Deoxyribonuclease I in Mammalian Tissues

SPECIFICITY OF INHIBITION BY ACTIN*

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Enzymes of the DNase I class, similar to bovine pancreatic DNase I with respect to molecular weight and ionic and pH requirements, were found in various tissues of the rat. Their analysis was facilitated by a method for detection of nucleases in crude extracts after polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and subsequent renaturation of the enzymes. High levels of DNase I were found in digestive tissues, such as the parotid and submaxillary salivary glands and the lining of the small intestine. Appreciable levels were present in the lymph node, kidney, heart, prostate gland, and seminal vesicle. No activity was found in pancreatic extracts. However, under some conditions, tissues rich in proteases gave poor recovery of DNase I. Fourteen other tissues showed little or no DNase I.

Inhibition of various DNase I enzymes by rabbit muscle actin was examined both in gels and in solution. Actin inhibited the bovine parotid DNase I as well as the bovine pancreatic enzyme, but actin did not inhibit any of the DNase I enzymes of the rat. This species specificity of actin inhibition makes it unlikely that the very strong association between monomeric actin and bovine DNase I is of general significance for cellular function.

Deoxyribonucleases in prokaryotic cells participate in a variety of metabolic functions including genetic recombination (Radding, 1978), repair of DNA damage (Grossman et al., 1975), restriction of foreign DNA (Arber, 1974), and transport of DNA into cells (Lacks, 1977). A number of mammalian DNases have been identified (Sierakowska and Shugar, 1977), but the two that have been best characterized appear to carry out, principally, digestive functions. Bovine pancreatic DNase I acts in the gut in a nutritional capacity (Laskowski, 1971). The lysosomal DNase II presumably serves a scavenging function in phagosomes (de Duve and Wattiaux, 1966). However, enzymes similar to pancreatic DNase I in pH optimum and ionic requirements have been reported in tissues where a digestive function would be unexpected (Cunningham and Laskowski, 1953; Shack, 1957).

One object of the present work was to determine the distribution of DNase I-like enzymes in tissues of the rat. This information could ultimately help reveal a possible function of such enzymes in the differentiation or physiological role of particular cell types.

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A novel technique for rapidly screening tissues for nucleases (Rosenthal and Lacks, 1977), by renaturation of the enzymes after electrophoresis in polyacrylamide gels in the presence of SDS, has been used in this study. This technique, which isolates the nucleases electrophoretically, detects their activity in the gel, and identifies them by their molecular weight, is applicable to other enzymes, as well (Lacks and Springhorn, 1980). A particular advantage of the method is that it liberates the enzymes from inhibitors that may be associated with them in an extract. The effect of activators or inhibitors on the renatured enzyme in the gel can then be tested.

A variety of eukaryotic cells contain a specific protein inhibitor of bovine pancreatic DNase I (Cooper et al., 1960). Lindberg and co-workers characterized the inhibitor (Lindberg, 1967; Lindberg and Skoog, 1970), which is a major component of the cell protein, and identified it as monomeric actin (Lazarides and Lindberg, 1974; Hitchcock et al., 1976). The enzyme and actin form a 1:1 complex with a binding constant of $5 \times 10^6$ M$^{-1}$ (Manneker et al., 1975, 1980). The widespread occurrence of actin in cells and its high affinity for DNase I suggested that the interaction of these two proteins may be of general significance for either the function of actin in cell motility or the physiological function of DNase I. It was considered worthwhile, therefore, to examine the generality of actin inhibition of DNase I-like enzymes from different tissues and different species.

EXPERIMENTAL PROCEDURES

Preparation of Tissue Extracts—Male, 200-g Sprague-Dawley rats were anaesthetized with ether and bled from the heart. Blood cells were separated by centrifugation. Internal organs were identified (according to Greene, 1955), excised, weighed, and frozen. Samples were thawed, minced, and homogenized in a ground-glass tissue grinder with ~5 volumes of ice-cold 10 mM Tris, pH 7.6, containing PMSF, 0.3 mg/ml. Large particulate residues were removed manually, and the extracts were stored at $-20^\circ$C. Extracts of bovine pancreas and parotid glands, which were obtained from Pel-Freez, were similarly prepared, except that only the darker tissue of the parotid gland, which contains the DNase, was used. Protein was determined by the method of Lowry et al. (1951).

