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QUARTERLY

Does a natural DNase-actin complex exist in the carp liver cytosol ?

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The specific interaction of bovine pancreatic DNase I with skeletal muscle actin is well documented. It leads to the formation of a 1:1 complex, with concomitant inhibition of the DNA degrading activity of bovine pancreatic DNase I and the ability of actin to form high molecular mass polymers [1–3]. DNase I binds to the monomeric actin G and to the filamentous F actin [1]. The biological role of this interaction is unknown. Previous studies on DNase-actin interaction have been carried out mostly on the artificial model of DNase I from bovine pancreas and actin from rabbit skeletal muscle. A natural DNase-actin complex has been demonstrated so far only in the rat pancreatic juice by Rohr & Mannherz [4] and in L1210 leukemia cells by Malicka-Błaszkiewicz & Roth [5]. We have also some preliminary data on the presence of the natural DNase-actin complex in the rat liver nucleoplasm [6]. DNase I-like activities accompanying considerable actin concentration were demonstrated in the liver cytosol of various vertebrate species [7, 8]. They appear in the liver cytosol in a latent form and can be detected only after prior denaturation of actin.

Our previous studies have shown that carp liver cytosol is a rich source of DNase I-like enzyme. This enzyme, similarly to DNase I, was inhibited by rabbit skeletal muscle actin and endogenous actin isolated from the carp liver cytosol [9]. The aim of this study was to find out whether a natural DNase-actin complex exists in the carp liver cytosol.

For experiments, fresh livers of carp (*Cyprinus carpio*) were used.

Homogenates were made in conditions described previously [7] except that 0.1 mM phenylmethylsulfonyl fluoride (PMSF) was added to the homogenization buffer A (10 mM Tris/HCl, pH 7.4; 0.1 mM ATP; 1 mM dithioerythritol; 0.1 mM $CaCl_2$) containing 0.25 M sucrose. The supernatant (105000 × g) served as the cytosolic fraction and was used immediately or frozen at –70°C.

DNase activity was measured spectrophotometrically at pH 7.0 in 40 mM Hepes buffer (*N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid]) in the presence of 5 mM $MgCl_2$ and 1 mM $CaCl_2$, with highly polymerized calf thymus DNA (Sigma) as a substrate, according to Malicka-Błaszkiewicz & Roth [10]. One unit of DNase activity is the amount of the enzyme giving the absorption increase at 260 nm by 0.1.

Actin content was determined by the inhibition of added exogenous DNase I from bovine pancreas (Sigma) in the standard DNase assay conditions [10]. One unit of DNase I inhibitor is the amount which decreases the activity of 20 ng of DNase I by 10% [10].

Protein was estimated spectrophotometrically at 280 nm and at 290 nm in the case of buffers containing ATP or by the standard Lowry procedure [11].

Ion exchange chromatography of the carp liver cytosol was performed essentially in conditions described by Gordon *et al.* [12] except

[1]Abbreviations: M_m, molecular mass; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

that the cytosol was dialyzed for 24 h against buffer A before it was applied to the column. Endogenous DNase activity and DNase I inhibitor (actin) were estimated in elution profile (see Fig. 1). Free DNase activity (measured by the standard test tube assay [10]) was found only in the fraction of nonadsorbed proteins in the chromatographic conditions applied, whereas actin (measured as exogenous DNase I inhibitor) was eluted from the column at 0.2–0.4 M KCl concentration. Nonadsorbed fractions containing DNase activity (Fig. 1) were subjected to molecular filtration on Sephacryl S-300 (Fig. 2). DNase activity was separated during molecular filtration into two peaks, I and II. Protein of both peaks was separated in SDS-PAGE[1] (according to Laemmli) [13] and analysed by Coomassie brilliant blue staining (Fig. 3A) and with respect to their endogenous DNase activity (Fig. 3B). Two bands of DNase activity with a mobility close to that of standard DNase I were found in DNase zymograms in both peaks, one band with a mobility identical to DNase I (31 kDa) and another one with lower mobility (cf. Fig. 3B a, b, c). There was no activity of the inhibitor of DNase I (actin) in the corresponding part of the elution profile but in peak I, among many other proteins, a protein band with the mobility equal to M_m of α-rabbit skeletal muscle actin (43 kDa) was found.

Rabbit antiserum directed against carp liver DNase, previously purified by Malicka-Błaszkiewicz *et al.* [9], and goat antiserum directed against α-rabbit skeletal muscle actin were used to identify DNase and actin, respectively, in peak I and II after Sephacryl filtration (Fig.

