QUANTITATIVE DETERMINATION OF LYSOZYME IN BLOOD SERUM

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A method of quantitative determination of lysozyme in human and rat blood serum, based on the ability of lysozyme to undergo selective adsorption on chitin with the formation of a reversible enzyme—substrate complex, is suggested. The method is simple in its use and does not require expensive equipment, so that it can be used in clinical practice. Lysozyme was determined by this method quantitatively in human and male rat blood serum, and the results were several times greater than the values of its lytic activity. This shows that the suggested method can be used to obtain additional characteristics of lysozyme besides its lytic activity.

KEY WORDS: lysozyme; chitin; adsorption.

The principal methods used for the quantitative determination of lysozyme are based on its ability to produce lysis of living and killed cultures of *Micrococcus lysodeikticus* [1]. However, under these conditions only the lytic activity of this enzyme can be judged, and for that reason various workers have recently suggested quantitative methods of lysozyme determination in different sorts of biological material [4, 7, 8].

Continuing work in this direction, the present writers have developed a method of quantitative determination of lysozyme in blood serum which is based on its ability to be selectively adsorbed on chitin, with the formation of a reversible enzyme—substrate complex [5, 6].

Chitin (poly-B-1,4-N-acetylglucosamine) was obtained from crustacean shells by the method described in [2]: The shells were carefully washed with water, boiled in 5% NaOH solution for 6-8 h, washed to remove alkali, and dried at 120-140°C. They were then ground in a ball mill and screened into fractions. Chitin powder with a particle size of 50-100 mesh was used. The chitin powder was treated with 5% HCl solution for 30 min, washed with water, and deaminated: 10.3 g $NaNO_2$ was dissolved in 300 ml water and treated at 2-4°C with concentrated HCl to a final concentration of 0.15 M in the solution. Chitin (100 g) was added to the solution and stirred for 4-6 h at 2-4°C (until the liberation of nitrogen ceased completely). The resulting absorbent was washed with water, dried at 100°C, and equilibrated with 0.1 M sodium-phosphate buffer solution, pH 8.5. To achieve complete adsorption of the lysozyme on the chitin the contents of the tube were allowed to stand at room temperature for 30 min, with periodic shaking. After incubation, the mixture was centrifuged for 2-3 min at 3000 rpm, the supernatant was discarded, and the chitin residue was treated with 5 ml of a 0.1 M solution of sodium-phosphate buffer, pH 8.5.

Into the centrifugate containing 1 cm^3 of chitin (in a medium containing a 0.1 M solution of sodium-phosphate buffer) we introduced 1 ml of the investigated serum and 1 ml of 0.2 M sodium-phosphate buffer, pH 8.5.

The contents of the tube were stirred for 1-2 min, after which the buffer was removed by centrifugation. The operation was repeated five times. The chitin was washed with water in the same way to remove salt. As a result of the washings, pure lysozyme-chitin complex, reversible and readily breaking down in an acid medium, remained in the tube. Desorption of the lysozyme was thus carried out by adding 2 ml of 0.2 M acetic acid solution to the chitin and allowing the mixture to stand at room temperature for 30 min with periodic stirring. The contents of the tube were then centrifuged for 5 min at 3000 rpm and the pro-

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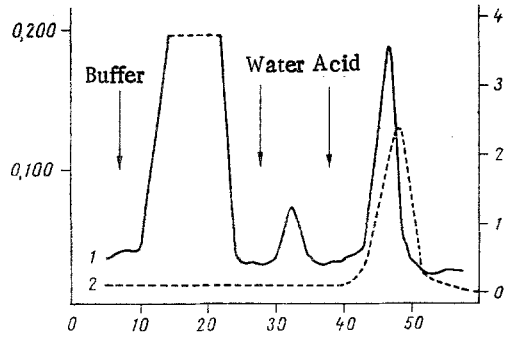

Fig. 1. Chromatography of blood serum on chitin. Abscissa, volume of eluate (in ml); ordinate: on left — optical density at 280 nm; on right — lysozyme activity (in μg/ml). 1) Optical density of test fractions. 2) Lysozyme activity of test fractions.

TABLE 1. Lysozyme Content in Normal Human and Male Rat Serum (M ± m)

Test material	No. of determinations	Lysozyme content, μg/ml	
		turbidimetric method	adsorption on chitin
Blood serum:			
human	30	2,39±0,08	26,68±0,78
rat	30	3,44±0,18	12,21±0,54

tein concentration in the supernatant was then determined by Lowry's method. Egg albumin lysozyme was used as the standard protein.

During isolation of the lysozyme all intermediate fractions were tested for optical density at 280 nm and for lysozyme activity by a turbidimetric method [3]. A chromatogram of human blood serum on chitin is shown in Fig. 1. Rinsing the chitin with buffer removed most of the accompanying serum constituents, and washing with water removed traces of protein and salt. During desorption with acetic acid solution a protein fraction with lysozyme activity was obtained.

Rinsing the chitin thus removed accompanying organic matter and salts, and all the lysozyme was desorbed with acetic acid solution.

Lysozyme was determined quantitatively in human and rat blood serum by the suggested method. The results are given in Table 1 together with values of lysozyme activity obtained by a turbidimetric method for comparison [3].

The results show that the lysozyme content in human serum, determined by the method of adsorption of chitin, is 11.2 times higher than the corresponding index obtained by the turbidimetric method. The ratio in rats was 3.5.

By the suggested method lysozyme can thus be determined quantitatively in blood serum. The absolute content of lysozyme is an additional and highly informative index, supplementary to determination of its lytic activity.

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