Enzyme Detection after SDS-Gel Electrophoresis—Polyacrylamide gel electrophoresis was carried out in the presence of SDS, and nuclease activity was detected essentially as previously described (Rosenthal and Lacks, 1977). Either calf thymus DNA (Worthington) or $^{32}$P-labeled DNA prepared from *Escherichia coli* (Lacks et al., 1967), at 10 μg/ml, and BSA (Relheis), also at 10 μg/ml, were incorporated into the separating gel, which contained 10% acrylamide and 0.54% bisacrylamide. Tissue extracts were diluted in 10 mM Tris, pH 7.6. Purified enzymes, bovine pancreatic DNase I (Worthington) and micrococcal nuclease (Worthington), were diluted in the same buffer containing BSA at 0.1%. Samples were denatured by heating for 2 min at 100°C in 1% SDS and 1% 2-mercaptoethanol. Electrophoresis was carried out with 0.05% SDS (Matheson, Coleman and Bell, lot 27) in the

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1 The abbreviations used are: SDS, sodium dodecyl sulfate; PMSF, phenylmethylsulfonyl fluoride; BSA, bovine serum albumin.
electrode buffer, after which the gels were washed for four 30-min periods with 200-ml portions of 40 mM Tris, pH 7.6, to remove the SDS (Lacks et al., 1979). The gels were then incubated at room temperature (22°C) in 40 mM Tris, pH 7.6, 2 mM MgCl₂, 2 mM CaCl₂ (except when indicated otherwise). Ethidium bromide was added to 2 µg/ml, and the gels were photographed under ultraviolet light. Nuclease activity appeared as dark bands against a fluorescent background of DNA-bound ethidium bromide. Gels containing [³²P]DNA were also dried and radioautographed.

Protease was assayed in gels as previously described (Lacks and Springhorn, 1980). The separating gel contained 15% acrylamide, 0.4% bisacrylamide, and fibrinogen (Sigma) at 170 µg/ml. Samples were heated for 2 min at 100°C in 1% SDS, but without 2-mercaptoethanol. The gels were washed and incubated as indicated above. After 3 days they were stained with Coomassie blue.

**Inhibition by Actin**—Rabbit skeletal muscle actin (Worthington) was dissolved at 1 mg/ml in 4 mM Tris, pH 8.0, 0.5 mM CaCl₂, 0.5 mM ATP, and diluted further in 10 mM Tris, pH 7.6. Gel electrophoresis and nuclease detection were carried out as above, except that after washing, the slab gel was divided into sections and incubated in 40 mM Tris, pH 7.6, with or without actin at 10 µg/ml for 4 to 16 h. Then MgCl₂ and CaCl₂ were added to each dish to 2 mM, to allow nuclease activity, and incubation was continued. Ethidium bromide was added, and the gels were photographed under ultraviolet illumination.

DNAse was assayed by the release of perchloric acid-soluble radioactivity from substrate mixtures (200 µl) containing 10 mM Tris, pH 7.6, 50 mM NaCl, 2 mM MgCl₂, 2 mM CaCl₂, 80 µg of BSA, and 1 µg of [³²P]DNA. Enzyme samples were diluted in 10 mM Tris, pH 7.6, containing 0.1% BSA. Actin was added to the DNAse immediately before incubation with the substrate at 30°C for 90 min.

**RESULTS**

**Rat Tissue DNase I Content**—Crude extracts of various rat tissues were analyzed for DNAse activity by subjecting them to electrophoresis in the presence of SDS in polyacrylamide gels containing DNA. Polypeptide components of proteins in the extract, which were separated by the electrophoresis, were renatured upon removal of the SDS. Incubation under appropriate ionic conditions and pH allowed DNAse action, which was detected by staining DNA in the gel with ethidium bromide and illumination with ultraviolet light. DNAse activity was revealed as dark bands against a fluorescent background. Because electrophoresis was in the presence of SDS, the position of the activity band indicated the molecular weight of the active polypeptide. Furthermore, the SDS would have dissociated any protein inhibitor bound to the enzyme in vivo.

Extracts of 21 different rat tissues or organs and 2 bovine tissues were examined. The only DNAse activity observed at neutral or alkaline pH with double-stranded DNA in the gel was similar to purified bovine pancreatic DNAse I. Fig. 1 shows that tissues of 7 rat organs contained DNAse of approximately 31,000 molecular weight. Tissues from the small intestine, lymph node, kidney, prostate gland, and seminal vesicle (lanes 4 to 8) gave bands that migrated identically with the bovine pancreatic enzyme. Slightly slower moving bands were seen in the parotid and submaxillary glands, and as secondary bands in the intestine and lymph node (lanes 2 to 5). A still slower moving band seen in the kidney (lane 6) corresponded in mobility to one observed in bovine parotid tissue (see Fig. 2, below lane 2). Different bands may represent different forms of DNAse I, which could arise from variation in the carbohydrate moiety attached to the enzyme polypeptide. Such variation has been found in bovine pancreatic DNAse I (Salnikow et al., 1970).

