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Measurement of deoxyribonuclease I (DNase) in the serum and urine of systemic lupus erythematosus (SLE)-prone NZB/NZW mice by a new radial enzyme diffusion assay

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SUMMARY

A new radial enzyme diffusion (RED) method for the measurement of DNase activity in serum and urine is described. The sensitivity of the assay is in the range of 15·6–500 ng/ml. The assay is based on the hydrolysis of double-stranded (ds) DNA (or nucleosomes) in agarose. The specificity of the reaction for DNase I was established by showing that either EDTA in the reaction buffer or G-actin abolished DNase activity. Being a functional assay, RED has advantages over radioimmunoassay (RIA) or ELISA, since antigenic assays may also measure complexes of DNase with actin. This method was used to measure DNase activity in the sera and urine of lupus-prone mice (NZB/NZW F_1 hybrids, aged 4–6 weeks). Serum DNase activity in these mice was significantly lower (mean 9 ng/ml) than in control, normal mice of the same age and sex (mean 37 ng/ml). Concentration of DNase in the urine of 4–6-week-old female NZB/NZW F_1 hybrids (24 ng/ml) was significantly lower then in control mice (521 ng/ml). The RED method was used to measure the concentration of actin as the DNase inhibitor in serum. G-actin in the presence of ATP binds DNase and inhibits its nucleolytic activity. Since ATP is necessary for the actin inhibition of DNase I, this shows that there is actin as well as DNase I in the serum. Actin is not only ATP-dependent, but also heat-labile. Heating the sera for 10 min at 50°C increases DNase activity. This is an alternative method for measuring the concentration of actin in the serum. An almost identical estimate of actin concentration in sera of normal mice was found from the difference of DNase activity in the presence or absence of ATP (mean actin concentration = 21 ng/ml) or from the difference of DNase activity in heated and non-heated serum (mean actin concentration 18 ng/ml). We were not able to demonstrate DNase inhibitors in the urine of either control or NZB/W F_1 hybrid mice.

Keywords deoxyribonuclease systemic lupus erythematosus radial enzyme diffusion assay NZB/NZW F_1 hybrids

INTRODUCTION

DNase I is a specific endonuclease that hydrolyses double-stranded (ds) DNA to short oligonucleotides having 5′-phosphate and 3′-hydroxyl termini. For full enzymatic activity a pH of around 7·5 and Ca^{2+}, Mg^{2+} or Mn^{2+} in micromolar concentrations are required. Human serum DNase has a molecular weight of 33–38 kD. Primary structures of DNase of various species have been described, and cDNA sequences are available for human [1], rat [2] and mouse [3].

Methods for the quantification of DNase I include colorimetry [4,5], precipitation, fluorometry and viscometry. There are drawbacks with these methods due to difficulties in spectroscopy as a result of turbidity of the solution and the need for large amounts of material. Immunoreactive DNase I (immunochemical concentration) has been measured by radioimmunoassay [6]. Enzymatic activity has been assayed using ^{32}P-labelled *Escherichia coli* DNA as the substrate [7]. DNase activity in murine serum has also been assayed by a synthetic end-labelled oligonucleotide substrate assay (D. Sinicropi, personal communication). Chitrabamrung *et al*. [8] and Nadano *et al*. [9] have reported the measurement of DNase I activity in human tissues and body fluids by single radial enzyme-diffusion methods. The method is based on the hydrolysis of DNA in a DNA-agar plate. The area of hydrolysed DNA was identified by either 0.1 amido black [8] or ethidium bromide [9] staining and was proportional to the amount of enzyme in the wells.

Distribution studies show high levels of the enzyme in digestive tissues such as the parotid, the submaxillary glands, the pancreas and the lining of the small intestine. Appreciable levels of DNase I can be found in the kidney, where the enzyme probably plays a scavenging role, and in the lymph nodes and thymus [10]. Urine also contains DNase in substantial amounts. Human serum DNase concentrations have been reported in the range of 3 ng/ml

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[11] to 23·9 ng/ml [7]. Previous studies showed that abnormal DNase levels occurred in association with a variety of diseases. A high serum DNase concentration was found in patients with renal failure [6,7]; advanced liver diseases [12]; acute haemorrhagic pancreatitis [13]; breast cancer [14]; oral cancer [15] and genitourinary cancer [16]. A low concentration of urine DNase was found in patients with xeroderma pigmentosum [17]. The reduced serum activity of DNase I found in patients with chronic pancreatitis or pancreatic cancer [18] may be due to the presence of DNase inhibitors.

