Rat Liver DNase I – Like Activity and Its Interaction with Actin

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Monomeric G actin, total actin and the F:G actin ratio were determined in the rat liver cytosol and nucleoplasm by measuring the inhibition of standard crystalline DNase I. Actin was purified from rat liver nucleoplasm by Sephadex filtration. Accompanying the considerable amount of actin an endogenous DNase I like activity was found in rat liver cytosol and nucleoplasm. It was shown, that similarly to DNase I from bovine pancreas the liver DNase was inhibited by mammalian and avian skeletal muscle actin as well as by endogenous liver actin, as verified by electrophoresis of DNase containing extracts on polyacrylamide gels with incorporated DNA.

Introduction

Pancreatic DNase I associates with globular muscle actin to form a highly stable and catalytically inactive complex [1–5]. The biological role of this interaction is still unknown. DNase I is considered as one of the many actin binding proteins with its potential ability of involvement in the regulation of actin polymerization, which is so important for many cellular functions, *e.g.* movements, division, maintenance of cell shape and secretion [6, 7].

Our interest has been focussed on the occurrence of DNase I like activities in different cells and tissues and their ability to interact with endogenous actin. In our earlier studies we observed the presence of latent DNase activity together with a considerable amount of actin in the liver cytosol of some vertebrate species [8, 9]. We thus suggested, that the DNase I like enzymes may commonly appear in eucaryotic cells in a latent form, as a result of their interaction with endogenous actin.

The question arises whether one of the many functions of actin in the cell is to control the intracellular DNase activity towards cellular DNA by forming a natural DNase-actin complex. A naturally occurring DNase-actin complex has been demonstrated so far only in the rat pancreatic juice by Rohr and Mannherz [10] and in the L 1210 lymphocytes by Malicka-Błaszkiewicz and Roth [11].

In these studies we present evidence for the existence of a DNase I like enzyme in the rat liver and its ability to interact with endogenous actin, as well as with muscle actin. Some preliminary results suggests that natural DNase-actin complex is present also in the rat liver nucleoplasm.

Materials and Methods

Studies were carried out on livers from two months old rats, *Rattus rattus* (Wistar strain). Fresh livers were excised, rinsed and homogenized with 3 volumes of freshly made 10 mM Tris-HCl buffer pH 7.4, containing 0.25 M sucrose, 1 mM DTT, 0.1 mM ATP and 0.1 mM $CaCl_2$ (buffer A). The homogenates were filtrated through gauze and centrifuged at 105,000 × *g* for 1 h. The precipitate was discarded and the supernatant was used as the cytosolic fraction, which was used immediately for experiments or frozen at −20 °C.

Nuclei were prepared from fresh rat liver using a partially modified method of Wallace *et al.* [12] and stored in glycerol suspension at −20 °C, not longer than one month.

Nucleoplasm preparation. From the glycerol suspension the nuclei were recovered by centrifugation at 1500 × *g*, washed twice with ice cold buffer A and homogenized with three volumes of buffer A, using an electrically driven Potter homogenizer. The homogenates were centrifuged at 16,500 × *g* for 10 min. The supernatant was taken as the nucleoplasm fraction and used immediately for experiments or frozen at −20 °C. Frozen por-

Abbreviations: DNase I, deoxyribonuclease I from bovine pancreas (EC 3.1.21.1); SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; DTT, dithiothreitol; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.

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tions of the nucleoplasm did not change their activities within one month.

Sephadex filtration of nucleoplasm was carried using the conditions described earlier for the isolation of actin from the cytosol of leukemia L1210 lymphocytes [11]. The nucleoplasm was dialyzed against buffer A (without sucrose) for 24 h and applied to the column of Sephadex G-150 superfine (1 × 90 cm) equilibrated with the same buffer; the 1.5 ml fractions were collected at a flow rate 3ml/h. All operations were done at 4 °C.

Ion exchange chromatography was performed on DEAE cellulose at conditions described by Rohr and Mannherz [10]. The nucleoplasm was dialyzed for 24 h against 0.004 M phosphate buffer pH 8.0 and applied to a DEAE-cellulose (DE-52) column (1 × 20 cm) equilibrated with the same buffer. The adsorbed proteins were eluted with increasing concentration of phosphate buffer, pH 8.0 (from 0.004–0.4 M); the 1.5 ml fractions were collected at a flow rate 30 ml per hour.

