Isolation of Streptococcal Nuclease B by Batch Adsorption

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A method has been developed for the preparation of streptococcal nuclease B by batch adsorption to diethylaminoethyl-cellulose. The enzyme is homogeneous with respect to nuclease activity and is suitable for use as an antigen in measurement of anti-deoxyribonuclease B levels in sera.

Studies of group A streptococcal infections and their complications, rheumatic fever and and glomerulonephritis, are greatly facilitated by the availability of tests for the determination of antistreptococcal antibodies. The levels of antibodies against streptolysin O have been commonly measured although it has been shown that, in some streptococcal infections, especially pyoderma, this antigen may not induce significantly elevated levels of antibody (1-3, 5, 8). Streptococcal nuclease B is an extracellular product that is produced by a large number of strains and elicits antibodies that have proven to be a useful index of streptococcal infection (1-3, 5, 8). The measurement of anti-streptolysin O and antistreptococcal nuclease B (anti-deoxyribonuclease B) is much more effective in the establishment of a diagnosis of streptococcal infection (1, 2, 5). An obstacle to this strategy in the past has been the difficulty of preparing nuclease B antigen homogeneous with respect to nuclease activity. Starch gel electrophoresis and column chromatography on ion exchange cellulose have been useful and effective methods for the purification of nuclease B. A simplified method for separating the four streptococcal nucleases by polyacrylamide gel electrophoresis has recently been described (6). The present report details a further simplified procedure for the isolation of nuclease B by batch treatment with ion exchange cellulose, which should facilitate the ready availability of this antigen.

MATERIALS AND METHODS

Preparation of extracellular nucleases. Previously frozen 1-ml cultures of *Streptococcus pyogenes* strain C203S (originally furnished by Allan Bernheimer) were grown for 6 to 8 h in 5 ml of sheep blood Todd-Hewitt broth (Difco) at 37 C and then transferred to 500 ml of Todd-Hewitt broth and incubated overnight at 37 C. The stationary-phase culture was cooled in an ice bath (all subsequent operations were carried out at 4 C), and the cells were removed by centrifugation for 15 min at 10,000 $\times g$. The supernatant fluid was made 85% saturated

with $(\text{NH}_4)_2\text{SO}_4$ by the addition of solid $(\text{NH}_4)_2\text{SO}_4$ and was stirred for 1 h.

The precipitate that formed was collected by centrifugation for 10 min at 7,000 $\times g$. The portion of the insoluble material that floats on the surface of the ammonium sulfate solution was combined with that which sediments to the bottom of the centrifuge tube, and the clear fluid was discarded. The precipitate was dissolved in 25 to 40 ml of 0.001 M glycine, pH 9, and dialyzed for 3 h against 15 to 20 volumes of the same buffer with several changes. After dialysis, the crude preparation of extracellular products (routinely containing approximately 30 mg of protein per ml) was either immediately fractionated or frozen and stored at -20 C.

Preparation of diethylaminoethyl-cellulose. DE-23 microfibrous diethylaminoethyl-cellulose (Reeve-Angel) was stirred for 30 min in 0.5 N HCl, washed with water, and then stirred for 30 min in 0.5 N NaOH. After washing with water until neutral, the DE-23 was washed once with TMC buffer [0.001 M tris(hydroxymethyl)aminomethane-hydrochloride, pH 7, 0.001 M MgCl_2 , and 0.001 M CaCl_2] and equilibrated overnight in the same buffer. Before use, the buffer was removed by vacuum filtration, and the resultant moist powder was employed in the subsequent procedure.

Isolation of streptococcal nuclease B. To each milliliter of crude extracellular extract was added 1.5 to 2 g (wet weight) of DE-23 cellulose prepared as described and 5 ml of TMC buffer, and the suspension was stirred for 20 min. The adsorption and all of the following operations were carried out at 4 C. The cellulose was removed by low-speed centrifugation, and the supernatant was reserved. The cellulose was resuspended in the same volume of TMC, stirred for 20 min, and sedimented as before. The two combined supernatants represent a preparation of nuclease B free of the other streptococcal nucleases. The yield of nuclease B can be slightly increased by another buffer elution of the DE-23. A mixture of the other three streptococcal nucleases A, C, and D with a small amount of nuclease B can be eluted from DE-23 in the same manner using TMC containing 1 M NaCl. These may be separated by polyacrylamide gel electrophoresis (6).

