

Evidence for the presence of DNase-actin complex in L1210 leukemia cells

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L1210 leukemia cell cytosol was analysed for the presence of DNase I activity. No free activity was determined in crude cytosol. DNase I enzyme was found to occur in a latent form bound to cytoplasmic actin. DNase-actin complex was partially isolated by Sephadex filtration and DNase I-like activity was demonstrated after SDS gel electrophoresis of the complex and enzyme renaturation. The results were compared with those for synthetic complex of pancreatic bovine DNase I and chicken muscle actin.

<i>DNase I</i>	<i>Actin</i>	<i>DNase-actin complex</i>	<i>DNase inhibition</i>
	<i>Latent DNase</i>	<i>Leukemia L1210</i>	

1. INTRODUCTION

DNase I-actin complex formation has attracted attention [1-4] as a possible regulatory mechanism for actin polymerization which is important in cell movement, maintaining cell shape and helping in cell division [5].

DNase I binds to purified monomeric actin in a 1:1 ratio and prevents actin polymerization [1,2,5]. DNase I was found to react also with F actin polymer by direct interaction with protomers in the polymer, probably changing the polymer conformation and resulting in the release of actin monomer even from the middle of the molecule. In both cases the equimolar DNase-actin complex appears [6,7]. The above studies have been carried out with bovine pancreatic DNase I, and rabbit muscle actin, and there is no convincing evidence

that this particular complex has a physiological role in cells.

The questions of whether there are DNase I-like enzymes in cells and whether actin functions to control the degradative activity of such enzymes on cell DNA are, however, interesting ones. Further questions of whether DNase I-actin complexes exist in normal and cancer cells and whether the complex has a function in DNA metabolism are also of great interest. Thus far, a natural DNase I-actin complex has been observed only in bovine pancreatic juice [4].

Published results on DNase activities in neoplastic cells are controversial. DNase I-like activity has been found to be diminished or absent [8]. It appears to be absent in leukemic cells from humans and mice [9-11].

We present evidence for the existence of a natural complex between DNase I-like enzyme(s) and actin, supported by model studies of the interaction of bovine DNase I and chicken muscle actin.

2. MATERIALS AND METHODS

L1210 leukemic cells were carried in DBA mice by intraperitoneal transfer once a week. Ascites

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Abbreviations: SDS, sodium dodecyl sulfate; pCMB, *p*-chloromercuribenzoic acid

fluid was removed and lymphocytes isolated and homogenized in 10 mM Tris-HCl buffer (pH 7.4), 0.25 M sucrose, 1 mM dithiothreitol, 0.1 mM ATP and 0.1 mM CaCl_2 (buffer A). DNase activity was measured spectrophotometrically at pH 7.0 in 40 mM Hepes buffer, in the presence of 5 mM MgCl_2 . Salmon sperm DNA (Calbiochem-Behring) was used as substrate. Actin content was measured by inhibition of added DNase with the conditions as above. For details of lymphocytes and homogenate preparations, DNase activity and actin content measurements, see [11].

Polyacrylamide slab gel electrophoresis was performed according to Laemmli [12] in 10% gels with current 30 mA/slab and 0.05% SDS in electrode buffer.

DNase activity was detected after electrophoresis essentially as in [13,14]. Salmon sperm DNA, 10 $\mu\text{g}/\text{ml}$, and bovine serum albumin, 10 $\mu\text{g}/\text{ml}$, were incorporated in the separating gels before their polymerization. Samples were diluted 1:1 with 10 mM Tris-HCl buffer (pH 7.4), containing 1% SDS, 2% β -mercaptoethanol, 10% glycerol and 0.1% bovine serum albumin and denatured 2 min at 100°C before they were applied to the gel.

Inhibition of DNase activity by actin on the gels after electrophoresis was as in [13], but lyophilized chicken actin was used instead of rabbit muscle actin.