Several studies have found a connection between DNase I activity and the development of human or animal SLE. The hallmark of SLE is the production of anti-nucleosome antibodies and immune complexes involving DNA-containing antigens. The mechanisms responsible for the production of antibodies to DNA nucleoproteins remain obscure, although it is clear that the autoantibody response is antigen-driven [19]. A defect in nucleosome breakdown may stimulate anti-DNA nucleoprotein antibody production. Therefore, the studies of DNase and inhibitor levels in human or experimental SLE may provide an insight into the abnormalities which underlie the autoantibody formation. The possibility that the prolonged survival of DNA nucleoproteins in the extracellular fluid could be involved in the production of antinuclear antibodies was raised by Lachmann [20,21], who showed that the serum levels of heat-labile DNase I inhibitor were significantly higher in patients with SLE than in sera of normal individuals. Frost & Lachmann [22] demonstrated an increased amount of an inhibitor of DNase I in SLE sera, and Hadjiyannaki & Lachmann [23] using a viscometric technique found significantly higher concentrations of heat-labile DNase 1 inhibitor in the serum of NZB/NZW F_1 mice at the time when they develop antinuclear antibodies. The inhibitor level in hybrid mice without antinuclear antibodies was not significantly different from that found in the control groups of NZB, NZW and BALB/c mice. These findings support the hypothesis that excessive inhibition of DNase may play a part in rendering DNA nucleoproteins antigenic. Serum DNase I activity in SLE patients was shown to be lower than in healthy people or patients with rheumatoid arthritis or scleroderma [8]. DNase I has recently been implicated in cell death by apoptosis [3], a process which is accompanied by chromatin DNA degradation to 180–200 bp oligonucleosomal fragments that form a distinctive ladder in agarose gels [24]. Apoptosis-specific endonuclease, extractable from both thymocytes and lymph node cells, is functionally and antigenically indistinguishable from DNase I [25]. Impairment of the effector pathway of apoptotic processes may result in the extracellular escape of nucleosomes and stimulate anti-DNA antibody production.

Therefore, the quantification of DNase in tissues and fluids has many applications. The aim of this work is to describe a simple, reproducible and sensitive assay method for the quantification of DNase in serum and urine. The assay has been applied to the study of the serum and urine DNase concentration in lupus-prone NZB/W mice and the relevance of the detected DNase I deficit to the pathogenesis of SLE in mice.

MATERIALS AND METHODS

Agarose

Agarose was ultra pure, electrophoresis grade, low melting point (LMP) from BRL (Life Technologies Inc., Gaithersburg, MD). Agarose (2%) was dissolved in distilled water.

Calf thymus deoxyribonucleic acid

Calf thymus DNA was from Sigma-Aldrich Co. Ltd (Poole, UK). It was dissolved in distilled water at a concentration of 5 mg/ml and was stored as 0·93-ml aliquots at −70°C.

Nucleosomal DNA

Nucleosomal DNA (146 bp) was a gift from Mrs Sandra Searles (Structural Division Studies, Laboratory of Molecular Biology, Cambridge, UK). Deoxyribonuclease I (DNase I) was from bovine pancreas (Sigma-Aldrich Co.). It was dissolved to 1 μg/ml in buffer (0·05 M Tris–HCl pH 7·2 containing 0·05 M $MgCl_2$, 0·025 M $CaCl_2$, 0·0015 M NaN_3) and was kept in aliquots of 100 μl at −70°C.

Adenosine 5′-triphosphate disodium salt

Adenosine 5′-triphosphate disodium salt (ATP) was obtained from Sigma-Aldrich Co. and dissolved in distilled water to 0·004 M.