Protein was determined spectrophotometrically at 280 nm or by the standard Lowry procedure [13].

DNase activity was measured spectrophotometrically at pH 7.0 in 40 mM HEPES buffer in the presence of 5 mM $MgCl_2$. A highly polymerized salmon sperm DNA (Calbiochem) was used as substrate according to Malicka-Błaszkiewicz and Roth [14].

The actin content was determined by the inhibition of added exogenous DNase I from bovine pancreas (Sigma) in standard DNase assay conditions [14]. The concentration of G-actin was estimated by DNase I inhibition directly in the crude sample. Total actin (T) content was measured after dilution of the sample with buffer A. For the expression of maximal inhibition a specific dilution has to be found, as we described earlier for leukemia L 1210 lymphocytes [14] and liver of some vertebrate species [9].

F-actin was calculated by subtracting the amount of G-actin from total actin (F = T–G). One unit of actin inhibitor is the amount, which decreases the activity of 20 ng of DNase I by 10%.

SDS-PAGE was performed according to Laemmli [15] on 10% gels at 30 mA per slab using 0.05% SDS in the electrode buffer. Electrophoresis under native conditions was carried out as described by Brown *et al.* [16].

DNase activity on the slab gels was detected after electrophoresis in native and denaturing conditions essentially as described by Lacks [17] in 40 mM Tris-HCl buffer pH 7.6 with 2 mM $MgCl_2$ and 2 mM $CaCl_2$. Gels were stained with ethidium bromide and examined under UV light.

Inhibition of DNase activity in the gels by actin was tested as described by Lacks [17]. Chicken and rabbit muscle actin (Sigma) as well as actin purified from rat liver nuclei and crude liver cytosol were used.

Results

Actin content and DNase activity

DNase activity, monomeric G actin, total actin and the state of actin polymerization (defined by the ratio of F actin to G actin) were examined in the rat liver cytosol and nucleoplasm.

Both subcellular fractions were found to be a rich source of actin. As shown in Table I actin appears in twice higher concentration in nucleoplasm, than in the cytosol, however, there is no difference in the state of actin polymerization be-

Table I. Comparison of actin content in the rat liver cytosol and nucleoplasm*.

	Protein [mg/ml]	Actin G[a]	Actin Total[b] units per mg protein	Actin F[c] units per mg protein	F:G	G % of total	DNase equivalent of DNase I per mg protein
Cytosol	24.8	42.5	900	857.5	20	4.7	0
Nucleoplasm	1.25	88	2004	1916	22	4.4	0

* Actin content based on inhibition of standard DNase I.
[a] Inhibition measured directly in the sample.
[b] Inhibition measured after dilution of the sample with buffer A (see Methods).
[c] F actin = total actin – G actin. DNase activity measured in standard assay conditions described earlier [14].

tween both subcellular fractions. The monomeric G actin comprises about 4.5% of the total cytosolic, as well as nucleoplasmic actin.

As we expected on the basis of our former observation [9], the endogenous hydrolytic activity towards highly polymerized DNA could not be detected directly in the test tube assay at pH 7.0 neither in the crude liver cytosol, nor nucleoplasm but it appeared in zymograms prepared after electrophoresis of the crude cytosol (Fig. 1 Ac and 1 Be) and nucleoplasm (Fig. 1 Bg). However, detection of DNase activity in the rat liver cytosol requires a preliminary treatment of the sample at pH 3.0, to denature the considerable excess of actin present in this fraction (compare Fig. 1 Ab and c and 1 Bd and e).

Analysis of the zymograms confirms the presence of DNase I like activities in the rat liver cytosol and nucleoplasm. This DNase resembles the DNase I from bovine pancreas by molecular

