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The inhibition of bovine and rat parotid deoxyribonuclease I by skeletal muscle actin

A biochemical and immunocytochemical study

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Rat and bovine parotid gland and pancreas contain deoxyribonuclease I (DNAase I) activities in different amounts. The DNAase I activity in tissue homogenates of bovine and rat parotid gland can be inhibited by addition of monomeric actin, as with the enzyme of bovine pancreas. The isolated DNAase I species from bovine and rat parotid gland differ in their molecular weights and also in their affinities for monomeric actin, being lowest for rat parotid DNAase I ($5 \times 10^6 M^{-1}$). Antibodies raised against rat and bovine parotid and bovine pancreatic DNAase I can be used to study the subcellular localization of DNAase I in these tissues by indirect immunofluorescence. DNAase I was found to be confined solely to the secretory granules of the tissue from which it was isolated.

The interaction of bovine pancreatic DNAase I and actin from different sources has recently attracted much interest. Both proteins interact with highly affinity, forming a stoichiometric 1:1 complex leading to an inhibition of the DNA-degrading activity of DNAase I and of the ability of actin to form high molecular weight polymers (Lazarides & Lindberg, 1974; Mannherz *et al.*, 1975; Hitchcock *et al.*, 1976). The complex from bovine pancreatic DNAase I and rabbit skeletal muscle actin (synthetic complex) has been crystallized and its three dimensional structure has been determined to 0.6 nm resolution (Suck *et al.*, 1981). Little, however, is known about the physiological significance of this interaction, although the natural occurrence of a stoichiometric complex has been demonstrated in rat pancreatic juice (Rohr & Mannherz, 1978). The DNA-degrading activity of the complex isolated from rat pancreatic juice was found to be reactivated after incubation with rat bile for several hours (Rohr & Mannherz, 1978). The reactivating factor has been identified to be 5′-nucleotidase, which also reactivated the synthetic actin–DNAase I complex, leading to a separation of the otherwise very stable complex (Mannherz & Rohr, 1978).

Although these results do not indicate a biological significance of the actin–DNAase I interaction, they may demonstrate a general property of DNAase I, namely its ability to interact with actin, since an inhibitory effect of actin on human pancreatic DNAase I has also been found (Finakoshi *et al.*, 1977). However, the ability of DNAase I from rat tissue to interact with actin has been questioned (Lacks, 1981). Since we are presently examining the subcellular localization of DNAase I in relation to actin and other actin-binding proteins in rat and bovine tissue (D. Drenckhahn, U. Gröschel-Stewart & H. G. Mannherz, unpublished work), we therefore re-evaluated the ability of actin to inhibit DNAase I from rat tissues. The results obtained indicate that the interaction of actin and rat parotid DNAase I also occurs. This could be demonstrated for the DNAase I activity present in tissue homogenates of rat parotid gland and for the purified DNAase I from this source.

Abbreviations used: DNAase, deoxyribonuclease; AMPase, adenosine 5′-monophosphatase; Ig, immunoglobulin; p[CH_2]pA, adenosine 5′-[α,β-methylene]diphosphate; SDS, sodium dodecyl sulphate; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

Experimental

Male Wistar rats were anaesthetized with diethyl ether and bled from the heart. Organs were excised and either frozen immediately in liquid N_2 or used

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for tissue extract preparation. Bovine tissues were obtained from the local slaughter-house within about 15 min of the stunning of the animal and were frozen in liquid N_2. Frozen tissues were taken up in about 5 vol. of extraction buffer A (1 mM-$MgCl_2$, 0.1 mM-$CaCl_2$, 1 mM-NaN_3, 0.3 mM-phenylmethanesulphonyl fluoride, 1% Triton X-100 and 10 mM-Tris/HCl, pH 8.0), ground with an Ultraturax (IKA, Freiburg, Germany), and centrifuged for 30 min at 100000 *g*. The supernatants were carefully removed, avoiding mixing with a top layer of fat. Tissue homogenates were either used immediately for experimental procedures or were stored at −20°C.

Protein concentration was determined according to Bradford (1976). Rabbit skeletal muscle actin was prepared as reported earlier (Mannherz *et al.*, 1975). DNAase I and 5′-nucleotidase activities were determined by using the optical assay systems according to Kunitz (1950) and Ipata (1967), respectively, using a Schimadzu UV 300 spectrophotometer. Test solutions for DNAase I activity contained 50 μg of salmon sperm DNA (Sigma type III) in 1 mM-$MgCl_2$/0.1 mM-$CaCl_2$/10 mM-Tris/HCl, pH 8.0. Activity is expressed in Kunitz units (1 Kunitz unit equals a ΔA_{260} of 0.001 min^{-1}). The test solution for 5′-nucleotidase activity contained 0.1 mM-5′-AMP in 0.1 mM-$CaCl_2$/5 mM-Hepes, pH 7.4, supplemented with 2 μg of adenosine deaminase/ml. Proteinase activity in tissue homogenates was tested by the semiquantitative diffusion procedure using casein as substrate (Sevier, 1976). Chymotrypsin activity was tested directly according to Hummel (1959).