Sephadex filtration was carried out with a 1 $\times$ 58 cm column of Sephadex G-150 superfine equilibrated with buffer A without sucrose. L1210 cell homogenates were centrifuged at $105000 \times g$ for 1 h and the supernatant fraction was immediately dialysed with buffer A without sucrose with constant stirring and buffer changes. The same column was calibrated with purified DNase I (crystalline bovine pancreatic M_r 32000, from Sigma), purified chicken muscle actin (M_r 43000, from Sigma) and their synthetic complex prepared by mixing DNase I and actin stock solution (1 mg/ml) in 3 different ratios (1:1, 1:5, 1:10) followed by overnight incubation in the presence of buffer A without sucrose. All operations were done in a cold room. Protein was estimated by the standard Lowry procedure [15].

Fig.1. Sephadex filtration (G-150 superfine): I, L1210 cell cytosol (1 ml samples applied, containing 2.5 mg protein); II, DNase I-crystalline from bovine pancreas (1 mg); III, actin lyophilized from chicken muscle (1 mg); IV, DNase I (0.2 mg)-actin (2 mg) synthetic mixture. $\longrightarrow$

3. RESULTS AND DISCUSSION

3.1. Identification of DNase-actin complex

Since our previous results suggested that the lack of endogenous DNase activity observed in L1210 cells cytosol could be caused by DNase binding to cytoplasmic actin which comprises 10% of L1210 cytosol protein [11], the following experiment was performed to check this possibility.

L1210 cell cytosol was dialysed against 10 mM Tris-HCl (pH 7.4), 0.1 mM ATP, 1 mM dithiothreitol and 0.1 mM CaCl_2 to depolymerize actin filaments and then allowed to pass through a Sephadex G-150 column with the same conditions (these were found earlier to be the best for the actin inhibition of bovine pancreatic DNase I). Fractions from the elution profile were analyzed for actin content and endogenous DNase activity (fig.1,I). The results were compared with those for purified DNase I, purified actin and their synthetic mixture (fig.1,II-IV, respectively).

No free DNase activity was detected in L1210 cytosol and, as expected, the actin content measured by the inhibition of added bovine DNase I was high (fig.1,I). DNase activity in the cytosol can be demonstrated after addition of pCMB which was found earlier to affect the combination between bovine pancreatic DNase I and actin [11]. The DNase activity appears earlier when compared to purified bovine pancreatic DNase I alone (fig.1,II) and this is true of DNase I in the synthetic mixture (fig.1,IV). This would be expected if the DNase were bound in the higher- M_r complex.

Sephadex filtration of purified DNase, actin mixtures prepared with different ratios of actin to DNase always showed DNase activity in the same place in the eluate with the maximum in fraction no.12 (fig.1,IV). DNase activity in synthetic DNase-actin complex was also observed in [3].

A representation of polyacrylamide gel electrophoresis of some fractions from experiments illustrated in fig.1 is shown in fig.2. In fig.2a, the lower 4 bands represent two fractions with standard actin mobility (fig.2d) and two fractions with standard DNase mobility (fig.2c). Possibly there are two major varieties of actin and DNase, each in the natural complex. The synthetic mixture con-