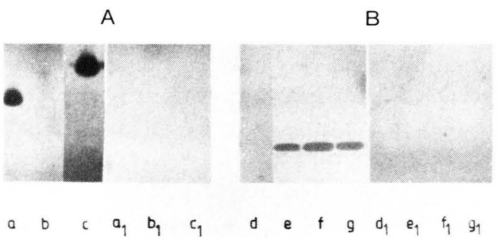

Fig. 1. DNase zymograms. Inhibition of DNase on the gels.
A. Slab PAGE after Brown *et al.* [16], with salmon sperm DNA included to the separating gels (20 μg/ml of the gel). After electrophoresis DNase activity was allowed to develope (15 h), as described by Lacks [17], *i.e.* gels were incubated with 40 mM Tris-HCl buffer pH 7.6, with 2 mM $MgCl_2$ and 2 mM $CaCl_2$. Gels were stained with ethidium bromide (2 μg/ml of buffer) and photographed under UV illumination. a,a_1 – standard DNase I from bovine pancreas – 10 pg of protein; b,b_1 – crude liver cytosol 1000 μg; c,c_1 – rat liver cytosol treated with H_2SO_4 to pH 3.0 to denature actin – 250 μg.
B. Slab SDS-PAGE according to Laemmli [15]. 20 μg of DNA and 10 μg of bovine serum albumin included per ml of separating gels. After electrophoresis the gels were washed to remove SDS and DNase activity was developed in the conditions as in A. d,d_1 – crude liver cytosol – 1000 μg; e,e_1 – rat liver cytosol treated with H_2SO_4 as in points c,c_1 – 250 μg; f,f_1 – standard DNase I – 50 pg; g,g_1 – crude rat liver nucleoplasm – 80 μg.
Aa_1–c_1 and Bd_1–g_1 – gels were preincubated (4 h) with actin added to the buffer (10 μg/ml) before $MgCl_2$ and $CaCl_2$ were added. The same results were obtained with rabbit, bovine and chicken skeletal muscle α actin or actin isolated from rat liver nucleoplasm or crude liver cytosol.

weight, pH and ion requirements, sensitivity to denaturing reagents, and the ability to form DNA degradative products which diffuse from the polyacrylamide gels (low molecular weight). However the rat liver cytosol DNase differs from DNase I in the charge of the molecule, as it exhibits different mobility during electrophoresis in native conditions (see Fig. 1 Aa, c).

The ability of endogenous liver DNase to interact with actins from different sources was tested. For this purpose it was necessary to isolate actin from the rat liver. Rat liver nucleoplasm was found to be a good source to purify actin.

Gel filtration of rat liver nucleoplasm

The nucleoplasm was filtered on Sephadex G-150 superfine in conditions described by Malicka-Błaszkiewicz and Roth [11]. The fractions were analyzed for the presence of endogenous DNase and inhibition capacity for DNase I from bovine pancreas (actin).

a) Isolation of actin

The inhibitor of DNase I was localized in one peak. The fractions with the highest inhibitory activity (from 53 to 64, Fig. 2) were pooled and used as a preparation of purified nuclear actin. It contains 13 times more units of DNase I inhibitor per mg of protein than the crude liver nucleoplasm and was shown to be homogenous by SDS-PAGE. Its mobility was found to be equal to the mobility of actin from rabbit skeletal muscle (Fig. 2, b and c in insert).

Although gel filtration of the nucleoplasm allowed the isolation of actin in one step the yield was only 10% of the starting DNase I inhibitory activity. This was probably caused by the loss of the ability of the purified nuclear actin to inhibit DNase I, most probably due to its denaturation during the purification procedure, even at 4 °C [14].

b) Latent DNase

No free endogenous DNase activity towards highly polymerized DNA of salmon sperm was found to be present in the collected fractions when using the test tube assay [14]. DNA degrading activity appears however in zymograms (not shown) of aliquots taken from fractions 40–45 (Fig. 2),

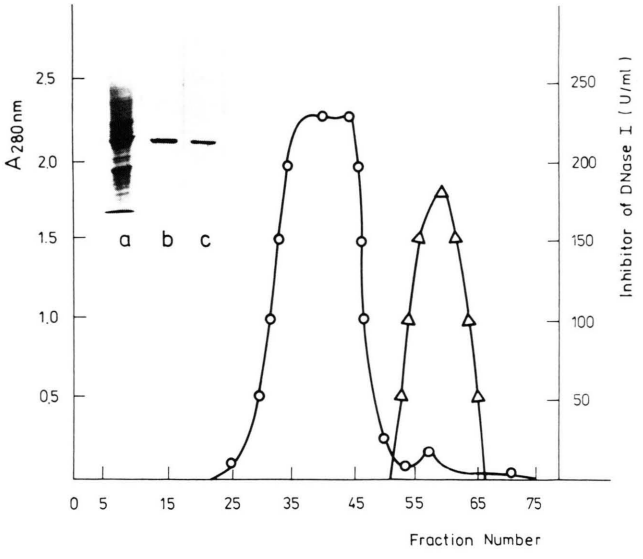

Fig. 2. Molecular filtration of rat liver nucleoplasm. Nucleoplasm was dialyzed for 24 h against buffer A (without sucrose, see Methods) and applied to the Sephadex G-150 superfine (column 1 × 90 cm). The column was equilibrated and elution carried with the same buffer. 1.5 ml fractions were collected at a flow rate 3 ml/h. Protein patterns obtained after SDS-PAGE [15], stained with silver method [22]. a – crude rat liver nucleoplasm – 40 μg protein; b – standard rabbit skeletal muscle actin – 5 μg; c – pooled fractions from 53 to 65 – 5 μg. (○–○) – absorption at 280 nm, (△–△) – inhibition capacity of DNase I.

