Isolation and Purification of an Extracellular Keratinase of *Trichophyton mentagrophytes*

R. J. YU, S. R. HARMON, AND F. BLANK

The Skin and Cancer Hospital, Temple University Health Sciences Center, Philadelphia, Pennsylvania 19140

Received for publication 25 July 1968

The keratinolytic activities of dermatophytes which parasitize skin, nails, and hair have been described previously (1, 3, 6, 8); however, the isolation of a keratinase from these organisms has not yet been reported.

Trichophyton mentagrophytes var. *granulosum* (*T. granulosum* Sabouraud, 1909) was grown in a keratin medium composed of (per liter): horse hair, 2.5 g; glucose, 0.9 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.6 g; thiamine, 0.01 g; pyridoxine 0.01 g; and inositol, 0.05 g in 0.028 M phosphate buffer (pH 7.8). Media ingredients were sterilized at 121 C and 15 psi for 20 min. After inoculation, the cultures were allowed to stand for 5 days at 25 C and then were shaken for 7 days. At the end of the growth period, the mycelium and residual hair were removed from the culture fluid by filtration.

Keratinolytic activity was assayed by a modification of the method of J. J. Noval and W. J. Nickerson (4). The substrate was white guinea pig hair, Hartley strain, extracted with chloroform-methanol and air-dried. The unsterilized hair (1 to 3 mm long, 50 mg) was suspended in 0.028 M phosphate buffer containing 1 mM MgSO_4 (pH 8.0) to which 45 μg of enzyme material was added; the final volume was 5 ml. Each assay included the following controls: (i) enzyme material in buffer solution, and (ii) hair in buffer solution. The reaction vessels, all tubes in duplicate, were incubated at 37 C for 1 hr and then were immersed in ice water for 5 min. The remaining hair was removed by filtration. Cor-

rected absorbance values at 280 nm were converted to keratinase units (1 KU = 0.100 corrected absorbance), and the specific activity was expressed as KU per milligram of protein. Protein contents of solutions were determined spectrophotometrically from the absorbance at 280 and at 260 nm according to the method of Layne (2).

The purification of the keratinase is summarized in Table 1. The culture filtrate was passed through a diethylaminoethyl (DEAE) cellulose column (Whatman DE1) and the effluent, adjusted to pH 6.5, was transferred onto a carboxymethyl (CM) cellulose column (Whatman CM1). The crude enzyme was eluted from the latter column with 1 M NaCl in 0.028 M phosphate buffer and was dialyzed and lyophilized.

The elution diagram of the first gel filtration (Sephadex G-100) is shown in Fig. 1. Fraction II showed a specific activity of 30.1 KU/mg. A second gel filtration of fraction II showed a single, symmetrical peak but resulted in a considerable loss of activity.

Disc electrophoresis was carried out according to the method of L. Ornstein and B. J. Davis (5). The purified enzyme (after the first or second gel filtration) gave a single band on 7% gel run at pH 6.

The molecular weight of the keratinase estimated on Sephadex G-100 according to the method of Whitaker (7) was approximately 48,000.

TABLE 1. Purification of keratinase

Procedure	Vol (ml)	Total units	Specific activity ^a (KU/mg)	Yield (%)
Culture filtrate.....	27,000	7.30×10^3	1.35	100
DEAE-cellulose.....	27,000	8.14×10^3	4.02	111.5
CM-cellulose.....	1,740	2.88×10^3	6.03	39.5
Gel-filtration fraction II.....	94	1.31×10^3	30.1	17.9

^a The value for trypsin (two times crystallized, Mann Research Laboratories, Inc., New York, N.Y.) was 1.21 KU/mg under the same conditions.

FIG. 1. Gel filtration of crude, dialyzed enzyme (214 mg) on Sephadex G-100 column (2.5 by 90 cm) eluted with 0.007 M phosphate buffer, pH 7.6. Eluate was collected at 30 ml/hr, 10 ml per fraction. Symbols: ○—○, OD at 280 nm, ○---○ keratinase activity (KU/ml).

Hydrolysis of guinea pig hair by the keratinase was also demonstrated by examination of the hydrolysis products on thin-layer chromatography (solvent system: *n*-butyl alcohol-acetic acid-water, 3:1:1). Significant amounts of ninhydrin-positive compounds were observed after incubation for 30 min. Under similar conditions, trypsinization of guinea pig hair did not reveal any products of hydrolysis which could be detected with ninhydrin.

This investigation was supported by a grant from the John A. Hartford Foundation, Inc., New York, N.Y., and by Public Health Research grant 5 RO1 GM10212 from the National Institute of General Medical Sciences, and is based upon earlier studies by William C. Day.

LITERATURE CITED

1. Daniels, G. 1953. The digestion of human hair keratin by *Microsporium canis* Bodin. *J. Gen. Microbiol.* **8**:289-294.
2. Layne, E. 1957. Spectrophotometric and turbidimetric methods for measuring proteins, p. 447-454. In S. P. Colowick and N. O. Kaplan (ed.), *Methods in enzymology*, vol. 3. Academic Press, Inc., New York.
3. Mathison, G. E. 1964. The microbiological decomposition of keratin. *Ann. Soc. Belge Med. Trop.* **44**:767-792.
4. Noval, J. J., and W. J. Nickerson. 1959. Decomposition of native keratin by *Streptomyces fradiae*. *J. Bacteriol.* **77**:251-263.
5. Ornstein, L. 1964. Disc electrophoresis. I. Background and theory. *Ann. N. Y. Acad. Sci.* **121**:321-349.
6. Davis, B. J. 1964. Disc electrophoresis. II. Method and application to human serum proteins. *Ann. N. Y. Acad. Sci.* **121**:404-427.
7. Weary, P. E., and C. M. Canby. 1967. Keratinolytic activity of *Trichophyton schoenleinii*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes*. *J. Invest. Dermatol.* **48**:240-248.
8. Whitaker, J. R. 1963. Determination of molecular weights of proteins by gel filtration on Sephadex. *Anal. Chem.* **35**:1950-1953.
9. Ziegler, H. 1967. Vergleichende Untersuchungen über den Stoffwechsel von Schimmelpilzen und Dermatophyten. *Z. Allgem. Mikrobiol.* **7**:53-75.