Deoxyribonuclease II (DNase II) Activity is Regulated Systemically by a Single Locus in Mice

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Abstract: A survey of inbred strains of mice revealed genetic variation in DNase II activity in the spleen, liver, kidney, lung, heart, coagulating gland and preputial gland. Since DNase II activities in these tissues were closely correlated to each other, systemic regulation of the enzyme was suggested. The inheritance of quantitative variation in the DNase II activity was studied in a test cross using the spleen DNase II activity level as an indicator of progeny phenotypes. The results showed that the strain difference in DNase II activity was controlled by a single autosomal locus. The locus for the mouse DNase II activity level, designated as *Dnl2a*, had at least two alleles. The allele *Dnl2a^h* determines high DNase II activity and occurs in C57BL/6 and C3H/He strains. The other allele *Dnl2a^l* determines low enzyme activity and occurs in BALB/c and DBA/2 strains.

Key words: Deoxyribonuclease II, DNase II, DNase lysosomal 2, *Dnl2a* (DNase lysosomal 2 activity level), mice

Introduction

DNase II (EC.3.1.22.1) is one of the endonucleases observed in mammalian tissues and hydrates DNA to 3'-phosphoryl oligonucleotides under an acid condition (pH 3-7). DNase II has been found in several mammalian sources [1, 4, 7, 10, 12–15] and high activity in lymphatic and tumor tissues is reported [7]. Recently, a possible role of DNase II in apoptosis (programmed cell death) was suggested [2–3, 9], but the biological role of DNase II is still unclear. This is partly because of the low concentration of the enzyme in tissues and body fluids, and the lack of a simple and sensitive method for quantitative and zymogram detection.

Recently, Yasuda *et al.* [14] established a sensitive and simple method for quantitative and zymogram detection of DNase II and revealed tissue distribution of the enzyme activity and a single locus control of the enzyme activity in human urine [15]. Bruns *et al.* [6] and Brook *et al.* [5] showed the chromosomal location of the DNase II structural gene on human chromosome 19p13.2-q13 by an electrophoretic analysis in man-mouse somatic cell hybrid clones expressing lysosomal DNase (DNase II) activity. Since polymorphism has not been observed in the human DNase II structural gene, the relationship between the loci for the structural gene and for the regulator (*DNASE2*) which controls the DNase II activity level in human urine [15]

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is unclear.

In the present study, the genetic variation in DNase II activity was studied in mice to evaluate the usefulness of the mouse as an animal model for the genetic study of DNase II. This report describes the systemic regulation of DNase II activity by an autosomal single locus in mice.

Materials and Methods

Animals: Specific pathogen-free BALB/c, C57BL/6, DBA/2, C3H/He, (C57BL/6 × DBA/2) F1 (BDF1) and (BALB/c × DBA/2) F1 (CDF1) mice of both sexes were obtained from Japan SLC Inc.(Hamamatsu). These mice were bred and maintained under the standard condition previously described [10].

DNase II determination: The DNase II activity was determined by the single radial enzyme-diffusion (SRED) method previously described [10, 14], and expressed as Kunitz units [11] per g wet weight of tissues or per ml of urine. Samples for quantitative DNase II assay were prepared as previously described [10].

Data analysis: Statistical analyses were performed with JMP version 3 software (SAS Institute Inc., Cary, North Carolina, USA). Differences in DNase II activities between strains and between sexes were analyzed by one-way analysis of variance and Turkey test. A relationship among DNase II activity levels in various tissues was sought by standard least squares model fitting. The Chi-square test was used to examine the

segregation of the spleen DNase II activity levels for fit with the single locus control model. A p value less than 0.05 was considered statistically significant.

Results

Strain differences in DNase II activity: The enzyme activity was assayed in 11 kinds of tissues in mice at 12 to 14 weeks of age: liver, salivary glands, kidney, pancreas, thymus, coagulating gland, preputial gland, and seminal vesicle. All of these tissues had been shown to contain moderate to high DNase II activity levels in a previous study [10]. A clear strain variation was observed in the DNase II activity in the spleen, kidneys, liver, lungs, heart, preputial gland and coagulating gland. The DNase II activities of C3H/He and C57BL/6 mice were significantly higher than those of BALB/c and DBA/2 mice (Table 1). Males had a significantly higher DNase II activity in the urine, kidneys and salivary glands than females in all mouse strains tested. Such sexual dimorphism has been observed previously [10].

Correlation of DNase II activity level among tissues: The DNase II activity levels in tissues from 60 mice of four inbred strains (Table 1) and two F1 hybrid strains, BDF1 and CDF1, were compared. The spleen DNase II activity levels were closely correlated with those of the liver, kidneys, coagulating gland and preputial gland (Fig. 1), but there was no correlation between DNase II activity levels in the spleen and urine. The DNase II

Table 1. Mouse strain variation in DNase II activity level (units/g wet weight)

