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PURIFICATION OF A URIDINE-SPECIFIC ACID NUCLEASE FROM CHICKEN LIVER BY FAST PROTEIN LIQUID CHROMATOGRAPHY

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SUMMARY

A rapid purification procedure for a novel uridine-specific nuclease from chicken liver based on the Pharmacia fast protein liquid chromatography (FPLC) system is presented. The purification was achieved by applying crude extract to a Mono S cation-exchange column equilibrated with 10 m*M* potassium phosphate buffer (pH 6.0). The enzyme was eluted in 20 min with a potassium chloride gradient at a flow-rate of 2 ml/min. The enzyme was then chromatographed on a Superose 12 size-exclusion column in less than 1 h at a flow-rate of 0.5 ml/min (K_{av} = 0.77). The enzyme was re-chromatographed on a second Mono S column to concentrate the protein. The uridine-specific nuclease hydrolyzed poly(U) and *Escherichia coli* 5S RNA. Poly(A) was hydrolyzed by the nuclease less efficiently (about 10% of the poly(U) activity). No hydrolysis was detected with poly(C), poly(G), poly(dT) or poly(dA) as substrate.

The speed with which each purification step could be carried out facilitated the determination of optimal chromatographic conditions. We found that the resolution of the Mono S and Superose 12 columns was superior to that of conventional ion exchangers and size-exclusion columns respectively.

INTRODUCTION

Acid nucleases are acid-stable enzymes found in a variety of animal tissues[1–7]. Some of these enzymes are of interest because they exhibit a base specificity which makes them potentially useful for RNA sequence analysis[8,9]. One such enzyme is the cytosine-specific nuclease from chicken liver, described by Levy and Karpetsky[6]. During the purification of this enzyme, we observed a uridine-specific activity which was partially separated from the cytosine-specific nuclease by gel chromatography on a Sephacryl S-200 column. To characterize this uridine-specific activity, we devised a rapid purification procedure based on the Pharmacia fast protein liquid chromatography (FPLC) system with a Mono S cation-exchange column and a Superose 12 size-exclusion column.

A uridine-specific enzyme has been purified from Hela cell lysosomes by Saha[9]. They demonstrated that this enzyme could be used for RNA sequencing. The presence of a similar enzyme in chicken liver would provide an inexpensive and readily available source of this activity.

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The behavior of this enzyme on ion-exchange and size-exclusion columns provided useful information for future scale-up and procedural refinements. The method described also yielded sufficiently pure protein for further characterization.

MATERIALS AND METHODS

Chromatography system

A Pharmacia (Uppsala, Sweden) FPLC system was used. The system included a GP-250 gradient programer, two P-500 pumps, two V-7 injector valves with a 0.5-ml loop and a 10-ml superloop, a UV-1 monitor with REC-481 recorder and a FRAC-100 fraction collector. Cation-exchange chromatography was performed on a Mono S column (50 × 5 mm I.D.). A Superose 12 column (300 × 10 mm I.D.) was used for size-exclusion chromatography.

Reagents

Buffer components were obtained from Fisher Scientific. Polynucleotides were from Pharmacia P-L Biochemicals. All FPLC buffers were filtered through a 0.2-μm Metricel membrane (Gelman, Ann Arbor, MI, U.S.A.) and degassed before use.

Enzyme source

The preparation of chicken liver extract was according to Levy and Karpetsky[6]. Frozen chicken livers were purchased locally. They were mixed with 0.25 *N* sulphuric acid (2 ml/g) and homogenized in a blender at high speed for 1 min. The homogenate was centrifuged in a Beckman J2-21 centrifuge with a JA-10 rotor at 10000 rpm for 40 min at 4°C. The supernatant was titrated to pH 6.0 with concentrated potassium hydroxide and centrifuged in a Beckman L8-55 ultracentrifuge with a Ti50.2 rotor for 1 h at 40000 rpm at 4°C. The supernatant was dialyzed against 10 m*M* potassium phosphate buffer (pH 6.0) (column buffer). The crude fraction was filtered through a 0.2-μm Acrodisc filter (Gelman) prior to chromatography.

