FURTHER PURIFICATION OF MICROCOCCAL NUCLEASE FROM THE STRAIN SA-B*[1]

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We have described (Ohsaka, et al., 1964) a method for the preparation of micrococcal nuclease with a specific activity in the range from 6 to 10. At this stage the enzyme is sufficiently free from contaminating phosphatases to be suitable for the analysis of the terminal groups in oligonucleotides bearing 5'-monophosphate. Recently, Dr. C. B. Anfinsen kindly sent us a sample of micrococcal nuclease prepared by a different method (Anfinsen, et al., 1963) from a different strain of Staphylococcus aureus. Measured under our conditions, Anfinsen's preparation had a specific activity of 65. Strain V-8 used by Anfinsen, et al. (1963) produces a crude enzyme many-fold richer in nuclease and alkaline phosphatase as well (Anfinsen, personal communication, 1964). As yet this crude material is not commercially available. We are now reporting that an equally potent preparation can be obtained (and preserved) from a commercially available source (using Dr. Catlin's strain

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1 The isolation and characteristics of strain SA-B have been described (Weckman and Catlin, 1957).

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SA-B), with an overall yield of about 1% (Fig. 3). An enzyme with a specific activity around 100 can be obtained with a yield of about 0.3%, but as yet cannot be preserved (Fig. 4).

The crude enzyme was purchased from the Worthington Biochemical Corporation. The first 5 steps were identical with those previously described (Ohsaka, et al., 1964). Step 6. The accumulated enzyme lyophilized after Step 5 was dissolved in a minimum amount of 0.01 M Tris-HCl, pH 9.0, and was dialyzed against this buffer for 6 hours. It was then passed through a 150 cm column of Sephadex G-75. Fig. 1 shows that a partial separation of the 2 active peaks was achieved. Each of the peaks was pooled separately and lyophilized as peak 6.1 and 6.2 respectively. Step 7. Two batches carried through Step 6 were combined, and peaks 6.1 and 6.2 were chromatographed separately on CM-cellulose, Fig. 2. It is obvious that peak 6.1 (bottom) contains 2 active components of which component A (7.1.A) predominates over B (7.1.B). Peak 6.2 (top) has the reverse composition, little A (7.2.A) and predominantly B (7.2.B). The last is the most active, with a specific activity reaching 58. Since the separation by gel filtration (Fig. 1) and by ion-exchange chromatography (Fig. 2) led to a resolution into essentially the same 2 components, the hypothesis that the strain SA-B produces 2 isonucleases was considered. This hypothesis, however, was contradicted by the next step.

Step 8. Each of the major peaks 7.1.A and 7.2.B was dialyzed, lyophilized, dissolved in a small amount of water and again dialyzed against water. It was then chromatographed on a DEAE-cellulose column, adjusted to 0.01 M Tris-HCl, pH 9.0, Fig. 3. Component 8.2.B.1 (top) appears with the front and shows the highest specific activity equal to Anfinsen's preparation. Tubes 14-17 after being pooled and lyophilized retained the same specific activity of 65. In the experiment 8A (Fig. 3, bottom) five different peaks are seen. This rules out the assumption of two isoenzymes. The most potent 8.A.1 appears with the front. The following four peaks show decreasing specific activities. It seems

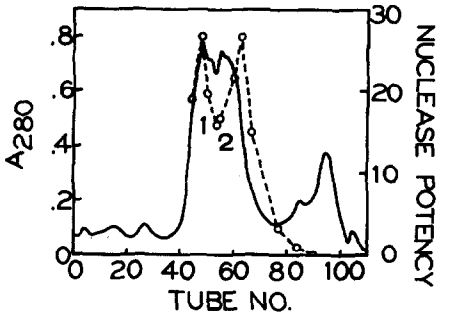
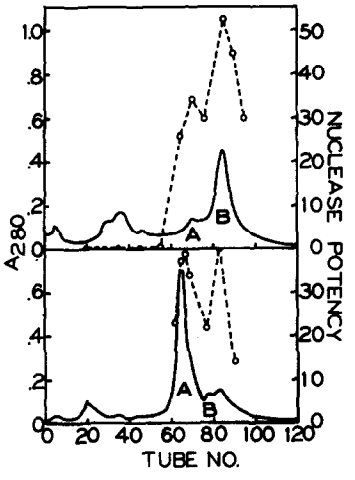


Figure 1. Step 6. Gel filtration on Sephadex G-75, coarse, column 1.9 x 150 cm, adjusted to 0.01 M Tris-HCl, pH 9.0. Total amount of protein charged was 100 A_{280} units. Elution with the original buffer. Flow rate 6 ml per tube, 5 tubes per hour. Solid line, absorbance at 280 mμ; dashed line, nuclease potency.