Method for the assay of deoxyribonuclease I

The method for the assay of DNase I was based on radial enzyme-diffusion in agarose gel containing the substrate (dsDNA or in an additional experiment nucleosomal DNA). To 7·9 ml of 0·05 M Tris Ca/Mg buffer, 1 ml of 0·004 M ATP, 0·93 ml of DNA at 5 mg/ml, 0·047 ml of ethidium bromide at 10 mg/ml and 10 ml of melted 2% agarose in distilled water, were added. The assay was also done without ATP in DNA/agarose buffer; 8 × 8 cm plates were poured. Wells were cut of 1 mm diameter (6 × 6 wells/plate), filled with 2-μl samples and the plates were incubated at 37°C in a plastic box, overnight. The plates were then overlaid with 0·05 M EDTA (to stop the reaction) and photographed on a UV Transilluminator (UVP Inc, San Gabriel, CA). The area of dark circles of hydrolysed DNA was scanned on an Optomax Image analyser (UM Micromeasurements, Pampisford, Cambridge, UK). The values for standards and samples were plotted and the concentration of DNase in samples was calculated using a Microplate manager computer program. The identical protocol was used for testing the effects of DNase I on nucleosomes. Nucleosomes were incorporated into agarose gel in Nunc TC plate wells of 17 mm diameter.

Standards

Two microlitres of six double dilutions of DNase in Tris/Ca/Mg buffer from 500 to 15·62 ng/ml were used.

G-actin

G-actin is a potent and specific inhibitor of DNase. Monomeric G-actin binds to and almost completely inhibits the nucleolytic activity of DNase I. Rabbit G-actin, mol. wt 43 kD, 130 mM (5·59 mg/ml) was a gift of Dr Sutherland Maciver (Structural Studies Division, Laboratory of Molecular Biology, Cambridge, UK). Actin was dissolved in 2 mM Tris at pH 8·0, 0·2 mM ATP, 0·5 mM DTT, 0·2 mM $CaCl_2$ and 1 mM NaN_3. Double dilutions of actin were made (5590 μg/ml to 5 μg/ml) and the wells of DNA-agarose plate were filled either with (i) 2 μl of each actin dilution and the plates left for a few minutes to adsorb. Then 2 μl of DNase (500 ng/ml) were poured in the wells; or (ii) 2 μl of a solution consisting of 50 μl of each actin solution (dilutions 5590–5 μg/ml) and 50 μl of DNase at 1 μg/ml. Double dilutions of DNase 500–15·6 ng/ml were used as standards.

The plates were incubated overnight at 37°C.

EDTA inhibition of DNase

EDTA inhibition of DNase was tested by chelating Ca/Mg in Tris

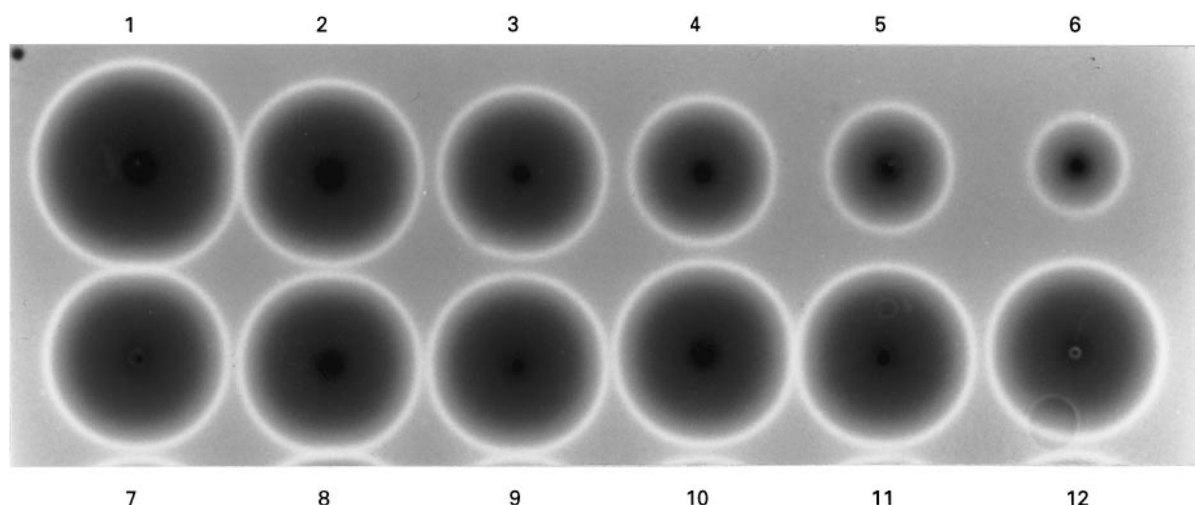

Fig. 1. Measurement of DNase activity by radial enzyme diffusion (RED) method. 1–6, DNase standards 500–15·62 ng/ml; 7–12, DNase in the urines of six control (C3H/km) mice, diluted 1 : 2.

buffer with 0·05 M EDTA. Standard DNase solutions (500–15·6 ng/ml) and normal mice sera were tested.