Chromatographic conditions

The Mono S column was equilibrated with 10 m*M* potassium phosphate buffer (pH 6.0). Elution buffer contained 10 m*M* potassium phosphate (pH 6.0) and 2 *M* potassium chloride. Flow-rate was 2 ml/min.

The Superose 12 column was operated at 0.5 ml/min with 10 m*M* potassium phosphate buffer (pH 6.0). Chromatography was done at room temperature.

Enzyme assays

The assay procedure is a modification of the procedure of Levy and Karpetsky[6]. Reaction mixtures (250 μl) contained 10 m*M* potassium phosphate (pH 6.0), 0.6 μmole homopolymer or 0.125 mg 5S RNA and 0.2–1 units enzyme. A control reaction was performed with water in place of enzyme. The reaction mixtures were incubated at 37°C for 15 min, then terminated by the addition of 250 μl 2 *N* perchloric acid containing 20 m*M* lanthanum chloride. After 10 min on ice, the mixture was centrifuged at 12000 *g* for 5 min, then the absorbance of the supernatant at 260 nm was measured. One enzyme unit is the amount of enzyme necessary to produce a change of one A_{260} unit in 15 min.

Protein concentration was determined by the method of Bradford[10].

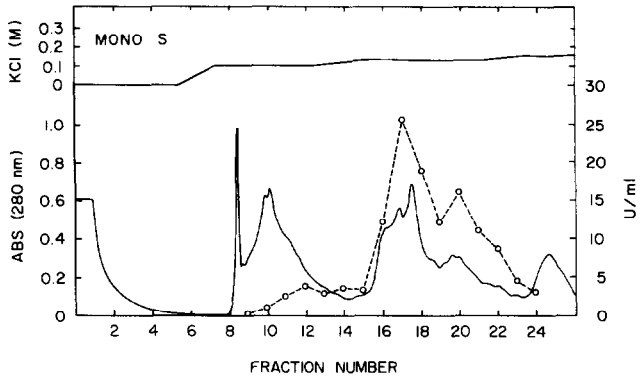

Fig. 1. Mono S cation-exchange chromatography. Crude chicken liver extract (20 ml, 2.2 mg/ml) in 10 m*M* potassium phosphate buffer (pH 6.0) was applied to a Mono S cation-exchange column (50 × 5 mm I.D.). Flow-rate, 2 ml/min; fraction size, 1 ml; elution buffer, 2 *M* potassium chloride in 10 m*M* potassium phosphate (pH 6.0). Dashed line represents enzymatic activity. Solid line is absorbance at 280 nm.

RESULTS

A 20-ml volume of chicken liver extract, containing 2.2 mg/ml protein, was applied to a 1-ml Mono S column (Fig. 1). Approximately 50% of the applied protein was bound to the column. Maximum resolution was achieved by first increasing the potassium chloride concentration rapidly to 0.1 *M*. A significant amount of protein was eluted which contained little or no nuclease activity. When the potassium chloride concentration was increased to 0.14 *M*, a major and minor peak of uridine-specific activity were eluted. These peaks, which were eluted between fractions 16 and 21, contained 67% of the total applied poly(U) hydrolyzing activity. There was no detectable activity against poly(C) in these fractions. The cytosine-specific activity present in the crude extract was eluted at a higher potassium chloride concentration (data not shown). We did not characterize the minor activity peak.

Peak fractions from the Mono S column were chromatographed individually

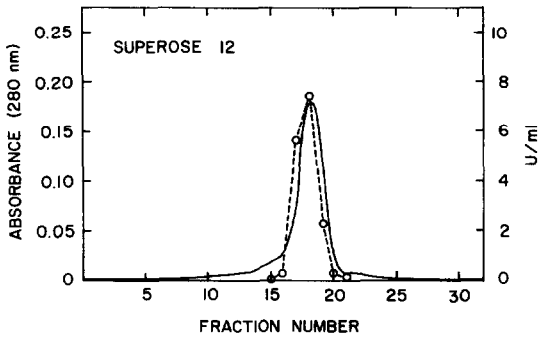

Fig. 2. Superose 12 size-exclusion chromatography. Fraction 17 (0.7 ml) of Mono S column (Fig. 1) was applied to a Superose 12 size-exclusion column (300 × 10 mm I.D.). Column buffer, 10 m*M* potassium phosphate (pH 6.0); flow-rate, 0.5 ml/min; fraction size, 0.5 ml. Solid and dashed lines represent absorbance at 280 nm and enzyme activity, respectively.

