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Note

Antibody affinity chromatography of hog and bovine spleen DNase II

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The purification of DNase II from a number of mammalian sources has been reported[1–3]. The procedures are relatively time-consuming and require repeated chromatography after preliminary extraction of enzyme. We report here a simple one-step procedure for purification of DNase II from crude extracts.

EXPERIMENTAL

Materials

DNase II was separated from hog or bovine spleens by chromatography on CM-cellulose and Sephadex G-100 as described before[1]. The enzyme was homogeneous as judged by polyacrylamide gel electrophoresis at pH 4.5 (ref. 4) and by the appearance of only a single line of immunodiffusion and immunoelectrophoresis against antibodies produced in rabbits. Non-specific phosphodiesterase was obtained as a by-product of the preparation.

Antisera were produced in sheep or rabbits and the IgG was separated[5]. This was immunoelectrophoretically homogeneous.

Antibody columns were prepared from the IgG and Sepharose 2B (ref. 6). The columns were prepared from 10 ml of activated Sepharose reacted with 280 mg of rabbit antihog spleen DNase II. The IgG Sepharose was washed with water and 0.1 *M* borate buffer, pH 7.5, and poured into a 1.1-cm-diameter plastic column and washed with 250 ml of borate-buffered saline, pH 7.5.

Enzyme assays

These were performed as previously described[1,7].

Antibody affinity chromatography

Antigens were applied to the columns and the latter washed with borate-buffered saline, pH 7.5, until the A_{280} of the eluate returned to baseline. The antigens were then eluted with 0.1 *N* acetic acid, and the pH of the elutate adjusted to 6.0 with glacial acetic acid or 4 *N* NaOH as necessary.

RESULTS AND DISCUSSION

The preparations and the results of antibody affinity chromatography are summarized in the Figs. 1–4 and Table I. Conventional chromatography on CM-cellulose and Sephadex G-100 resulted in a preparation of hog spleen DNase II essentially free of contaminating enzymes and similar to the bovine spleen preparations previously reported from this laboratory[1]. IgG prepared from rabbits immunized with the hog spleen DNase II reacted specifically in immunoprecipitation tests with the homologous enzyme, showed some cross-reaction with beef spleen DNase II, and

TABLE I

CHARACTERISTICS OF THE ENZYMES

	Applied			*Wash*		*Eluate*		
	DNase		*Phospho-diesterase*	*DNase*	*Phospho-diesterase*	*DNase*		*Phospho-diesterase*
	Units	*Specific activity*	*Units*	*Units*	*Units*	*Units*	*Specific activity*	*Units*
Crude hog DNase II	201	0.67	30	75.5	32.1	98	52.4	0
Crude bovine DNase II	340	0.76	38	306	39.7	19	30.6	0
Hog DNase II	195	67.2	0.31	87.4	0.36	93	110.7	0
Hog phospho-diesterase	0		7.4	0	8.1	0		0

