Rapid purification of deoxyribonuclease I using fast protein liquid chromatography

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Deoxyribonuclease I finds extensive application in the fields of both nucleic acid and cell motility research. This paper describes the use of the cationic exchange column MonoQ, marketed by Pharmacia as part of their Fast Protein Liquid Chromatography system, for further purification of the enzyme from commercially available material. Up to 7 mg DNase I of high purity can be obtained in a single separation step taking about 20 min to perform. The quality of the product is documented using 3 independent assay criteria.

Deoxyribonuclease I *MonoQ chromatography* *Actin*

1. INTRODUCTION

Since the demonstration in 1974 [1] that actin is a specific inhibitor of deoxyribonuclease I, a secretory endonuclease from pancreas, the enzyme has found extensive application in the field of cell motility research. Even before identification of the DNase inhibitor as actin, immobilised DNase was introduced as an affinity purification medium for the inhibitor [2], and this technique has been of particular value in isolating and studying proteins which interact with actin (see [3–5]). Antibodies against DNase have been used together with DNase itself in a sandwich immunofluorescence method for localisation of actin in cells (see [6]). Inhibition of the enzyme by actin has also been exploited in a rapid assay which discriminates between monomeric and filamentous actin in cell extracts [7].

Bovine deoxyribonuclease I is commercially available from many suppliers, but pure material is discouragingly expensive for purposes requiring stoichiometric rather than enzymatic amounts. Impurities in the cheaper grades often include proteases and other nucleases, particularly unwelcome in the fields of actin and DNA research, respectively. Chromatographic procedures for further purification of DNase I have been reported [8,9], but experience in our laboratory has not found these methods satisfactory for routine purification of the enzyme from the 'DN-100' grade material supplied by Sigma, which we have used as our major source of DNase. Acceptable purification can sometimes be obtained by a single-step gel filtration on Sephadex G100 [10], but inactive components in the DN-100 are not always resolved from the enzyme on this medium. Good separation of the enzyme from impurities can be obtained by gel filtration on Sephadex G75 Superfine (L. Carlsson and S. Eriksson, unpublished), but application of this method on a useful scale requires a large column, and the low flow rates make preparation a long process.

I report here that DNase I can be purified rapidly on the MonoQ cationic exchange column marketed by Pharmacia as a component in their Fast Protein Liquid Chromatography (FPLC) system. Starting with Sigma DN-100 containing not more than 35% DNase, high recovery of activity in electrophoretically homogeneous material is achieved in a single separation step taking about 15–20 min to perform. The commercially available MonoQ column has sufficient capacity to

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prepare at least 7 mg of purified DNase in one step. Re-equilibration of the column in preparation for a repeated run takes no more than 5 min, so that very large amounts of DNase can be extensively purified in one working day.

2. EXPERIMENTAL

Deoxyribonuclease I, grades DN-100 and DN-EP, was obtained from Sigma (St. Louis, MO). Purification of DNase I by gel filtration on Sephadex G75 Superfine was performed in 10 mM Tris–HCl, pH 7.6, 150 mM NaCl, 0.5 mM $CaCl_2$: the gel-filtered sample used here had been stored for about 3 years at −20°C in this buffer.

Chromatography on a ready-packed MonoQ cation exchange column (Pharmacia HR5/5) used the complete Pharmacia FPLC system with programmable FRAC-100 fraction collector. The only components essential to the separation are the column itself and a pump capable of delivering sufficient pressure (about 2.5 MPa is required for a flow rate of 1.5 ml/min). A gradient programmer facilitates reproducible separations, and a monitor and 'intelligent' fraction collector permit automated handling of large batches.

Amounts of protein were determined from absorbance at 280 nm, assuming an extinction of 1.2 for a solution of 1 mg/ml (the correct value for DNase I [10]).

Nuclease activity was measured in terms of the rate of increase in A_{260} in a sample of calf thymus DNA type I (Sigma) as in [7], and expressed as change in absorbance/min. No attempt was made to correlate measured activities with the supplier's specifications in Kunitz units.

The conditions for the assay of inhibition of DNase by actin have been described [7]. The assay, initially designed to measure actin, was used to estimate the amount of DNase I in a sample as follows. The percentage inhibition of the DNase sample by a known amount of pure calf spleen actin (obtained as in [11]) was first measured, and used to calculate the amount of actin needed for complete inhibition of the DNase on a linear basis. This figure was then converted to amount of DNase I assuming equimolar binding of actin to DNase [12,13] and M_r of 31 000 [10] and 42 000 [14] for DNase and actin, respectively.