The nuclease B pool was assayed by either the acid-soluble method (6) or by the deoxyribonucleic acid-methyl green procedure as described by Nelson et al. (7). The preparation may be stored frozen in

aliquots at -20°C or may be lyophilized. In this case, bovine serum albumin is added to a final concentration of 0.1% before freeze drying. The lyophilized enzyme is stable for prolonged periods (at least 1 year) at -20°C .

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis of crude and fractionated extracellular products was carried out as previously described (6).

Antibody neutralization. Antibody neutralization tests of purity of nuclease B preparation were performed by the microtiter method of Nelson et al. (7).

RESULTS

Polyacrylamide gel electrophoretic profiles before and after fractionation of a crude streptococcal extracellular preparation by the batch adsorption procedure are shown in Fig. 1. The streptococcal nucleases are well separated by polyacrylamide gel electrophoresis, and the preparation depicted shows a typical distribution of the four enzymes (Fig. 1, upper panel). Nuclease B represents approximately 60% of the total nuclease activity in the crude preparation. After treatment with DE-23 (Fig. 1, lower panel), nucleases A, C, and D have been completely removed, leaving only nuclease B. Nuclease B is composed of various amounts of at least three enzyme species (4), accounting for the complexity of the nuclease B peak in the crude extract. The electrophoretic profile of nuclease B in the fractionated extract indicates much less heterogeneity and suggests that

FIG. 1. Fractionation of streptococcal nucleases by batch (diethylaminoethyl) treatment. A crude extracellular extract was fractionated as described and subjected to polyacrylamide gel electrophoresis. The gels were sliced and the nucleases were eluted and assayed by the acid-soluble method (6) as previously noted. The upper panel represents the nuclease activity of the unfractionated extract, and the lower panel represents that of the fractionated nuclease B. DNase, Deoxyribonuclease.

some fractionation of the nuclease B species may have occurred. Nuclease B prepared by batch adsorption is neutralized by specific rabbit antisera to nuclease B and is unaffected by specific rabbit antisera to nucleases A, C, and D. The final preparation is, therefore, homogeneous with respect to nuclease activity by immunological, as well as biochemical, criteria. It contains medium components and other streptococcal products, but these do not interfere with its use in the determination of serum antinuclease B titer by specific enzyme neutralization.

The dependence of the fractionation procedure on the relative amounts of DE-23 for adsorption is shown in Fig. 2, which represents the proportion of nucleases A, C, and D present in the supernatant. Until the addition of approximately 1 g of DE-23 per ml of extract, there are still traces of the other unadsorbed nucleases. At higher amounts of ion exchange cellulose, however, only nuclease B is left in the supernatant fraction. This relationship emphasizes the necessity of using an excess of absorbant to assure the homogeneity of the preparation with regard to nuclease activity. For routine preparation of nuclease B, 1.5 to 2.0 g of DE-23 per ml of extract is utilized to ensure complete removal of the other nucleases and to allow for possible variation of protein concentration. This procedure does not affect the efficiency of recovery of nuclease B and eliminates the necessity for measurement of protein in crude extract before fractionation.

FIG. 2. Adsorption of nucleases A, C, and D by diethylaminoethyl (DEAE)-cellulose. Crude extract (30 mg of protein/ml) was fractionated using various amounts of DE-23. The supernatants from the fractionation were subjected to polyacrylamide gel electrophoresis, and the nucleases were eluted and measured as described in the legend to Fig. 1. The amounts of nucleases A, C, and D present in the supernatants are expressed as a proportion of the total nuclease activity.

DISCUSSION

The batch adsorption method is an efficient and effective procedure for the separation of nuclease B from the other streptococcal nucleases. It allows the ready preparation of antigen for anti-deoxyribonuclease B determination by any of several specific neutralization techniques (7). The product is homogeneous with regard to nuclease activity by immunological and biochemical criteria. The apparent fractionation by batch adsorption of the multiple species of nuclease B does not affect the titration of antinuclease B antibodies in serum, since all species of nuclease B are inhibited by these immunoglobulins.

The homogeneity of the nuclease B preparation should be verified by immunological neutralization with specific antisera or by gel electrophoretic analysis. If, however, the preparation is subjected to an additional cycle of adsorption with fresh ion exchange cellulose, there would be very little chance of contamination of nuclease B with any of the other nucleases.

The procedure should allow the wider utilization of a valuable diagnostic tool.

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