Despite the slight differences in mobility, the enzymes corresponding to all of the bands of molecular weight approximately 31,000 showed similar requirements for activity, so they will be referred to as DNAse I. They also degraded single-stranded DNA in the gel. Their requirements for divalent cations and pH are illustrated in Fig. 2. Here, purified bovine pancreatic DNAse I and the enzymes in crude extracts of bovine parotid tissue and two rat organs were compared. The enzymes were all much more active at pH 7.6 (Fig. 2A) than at pH 5.4 (B). For optimal activity they required both magnesium and calcium ions (A). They were much less active with magnesium alone (C), and showed no activity with calcium alone (D). The rat enzymes appeared to be slightly more active than the bovine with only magnesium ions present.

In addition to the activities shown in Fig. 1, DNAse I was found in rat heart tissue, and trace amounts were observed.
Subsequent to SDS-polyacrylamide gel electrophoresis and renaturation, the intensity and width of DNase bands of $M_c \approx 31,000$ were compared to that given by 40 pg of pure bovine pancreatic DNase I after various periods of incubation. Relative activity was estimated over the range 0.02 to 2.0 (equivalent to 0.8 to 80 pg). This value was divided by the amount of protein in the tissue extract applied.

<table>
<thead>
<tr>
<th>Source of tissue</th>
<th>DNase I content (pg/μg protein)</th>
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</thead>
<tbody>
<tr>
<td>Parotid gland</td>
<td>30,000.</td>
</tr>
<tr>
<td>Small intestine</td>
<td>200.</td>
</tr>
<tr>
<td>Submaxillary gland</td>
<td>40.</td>
</tr>
<tr>
<td>Kidney</td>
<td>8.</td>
</tr>
<tr>
<td>Lymph node</td>
<td>4.</td>
</tr>
<tr>
<td>Heart</td>
<td>1.</td>
</tr>
<tr>
<td>Prostate gland</td>
<td>1.</td>
</tr>
<tr>
<td>Seminal vesicle</td>
<td>0.2</td>
</tr>
<tr>
<td>Lung</td>
<td>0.04</td>
</tr>
<tr>
<td>Blood cells</td>
<td>0.04</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.02</td>
</tr>
<tr>
<td>Medulla</td>
<td>0.02</td>
</tr>
<tr>
<td>Thymus, spleen, pancreas, liver, skeletal muscle, stomach, testis, adrenal gland, cerebrum</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

(with several days of incubation) in rat lung, cerebrum, medulla, and blood cells (data not shown). No DNase I was observed in thymus, spleen, pancreas, or liver (Fig. 1, lanes 10 to 13), nor in a number of other tissues listed in Table I. The slightly dark bands in the lower part of lanes 9 to 11 (Fig. 1) result from binding of DNA to histones (Rosenthal and Lacks, 1978). They were not evident in the radioautogram of the dried gel (not shown) in which DNase bands appeared as clear areas lacking [32P]DNA. A semiquantitative estimate of the amounts of DNase I in various tissues, which was determined from the quantity of protein applied and the intensity of the activity band in the gel compared to the pure DNase I standard, is given in Table I. The largest amounts were found in tissues that secrete enzymes into the gut, the parotid and submaxillary glands, and the intestine. However, lymph nodes and urogenital tissues also showed significant activities. Multiple samples of these tissues, from the same and different animals, were compared. Although differences as great as 2-fold were encountered, the results listed in Table I can be considered representative.

When crude extracts were treated with SDS prior to dilution and sampling, for most tissues no difference in activity was observed compared to samples of the native extracts that were treated with SDS after dilution. However, prior treatment of extracts of the intestine and the submaxillary gland with SDS caused rapid loss of DNase I activity (data not shown). Both of these extracts were found to contain large amounts of proteolytic activity (Fig. 3, lanes 1 and 2). The assay used to demonstrate proteases after SDS-polyacrylamide gel electrophoresis depended on hydrolysis of fibrinogen incorporated in the gel (Lacks and Springhorn, 1980). Digestion by proteases gave colorless bands in a blue background after staining by Coomassie blue. Prior inhibition of serine proteases with PMSF did not prevent their detection (data not shown) because the reaction with PMSF is reversible on denaturation of the protease (Gold and Fahmey, 1964). Most tissue extracts contained little protease activity (Fig. 3, lanes 5 to 11). Presumably, the loss of DNase activity in submaxillary and intestinal extracts was due to proteolytic destruction of the enzyme polypeptide when it was unfolded by SDS in the concentrated extracts. The rat pancreas, also, contained considerable protease activity (lane 4). No DNase I was detected in this tissue with or without prior SDS treatment. However, another enzyme, a-amylase, was readily detected in pancreatic extracts, and 20 pg of pure bovine pancreatic DNase I was still detected as a band, although of diminished intensity, after mixing with a crude rat pancreatic extract containing 20 μg of protein (data not shown).

