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Genetic variation in esteroproteases in the mouse submandibular gland

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Summary. 9 isozymes of esteroproteases were detected by column isoelectric focusing of submandibular gland extracts from four inbred strains of male mice. A marked strain variance in the esteroprotease isozymes was found among the strains.

The submandibular gland of mice is a rich source of biologically active proteins, such as nerve growth factor (NGF) and epidermal growth factor (EGF). In addition, this organ contains large quantities of trypsin-like enzymes with arginine-specific esteroprotease activity[1,2]. These enzymes and growth factors are androgen-dependent and are localized in serous-like granules in the cells of the secretory tubules[3-5]. Induction of the esteroproteases by androgen is good marker to use in studies on the mechanism of hormone action[6,7]. Several esteroproteases in the mouse submandibular gland have been isolated and their biochemical and immunological properties have been reported[8-10]. Some of the enzymes are thought to be involved in the processing of precursors of NGF and EGF[11-13]. Recently it has been shown that one of the esteroproteases in the mouse submandibular gland is under the control of genetic factors[14]. Here we report variations of several esteroproteases in different inbred strains of mice.

Materials and methods. Inbred mouse strains, BALB/cA, C57BL/10N, C3H/HeN and DBA/2N were obtained from the Central Institute for Experimental Animals (Kanagawa, Japan) and were given standard laboratory chow and water ad libitum. Mice were killed by cervical dislocation between weeks 10 and 12 after birth and their submandibular glands were removed and homogenized with 9 volumes of 20 mM phosphate buffer (pH 7.0) in a glass-teflon homogenizer. The homogenate was centrifuged at $5000 \times g$ for 30 min and the supernatant was used for enzyme assay and isoelectric focusing.

Esteroprotease activity was measured by the method of Trautschold and Werle[15] with α-N-benzoyl-L-arginine ethyl ester (BAEE) as substrate. The reaction mixture contained 0.5 mM BAEE, 1 mM NAD, 130 mM semicarbazide, 130 mM pyrophosphate-380 mM glycine buffer (pH 8.7), 100 units of alcohol dehydrogenase and the enzyme sample in a total volume of 1 ml. The reaction was followed by measuring the change in absorbance at 340 nm and 1 unit of enzyme activity was defined as the amount which hydrolyzed 1 µmole of substrate per min. Protein was determined by the method of Lowry et al.[16]. Column isoelectric focusing was carried out in a column with 50 ml capacity. A density gradient of 0-50% sucrose containing 1% ampholine (pH3.5-10) was prepared and the sample was layered on the middle of the gradient. After focusing for 24 h at 700 V at 0-2 °C, 1-ml fractions were collected.

Results and discussion. The table shows the esteroprotease activities in the submandibular glands of 4 inbred strains of mice. The activity was much higher in males than in females. In males, the specific and total activities of BALB/cA and C57 BL/10N strains were about twice those in C3H/HeN and DBA/2N strains.

Figure 1 shows the isoelectric focusing pattern of the esteroproteases in males. 9 isozymes with different isoelectric points were detected in the 4 strains examined, and these were named esteroproteases I-IX on the basis of isoelectric points (I, 4.7; II, 5.2; III, 5.6; IV, 5.9; V, 6.8; VI, 7.6; VII, 8.1; VIII, 8.8; IX, 9.8). Esteroproteases I, II, VII and VIII were found only in strain DBA/2N; III and V in strains BALB/cA, C57 BL/10N and C3H/HeN; IV in strains BALB/cA and C57 BL/10N; VI in strains BALB/cA and DBA/2N, and IX only in strain BALB/cA. Similar

Esteroprotease activity in the submandibular gland of mice

Strain	Sex	BAEE hydrolytic activity Units/mg tissue	Units/mg protein
BALB/cA	♂	19.5±0.56	169±3.9
	♀	0.8±0.09	7±0.4
C57BL/10N	♂	21.6±1.12	180±5.9
	♀	1.1±0.09	15±0.6
C3H/HeN	♂	10.5±0.94	91±3.7
	♀	1.8±0.08	17±0.8
DBA/2N	♂	12.4±1.01	104±3.1
	♀	1.4±0.06	13±0.8

Values are mean±SE for 6 mice.