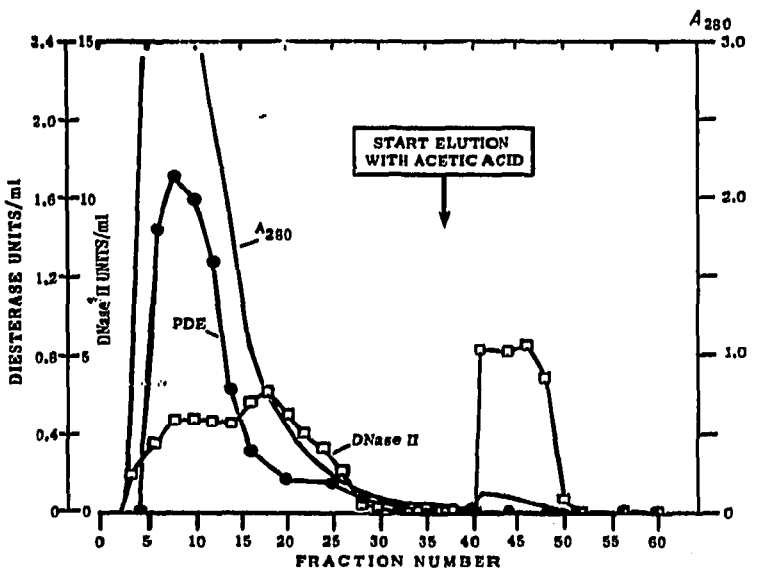

Fig. 1. Antibody affinity chromatography of crude, dialyzed hog spleen extract. The extract (8 ml) was applied to the column, washed with borate-buffered saline, pH 7.5, and eluted with 0.1 *N* acetic acid. Fraction size, 2 ml. PDE = Phosphodiesterase.

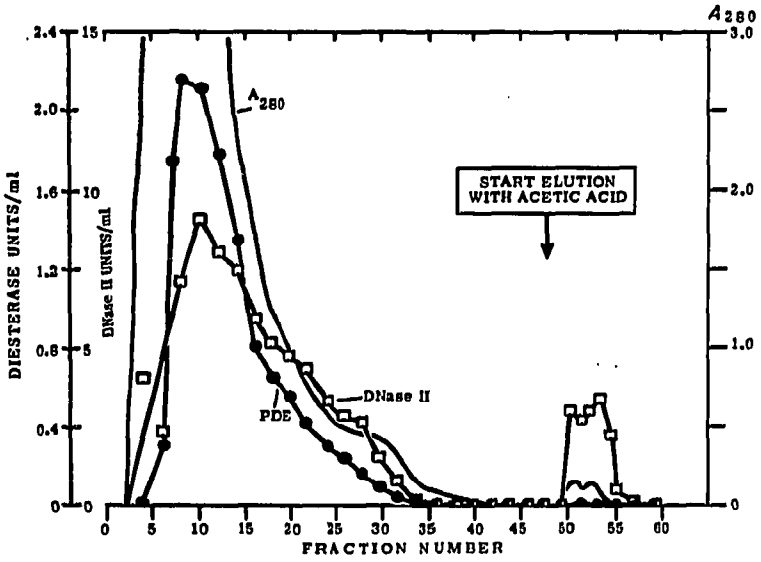

Fig. 2. Antibody affinity chromatography of crude, dialyzed beef spleen extract. 4 mg of lyophilized material were dissolved in 4 ml of borate-buffered saline and applied to the column. Washing and elution were as described in the legend to Fig. 1.

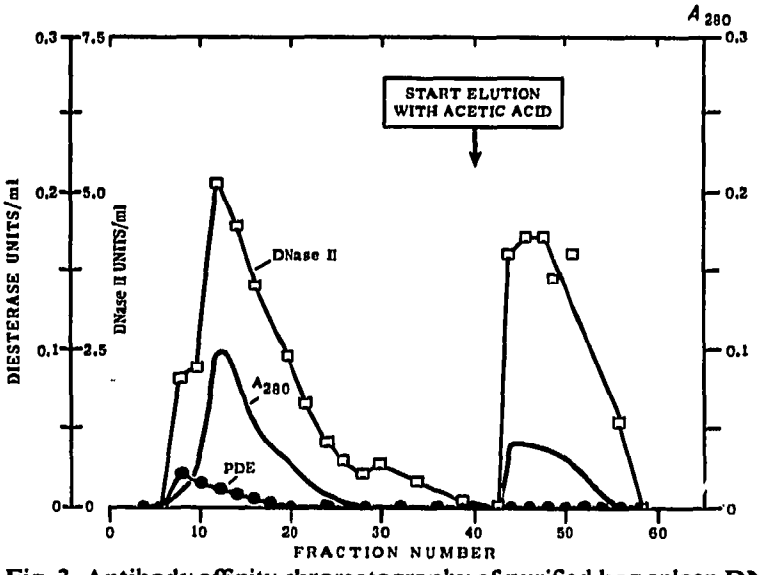

Fig. 3. Antibody affinity chromatography of purified hog spleen DNase II. 4 mg of lyophilized material in 4 ml of buffer were applied and eluted as described in the legend to Fig. 2.