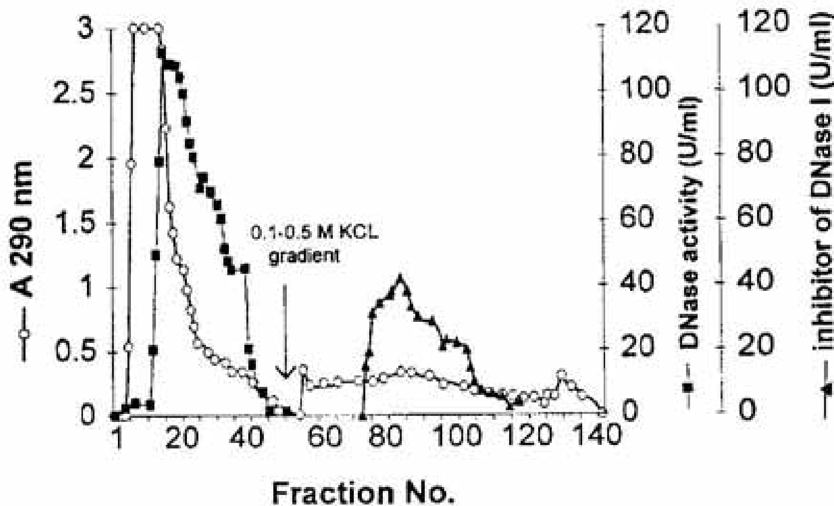

Fig. 1. Ion exchange chromatography of carp liver cytosol. DEAE-cellulose column (2.5 × 18 cm) was equilibrated with buffer A containing 0.1 M KCl. Elution was carried out with the same buffer and then with a linear KCl concentration gradient (0.1–0.5 M). Nonadsorbed fractions containing DNase activity (12–42) were concentrated with $(NH_4)_2SO_4$ (80% saturation) and subjected for further experiments.

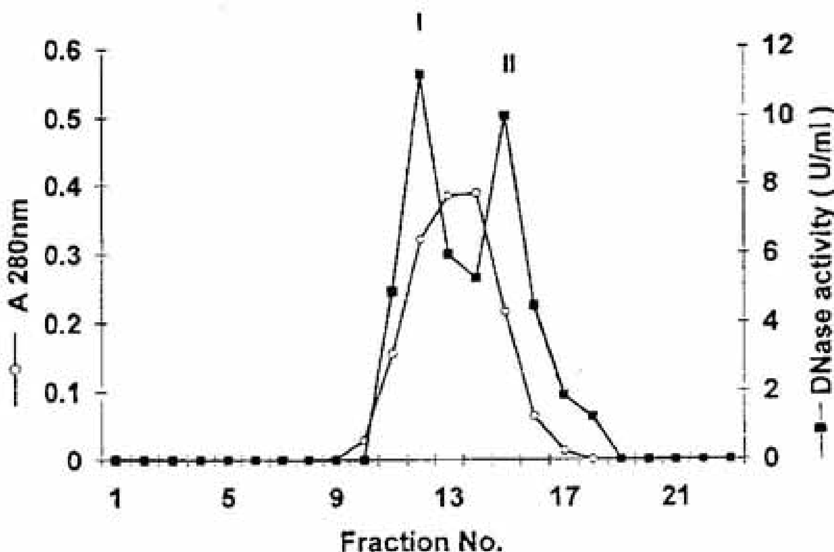

Fig. 2. Molecular filtration of fractions with DNase activity. Sephacryl S-300 column (0.9 × 48 cm) was equilibrated with buffer A without ATP and elution was carried with the same buffer. Active fractions of peak I (11–14) and peak II (15–17) were pooled and subjected for further analysis.