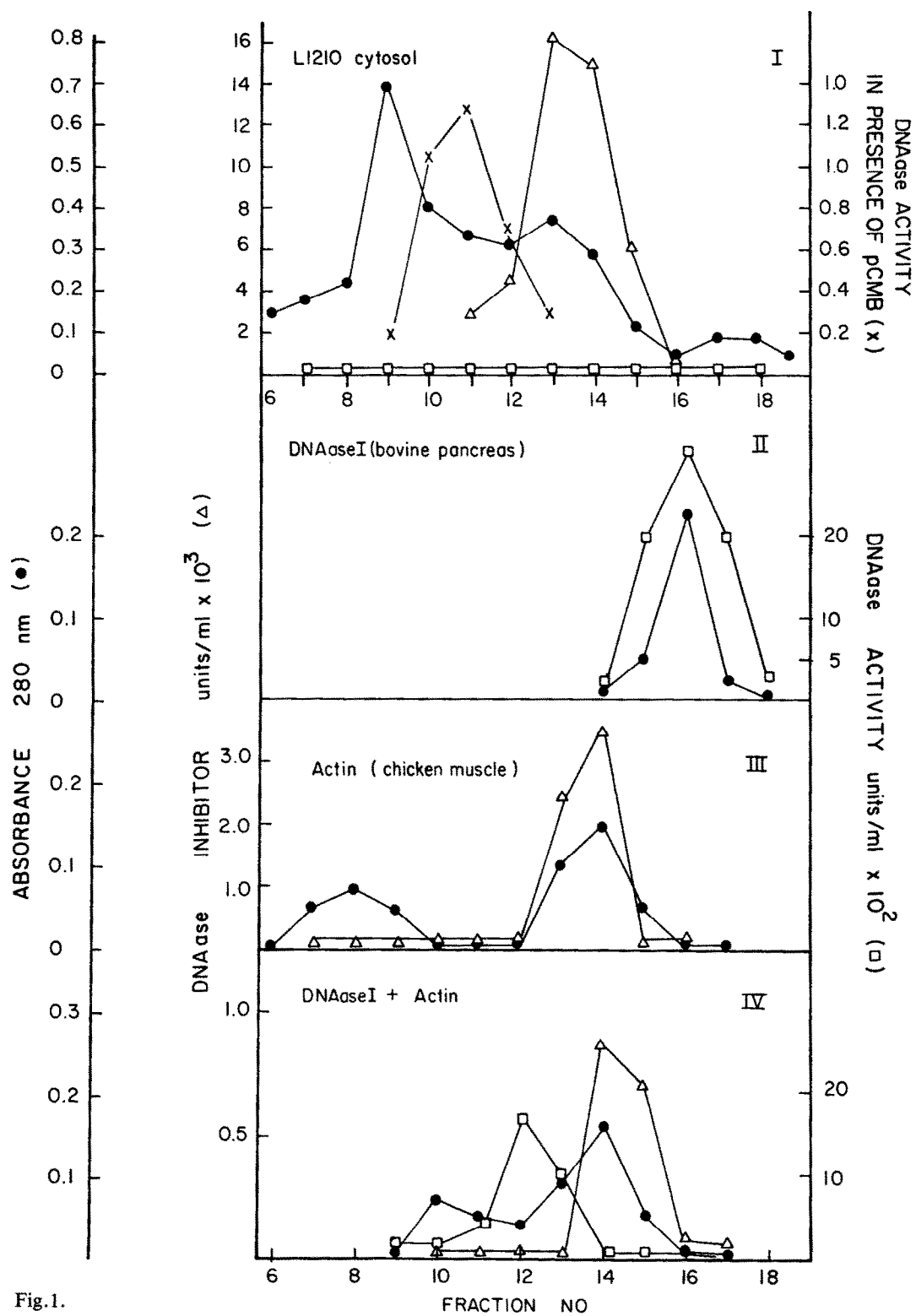


Fig.1.

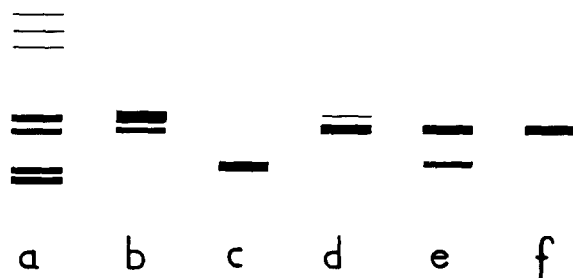


Fig. 2. SDS slab gel electrophoresis. Gels stained for protein with Coomassie brilliant blue according to Laemmli [12]: (a) presumable natural DNase-actin complex in L1210 cells, 27 μ g protein (frac. 11, fig. 1, I); (b) L1210 cells actin, 18 μ g (frac. 13-15, fig. 1, I); (c) DNase I, 10 μ g (frac. 15-17, fig. 1, II); (d) chicken muscle actin, 10 μ g (frac. 13-15, fig. 1, III); (e) synthetic DNase I-actin complex, 20 μ g (frac. 12, fig. 1, IV); (f) actin peak, 10 μ g (frac. 14-15, fig. 1, IV).