The effect of circulating anti-DNA antibodies on the hydrolysis of DNA by DNase

Sequential caprylic acid and saturated ammonium sulphate precipitation [26] were used to isolate IgG from 30 ml of effluent obtained after plasmapheresis treatment of two patients with active SLE and high serum anti-DNA antibody titre. The final IgG preparation was tested for the presence of anti-DNA antibodies by an indirect immunofluorescence test (antinuclear factor (ANF)) and anti-DNA antibody ELISA. IgG at 1 mg/ml was double-diluted with Tris/Mg/Ca buffer (final dilutions 0·5–0·015 mg/ml). To 50 μl of IgG solution, 50 μl of DNase at 250 ng/ml were added (final DNase concentration was 125 ng/ml) and incubated 15 min at 37°C. Of this solution, 2 μl were added to the wells of DNA-agarose plates. The hydrolysis of DNA was read after 12 h and compared with standard dilutions of DNase (500–15·6 ng/ml).

Serum concentration of DNase

The serum concentration of DNase in normal (24 female C3H/km mice 5–12 weeks old) and sera from lupus mice (24 female NZB/NZW F_1 hybrids 5–11·5 weeks old) was investigated. F_1 hybrids at this age had no serum anti-DNA antibodies either by ANF test, Farr assay or ELISA.

The effects of ATP in the Tris buffer

Serum DNase concentration was measured in the presence of 0·2 mM ATP in the reaction buffer and without ATP. Thirty normal mice sera were tested.

Detection of DNase inhibitors in sera

The radial enzyme diffusion (RED) method was applied for detecting DNase inhibitors in sera. The presence of heat-labile DNase inhibitors was examined directly in 30 unheated and heated (10 min at 50°C) normal mice sera. Of each serum sample, 2 μl were tested by the standard method for estimating DNase activity.

Urinary DNase

Concentration of DNase in the urine of control, healthy C3H/km female mice (6 weeks of age) and female NZB/NZW F_1 hybrids (4–6 weeks, 3, 4, 5, 6 and 7 months of age) was estimated by the RED method. Urine of normal mice and NZB/W F_1 hybrids (4–6 weeks old) were also tested after heating at 50°C for 10 min). Double dilutions of DNase (500–15·6 ng/ml) were used as standards. Urine was tested in dilutions of 1 : 2.

Statistical analysis

Statistical analysis was done using Student's *t*-test for two samples assuming equal variances.

RESULTS

RED

The RED method is based on the fact that ethidium bromide fluoresces only with unhydrolysed DNA and not with DNA digested by DNase. A dark circular zone, visible under UV light, is formed as DNase diffuses from the well into the agarose gel containing DNA and ethidium bromide (Fig. 1). Longer incubation increases the assay sensitivity. The diffusion radius is linearly proportional to the DNase concentration. The concentration of DNase I determined by the RED method was expressed in ng/ml using specific activity of double dilutions of purified bovine pancreatic DNase I as standard (Fig. 2).

Identical rings of DNA hydrolysis (slightly delayed) were observed when calf thymus DNA was substituted by nucleosomes (Fig. 3).

G-actin

G-actin caused no change in the immunoreactivity of DNase I, whereas it caused a striking decrease in its activity. Identical inhibition of DNase was achieved either by first adding actin to the wells of the DNA/agarose plate and then adding the DNase solution; or by pre-incubating actin and DNase and then filling the wells of the DNA/agarose gel with the mixture. Actin in concentrations of 60 ng/ml or higher completely inhibited DNase (1 ng/ml). If the concentration of actin in the wells was decreased, DNase activity increased (Fig. 4).