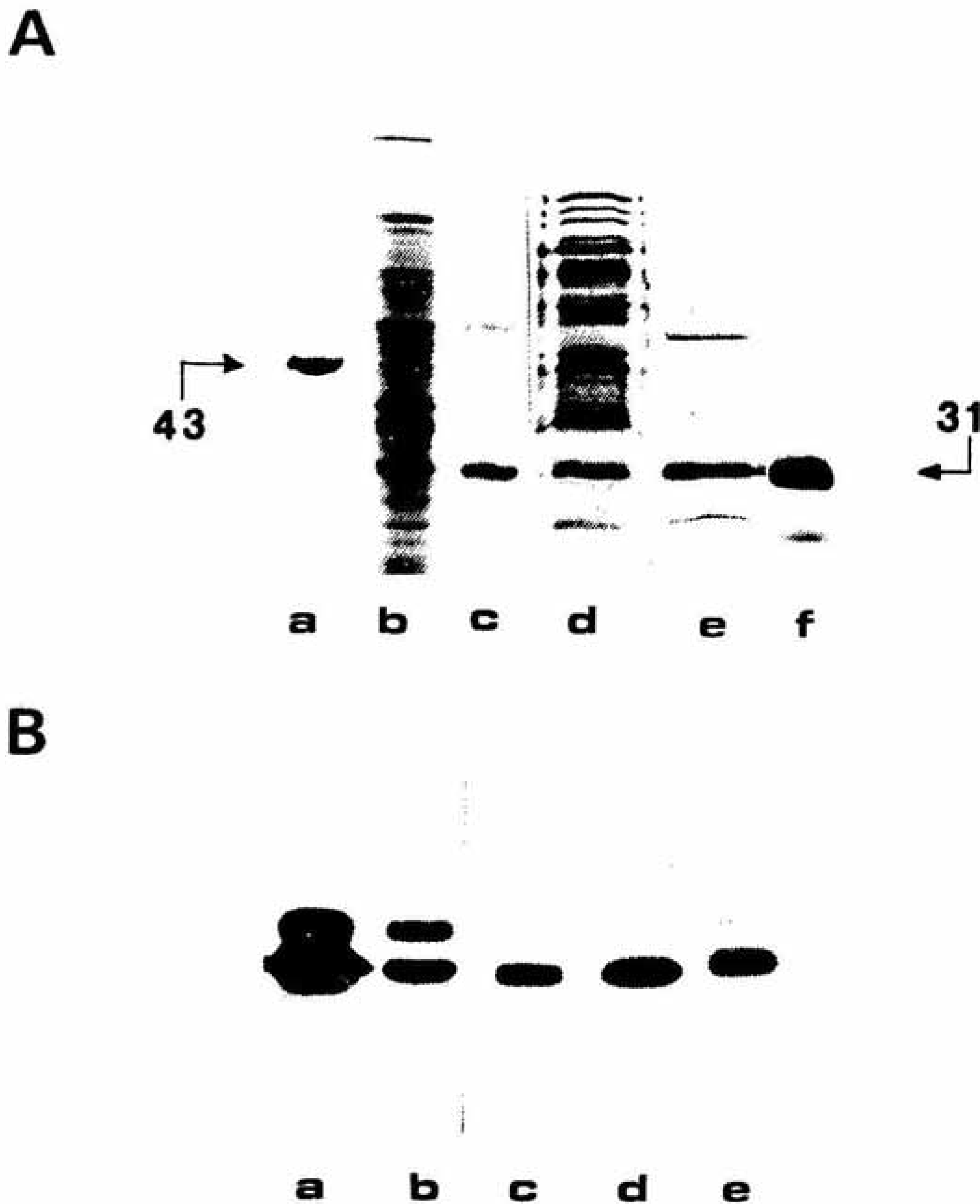

Fig. 3. Electrophoretic analysis of the examined samples.
A. Coomassie brilliant blue staining. SDS-PAGE [13] in 10% polyacrylamide gel; a, α-rabbit skeletal muscle actin, 10 µg; b, carp liver cytosol, 33 µg; c, nonadsorbed fractions (Fig. 1), 47 µg; d, peak I (Fig. 2), 78 µg; e, peak II (Fig. 2), 43 µg; f, DNase I from bovine pancreas, 24 µg.
B. DNase zymograms. SDS-PAGE, as described in part A [13]. Calf thymus DNA 20 µg and 10 µg of bovine serum albumin per 1 ml of separating gels. After electrophoresis the gels were washed to remove SDS and DNase activity was developed in the conditions described by Lacks [15]; a, peak II (Fig. 2), 51 µg; b, peak I (Fig. 2), 55 µg; c, DNase I from bovine pancreas, 0.8 ng; d, nonadsorbed fractions (Fig. 1), 47 µg; e, carp liver cytosol, 33 µg.

4A and B). Both the collected fractions of peak I and peak II (Fig. 2) showed the presence of DNase on immunoblots (Fig. 4A b, c) but actin was identified only in fractions of peak I (Fig. 4B b).

DNase of peak I appeared earlier during Sephacryl filtration than the rest of DNase activity, suggesting to be bound into a higher molecular mass complex with an other protein, which was presumably actin.