**Inhibition by Actin**—The ability of rabbit muscle actin to inhibit bovine pancreatic DNase I after its renaturation within a polyacrylamide gel is shown in Fig. 4, lane 3. Although high concentrations of actin were necessary, actin molecules were able to enter the gel and inhibit the enzyme. Micrococcal nuclease, separated in the same lane, was not inhibited, as expected from earlier work (Lindberg and Skoog, 1970). How-

![Fig. 3. Proteases in rat tissues.](image)

![Fig. 4. Actin inhibition of DNases in gels.](image)
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Fig. 5. Actin inhibition in gels of DNase I from bovine and rat tissues. Parallel samples containing DNase I were subjected to electrophoresis in sections of an SDS-polyacrylamide gel containing bovine thymus DNA. Section A was incubated without actin in 40 mm Tris, pH 7.6, at 22°C. Section B was incubated in the same buffer, but with rabbit muscle actin at 10 μg/ml. After 4½ h, 2 mg each of MgCl₂ and CaCl₂ were added, and incubation was continued for 19 h. Lanes 1, pure bovine pancreatic DNase I, 0.2 ng. Lanes 2 to 5, crude tissue extracts containing protein as follows: Lane 2, bovine pancreas, 40 ng; 3, bovine parotid gland, 6 ng; 4, rat parotid gland, 20 ng; 5, rat small intestine, 15 μg; 6, rat submaxillary gland, 25 μg.

FIG. 6. Actin inhibition in solution of DNase I from different sources. Various amounts of rabbit muscle actin were added to samples of enzyme immediately before addition of the substrate solution containing 1 μg of [³²P]DNA, specific activity, 250 cpm/μg. The reaction mixtures (200 μl) were incubated for 60 min at 30°C. The reaction was terminated by addition of perchloric acid to 1.75% (w/v), and the acid-soluble radioactivity was determined with a low background (2 cpm) planchet counter. The enzyme samples were tested at the protein concentrations indicated below. Symbols: (●—●) pure bovine pancreatic DNase I, 3 ng/ml; (○—○) crude bovine pancreatic extract, 1.2 μg/ml; (Δ—Δ) crude bovine parotid extract, 0.5 μg/ml; (□—□) crude rat parotid extract, 0.8 μg/ml. Without actin the proportion of substrate hydrolyzed was, respectively, 18, 26, 30, and 43%.

ever, the DNase I enzymes from rat lymph node and seminal vesicle, also, were not inhibited (lanes 1 and 2).

To see whether the lack of inhibition of rat tissue DNase I was related to its presence in a crude extract, a similarly prepared extract of bovine pancreas was examined (Fig. 5, lane 2). The enzyme in this extract was inhibited as readily as its purified counterpart (lane 1). The possibility of tissue specificity was examined by comparing the bovine parotid DNase I (lane 3). This enzyme was inhibited similarly to the pancreatic enzyme of the same species. The parotid DNase I of the rat, however, was not inhibited (lane 4), and neither were the enzymes from the intestine nor submaxillary gland of the rat. Thus, it is concluded that actin inhibition of DNase I is not general. Neither is it tissue-specific. It is species-specific in that it is limited to DNase I of the cow as opposed to the rat.

The validity of the analysis of actin inhibition in gels was confirmed with a more conventional assay of DNase activity. Fig. 6 shows that with such an assay, DNase I in crude extracts of bovine tissues was indeed inhibited, whereas the corresponding enzyme in a rat parotid extract was not. Furthermore, this experiment, in which the native rat parotid DNase I was not inhibited, showed that the absence of actin inhibition of rat enzymes in gels did not result from the cycle of denaturation and renaturation.

DISCUSSION

The identification, here, of rat tissue enzymes as DNase I, a classification introduced by Cunningham and Laskowski (1953), was based on five criteria: substrate specificity, nature of products, pH dependence, ionic requirements, and molecular weight. All of the enzymes acted on both double-stranded and single-stranded DNA, formed low molecular weight (diffusible) products, and were most active at pH 7 to 8. They required both Mg²⁺ and Ca²⁺ for activity, as does the bovine pancreatic DNase I prototype (Wiberg, 1958; Price, 1975). Their molecular weights were close to that of the bovine pancreatic enzyme, 31,000 (Lindberg, 1967b).