which contain proteins of higher molecular weight than actin (*i.e.* eluted from the column in front of actin). This, as well as the analysis of the zymograms of crude nucleoplasm after SDS-PAGE suggests the presence of latent DNase activity due to its complexation with an inhibitory protein of higher molecular weight, which might be actin. This complex may dissociate under the conditions of the electrophoretic separation or the actin may denature and allow the DNase activity to appear.

Ion exchange chromatography

Assuming differences between the molecular charge of DNase, actin and presumptive DNase-actin complex present in rat liver nucleoplasm ion exchange chromatography on DEAE cellulose was performed under the conditions described by Rohr and Mannherz [10] used for the isolation of actin-DNase complex from rat pancreatic juice.

Four protein peaks appear in the elution profile (Fig. 3), but the DNA degradative activity was de-

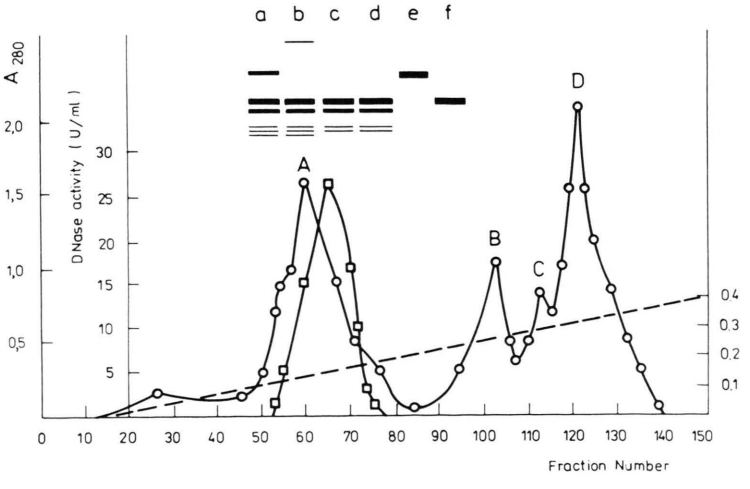

Fig. 3. Ion exchange chromatography of rat liver nucleoplasm. DEAE cellulose column (2 × 20 cm) equilibrated with 0.004 M phosphate buffer, pH 8.0. Elution carried in linear concentration gradient from 0.004–0.4 M phosphate. 1.5 ml fractions were collected at a flow rate 30 ml/h. Protein patterns obtained in the same conditions as in Fig. 2. a, b, c, d – fraction 52, 58, 65, 75 respectively, 70 μl of the sample applied to the gel; e – standard rabbit skeletal muscle actin – 5 μg; f – standard DNase I from bovine pancreas – 5 μg; (○–○) – absorption at 280 nm; (□–□) – DNase activity.

tected only in fractions of peak A. The maximum of this activity, when examined directly in the test tube assay, was eluted in fraction 65 of the descending arm of the protein peak A (Fig. 3). However, analysis of the fractions of peak A after SDS-PAGE followed by the zymogram technique, shows that the DNase activity is highest in fraction 52 of the ascending arm of peak A (compare Fig. 4c and d). Silver staining of the fractions from peak A showed that all of them contained a predominant protein band corresponding to standard DNase I (Fig. 3, f in insert), but only fraction 52 showed an additional protein band corresponding to standard rabbit skeletal muscle actin (Fig. 3, e in insert). This suggests that DNase is complexed with actin in this fraction.

The inconsistency between the DNA degrading activity shown in fraction 65 of the activity profile (Fig. 3) and fraction 52 of the zymograms results from the fact that after column chromatography only free DNase was determined in the sample while in the zymogram technique total DNase was visualized. Thus, the higher activity on the zymogram of fraction 52 is further evidence that some of the DNase is complexed with actin and therefore could not be estimated in the test tube assay conditions.