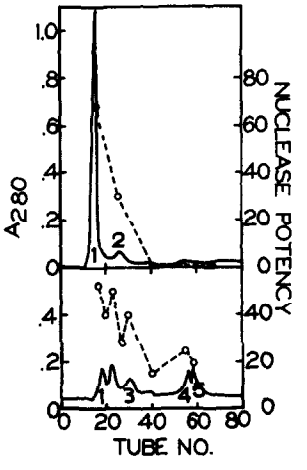


Figure 2. (left) Step 7. A composite of 2 chromatographic experiments. The same column of CM-cellulose in both. Elution with a linear gradient: 500 ml of 0.1 M and 500 ml of 1.0 M ammonium acetate, pH 6.0. Flow rate 6 ml per tube, 5 tubes per hour. Solid line, absorbance at 280 mμ; dashed line, nuclease potency. Expt. 6.1 (bottom, the first peak from the previous step) 74 A_{280} units were charged. Expt. 6.2 (top, the second peak from the previous step) 52 A_{280} units were charged.

Figure 3. (right) Step 8. A composite of 2 chromatographic experiments. The same column of DEAE-cellulose 1.9 x 50 cm in both. Elution with a linear gradient: 500 ml of 0.01 M and 500 ml of 0.1 M Tris-HCl, pH 9.0. Flow rate 6 ml per tube, 5 tubes per hour. Expt. 7.1.A (bottom, the first peak from the previous step) 20 A_{280} units were charged. Expt. 7.2.B (top, the second peak from the previous step) 28 A_{280} units were charged. Solid line, absorbance at 280 mμ; dashed line, nuclease potency.

more probable that strain SA-B produces only one nuclease which forms fairly stable complexes with a number of inactive proteins. Two of these complexes are predominant.

This conclusion is further supported by the next experiment. The crude enzyme (200 gm) was worked through Steps 1 and 2 (Ohsaka, et al., 1964), but Step 3 was modified. After precipitating the enzyme with 50% alcohol, the precipitate was thoroughly washed with cold 50% alcohol, suspended in 0.1 M Tris-HCl, pH 9.0 and centrifuged in the Spinco Model L centrifuge at 40,000 for 1 hour. The fatty layer on the top and the pellet were discarded. This resulted in a decreased yield but an increased potency (5 instead of the previous 1.5). Subsequent chromatography on DEAE-cellulose (Step 4) was also improved. It led to a double headed active peak, each part having a potency of about 35. The second part, after heating and lyophilization (Step 5) was chromatographed on CM-cellulose, Fig. 4. The pattern shows several poorly separated active

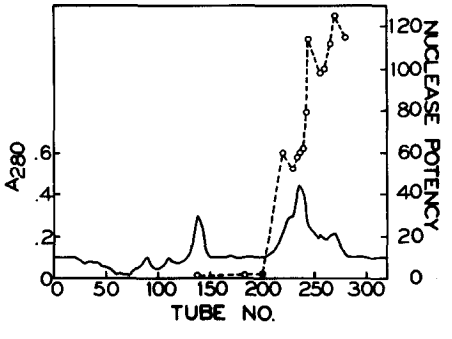


Figure 4. Chromatography on CM-cellulose. Column 2.7 x 95 cm. Load 15.9 A_{280} units of enzyme. Elution with a linear gradient: 750 ml of 0.1 M and 750 ml of 1.0 M ammonium acetate, pH 6.0. Flow rate 5 ml per tube, 15 tubes per hour.

peaks. The last peak (tubes 260-280) shows a potency of about 120. After pooling, the whole peak still showed a potency over 100. At this dilution the accuracy of determination is probably less than 20%. Di-

alysis and lyophilization of so dilute a solution resulted in a loss of 50% of activity.

The pure enzyme when obtained, should therefore have a specific activity of no less than 100. Our findings do not imply that the pure enzyme from strain V-8 should exhibit a similar potency.

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