Electrophoresis on SDS-15% polyacrylamide gels was performed as in [15]. Gels were stained with Coomassie blue R250 and scanned on an LKB Ultroscan laser densitometer.

3. RESULTS AND DISCUSSION

The starting material for the experiments described below was a batch of DNase I from Sigma, grade DN-100, of relatively high purity according to the product description (1505 Kunitz units/mg protein, compared with about 2400 units/mg for Sigma's electrophoretically pure DN-EP). Fig.1 shows chromatography of a sample of this DN-100 on MonoQ cationic exchanger, applied in 10 mM Tris–HCl (pH 7.6), 0.5 mM $CaCl_2$, and eluted with a gradient of NaCl as shown. Several peaks of absorbance at 280 nm are apparent, with the bulk of the material divided between the flowthrough and a complex of two major and several minor peaks eluting between about 150 mM and 250 mM NaCl. The bulk of the DNase activity was confined to the first major peak eluted with salt, although minor peaks of nuclease were also present in the flowthrough and in the second of the major eluted peaks. The recoveries of protein and nuclease activity are summarised in table 1.

Electrophoretic analysis (fig.1b) revealed that the starting material contained several protein components. On the basis of molecular mass alone, the largest major band at about 31 kDa (marked with an arrow in fig.1b) is expected to represent DNase I. This band is essentially the sole constituent of the first major eluted peak, where the bulk of the nuclease activity was recovered, confirming the identification of the 31 kDa band as DNase I. Trace amounts of the 31 kDa component are also eluted in the flowthrough and the second eluted peak.

The chromatogram in fig.1 was loaded with about 4 mg DN-100 for analytical purposes. In 5 successive applications of 7 mg each with no intervening elution, the amount of unadsorbed material remained unchanged, indicating that the column capacity was sufficient for at least 35 mg DN-100. Subsequent elution of this amount gave resolution comparable with that in fig.1, and yielded 7 mg protein in the active peak.

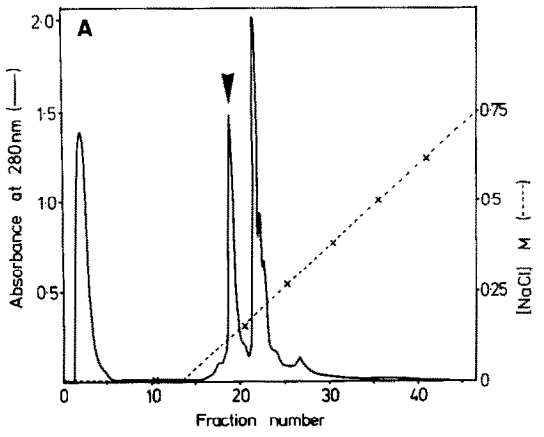

Fig.1. Chromatography of DNase I on MonoQ ion exchanger. A sample containing 4.1 mg DNase I (Sigma DN-100) in 1 ml 10 mM Tris–HCl (pH 7.6), 0.5 mM $CaCl_2$ was applied to a prepacked column of MonoQ (Pharmacia HR5/5) equilibrated with the same buffer, and was eluted with a linear gradient of NaCl from 0–0.75 M at a flow rate of 1.5 ml/min. (A) Absorbance at 280 nm (——) was monitored continuously, and fractions of 0.75 ml were collected. The salt gradient (- - -) was checked by conductivity measurements of selected fractions (marked ×). The vertical arrow (▾) marks the major peak of nuclease activity. (B) Aliquots of the starting material and of each fraction were precipitated with cold acetone (final concentration 70%), and analysed by electrophoresis on SDS-15% polyacrylamide gels. The sample marked 'S' is the material applied to the column: the remaining samples are identified by their fraction numbers. The band corresponding to DNase I (31 kDa) is marked with an arrow.