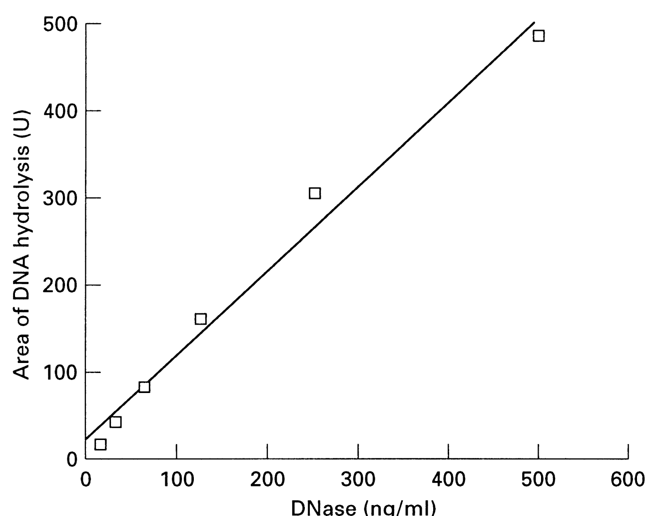

Fig. 2. Dose–response curve of bovine DNase I in DNA agarose plates. Values (ng/ml) of DNase concentration in the wells (abscissa) are plotted against means (in arbitrary units) of duplicate measurements of the area of hydrolysis. Radial enzyme diffusion method.

EDTA
EDTA abolishes DNase I activity. EDTA is a specific inhibitor of DNase, as this enzyme requires Mg^{2+} and Ca^{2+} for maximal activity. When 0·05 M EDTA was added to Tris–HCl buffer, DNase activity was inhibited and no DNA hydrolysis was seen in the gels either in the DNase standards or in normal mice sera.

Effects of anti-DNA antibodies
IgG was isolated from 30 ml of effluent from the plasmapheresis treatment of two patients with active SLE. The final IgG preparation was strongly positive for anti-DNA antibodies in the indirect immunofluorescent test (ANF = +4) and also in anti-DNA antibody ELISA (317 and 332 anti-DNA antibody units at IgG concentration of 28 and 21 mg/ml). Equal volumes of DNase (final concentration 125 ng/ml) and IgG at various dilutions were incubated for 15 min at 37°C. Afterwards, the solution was tested for DNase activity. No reduction of DNase activity was demonstrated in any of the anti-DNA-containing IgG dilutions (Table 1).

Table 1. Effect of anti-DNA-containing IgG on DNase activity

IgG, mg/ml	DNase, ng/ml
0·5	121
0·25	104
0·125	111
0·062	107
0·031	114
0·015	102
0	125

The mean serum concentration of DNase in normal mice was 37 ng/ml (coefficient of variation = 39·38%). The mean serum concentration of DNase in NZB/NZW F_1 hybrids was 10 ng/ml (coefficient of variation = 85·2%). There was a significant difference ($P < 0·0001$) between the mean serum concentration of DNase in normal mice and that in NZB/NZW hybrid mice (Table 2). The mice in both groups were 5–12 weeks old.

Effects of ATP in Tris buffer
If the assay was done without ATP in the DNA/agarose buffer, values for serum concentration of DNase I in mice sera were over 70% higher (Table 2). Serum DNase concentrations in the absence and presence of ATP were significantly different ($P < 0·001$).

Detection of DNase inhibitors in sera
The RED method was used for measuring the presence and the effects of DNase inhibitors in sera of NZB/NZW F_1 hybrids. Sera from 30 normal mice or 30 NZB/NZW F_1 hybrids, 5–12 weeks old, were tested directly in DNA/agarose plates before and after heating for 10 min at 50°C. Heated sera showed a significant ($P < 0·001$) increase in DNase activity (Table 2).

DNase in urine
The RED method was used to detect DNase in urine. Circular rings of DNA hydrolysis were visualized in urine dilutions 1 : 1–1 : 125. The urinary concentration of DNase in 6-week-old female C3H/km mice was 521 ng/ml (mean value of six tests on pooled 12 h