none with hog spleen phosphodiesterase (data not shown). When insolubilized by linkage to Sepharose, the rabbit antihog spleen DNase II IgG was able to separate DNase II from other proteins in preparations from either hog or beef spleen (Table I and Figs. 1–3). The column had no affinity for non-specific phosphodiesterase (Table I and Fig. 4). The hog spleen DNase II eluted from the column after application of

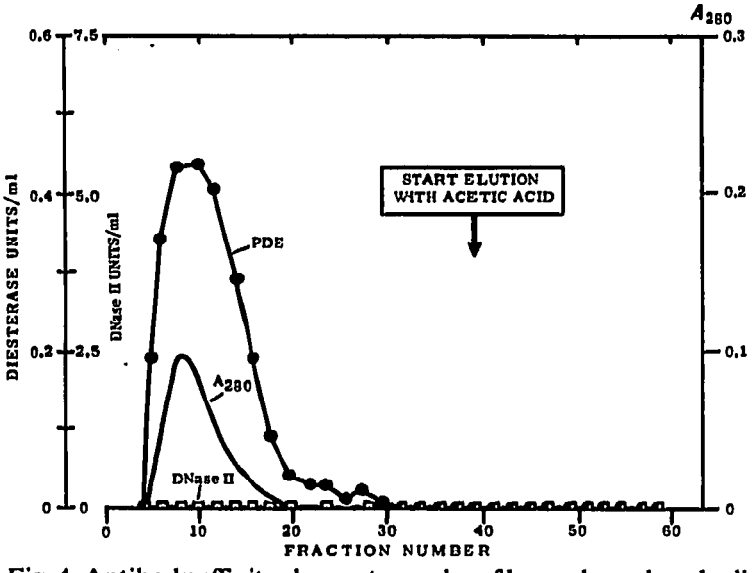

Fig. 4. Antibody affinity chromatography of hog spleen phosphodiesterase. 4 mg of lyophilized enzyme dissolved in 4 ml of buffer were applied and eluted as described in the legend to Fig. 2.

crude extract was identical on immunodiffusion in agar to the material prepared by conventional chromatography. The washes and the eluates together accounted for 86–110% of the enzyme units applied.

Affinity chromatography has provided a simple, convenient and rapid method for isolation of enzymes[8]. Recently the use of DNA bound to Sepharose has been proposed for purification of DNase I and II (ref. 9), but quantitative data were not presented, and presumably other proteins, such as DNA polymerase, binding to DNA would contaminate the DNases. Although antibody affinity chromatography offers a large measure of specificity, it has not been much exploited in the isolation of enzymes[10]. Chidlow *et al.*[11] used an immunoadsorbent to chromatograph lysozyme and Livingston *et al.*[12] chromatographed reverse transcriptase on antibody bound to Sepharose. Our work differs from that of Schabort[9] in the specificity of binding of the DNase II to the column. Non-specific phosphodiesterase was not retained at all and indeed could be separated readily from DNase II, as was the bulk of protein present in a crude cell extract.

The binding of bovine spleen DNase by the column is interesting. The capacity for the bovine enzyme was about 20% of that for the hog DNase II. Livingston *et al.*[12] noted that reverse transcriptase from both murine and feline leukemia virus was bound by rabbit antimurine leukemia virus antibodies. The column did not bind enzymes from avian leukemia virus, and the affinity for the feline enzyme was less than for the homologous antigen. The relationships among the enzymes (DNase II from two species and phosphodiesterase) revealed by the column are similar to those found by immunoprecipitation in tubes[13]. With some modification it will be possible to use the column method for quantitative immunological comparison of DNase II from different sources. This method can be useful also for purification of DNase II from different sources, for as long as the enzymes and antibodies cross-react purifica-

tion of the heterologous enzyme can be obtained. In the work reported here, a 40.3-fold increase in specific activity was obtained after a single passage of crude bovine spleen extract through the column of hog antibodies (compared to a 78.2-fold increase for hog spleen).

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