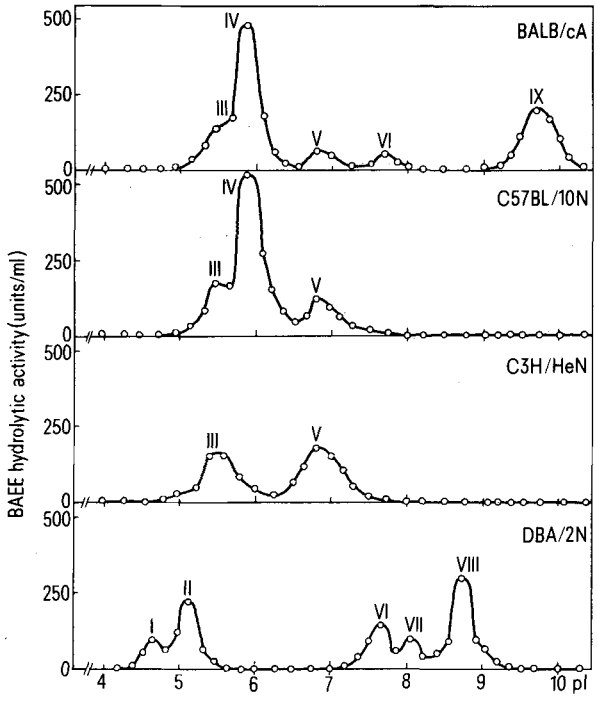

Figure 1. Isoelectric focusing pattern of esteroproteases. 1 ml of submandibular gland extract was focused as described in the methods section.

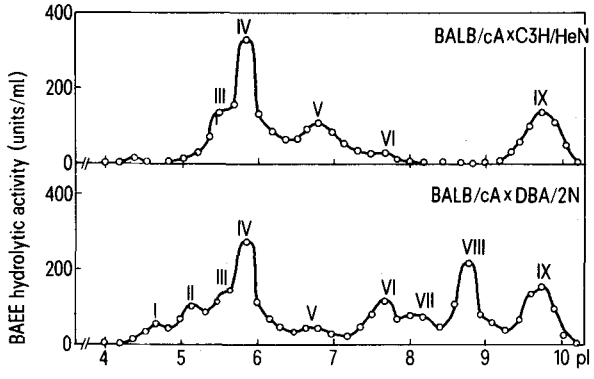

Figure 2. Isoelectric focusing pattern of esteroproteases in F_1 progeny. 1 ml of the gland extract was focused.

isozyme patterns were found in females, although the activities were much lower (data not shown).

The isoelectric focusing patterns of the isozymes in the male F_1 progeny (BALB/cA × C3H/HeN and BALB/cA × DBA/2N) are shown in figure 2. The isozymes in the parents were inherited by the F_1 progeny, indicating that strain variations of the esteroproteases are under genetic control.

Esteroproteases in the mouse submandibular gland are known to be exocrine proteins and their secretion into saliva is controlled by α-adrenergic receptors[17]. When the norepinephrine-induced saliva was analyzed by isoelectric focusing, similar variations of the isozyme patterns shown in figure 1 were observed; saliva was collected by washing the oral cavity with 1 ml of distilled water after treating the mice with norepinephrine (0.5 mg/kg, i.v.). These salivary esteroproteases may be useful as genetic markers in mice.

Several esteroproteases in the mouse submandibular gland have been isolated and their properties have been described. For example the proteases A and D reported by Boesman et al.[9] and the NGF endopeptidase reported by Wilson and Shooter[18] are well characterized. The r subunit of NGF- and EGF-binding protein also possesses enzyme activity[19,20] and the latter has been identified with the protease D[10]. Although the relationship between these enzymes and esteroproteases I–IX presented here is unclear, similarity of the isoelectric points suggests that esteroproteases III and IV may be identified with protease D and NGF endopeptidase, respectively (isoelectric point; 5.6 in the protease D and 5.8 in the NGF endopeptidase). These isozymes are not found in the strain, DBA/2N.

Recent reports have suggested that some of the esteroproteases are involved in the processing of precursors of NGF and EGF[11-13]. The results presented here indicate a new approach in studies of the biochemical properties and physiological roles of esteroproteases in the mouse submandibular gland.

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