Antisera against DNAase I isolated from rat and bovine parotid gland and bovine pancreas were raised in rabbits after three successive installations of the antigen. For the first injection, 0.1 mg of the antigen was mixed with 1 ml of complete Freund's adjuvant and injected subcutaneously in about 0.2 ml portions along the spine. After 3 and 5 weeks 0.1 mg of the antigen mixed with 1 ml of incomplete Freund's adjuvant was injected intraperitoneally. Usually the rabbits were bled 6–7 weeks after the first application of antigen. IgG fractions from antisera were prepared according to Maschler (1973).

Two-dimensional immunodiffusion of antisera or IgG fractions against DNAase I was performed according to Ouchterlony (1958). Tissue preparation and visualization of DNAase I by indirect immunofluorescence using antisera or IgG fractions was carried out with the procedure employed in the parallel study (Hoffmann & Drenckhahn, 1982) of the localization of DNAase I in relation to actin and actin-binding proteins (D. Drenckhahn, U. Gröschel-Stewart & H. G. Mannherz, unpublished work).

Adenosine deaminase was obtained from Boehringer Mannheim. Sepharose 4B containing immobilized 5′-AMP was obtained from Pharmacia. p[CH_2]pA was a commercial product from Miles, and 5′-nucleotidase from snake venom (*Crotalus adamanteus*) was obtained from Sigma.

Electrophoresis on polyacrylamide gels in the presence of sodium dodecyl sulphate was carried out according to Laemmli (1970).

Results

Inhibition of DNAase I in rat and bovine parotid gland tissue homogenates

Table 1 summarizes the distribution of DNA-degrading activity in tissue homogenates from rat and bovine parotid gland and pancreas. As tests for the identity of these activities with DNAase I, the DNA-degrading activity was tested in the presence of 10 mM-EDTA at pH 8.0 or by replacing the Tris/HCl buffer with 10 mM-sodium acetate, pH 4.7. Under these conditions there was no DNA-degrading activity detectable. Preincubation of the tissue homogenates at pH 3.0 for 10 min and 24 h after titration with 0.25 M-H_2SO_4 changed the DNA-degrading activity to the values also indicated in Table 1. Lowering the pH to 3.0 by addition of H_2SO_4 leads to a separation of any existing actin–

Table 1. *Specific DNAase I activity in rat and bovine parotid and pancreas tissue extracts expressed in Kunitz units*
Rabbit skeletal muscle actin at 0.1 mg/ml was included in 1 ml of DNAase I test solution before addition of tissue extracts. The pH of tissue extracts was lowered to 3.0 by addition of 0.25 M-H_2SO_4 at 4°C; the mixture was kept on ice.

	Enzyme activity (Kunitz units per mg of protein)				
Source	Homogenate	After addition of actin (0.1 mg/ml)	After 24 h preincubation at room temperature	Immediately after bringing pH to 3.0	24 h after bringing pH to 3.0 at 4°C
Bovine parotid	1323.5	0	442	3000	1765
Bovine pancreas	6462	0	404	7615	3924
Rat parotid	15600	750	26400	6000	825
Rat pancreas	250	0	180	350	0

DNAase I complex in the tissue homogenate. Since this treatment irreversibly denatures actin, an increase in DNA-degrading activity is observed after neutralization if the DNAase I was complexed with actin (Kunitz, 1950; Rohr & Mannherz, 1978).

The inhibitory effect of actin on the DNAase I activity of the tissue homogenates is also given in Table 1. In these experiments monomeric actin was added to the DNA-containing test solution just before the addition of the tissue homogenates. It can be seen that the DNA-degrading activity of all tissue homogenates is inhibited. This inhibition occurred immediately after adding the tissue homogenate to the actin-containing test solution.

If, however, the tissue homogenates were preincubated with increasing actin concentrations for different periods of time a reactivation of the initially inhibited DNAase I activity was observed. This effect is illustrated in Figs. 1(*a*), 1(*b*), and 1(*c*) for tissue extracts from rat and bovine parotid gland and bovine pancreas. A slow reactivation of the DNAase I activity occurred with time in all three tissue extracts, and was found to be maximal after 24h of incubation in bovine and rat parotid gland extract. Bovine pancreatic tissue homogenate exhibits a similar reactivation up to 5h; after 24h of incubation, however, no DNA-degrading activity was detectable at all. This latter effect was attributed to the action of proteinases, which are present in bovine pancreatic tissue extract in large excess over DNAase I (Keller *et al.*, 1958). In contrast, tissue homogenates of the parotid gland exhibited only low proteinase and chymotryptic activities when analysed as described in the Experimental section (Table 2).

Therefore an additional component in parotid gland homogenates was assumed to be responsible for the reactivation of the DNAase I activity after inhibition with actin. Since 5′-nucleotidase isolated from snake venom or rat bile has been shown to reactivate the DNAase I activity of synthetic actin–DNAase I complexes (Rohr & Mannherz, 1978; Mannherz & Rohr, 1978), the tissue homogenates were analysed for AMPase activity. Distinction of 5′-nucleotidase from phosphatase activity was achieved by selective inhibition of 5′-nucleotidase after addition of 10μM-p[CH_2]pA (Burger & Lowenstein, 1970). The results are summarized in

