Purification of splenic desoxyribonuclease II*

In conjunction with investigations carried out in this laboratory on the effect of X-irradiation on desoxyribonuclease II (DNase II) in urine[1], body fluids[2], and tissues[3] of rats, efforts have been made to obtain purified preparations of this enzyme. It has a pH optimum of 5.6 and has been separated from DNase I in human urine by paper electrophoresis[4]. A moderate degree of purification of DNase II from calf spleen has been achieved by other investigators[5].

In the present report a new method capable of achieving a 200-fold purification of DNase II will be described.

The initial steps in the purification of the enzyme were developed independently in this laboratory, but are similar in some respects to the method of McDONALD[6]. A water extract of fresh (or frozen) calf spleen was adjusted to pH 4.0 in the cold with 5 N H_2SO_4. The resulting precipitate was removed by centrifugation. The supernatant, adjusted to pH 7.0 with 5 N NH_4OH and warmed to 25° C, was made up to a 40% saturated solution by the addition of solid $(NH_4)_2SO_4$. The resultant precipitate was removed by centrifugation, and additional $(NH_4)_2SO_4$ was slowly added to the supernatant solution until the concentration was 80% of saturation. The dark red precipitate which appeared was dissolved in a small volume of water and dialyzed against 0.1M phosphate buffer, pH 6.6, at 2° C for 24 hours.

A column (1 × 6 cm) charged with IRC-50(XE-64) resin was equilibrated with 0.1M sodium phosphate buffer, pH 6.6, according to the method of BOARDMAN AND PARTRIDGE[7]. The buffered solution (5–10 ml) of enzyme (calculated on the basis of the conversion factor extrapolated by NEILANDS AND STUMPF[8]) containing 0.3–0.6 g of protein was placed on this column and the effluent solution collected for analysis. Elution was then started with 0.1M phosphate buffer, pH 6.6, and continued until most of the red pigment had been eluted from the column. The remaining protein was then eluted from the resin with 0.1M phosphate buffer, pH 8.0). Throughout the chromatographic procedure, fractions of 5–6 ml were collected with a mechanical fraction collector at a rate of 2–3 ml per hour. Each fraction was dialyzed against running tap water or, preferably, against 0.2M acetate buffer, pH 5.6, at 2° C, and analyzed for DNase II activity and total protein content. The protein content was determined by measuring the optical density of dialyzed effluent at 280 mμ. DNase II activity was assayed by the diphenylamine reaction, as modified by KOWLESSAR *et al.*[1,9], but with the omission of a determination of the "zero-time reading" or "tissue blank".

About 20% of the total DNase II activity initially applied to the column was eluted with 0.1M phosphate buffer, pH 6.6, together with a red pigment (presumably a derivative of hemoglobin) in the first 5–30 ml of the effluent. No purification of DNase II was achieved in this fraction. A second fraction containing DNase II activity and representing about 20% of the total activity initially placed on the column was collected after 70–90 ml of 0.1M phosphate buffer, pH 8.0, had passed through the column. The specific enzymic activity in this fraction was 50–200 times

TABLE I

DEGREE OF PURIFICATION AND YIELD OF DNASE II

Fraction	*Specific§ activity*	*Purification*	*% Yield*
Water extract	0.018	—	100
pH 4.0; Supernatant	0.064	3 ×	70
Ammonium sulfate-buffer dialysate	0.093	5 ×	51
0.1M Phosphate buffer, pH 6.6, eluates	0.09–0.10	5 ×	10
0.1M Phosphate buffer, pH 8.0, eluates	1.0–4.0	50–200 ×	10

§ Specific activity = $\frac{\text{optical density of diphenylamine color measured at 600 m}\mu}{\text{optical density of fraction at 280 m}\mu}$

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greater than that in the original aqueous splenic extract*. The results obtained with ion-exchange chromatography at 2° C were usually reproducible, but the purification was not as good under these conditions as it was in some experiments carried out at room temperature. Dialysis of the effluent solutions against 0.2*M* acetate buffer, pH 5.6, at 2° C, resulted in a better yield of enzymic activity than dialysis against running tap water at room temperature.

As is evident from Table I, the procedure yielded an enzyme preparation whose purity was up to 200 times greater than that of the original aqueous splenic extract.

Furthermore, the data shown in Fig. 1 suggest the existence of two separable DNase II activities, neither of which requires Mg^{++} for activation. These two fractions with DNase II activity might well represent distinct enzymic entities since rechromatography of the fraction emerging from the column in the initial 5–30 ml of effluent solution resulted in complete recovery of the DNase II activity in this same initial fraction. These findings suggest that the initial fraction of DNase II activity is not contaminated with the second fraction of DNase II activity eluted subsequently. Work is now in progress to establish whether or not these two chromatographically different fractions possessing DNase II activity are indeed two distinct enzymes.

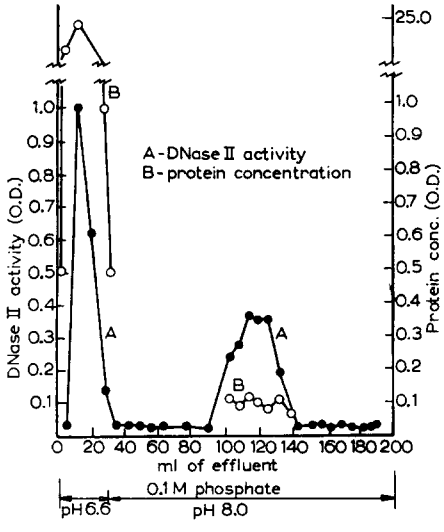

Fig. 1. Ion-exchange chromatography of buffered $(NH_4)_2SO_4$ fraction of calf spleen at room temperature. A = DNase II activity as optical density (O.D.) of diphenylamine color at 600 mμ. B = Protein concentration expressed as optical density reading at 280 mμ.

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* It should be pointed out that in this paper all values pertaining to the degree of purification of DNase II refer to the aqueous splenic extract rather than to the initial tissue homogenate as done by other investigators[5].