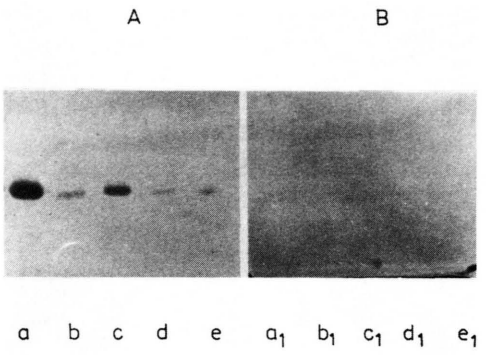

Fig. 4. Actin inhibition in the gels of DNase separated from rat liver. Slab SDS-PAGE was performed as described by Laemmli [15].
A. DNase zymograms obtained directly after electrophoresis and subsequent enzyme renaturation according to Lacks [17].
B. Before DNase activity was allowed to develope gels were incubated with actins from the same sources as in experiment shown in Fig. 1. a,a_1 – standard DNase I – 100 pg; b,b_1 – fraction 58; c,c_1 – fraction 52; d,d_1 – fraction 65; e,e_1 – fraction 75 from Fig. 3 – 70 µl was applied to the gel.

Inhibition of liver DNase by actin

In Fig. 1 and Fig. 4 DNase zymograms of crude liver cytosol, nucleoplasm and fractions a peak A are presented. The gels of Fig. 1 Aa_1-c_1, Bd_1-g_1 and Fig. 4B were preincubated (before DNase activity was allowed to develop) in buffers containing added purified actin from rabbit, bovine and chicken skeletal muscle, or actin isolated from rat liver nucleoplasm or crude liver cytosol (as a source of actin). The results have been compared with those for standard DNase I. As can be seen from Fig. 1A, B and 4, the endogenous DNase of crude rat liver cytosol and of the nucleoplasm and the DNase isolated from rat liver nucleoplasm are inhibited on the gels by mammalian and avian skeletal muscle as well as by endogenous liver actin, to the same extend as the control bovine pancreatic DNase I.

Discussion

Previous studies on the DNase-actin interaction have been carried out mostly on the artificial model of DNase I from bovine pancreas and actin from rabbit skeletal muscle. There are only a few informations on DNase I like activities occurring in cells other than pancreas and parotid and their ability to interact with endogenous actin [8, 10].

Lacks [17] examined this activity in different rat organs and tissues and demonstrated, that high concentrations of DNase I are only present in the secretory glands. He did not show DNase I like activities in the rat pancreas, thymus, spleen brain and liver. In his experiments DNase I like activities present in the rat tissues were not inhibited by rabbit skeletal muscle actin. Therefore the biological meaning of DNase-actin interaction was questionable, although it was shown later, that rat parotid DNase I is able to interact with actin [23].

Our studies using the same method (zymogram after SDS-PAGE) for the detection of DNase activity in crude samples have shown the existence of DNase I like activities in the liver cytosols of a number of different vertebrate species [8, 9]. In the rat liver this type of activity has been shown to be present in the cytosol and in the nucleoplasm. The high amount of actin present in the liver made it probably difficult to detect this enzyme in experiments of Lacks [17]. The use of electrophoresis in the presence of SDS causes actin denaturation and

allows DNase activity to appear (after former enzyme renaturation). But in the case of cytosol of rat liver, additional denaturation of actin was necessary, by adjustment of the pH of the sample to pH 3, with the preservation of DNase activity.

The DNase I like activity of rat liver is inhibited by muscle actin and actin isolated from the rat liver in an identical manner as DNase I from bovine pancreas. We also observed the ability of porcine and fish liver cytosol DNase to interact with rabbit muscle actin (not published).

The presence of actin in liver cell nuclei has been known for same time [18–20], but its functions is less well understood, than that of cytosolic actin. There have been previous reports indicating the presence of DNases in nuclei of eucaryotic cells, but its subclass and physiological function was not understood [21], here we corroborate these reports and in addition give evidence for its ability to interact with actin thus already proving that, the nuclear DNA-degrading activity is due to a DNase I like enzyme.

The results presented here are of qualitative character, but they suggest the possibility of the existence of a DNase-actin complex occurring naturally and intracellularly in rat liver and they encourage to perform studies on the possible involvement of actin on the regulation of the activity of endogenous DNases.

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