However, enzymes from different tissues, or even the same tissue, migrated to slightly different positions in SDS-polyacrylamide gels. At least four distinct mobilities were observed. They may correspond to polypeptides of identical length but linked to different carbohydrate moieties. This view is supported by the behavior of the bovine pancreatic extract, which gave two closely spaced bands (Fig. 5A, lane 2). These may correspond to the two forms of DNase I, identical in amino acid composition, but differing in carbohydrate side chains, that were isolated from the bovine pancreas by Salnikow et al. (1970). The bovine parotid DNase I corresponded, predominantly, to a more slowly migrating band (Fig. 5A, lane 2). This enzyme was shown to be immunologically distinct from the pancreatic enzyme (Lundblad et al., 1973), and this distinction could also result from a different carbohydrate moiety. However, the possibility that the multiple bands correspond to polypeptides of different composition or length, which could result either from multiple genes or from protein processing, cannot be ruled out.

Recovery of DNase I in the gel system was quite reproducible. Only with tissues of the intestine and submaxillary gland, which have high protease activity, were problems encountered. Here, DNase I bands were reproducible as long as SDS was not added to the concentrated extract. Addition of SDS to diluted extracts, followed immediately by boiling, apparently prevented protease attack on the nuclease. Actin did not inhibit the DNase I activity of either tissue. However, rat intestinal DNase I was reported to be inhibited by an extract of the crop gland of the pigeon (Lee and Zbarsky, 1967), but the nature of the inhibitor was not determined. The high level of DNase I in the parotid gland of rodents was previously observed (Sreebny et al., 1967; Ball, 1971). Together with the enzyme in another salivary gland, the submaxillary, and in the small intestine, the parotid enzyme presumably serves a digestive function in the gut.

No DNase I was found in the rat pancreas in this study or previously (Sreebny et al., 1967). Rohr and Manherz (1978) reported that rat pancreatic tissue extracts and pancreatic juice contained DNase activity that was inhibited by rabbit...
muscle actin. The discrepancy between their report and the present study, in which DNase I from various rat tissues was not inhibited by actin, remains unresolved. However, aside from its inhibition by actin, the DNase examined by Rohr and Mannherz (1978) was not specifically shown to be like DNase I in its enzymatic properties. No attempt was made in the present study to examine the enzyme in pancreatic juice. It is unlikely that differences in actin inhibition are attributable to different methods of extraction because the same procedure was used here to prepare both bovine and rat tissue extracts, although only the former were inhibited by actin.

The present study was limited to the examination of tissues in whole organs, but the approach should be applicable to selected tissue types and subcellular fractions, and it would complement histochemical techniques for DNase localization (Daoust, 1965). Such information could help elucidate the function of DNase I in a complex organ such as the kidney. Conceivably, the enzyme has a scavenging function in this organ. DNase I was consistently present in lymph nodes, although its level varied. It may be speculated that the enzyme plays a role in DNA recombination during differentiation of cells in the nodes. Specific DNA rearrangements accompany the differentiation of antibody-producing cells (Bernard et al., 1978; Davis et al., 1980). Treatment of chromatin with DNase I cleaves globin DNA at particular sites in a tissue-specific way (Stalder et al., 1980). This site-specific cleavage requires interaction of the chromatin with specific nuclear proteins. Similar cleavage, guided by proteins specific for DNA sequence and developmental stage, could conceivably initiate recombinational switches of immunoglobulin DNA. Alternatively, the lymph node enzyme may participate in the digestion of phagocytosed material, although the latter function is generally attributed to lysosomal DNase II (de Duve and Wattiaux, 1966).

In the only other tissues with significant activity, the prostate gland and the seminal vesicle, the DNase I produced may function in the resorption of sperm.

The results of the present study on the inhibition of DNase I by actin appear to be unequivocal. DNase I from either of the bovine tissues, but from none of the rat tissues, was inhibited. Actin inhibition, therefore, is species-specific. This specificity presumably resides in the amino acid sequence of the protein because differently migrating bands, which presumably are indicative of different carbohydrate side chains, responded similarly in each species. The lack of generality of actin inhibition of DNase I raises doubt that the interaction of the two proteins evolved to play a general role in cells with respect to the control of either cell motility or nuclease action. The possibility remains that such a function did evolve in a limited way in the cow. But it seems more likely that the very tight binding between bovine DNase I and monomeric actin from various sources is simply a coincidence.

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