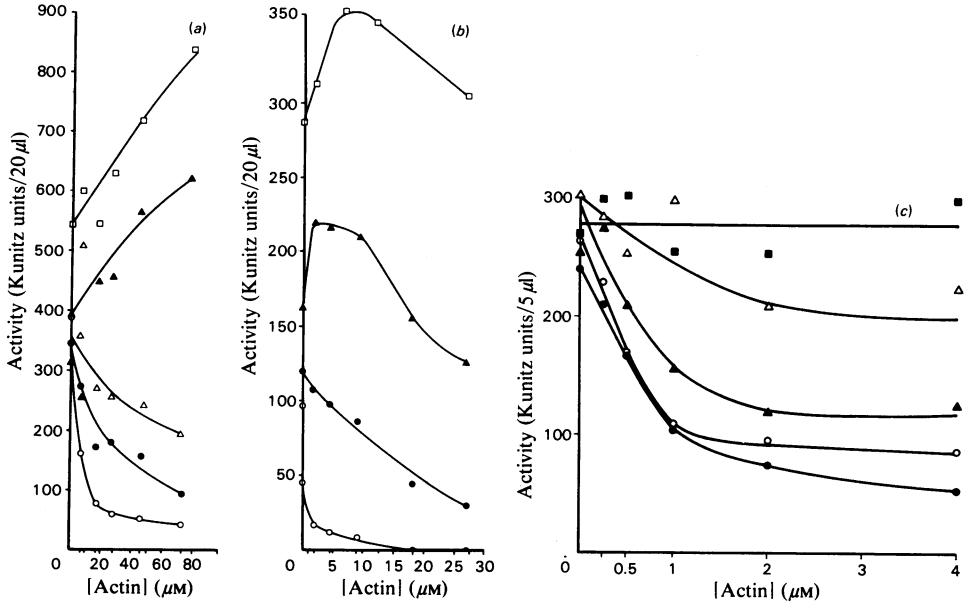


Fig. 1. *Time-dependent reactivation of DNAase I activity of tissue extracts after inhibition with increasing concentrations of rabbit skeletal muscle actin*

(*a*) Rat (1.2mg/ml) and (*b*) bovine (5.8mg/ml) parotid tissue extracts were preincubated with actin: (○) immediately after mixing tissue extract with actin; (●) after 125min; (△) after 220min; (▲) after 5.5h; and (□) after 24h of preincubation at room temperature. (*c*) Bovine pancreas homogenate (9mg/ml) was preincubated with actin: (●) immediately after mixing; (○) after 70min; (▲) after 140min; (△) after 210min; and (■) after 5h. After 24h no activity was detectable. DNAase I activity was determined as described in the Experimental section in 1ml of test solution at 25°C. Activity is expressed in Kunitz units per μl of incubation solution. One Kunitz unit equals a ΔA_{260} of 0.001 min^{-1}.

Table 2. *Chymotryptic and 5'-nucleotidase activities in rat and bovine tissue extracts*

Source	Chymotryptic specific activity in i.u.	5'-Nucleotidase activity (μmol of AMP hydrolysed/min per mg of protein)	
		No addition	After addition of 10^{-5} M-p[CH_2]pA
Bovine			
pancreas	76.15	0	0
parotid gland	2.18	0.07	0
Rat			
pancreas	12.2	0	0
parotid gland	1.53	0.186	0.015

Table 2. In order to demonstrate that the endogenous 5'-nucleotidase was responsible for the time-dependent reactivation of DNAase I activity after inhibition with actin, rat parotid extract was passed over a Sepharose 4B column containing immobilized 5'-AMP, which is known to retain specifically 5'-nucleotidase (2ml bed volume per ml of tissue extract) (Mannherz & Rohr, 1978; Dornand *et al.*, 1978). The fractions containing DNAase I activity were concentrated by precipitation with $(NH_4)_2SO_4$ at 70% saturation and, after dialysis against buffer A, were analysed for their specific DNAase I and 5'-nucleotidase activities. In the experiment shown in Fig. 2 the specific DNAase I activity increased from 27600 to 34270 Kunitz units per mg of protein whereas the specific AMPase activity decreased from 23 to 0.83pmol of AMP hydrolysed/min per mg of protein after passage over 5'-AMP–Sepharose 4B. From Fig. 2 it can be seen that the time-dependent reactivation of DNAase I activity after inhibition with skeletal muscle actin is almost abolished after removal of most of the 5'-nucleotidase activity.

The presence of proteinase activity in the tissue homogenates could also be made responsible for the relief of the inhibitory effect of actin on DNAase I due to a proteolytic degradation of the added actin. Although this possibility cannot be completely ruled out, a number of points make it appear unlikely that this is the major reason for the observed time-dependent reactivation of DNAase I activity in the bovine and rat parotid tissue homogenates. First, the tissue homogenates were treated with phenylmethanesulphonyl fluoride to block proteinase activity, unless chymotrypsin was tested (Table 2). Second, passage of rat parotid tissue homogenate over 5'-AMP Sepharose 4B should only have led to a retention of 5'-nucleotidase and not of contaminating proteinases, yet the time-dependent reactivation of actin-inhibited DNAase I was lost for the rat parotid homogenate.

