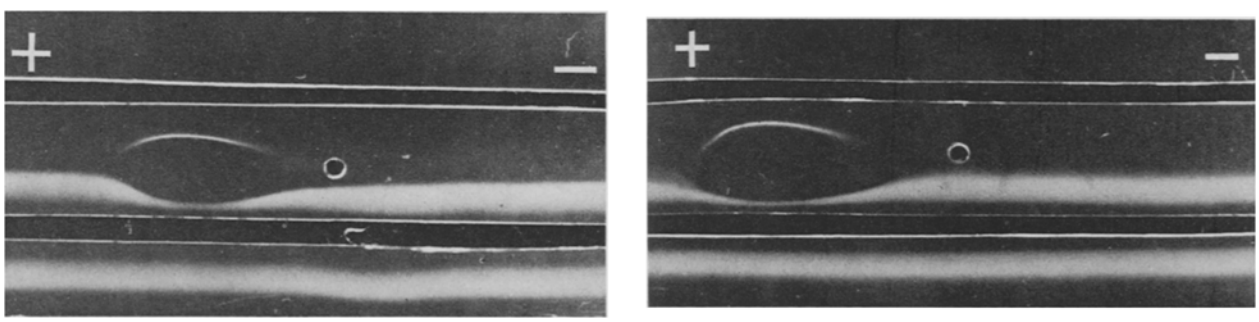
We isolated the α_2-macroglobulin from human newborn serum. Ammonium sulfate was added to 50 ml pooled baby serum to a 30% saturation and the precipitate was removed by centrifugation. The supernatant was then brought to a 50% saturation with ammonium sulfate, and after centrifugation, the precipitate was dissolved in 10 m*M* phosphate buffer, pH 7.6, dialyzed and lyophilized for storage. The lyophilized powder was dissolved in buffer (50 ml) and put on a Sephadex G-200 column (5.0 × 58 cm). The first distinct component (198 mg) eluted with buffer was lyophilized. This compound gave a single band on polyacrylamide disc electrophoresis using amido black as stain (Figure a) and on immunoelectrophoresis using rabbit anti-human serum. It was identified as α_2-macroglobulin using an immunoelectrophoretic modification of the casein precipitating test of FOSSUM[3] (Figure b).

For initial screening of sera for inhibitors, guinea-pig hair was used as the substrate for the 3 keratinolytic enzymes of *T. mentagrophytes*. It was then shown that inhibition also occurs with casein as substrate so that all further studies were carried out as follows.

A mixture of 0.1 ml enzyme and 0.1 ml α_2-macroglobulin solution and 0.8 ml 0.05 *M* phosphate buffer pH 7.5 was preincubated at room temperature for 10 min and the proteolytic activity assayed by the method of KUNITZ[4]. As control, the enzymatic hydrolysis of casein without α_2-macroglobulin was carried out by preincubation of 0.1 ml enzyme and 0.9 ml buffer at room temperature for 10 min then followed by the same procedure.

The inhibition of enzymatic activity by α_2-macroglobulin at various concentrations is shown in the Table. The degree of inhibition in percentage can be plotted arithmetically against the concentration of α_2-macroglobulin to determine the concentration giving 50% inhibitition. This concentration is doubled to estimate the amount needed for 100% inhibition. Based on molecular weights of keratinase I, 48,000; keratinase II, 440,000; keratinase III, 20,300; trypsin, 23,030 and α-chymotrypsin, 22,500, 1 mole of α_2-macroglobulin inhibits 0.11 mole of keratinase I, 0.11 mole of keratinase II, 1.39 moles of keratinase III, 0.70 mole of trypsin and 0.24 mole of α-chymotrypsin.

As shown in Figure b, this is the only component of newborn baby serum which inhibits the proteolytic activity of these enzymes. The biological significance of these findings is under investigation[5].



Immunoelectrophoretic casein precipitating test. a) Identification of inhibitor: Top trough: Rabbit anti-human-α_2-macroglobulin; well: newborn baby serum; bottom trough: Keratinase II, 100 µg/ml. b) Demonstration of purity of isolated inhibitor: Top through: rabbit anti-human serum; well: inhibitor (α_2-macroglobulin) from newborn baby serum, 10 mg/ml; bottom through: Keratinase II, 100 µg/ml.

Inhibition (%) of enzymatic activity by α_2-macroglobulin

Enzyme	α_2-macroglobulin (µg/ml) 72	217	542	900
Keratinase I (5 µg/ml)	5	17	78	86
Keratinase II (20 µg/ml)	12	60	83	86
Keratinase III (8 µg/ml)	22	78	82	87
Trypsin (5 µg/ml)	18	78	87	94
α-chymotrypsin (2.5 µg/ml)	29	54	69	76

Zusammenfassung. Sera von Patienten mit Dermatophytosis und von Gesunden (Säuglinge und Erwachsene) hemmten die Keratinasen von *Trichophyton mentagrophytes*. Der Inhibitor wurde von Neugeborenen-Serum isoliert und als α_2-Makroglobulin identifiziert.

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