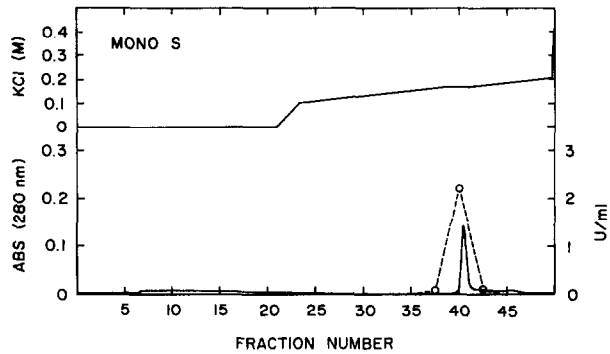

Fig. 3. Concentration of enzyme activity by means of Mono S column. Fractions containing activity from two separate Superose 12 columns were pooled (7 ml), then loaded onto a Mono S column (50 × 5 mm I.D.) and chromatographed as described in Fig. 1. The elution pattern is identical to that obtained in Fig. 1. The number of fractions is greater than in Fig. 1 because the applied enzyme volume was collected as fractions while in the experiment described in Fig. 1 the applied enzyme volume was collected in bulk before individual fractions were collected.

on the Superose 12 size-exclusion column. A single protein peak was eluted, which coincided with the activity peak (Fig. 2). Its elution volume was 19 ml. Column performance was reproducible if samples did not exceed 6.5 mg of protein and 700 μl load-volume. The recovery of enzymatic activity approached 100%.

Fractions from several Superose columns were concentrated on a second Mono S column (Fig. 3) and eluted as described in Fig. 1. Activity was eluted as a single peak.

The base specificity of the acid nuclease was examined (Table I). Besides poly(U), poly(A) was the only synthetic homopolymer degraded. The enzyme hydrolyzed *Escherichia coli* 5S RNA at about half the rate of poly(U) hydrolysis.

DISCUSSION

We have described the purification of a uridine-specific nuclease from chicken

TABLE I

SPECIFICITY OF CHICKEN LIVER NUCLEASE

The nuclease was assayed in a reaction mixture, containing 0.125 mg of *E. coli* 5S RNA or 0.6 μmoles of synthetic homopolymer, as described in Materials and methods. Each reaction mixture contained 0.008 units of enzyme, as determined with poly(U) as substrate. Activities with each substrate are listed as percent of activity observed with poly(U).

Substrate	*Poly(U) activity (%)*
Poly(U)	100
5S RNA	56
Poly(A)	12
Poly(G)	0
Poly(C)	0
Poly(dT)	0
Poly(dA)	0

liver. The purification procedure utilizes the Pharmacia FPLC system with a Mono S cation-exchange column and a Superose 12 size-exclusion column. The advantages of this system were two-fold. First, chromatography is so rapid that optimal chromatographic conditions can easily be determined within one day, or overnight if an automatic LCC 500 controller is used. In contrast, it is often impractical to evaluate many different chromatographic conditions using conventional ion-exchange or size-exclusion columns because of the long time involved in each experiment. Secondly, the chromatographic steps are so rapid it is possible to purify the uridine-specific nuclease in one day, once optimal chromatographic conditions are known. This contrasts with the conventional procedure used by Saha[9] to purify a similar nuclease from Hela cells. This procedure involves Sephadex G-100 gel filtration, DEAE-cellulose chromatography and concanavalin A-agarose affinity chromatography[9] and would take at least one week to complete. In addition, Hela cells are not a practical starting material for large scale enzyme purification. Thus, the presence of a uridine-specific nuclease in chicken liver provides an inexpensive and readily available source of this activity.

The uridine-specific nuclease from Hela cells has been shown by Saha[9] to have specificity useful for the RNA sequence determination of *E. coli* 5S RNA. The uridine-specific nuclease we describe from chicken liver also has sufficient specificity to yield RNA sequence information using *E. coli* 5S RNA as substrate (data to be published elsewhere).

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