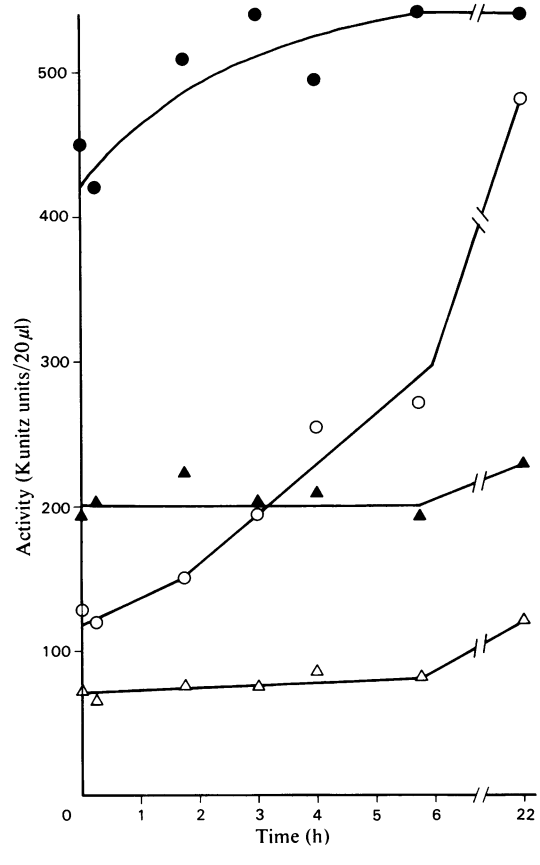


Fig. 2. *Time-dependent reactivation of DNAase I activity of rat parotid tissue extract before and after passage over a Sepharose 4B column containing immobilized 5'-AMP*

Rat parotid homogenate (0.6mg/ml) before chromatography without (●) and (○) with preincubation with 51μM-actin at room temperature. Rat parotid homogenate (0.29mg/ml) after chromatography without (▲) and (△) with preincubation with 51μM-actin at room temperature.

Inhibitory action of actin on isolated rat parotid gland DNAase I

To support further the finding of an inhibitory effect of monomeric actin on rat parotid gland DNAase I, this enzyme was isolated following a protocol initially developed for the isolation of bovine parotid gland DNAase I (V. Kreuder & H. G. Mannherz, unpublished work). In brief, this procedure involves chromatography of the tissue homogenates first over a DEAE-cellulose column, from which DNAase I activity is eluted at about 60mM-sodium phosphate buffer, pH8.0, using a linear gradient from 30mM to 0.2M phosphate buffer. The fractions containing DNAase I are concentrated by precipitation with 70% saturation of $(NH_4)_2SO_4$ and applied to a Sephadex G-75 column eluted with buffer A. Finally, the fractions containing DNAase I are chromatographed on a hydroxyapatite column from which the enzyme can be eluted in pure form at 30mM-sodium phosphate, pH6.8, again using a linear gradient ranging from 10 to 100mM-phosphate buffer, pH6.8. This procedure yields almost pure DNAase I as judged by SDS/polyacrylamide-gel electrophoresis. Fig. 3 illustrates the purity of DNAase I isolated from various sources on SDS/polyacrylamide-gel electrophoresis. DNAase I isolated from bovine and rat parotid gland exhibits a slightly higher molecular weight as judged by this technique. At present, however, the possibility of this difference being due to an altered degree or composition of glycosylation cannot be excluded. To confirm that this enzyme was DNAase I, the dependence of its enzymic activity on pH and ionic composition was tested. Its ability to hydrolyse native double-stranded DNA was optimal over a pH range from 7 to 8 and completely depended on the presence of divalent cations, with identical characteristics to bovine pancreatic DNAase I, i.e. its activity is completely inhibited when 2mM-EDTA (final concn.) was added to the DNA test solution or when the Tris buffer, pH8.0, was replaced by 10mM-sodium acetate, pH4.7. Furthermore, at optimal conditions its DNA-degrading activity is clearly inhibited by rabbit skeletal muscle G-actin (Fig. 4). The inhibitory action of G-actin on rat parotid gland DNAase I occurs immediately after mixing both proteins in a cuvette containing the DNA test solution. When both proteins are incubated at room temperature for 24h, no reactivation of the actin-inhibited DNAase I activity was observed (Fig. 5). No inhibition of rat parotid DNAase I was observed when actin polymerized in the presence of an equimolar concentration of the toadstool phalloidin was added, whereas when F-actin was added only a slight instantaneous inhibition was observed which remained constant even after prolonged incubation (Fig. 5).

The latter result indicates that rat parotid gland DNAase I is not able to depolymerize F-actin. From Fig. 4 it can be seen that, at an equimolar concentration of actin, rat parotid DNAase I is only inhibited by 15%, whereas under identical conditions bovine pancreatic DNAase I is inhibited by 80–90% (Mannherz *et al.*, 1980). A possible explanation may be a reduced affinity of rat parotid gland DNAase I for rabbit skeletal muscle actin (in comparison with the bovine pancreatic enzyme). Since rat parotid gland DNAase I is inhibited by rabbit skeletal muscle actin in a competitive manner, as is bovine pancreatic DNAase I (Mannherz *et al.*, 1980), the K_i for actin was determined to be 1.8×10^{-7}M from the experiment shown in Fig. 6. For bovine pancreatic DNAase I it was shown that