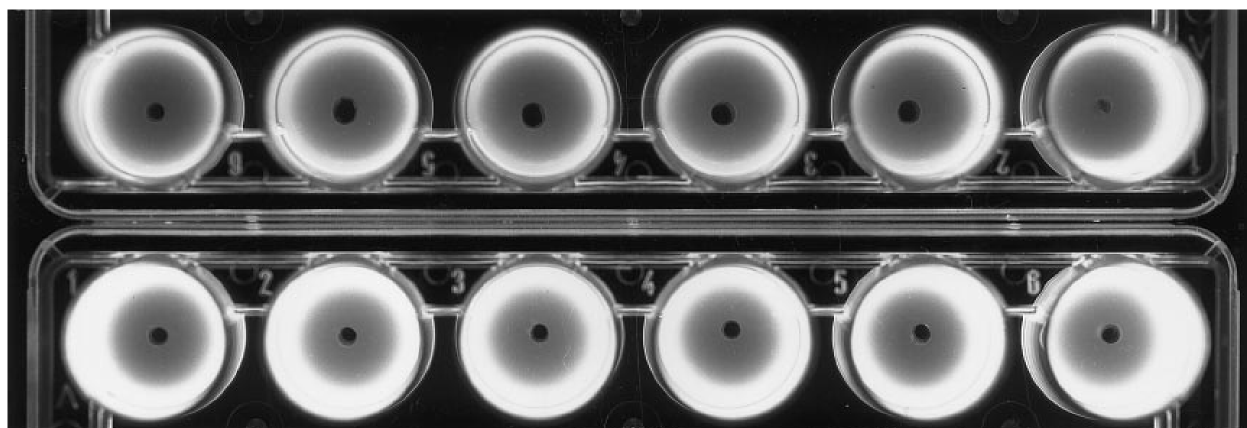

Fig. 3. Two microlitres of DNase (at 250 ng/ml)/well were incubated overnight at 37°C in calf thymus DNA/agarose gel (upper row) and 146 bp nucleosomal DNA/agarose gel (lower row). Hydrolysis of DNA was evident in both substrates, although slightly delayed in nucleosomal DNA.

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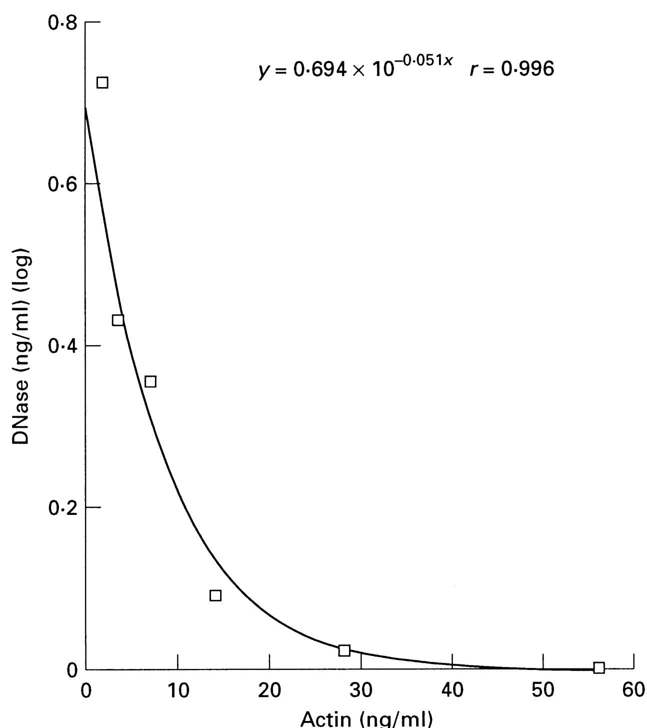

Fig. 4. Inhibition of DNase by actin. Two microlitres of actin (0·01–0·06 μg/well) were added to the wells of DNA/agarose plates. There was no actin in the first well. The plates were left for a few minutes for the actin to absorb. DNase I (0·001 μg) was then added to all the wells and incubated overnight at 37°C. Logarithmic DNase activity was determined and plotted against the concentration of actin. This gives the best fit to the data ($r = 0{\cdot}996$).

collection of urine from six cohorts, four mice/cohort). Pooled urine from NZB/NZW F_1 hybrids of the same age and sex (six cohorts, three with nine and three with 15 mice/cohort) had significantly less ($P < 0{\cdot}0001$) DNase in urine (mean = 24 ng/ml). DNase concentration in the urine pool from 10 7-month-old female NZB/NZW F_1 hybrids was even lower (15 ng/ml) (Table 3).

Heating the urine of normal mice for 10 min at 50°C did not significantly increase DNase activity.