Our results confirm the former observation [9] that DNase I-like enzyme occurs in a free form in the carp liver cytosol; however, some of its activity seems to be bound in a natural complex with cytoplasmic actin. Free DNase activity observed during molecular filtration in fractions of peak I, containing the presumable

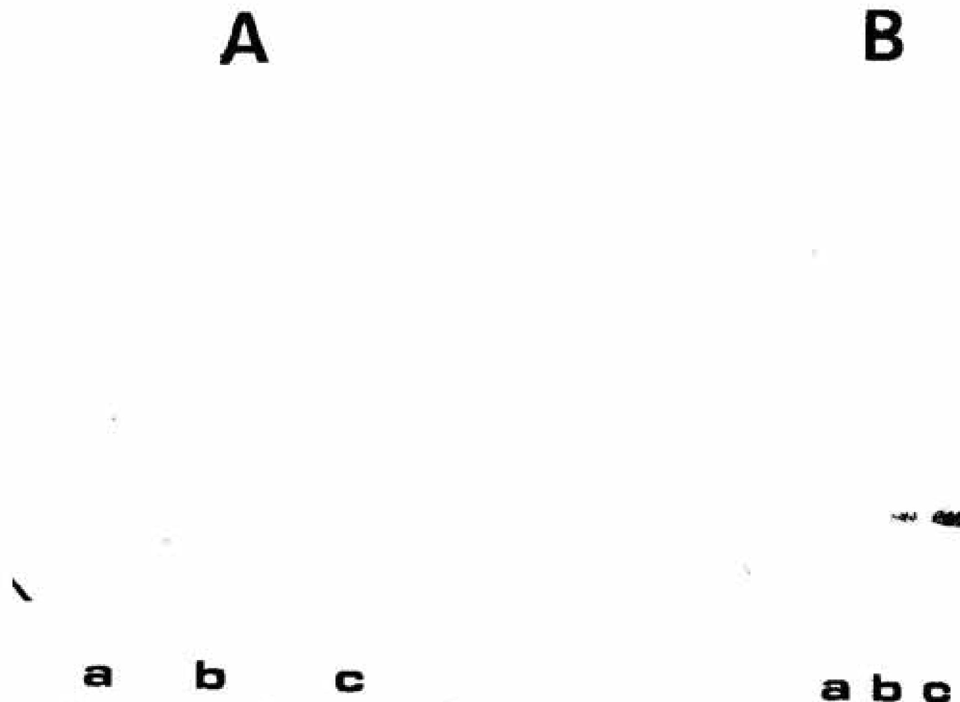

Fig. 4. Immunoblotting of carp liver DNase and actin.
Immunoblotting according to Towbin *et al.* [16] after SDS-PAGE. The following sera were used: A, rabbit antiserum against DNase isolated from carp liver; B, goat antiserum against α-actin from rabbit skeletal muscle; A, a, nonadsorbed fractions (Fig. 1), 94 µg; b, peak I (Fig. 2), 78 µg; c, peak II (Fig. 2), 42 µg; B, a, peak II (Fig. 2), 42 µg; b, peak I (Fig. 2), 78 µg; c, α-rabbit skeletal muscle actin, 10 µg.

DNase-actin complex, could be caused by actin denaturation in the absence of ATP in the elution buffer, and actin desensitization [14].

Further studies on isolation of the complex, its purification and identification of its components are in progress.

REFERENCES

1. Hitchcock, S.E., Carlson, L. & Lindberg, U. (1976) *Cell* **7**, 531–542.
2. Mannherz, H.G., Barrington Leigh, J., Leberman, R. & Pfrang, H. (1975) *FEBS Lett.* **60**, 34–38.
3. Mannherz, H.G., Kabsch, W. & Leberman, R. (1977) *FEBS Lett.* **73**, 141–143.
4. Rohr, G. & Mannherz, H.G. (1978) *Eur. J. Biochem.* **89**, 151–167.
5. Malicka-Błaszkiewicz, M. & Roth, J. (1983) *FEBS Lett.* **153**, 235–239.
6. Malicka-Błaszkiewicz, M. (1990) *Z. Naturforsch.* **45c**, 1165–1170.
7. Malicka-Błaszkiewicz, M. (1986) *Comp. Biochem. Physiol.* **84B**, 207–209.
8. Malicka-Błaszkiewicz, M. (1988) *Acta Universitatis Wratislaviensis* **1048**, 77–81.
9. Malicka-Błaszkiewicz, M., Majcher, I. & Nowak, D. (1992) *32nd Annual A.S.C.B. Meeting*, Abstracts, p. 154a, Denver, Colorado, U.S.A.
10. Malicka-Błaszkiewicz, M. & Roth, J. (1981) *Biochem. Biophys. Res. Commun.* **102**, 594–601.
11. Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951) *J. Biol. Chem.* **193**, 265–275.
12. Gordon, D.J., Eisenberg, E. & Korn, E.D. (1976) *J. Biol. Chem.* **251**, 4778–4786.
13. Laemmli, U.K. (1970) *Nature (London)* **227**, 680–685.
14. Dieckhoff, J. & Mannherz, H.G. (1985) *Biochim. Biophys. Acta* **829**, 209–220.
15. Lacks, S.A. (1981) *J. Biol. Chem.* **256**, 2644–2648.
16. Towbin, H., Staehelin, T. & Fordin, J. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350–4354.