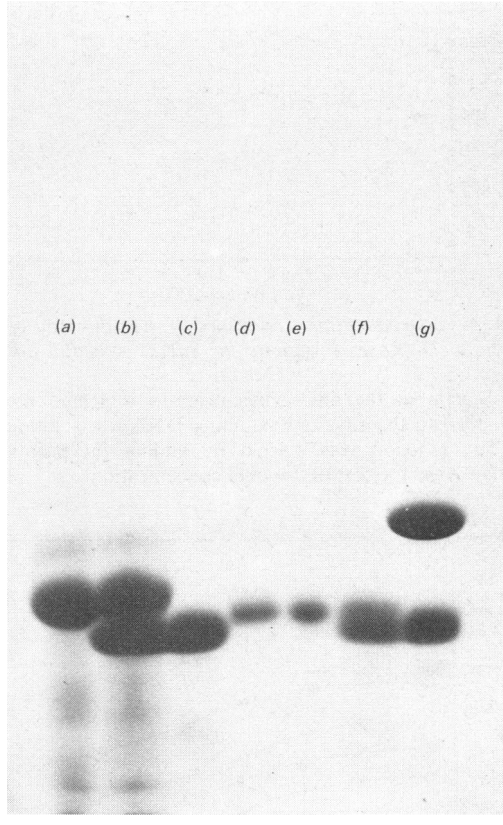


Fig. 3. *SDS/polyacrylamide-gel electrophoresis of DNAase I from various sources*

(*a*) DNAase I obtained from bovine parotid gland (apparent M_r 35000); (*b*) comigration of bovine parotid and pancreatic DNAase I; (*c*) bovine pancreatic DNAase I; (*d*) and (*e*) rat parotid DNAase I (apparent M_r about 32000), (*f*) comigration of rat parotid and bovine pancreatic DNAase I; (*g*) synthetic actin–DNAase I complex (bovine pancreatic DNAase I).

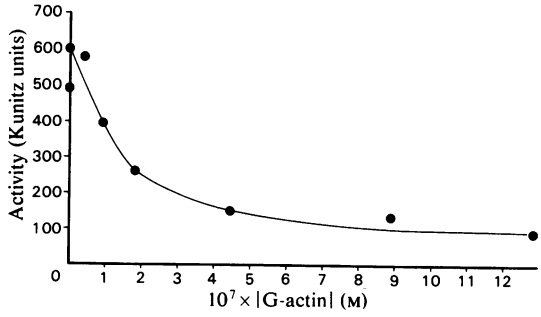


Fig. 4. *Concentration-dependence of the inhibition of rat parotid DNAase I activity to rabbit skeletal muscle G-actin*

G-actin at the final concentrations indicated was added to the cuvette containing DNA test solution. The reaction was started by adding rat parotid DNAase I to 5×10^{-8} M final concentration.

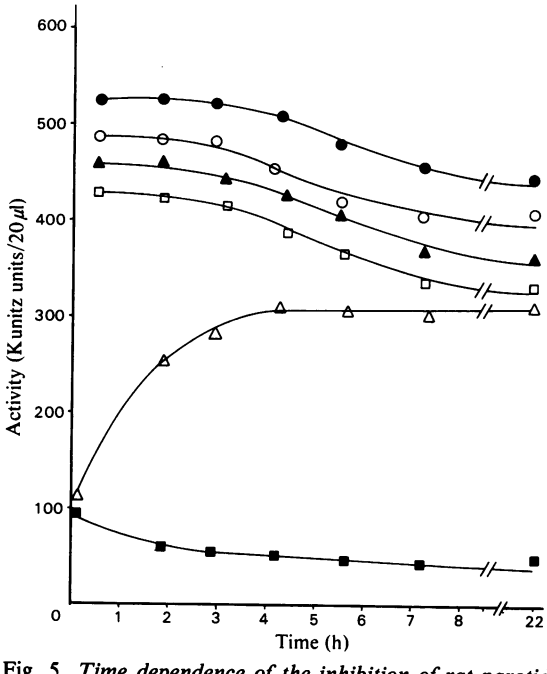


Fig. 5. *Time dependence of the inhibition of rat parotid DNAase I activity by various polymeric forms of rabbit skeletal muscle actin*

DNAase I (3.4 µM) was incubated with 68.5 µM-actin at 25°C. At the time intervals indicated 20 µl was used to test the DNA-degrading activity in 1 ml of DNA test solution at 25°C. (●) DNAase I alone; (○) DNAase I plus 1 mM-$MgCl_2$; (▲) DNAase I plus actin polymerized in the presence of 8.75 µM-phalloidin by addition of 1 mM-$MgCl_2$; (□) DNAase I plus F-actin (1 mM-$MgCl_2$); (■) DNAase I plus monomeric actin; (△) DNAase I mixed with monomeric actin for 5 min, followed by addition of 1 mM-$MgCl_2$ final concentration.

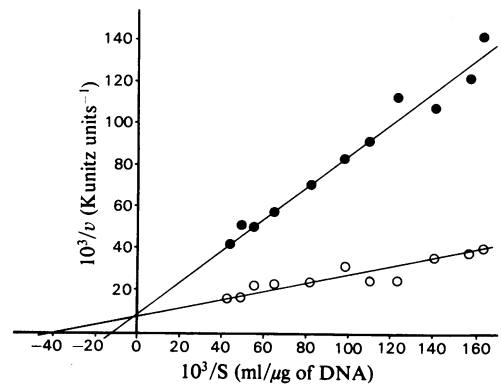


Fig. 6. *Lineweaver–Burk plot of the inhibition of rat parotid DNAase I by rabbit skeletal muscle G-actin*

G-actin was mixed into 3 ml test solutions of increasing DNA concentrations at 1.7×10^{-7} M final concentration. Reaction was started by adding DNAase I to a final concentration of 2×10^{-8} M.