Table 3. DNase in urine

Group	DNase, ng/ml (mean (s.e.m.))
Normal mice	521 (71)
Normal mice 10′ at 50°C	516 (99)
NZB/W F_1 (4–6 weeks old)	23 (3)
NZB/W F_1 (4–6 weeks old) 10′ at 50°C	N/A
NZB/W F_1 (3 months old)	12
NZB/W F_1 (4 months old)	29
NZB/W F_1 (5 months old)	37
NZB/W F_1 (6 months old)	32
NZB/W F_1 (7 months old)	15

DISCUSSION

The RED method described here is a simple, reproducible and sensitive technique for detecting DNase in body fluids. It was possible to detect DNase I in the range of 15·6–500 ng/ml. The test is similar in principle to the radial immunodiffusion method for measuring antigen concentration in agarose gels using a specific antibody [27]. A comparable method, but with different solutions and conditions, was used for measuring serum DNase I by Chitrabamrung *et al.* [8] and Nadano *et al.* [9]. Conditions to maximize detection of DNase—low salt concentration and a high ratio of DNA/DNase—were used. Measurement of DNase 1 under physiological conditions is more difficult, since DNA and DNA nucleoprotein are then not freely soluble and the enzyme itself shows some inhibition by salt (D. Sinicropi, personal communication). When whole nucleosomes were substituted for calf-thymus DNA, rings of hydrolysed DNA nucleoprotein of matching size were produced, showing that DNase I cuts native DNA nucleoprotein as efficiently as DNA alone.

The specificity of the method for DNase I was demonstrated *in vitro*, by showing inhibition by G-actin. The test was negative when actin was incubated with DNase prior to filling the wells of the DNA/agarose gel or when the actin solution was poured first, followed by DNase. This inhibition requires the presence of ATP in the reaction. If ATP is omitted, serum DNase levels increase by about 70%, showing the presence of actin in the serum. Lazarides & Lindberg [28] identified actin, the most abundant protein in mammalian cells, as the heat-labile inhibitor of DNase I. Actin is a 42-kD molecule with a pivotal role in the biology of cells, not only as an intracellular cytoskeletal element, but also as a protein that specifically binds to DNase I [24]. In this complex, DNase I activity is inhibited (about 95% inhibition at equimolar ratio)

Table 2. Activity of DNase and DNase inhibitor (actin) in sera of 5–12-week-old mice

Mice	No ATP	ATP		Heated	Unheated	
Normals						
DNase, ng/ml	66	37		53	27	
Log DNase	1·82	1·57	(Δ − 0·25)			(Δ − 0·29)
Actin, μg/ml			2·45			2·85
NZB/W						
DNase, ng/ml	21	9				
Log DNase	1·32	0·95	(Δ − 0·37)			
Actin, μg/ml			3·61			

Actin concentrations are calculated from the differences in DNase levels using the curve in Fig. 4. Units are given/ml of serum.

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and the actin is unable to repolymerize [9] even in the presence of high salt or other actin-binding proteins known to induce actin polymerization. Rat DNase I carries two mutations (E 13 to D and V 67 to I) responsible for the decrease in actin-binding of rat DNase I [3].

Contrary to the findings of Emlen *et al*. [29] and Pucetti *et al*. [30], we were unable to demonstrate any inhibitory or protective effect of anti-DNA antibodies on the reaction of DNase with DNA in agarose gels. Emlen *et al*. [29] have shown that a 35–45 base pair (bp) DNA fragment in SLE sera is protected from DNase digestion and remains bound to antibody, thereby forming a small, DNase-resistant DNA–anti-DNA immune complex. IgG from two of the SLE plasma exchange effluents that we tested did not block DNase digestion of DNA.

We used the RED method to detect and quantify DNase in mice sera and also in urine. Normal mice sera and sera of NZB/NZW F_1 hybrids were tested repeatedly. NZB/NZW F_1 hybrid mice (5–10·5 weeks old) had significantly lower serum concentrations of DNase (mean 10 ng/ml) than normal mice (mean 37 ng/ml). These mice develop anti-DNA antibodies (measured either by Farr assay or solid-phase ELISA) after the age of 12–16 weeks. Therefore, the effects of anti-DNA antibodies on the serum DNase concentration were excluded. Young F_1 hybrid mice have significantly less serum DNAse activity in comparison with normal mice of the same sex and age. This is true in the presence and absence of ATP, and is therefore a genuine reduction in enzyme level and not due only to the raised level of actin.