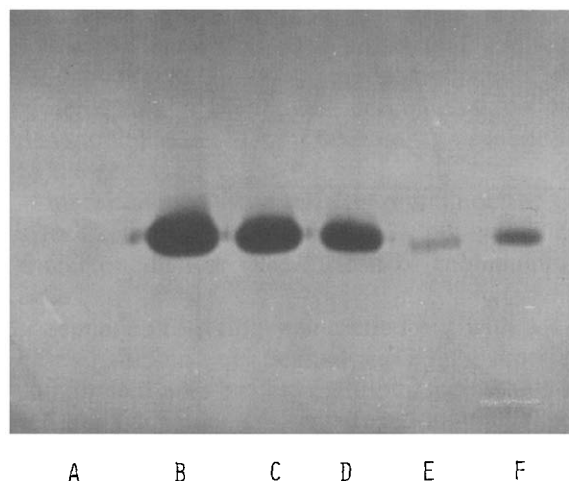


Fig. 3. DNase zymograms. After electrophoresis, SDS was washed out of the gel and DNase activity was detected as in [13,14], in 40 mM Tris-HCl buffer (pH 7.4), 2 mM CaCl_2 and 2 mM MgCl_2 after 20 h incubation: (A) L1210 crude cytosol, 240 μ g protein; (B-D) presumable natural DNase-actin complex (fig. 1, I, frac. 10-12 = 5 μ g, 3 μ g and 2 μ g, respectively); (E) DNase I from bovine pancreas, 50 pg; (F) synthetic DNase I-actin complex, 0.8 ng (frac. 12, fig. 1, IV).

taining the complex, however, showed only one band with DNase mobility and one with actin mobility (fig. 2e).

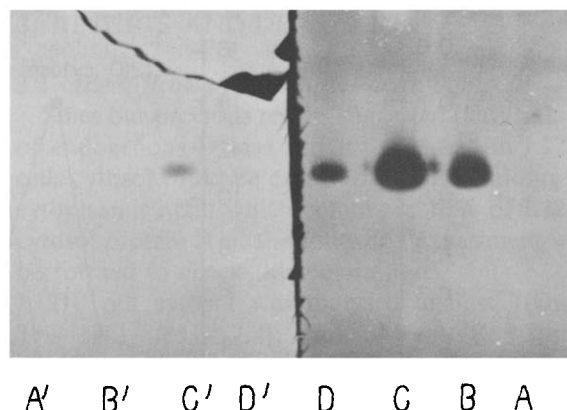


Fig. 4. Actin inhibition of DNases in the gel. SDS slab gel electrophoresis and DNase activity staining conditions as in fig. 3. Half of the slab (wells A'-D') was incubated with chicken muscle actin (10 μ g/ml) before DNase activity was detected: AA', L1210 crude cytosol, 240 μ g; BB', synthetic DNase-actin complex, 1 ng; CC', DNase-actin natural complex, 2.5 μ g; DD', DNase I from bovine pancreas, 75 pg.

3.2. Demonstration of endogenous DNase activity

Fig. 3 shows the results of DNase detection after SDS electrophoresis according to techniques in [13]. This method which allows detection of picograms of DNase, shows no activity is present in crude L1210 cytosol (fig. 3A), but partially separated fractions from the area of DNase-actin complex exhibit considerable activity (fig. 3B-D) with the same mobility as crystalline DNase I (fig. 3E). Presumably, the SDS electrophoresis either separates DNase from actin or irreversibly denatures the actin releasing DNase activity.

Both the DNase I-like activity from the natural complex and bovine pancreatic DNase I (also from synthetic complex) can combine with actin after SDS electrophoresis and DNase renaturation by the treatment in [14]. This is shown in fig. 4. Treatment with excess actin nearly completely removes all DNase activity. In the one sample in which some activity remained (fig. 4C') slightly higher concentrations of actin or longer times of treatment are apparently needed to inactivate all the DNase.

These observations lead to the conclusion that in L1210 cells cytosol DNase I-like activity exists in a latent form bound to cytoplasmic actin. This DNase is similar to DNase I with respect to M_r , ion

requirements, forming of low- M_r diffusible products and sensitivity towards denaturing reagents.

The widespread occurrence of actin in cells and its high affinity for DNase suggests that interaction of those two proteins may be of general significance for either the function of actin in cell motility, shape and division or the activity of DNase towards cellular DNA.

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