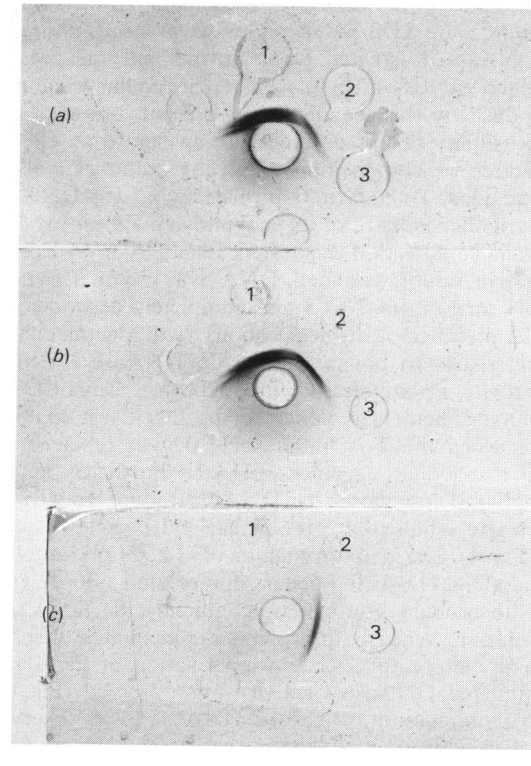
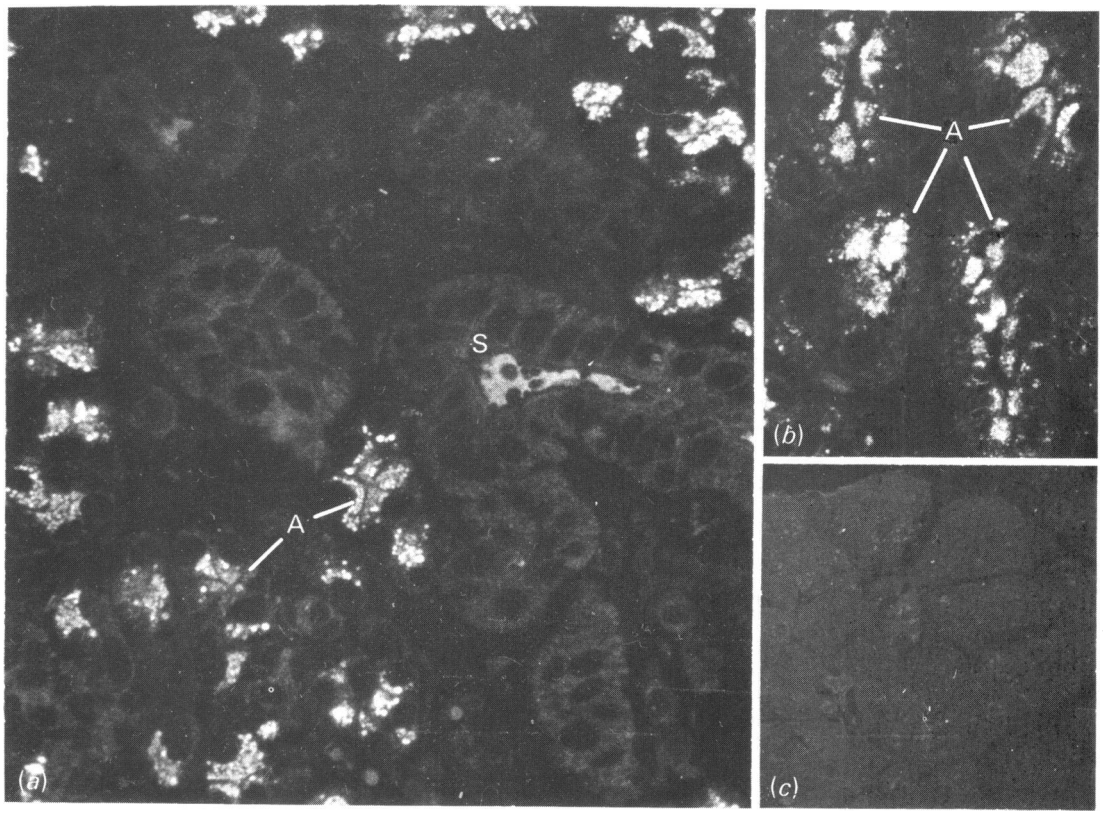


Fig. 7. *Double immunodiffusion according to Ouchterlony of antisera against (a) bovine pancreatic, (b) bovine parotid and (c) rat parotid DNAase I (centre well)*

Outer wells: (1) bovine pancreatic, (2) bovine parotid and (3) rat parotid DNAase I.



EXPLANATION OF PLATE 1

Immunolocalization of DNAase I

Tissue sections were incubated at room temperature with antiserum against rat parotid DNAase I after 20-fold dilution with phosphate-buffered saline for 30 min. After extensive washing with phosphate-buffered saline, fluorescamine-conjugated anti-(rabbit IgG) was used to visualize specifically attached antibodies. (*a*) Rat parotid gland: note fluorescence staining of the secretory granules of the secretory cells of the end pieces (A), no staining of the cells of the striated ducts, but staining of the secretory product present in the striated ducts (S). (*b*) Bovine parotid gland, similar distribution of anti-(DNAase I) but fluorescence much weaker. (*c*) Bovine pancreas, no visualization of DNAase I-like immunoreactivity.

