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# Isolation of Human Urinary Lysozyme*

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In 1966 Osserman and Lawlor reported that patients suffering from monocytic and monomyelocytic leukemia excreted a quantity of a basic protein which had enzymatic, immunological and physicochemical properties identical with those of human lysozyme. The identity of this urinary basic protein with lysozyme was then confirmed by comparison of the primary structures of these two proteins. Therefore the urine of leukemia patients excreting lysozyme is a good source for the preparation of human lysozyme, since the content in leucocytes, tissues, and secretions which normally contain this protein is relatively low. In fact, several methods have been devised for the isolation of urinary lysozyme; these include preferential adsorption on bentonite, CM-Sephadex C-50, and Bio Rex 70, gel-filtration on Sephadex G-25, and affinity chromatography. However the yield (~30%) was given only by Johansson and Malmquist for their preparation using the adsorption of lysozyme on CM-Sephadex C-50, though quantitative isolation was possible by means of a one-step adsorption of the protein on a special adsorbent utilizing lysozyme lysates of the cell walls of *micrococcus lysodeikticus*.

In this communication, we describe a new method for single-step separation of lysozyme by gel-filtration on Sephadex G-50 from the urine of leukemia patients.

Isolation of lysozyme in Urine——Frosen, pooled urine (20 liters) of leukemia patients was thawed at room temperature and the insoluble materials were removed through filter paper under good ventilation. Ammonium sulfate (7.9 Kg) was added slowly to the clear filtrate with stirring and the resulting suspension was stirred for 4—5 h below 10°C. The proteins salted out were then collected at 4°C by centrifugation (4,000 rpm for 30 min.).

All the procedures described below were carried out in a cold room at 4°C. The wet protein precipitates were suspended in 1% acetic acid containing 1% NaCl (300 ml), then the suspension was stirred overnight and centrifuged (8,000 rpm for 30 min). The pellets collected were again treated with acetic acid-NaCl (400 ml) under the same conditions. The collected supernatant containing acid-soluble proteins was divided into several parts and chromatographed on a Sephadex G-50 column (4.6×130 cm or 5.0×100 cm) equilibrated with 1% acetic acid containing 1% NaCl. A protein fraction with lysozyme activity was collected and rechromatographed on Sephadex G-50 under the same conditions directly, or after lyophilization subsequent to desalting on Sephadex G-10 with 1% acetic

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acid. The materials appearing in the lysozyme fraction were collected, desalted, and lyophilized. The lyophilized lysozyme sample weighted 4.35 g (73%).

Chromatography of crude lysozyme obtained above was carried out on a CM-Sepharose CL-6B column (1.8×140 cm) using 0.05 M acetate buffer (pH 5.25 or 5.35) with a linear concentration gradient of NaCl from 0.1 to 0.5 M. The materials in the main fraction with the same specific activity as human milk lysozyme were collected, desalted on Sephadex G-10 using 1% acetic acid and lyophilized, Since the recovery of lysozyme was 84% for this ion-exchange chromatography, the amount of lysozyme isolated in a pure state eventually corresponded to approximately 60% of the enzyme in the urine.

The present method was applicable to various volumes of urine (30 ml to 20 liters) and the isolation yield of pure lysozyme was at least 60%. The whole process usually required seven to ten days, and did not call for any special adsorbents for lysozyme.