Tissue	C57BL/6		C3H/He		DBA/2		BALB/c		Differences	
	Males	Females	Males	Females	Males	Females	Males	Females	Strain	Sex
Liver	0.96±0.27	0.72±0.13	1.31±0.19	1.12±0.41	0.51±0.07	0.52±0.09	0.74±0.05	0.60±0.07	P<0.001	NS
Lung	0.79±0.09	0.80±0.20	1.12±0.06	0.97±0.16	0.82±0.11	0.82±0.09	1.01±0.21	0.95±0.08	P<0.01	NS
Spleen	2.93±0.65	3.35±0.29	3.05±0.87	3.28±0.65	1.18±0.35	1.60±0.31	2.00±0.74	2.68±0.60	P<0.001	NS
Salivary gland	11.07±4.30	0.68±0.21	5.98±0.77	1.25±0.13	2.98±0.92	1.64±0.68	4.00±0.68	0.84±0.17	NS (P=0.065)	P<0.001
Kidney	0.71±0.09	0.42±0.05	0.96±0.05	0.65±0.16	0.43±0.09	0.28±0.06	0.52±0.04	0.46±0.07	P<0.001	P<0.001
Heart	0.35±0.03	0.36±0.08	0.49±0.04	0.51±0.03	0.29±0.06	0.41±0.07	0.35±0.02	0.36±0.02	P<0.001	P<0.05
Thymus	0.35±0.22	0.38±0.09	0.30±0.16	0.50±0.19	0.33±0.11	0.38±0.12	0.24±0.06	0.51±0.03	NS	P<0.001
Pancreas	0.71±0.13	0.59±0.05	0.73±0.24	0.60±0.16	0.68±0.18	0.84±0.10	0.66±0.04	0.69±0.06	NS	NS
Coagulating gland	1.69±0.09	-	1.95±0.28	-	1.13±0.13	-	1.14±0.09	-	P<0.001	-
Preputial gland	3.37±0.57	-	3.01±0.73	-	1.03±0.13	-	0.82±0.08	-	P<0.001	-
Seminal vesicle	1.67±0.42	-	1.77±0.16	-	1.73±0.49	-	1.52±0.10	-	NS	-

Data are the mean ± SD for five mice. NS: Not significant.

Fig. 1. Correlations between the DNase II activity in the spleen and that in the liver, preputial gland, coagulating gland, urine or kidneys. Each point represents a single mouse of BALB/c, C3H/He, DBA/2, C57BL/6, BDF1 or CDF1 strain with indicated DNase II activity (units/g wet weight or units/ml). The least square line is drawn (correlation coefficients is 0.76 for spleen vs. liver, 0.69 for spleen vs. kidney (male), 0.77 for spleen vs. coagulating gland, 0.72 for spleen vs. preputial gland and 0.65 for spleen vs. salivary gland (male)).

level in urine varied to a great extent from day to day (Data not shown).

Postnatal development of DNase II activity: The developmental pattern of DNase II activity was examined in the spleen, liver, kidneys and salivary glands. As shown in Fig. 2, the pattern in the spleen was similar to those in the kidneys and salivary glands which plateaued at four weeks of age. On the other hand, the pattern in the liver plateaued at one week of age.

Single locus control of DNase II activity level: The quantitative variation in DNase II activity levels in the spleen was studied in C57BL/6 (high activity) and DBA/2 (low activity) and their F1 hybrids and backcross progeny (BDF1 $\times$ D2 and D2 $\times$ BDF1). Since the spleen DNase II activity reached a plateau by four weeks of age and no daily variation was observed, the DNase II activities were determined at eight to 12 weeks of age. The DNase II activity levels in F1 hybrids were the intermediate levels of parental strains. The frequency of distribution of DNase II activity in backcross progeny was apparently bimodal (Fig. 3). Of 125 backcross progeny, 66 mice had F1 type DNase II activity, while 59 mice had DBA/2 type activity. The calculated ratio of F1 type: DBA/2 type was subjected to χ^2

analysis for a fit with the ratio expected for a single autosomal codominant inheritance ($\chi^2 = 0.392$, $df=1$, $0.25 < P < 0.5$).

Discussion

In the present study, strain variation in DNase II activity levels among inbred strains of mice was observed. Correlation of the DNase II activities in various tissues suggests that the DNase II activity level was regulated systemically in mice. A similar good correlation between DNase II activity levels in paired semen and leukocytes, and between urine and leukocyte samples from the same individuals has been observed in humans [14–15], but the DNase II activity in mouse urine was not correlated with those of other tissues in the present study (Fig. 1). The reason for this difference is unknown.

The cross study showed that a single autosomal locus with two alleles designated as *Dnl2al* (DNase lysosomal 2 activity level) controls the DNase II activities in mouse tissues. The allele *Dnl2al^h* determines high DNase II activity in the spleen, liver, kidneys, lungs, heart, coagulating gland and preputial gland and

Fig. 2. Postnatal developmental changes in DNase II activities in male C3H/He mice. Each point represents the mean DNase II activity of three to five mice.

occurs in C57BL/6 and C3H/He strains. The other allele *Dn12alb* determines low enzyme activity and occurs in BALB/c and DBA/2 strains.

The inheritance of a DNase II activity level in mice demonstrated in the present study is comparable with the observation reported by Yasuda *et al.* [15] in humans: that a single autosomal locus (*DNASE2*) with two alleles (H and L) controls the DNase II activity levels. Since no polymorphism was found in isoelectric focusing patterns in human DNase II [14], the relationship between the *DNASE2* locus which controls DNase II activity levels and the structural gene for human DNase II designated as DNL located on chromosome 19p13.2-q13.4 [5–6], is unclear. In the present study, we also could not detect electrophoretic variation in DNase II among the mouse strains used in the present study (Data not shown). It therefore remains to be determined whether the genetic variation in mouse DNase II activity levels is caused by variation in the structural gene or a regulatory gene.

Fig. 3. DNase II activity levels in the spleen of C57BL/6 (B6), DBA/2 (D2), BDF1 ((B6 × DBA/2) F1) and backcross progeny (BDF1 × D2 and D2 × BDF1) mice. Each point represents a single mouse with the DNase II activity level indicated (units/g wet weight). Since there was no statistically significant sex difference in the spleen DNase II activity, data from both sexes were combined.

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