We were able to confirm earlier findings on the presence of DNase inhibitors in sera of normal and lupus mice [22]. Low molecular weight inhibitors diffuse rapidly in the gel and do not interfere with the reaction. To detect actin, the heat-labile, high molecular weight DNase inhibitor, mice sera and urine were tested unheated and heated (10 min at 50°C). Heating restored up to 70% of serum DNase activity.

Analogous to the findings in the sera, we were also able to demonstrate the presence of DNase in the urine of normal and NZB/W F_1 hybrid mice. NZB/W hybrid mice were tested at the age of 6 weeks, before the appearance of anti-DNA antibodies in their sera, and between 3 and 7 months of age, at the height of their lupus disease activity. Lupus-prone mice and mice with active lupus nephritis had significantly less DNase in the urine. This finding may have implications for the clearance of immune complexes in lupus nephritis. Urinary DNase degradation of antigen may play a significant role in breaking down the complexes in the basement membrane. Low levels of DNase I may allow more immune complexes to persist and allow disease progression. To our knowledge, this is the first report on the low concentration of DNase in urine in SLE.

The implications of the finding of low serum DNase concentration (activity) in young NZB/NZW F_1 hybrids are manifold. It has been known for decades that circulating DNA–anti-DNA immune complexes are responsible for the systemic lesions in lupus patients and in NZB/NZW mice [31]. A great deal was learned about the physicochemical and immunologic properties of anti-DNA antibodies, but data on extracellular DNA appeared to be contradictory. No agreement was reached on the origin, immunological characteristics and concentration of DNA in the blood of healthy persons and lupus patients. Labelled DNA itself was cleared rapidly from the circulation by digestion with circulating nucleases and by the liver cell surface nucleases [32]. The half life of that DNA is 4 min.

The presence of free dsDNA in the form of nucleosomes in circulation might be due to a defective clearance mechanism either intracellularly (a defect in the effector pathway of apoptosis or the defective endonuclease digestion of chromatin in the cells and leakage of nucleosomes through the plasma cell membrane) or extracellularly (a decrease in serum DNase I) due to the presence of DNase inhibitors or low expression of the genes responsible for DNase production.

Is the presence of circulating DNA nucleoprotein responsible for the induction of anti-DNA antibodies and involved in the formation of circulating immune complexes? McCoubrey-Hoyer *et al*. [33] reported that the serum concentration of DNA in normal people was in the range of 4–13 ng/ml, and in SLE patients in the range of 4–400 ng/ml. Tan *et al*. [34] detected increased levels of native (ds) DNA in some lupus sera using the gel diffusion method. Lambert & Dixon [31] found circulating DNA in sera of NZB/NZW mice. In 1973, using an assay based on the inhibition of haemagglutination, circulating ssDNA (mean concentration 53 mg/ml) was reported to occur in about 50% of patients with SLE [35]. Sano & Morimoto [36] have isolated DNA from immune complexes in SLE sera. Results from this and follow-up studies further supported the view that extracellular DNA was present in the blood and that it may play a pathogenic role in the formation of DNA–anti-DNA immune complexes [37–39].

The rate of clearance of extracellular plasma DNA in man has important implications for the pathological mechanisms of SLE. Plasma DNA in SLE consists of oligonucleosome-like molecules of 200 bp unit size. These multimeric complexes of DNA are bound to histone [40]. A decrease of serum DNase activity may play a vital role in rendering DNA nucleoproteins antigenic. Therefore, the estimation of DNase activity may be important in the studies of complex immunopathological mechanisms of SLE. Chitrabamrung *et al*. [8] demonstrated that patients with SLE had lower serum DNase activity than healthy people. They also showed a relationship between DNase concentration and SLE activity, with patients with active lupus nephritis having the lowest levels of enzymatic activity. The method used in this work offered an opportunity to re-investigate the activity of serum DNase in lupus-prone mice. Compared with control animals, lupus-prone mice have lower enzyme levels not only in sera, but also in urine. We also demonstrated the presence of DNase inhibitors in the sera of lupus mice. Due to its simplicity and sensitivity, the method may find an application in numerous other conditions where DNase is involved in cell/tissue physiology or disease. Low serum concentrations of DNase in lupus-prone mice and high concentrations of DNase inhibitors in their sera may have a role in the complex pathogenic mechanism of murine SLE.

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