Table 1

Purification of DNase I on MonoQ ion exchanger

	% Protein	% Nuclease activity	Specific activity[a]
Start	100	100	39.5
Peak recovery[b]	24	65	110
Total recovery[c]	96	85	–

[a] Specific activity is expressed as nuclease activity/unit absorbance at 280 nm (see section 2)
[b] Peak recovery summed over fractions 19–21
[c] Total recovery summed over the whole chromatogram

The figures are calculated for the chromatogram in fig.1

Comparison of the product obtained from chromatography on MonoQ with DNase from other sources is shown in fig.2 and table 2. The other samples shown are Sigma DN-100 (the starting material for MonoQ chromatography), two separate batches of Sigma DN-EP ('elec-

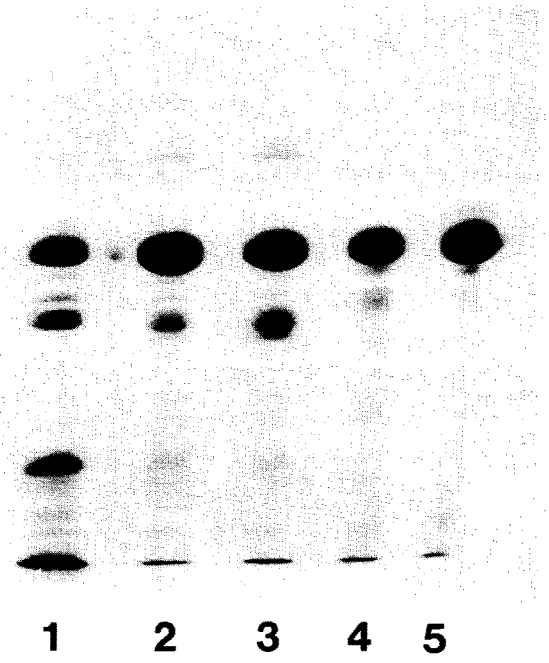

Fig.2. Comparison of different samples of DNase I by electrophoresis on SDS-15% polyacrylamide gels. The samples are: (1) Sigma DN-100; (2) and (3) different batches of 'electrophoretically pure' DNase (DN-EP, Sigma); (4) DNase purified from DN-100 by chromatography on Sephadex G75 Superfine; (5) DNase purified from DN-100 by chromatography on MonoQ.

trophoretically pure'), and a preparation obtained by gel filtration of DN-100 on Sephadex G75 Superfine. By SDS gel electrophoresis (fig.2), all the samples except DN-100 contain one dominant band in the position of DNase; however, the amount of minor contaminating bands is least in the product from MonoQ.

This is expressed quantitatively in table 2, which summarises the purity of the 5 preparations by 3 independent criteria. The first column gives the purity of the 31 kDa polypeptide measured by densitometry of the gel in fig.2. The second column shows the purity of each preparation estimated from the amount of actin required to inhibit the nuclease activity completely (see section 2). This measurement would overestimate the amount of DNase I if the sample contained components which interfered in any way with the actin–DNase interaction, and in particular if inactive DNase which retained its actin-binding activity were present. This latter source of error would not be revealed by a comparison of columns 1 and 2 as long as the inactivation did not involve a significant change in mobility on SDS gels.

The figures in the third column of table 2 are the specific nuclease activity, expressed in terms of the rate of DNA hydrolysis per unit of material absorbing at 280 nm (see section 2). These figures follow a similar pattern to the other two criteria, except that the activity of the G75 Superfine product is markedly lower than expected. By the argument above, a possible explanation of the discrepancy suggests that a gradual loss of nuclease activity without concomitant loss of actin-binding capacity might have occurred during the prolonged storage of the G75 sample at −20°C.

Table 2

Comparison of the purity of different samples of DNase I

Sample	% DNase I estimated by		Specific activity
	Electrophoretic analysis	Actin assay	
DN-100	28.8	33.0	39.5
DN-EP (1)	77.1	80.0	99.8
DN-EP (2)	68.5	78.5	87.4
G75 product	83.0	81.0	76.6
MonoQ product	87.4	85.0	113.0

The different assays are explained in section 2. The samples are DN-100 and two separate batches of DN-EP ('electrophoretically pure') from Sigma, and material purified from DN-100 by gel filtration on Sephadex G75 Superfine and MonoQ

The data in fig.2 and table 2 show that DNase of a quality only slightly lower than that obtained from MonoQ can also be prepared from DN-100 by conventional gel filtration chromatography on Sephadex G75 Superfine. However, separation on MonoQ has advantages in speed and capacity over Sephadex G75, and permits routine preparation of pure DNase on a scale impractical with conventional methods. The simplicity of the technique means that the amounts of DNase purified at one time can be better suited to specific needs, avoiding the possible problems inherent in long-term storage of the purified material. It seems reasonable to expect that the method, perhaps with minor adaptations, will be applicable to purification of DNase from other commercial sources than Sigma DN-100, and perhaps also from pancreas itself.

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