Table 3. *Scheme of the cross-reactivity of the antisera elicited against DNAase I from various sources*
Immunodiffusion (ID) and immunofluorescence (IF) techniques were used; abbreviation: n.d., not determined.

		Cross-reactivity					
	Antibody ...	Anti-(bovine pancreatic DNAase I)		Anti-(bovine parotid DNAase I)		Anti-(rat parotid DNAase I)	
Tissue or source of DNAase I	Technique ...	ID	IF	ID	IF	ID	IF
Bovine pancreas		++	++	++	++	0	0
Bovine parotid		++	++	++	++	0	+
Rat parotid		0	0	0	+	++	++
Rat pancreas		n.d.	0	n.d.	0	n.d.	0

$K_i = 1/K_B = 5 \times 10^8 \text{ M}^{-1}$, where K_B is the binding constant of actin to DNAase I (Mannherz *et al.*, 1980). Assuming that the same relationship is applicable to rat parotid DNAase I, $K_B = 5 \times 10^6 \text{ M}^{-1}$, indicating a reduced affinity of rat parotid DNAase I for rabbit skeletal muscle actin. DNAase I isolated from bovine parotid gland by the procedure outlined above exhibits an M_r of about 35000 as judged by SDS/polyacrylamide gel electrophoresis (Fig. 3; see also Lacks, 1981). Its enzymic activity is also inhibited by actin in accordance with Lacks (1981) (results not shown).

Subcellular localization of DNAase I in rat parotid gland and pancreatic tissue

Specific antibodies against DNAase I from bovine pancreas and parotid gland and against rat parotid gland were raised in rabbits with the protocol given in the Experimental section. A partial tissue- and species-specific behaviour of the antisera and purified IgG fractions obtained was observed: antibodies raised against DNAase I from bovine pancreas and parotid gland showed full crossreactivity as judged by immunoprecipitation, but no reactivity against rat parotid DNAase I as judged by the same criterion (Fig. 7 and Table 3) and vice versa.

The antibodies raised against DNAase I from the sources indicated were used to determine the subcellular localization of DNAase I in bovine and rat exocrine glands. For these experiments, animals fasted for 24 h were taken whose digestive exocrine glands contain a high concentration of secretory granules with large amounts of digestive enzymes. Thin sections of bovine and rat parotid gland and pancreas were stained for immunofluorescence with anti-(bovine pancreatic DNAase I), anti-(bovine parotid DNAase I), and anti-(rat parotid DNAase I). All three antibodies reacted strongly with the zymogen granules of the glands from which the antigens was isolated (Plate 1). The immunofluorescence could be suppressed after incubation of the antisera with the respective DNAase I in complex with actin. The antibody raised against bovine pancreatic DNAase I, however, exhibited only species-specific cross-reactivity.

In addition there was some degree of species crossreactivity using immunofluorescence as criterion, as also listed in Table 3. Antisera against bovine parotid DNAase I also slightly stained the secretory granules of rat parotid gland and vice versa. None of the antibodies obtained, however, exhibited any specific immunofluorescence staining of rat pancreas; see also Plate 1.

Discussion

The results presented clearly indicate that the DNAase I activity in both rat and bovine parotid tissue extracts is inhibited by monomeric actin. During prolonged incubation of parotid gland tissue extracts, however, the inhibitory action is reversed. This reversal appears to be due to the simultaneous presence of 5′-nucleotidase in the parotid tissue extracts of both species. Since this enzyme has been shown to reverse the inhibitory action of actin on DNAase I (Rohr & Mannherz, 1978; Mannherz & Rohr, 1978) we believe that an identical process occurs after mixing the tissue extracts with monomeric actin. This assumption is sustained by the observation that the slow reactivation of DNAase I activity is nearly completely abolished after selective removal of 5′-nucleotidase activity by affinity chromatography. Therefore the experiments presented can also be taken as additional evidence for the interaction of 5′-nucleotidase with the actin–DNAase I complex, although very little is presently known about the mode of this interaction.

This notion is fully corroborated by the results obtained with purified rat parotid gland DNAase I. Although the DNAase I from this source appears not to be identical in its molecular structure with bovine pancreatic DNAase I, as judged by its molecular weight and antigenicity, its enzymic activity can be similarly inhibited by monomeric actin. Its affinity for actin was preliminarily determined to be $5 \times 10^6 \text{ M}^{-1}$, which indicates a decrease in the actin-binding constant by a factor of 100 in com-

parison with bovine pancreas DNAase I (Mannherz *et al.*, 1980). This might explain its inability to depolymerize filamentous actin, if one assumes that the depolymerization of F-actin by DNAase I is due to a shift in the G–F equilibrium of actin (Mannherz *et al.*, 1980); the critical concentration of polymerization of actin under these conditions is below 1 μM at 1 mM-$MgCl_2$ in the presence of ATP (Engel *et al.*, 1977).

The results presented are in contrast to a recent report by Lacks (1981) that demonstrated the inability of actin to inhibit rat parotid DNAase I activity. The reasons for this discrepancy are difficult to evaluate, since different assay procedures and conditions for DNAase I were employed. We believe the simultaneous presence of 5′-nucleotidase in rat parotid extract to be responsible for the reported lack of actin inhibition after prolonged incubation, especially when low concentrations of actin are used (Lacks, 1981). In his report Lacks (1981) used an elegant technique of enzyme detection after gel electrophoresis in the presence of SDS, which involves denaturation and renaturation of the enzymes to be investigated (Rosenthal & Lacks, 1977). Although it is possible to detect DNAase I activity from rat and bovine tissue extract using this technique, it is possible that the actin-binding ability of DNAase I is changed in a species-specific mode after this procedure. Species differences in the resistance to low pH between DNAase I from rat and bovine sources are illustrated in Table 1. Furthermore, the reduced affinity of rat parotid DNAase I for monomeric actin and its inability to depolymerize filamentous actin might exclude by itself the interaction of actin and rat DNAase I entrapped in a polyacrylamide gel.

The subcellular localization of DNAase I in the rat parotid gland was also found to be confined to the secretory granules, as in the respective bovine organs (Kraehenbuhl *et al.*, 1977; D. Drenckhahn, U. Gröschel-Stewart & H. G. Mannherz, unpublished work). With the antisera available no specific staining of rat pancreas could be obtained. This result is in agreement with the published relative distribution of DNAase I activity in various rat organs (Lacks, 1981). In contrast, Rohr & Mannherz (1978) reported the presence of DNAase I in complex with actin in rat pancreatic juice, although with low specific activity, which might indicate that this enzyme is only present in minute amounts in rat pancreatic juice and the exocrine pancreatic cell. Taking into account the species and tissue specificity of the DNAase I antisera obtained, the lack of staining of rat pancreas could be explained by tissue differences in the types of DNAase I isolated and their respective antibodies or/and its low concentration in rat pancreas. Further experiments will be necessary to solve conclusively this question.

From these studies, however, it appears that DNAase I is a secretory protein that acts enzymically extracellularly in the digestive tract. Its ability to interact with actin remains therefore difficult to explain functionally, since an intracellular regulatory effect of DNAase I on the polymeric state of actin seems to be excluded by its segregation into secretory granules. Further subcellular localization studies of DNAase I in other tissues where its presence has been reported will be necessary before a final answer to this question can be given. It is interesting to note that 5′-nucleotidase activity could also be detected in bovine and rat parotid gland biochemically. Preliminary experiments using an antibody raised against 5′-nucleotidase from snake venom also stained the zymogen granules of rat parotid gland. Whether this enzyme is a true secretory product of this organ in rats, as in snakes, cannot presently be decided, since its presence in rat parotid secretion has not been reported yet. The possibility, however, remains that this enzyme can also exist in a secretory form as well as in its classical type, namely being incorporated predominantly within the plasma membrane (for a recent report see Widnell *et al.*, 1982).

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References

Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
Burger, R. M. & Lowenstein, J. M. (1970) *J. Biol. Chem.* **245**, 6274–6280
Dornand, J., Bonnafous, J. C. & Mani, J. C. (1978) *Eur. J. Biochem.* **87**, 459–465
Engel, J., Fasold, H., Hulla, F. W., Waechter, F. & Wegener, A. (1977) *Mol. Cell. Biochem.* **18**, 3–13
Finakoshi, A., Tsubota, Y., Wakasuki, H., Ibayashi, H. & Takagi, Y. (1977) *J. Biochem.* (*Tokyo*) **82**, 1771–1777
Hitchcock, S. E., Carlsson, L. & Lindberg, U. (1976) *Cell.* **7**, 531–542
Hoffmann, H. D. & Drenckhahn, D. (1981) *Cell Tissue Res.* **221**, 77–83
Hummel, B. C. W. (1959) *Can. J. Biochem. Physiol.* **37**, 1393–1399
Ipata, P. L. (1967) *Anal. Biochem.* **74**, 592–596
Keller, P. J., Cohen, E. & Neurath, H. (1958) *J. Biol. Chem.* **233**, 344–349
Kraehenbuhl, J. P., Racine, L. & Jamieson, J. D. (1977) *J. Cell Biol.* **72**, 406–423
Kunitz, M. (1950) *J. Gen. Physiol.* **33**, 349–362
Lacks, S. A. (1981) *J. Biol. Chem.* **256**, 2644–2648
Laemmli, U. K. (1970) *Nature* (*London*) **227**, 680–685
Lazarides, E. & Lindberg, U. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4742–4746
Mannherz, H. G. & Rohr, G. (1978) *FEBS Lett.* **95**, 284–289

Mannherz, H. G., Barrington-Leigh, J., Leberman, R. & Pfrang, H. (1975) *FEBS Lett.* **60**, 34–38
Mannherz, H. G., Goody, R. S., Konrad, M. & Nowak, E. (1980) *Eur. J. Biochem.* **104**, 367–379
Maschler, R. (1973) Ph.D. Thesis, Free University, Berlin
Ouchterlony, O. (1958) *Prog. Allergy* **5**, 1–78
Rohr, G. & Mannherz, H. G. (1978) *Eur. J. Biochem.* **89**, 151–157
Rosenthal, A. L. & Lacks, S. A. (1977) *Anal. Biochem.* **80**, 76–90
Sevier, E. D. (1976) *Anal. Biochem.* **74**, 592–596
Suck, D., Kabsch, W. & Mannherz, H. G. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 4319–4323
Widnell, C. C., Schneider, Y.-J., Pierre, B., Baudhuin, P. & Trouet, A. (1982) *Cell* **28**, 61–70