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Peptidases A, B, C, D and S in the American mink: polymorphism and chromosome localization

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Summary. An electrophoretic analysis of peptidases was carried out in a population of American mink. Based on substrate and tissue specificities, as well as subunit composition, homologies were established between mink peptidases A, B, C, D and S and human peptidases. Polymorphism for peptidases B and D was demonstrated for minks of three coat colour types. Breeding data indicated that the peptidase variations are under the control of allele pairs at distinct autosomal loci designated as PEPB and PEPD, respectively. Using a panel of American mink-Chinese hamster hybrid clones, the gene for PEPB was assigned to mink chromosome 9.

Key words: American mink – Peptidases – Polymorphism – Gene mapping

Introduction

Peptidases comprise a group of hydrolytic enzymes whose activities can be detected by means of synthetic di- and tripeptides (Harris and Hopkinson 1976). In spite of overlapping substrate specificities, the peptidases can be distinguished from one another on the basis of preferential affinities for particular peptides.

These preferences have made it possible to identify a set of peptidases, designated as A, B, C, D, E, F and S, in man (Harris and Hopkinson 1976). For instance, peptidase D is distinguished by high substrate specificity of proline-containing peptides, whilst peptidase B digests tripeptides, but not dipeptides. Another criterion for peptidase identification is tissue-specificity: all peptidases occur in the erythrocytes with the exception of peptidase S. Furthermore, peptidases differ in subunit composition: peptidases B, C and E are monomers, A and D are dimers, and S is a hexamer (Harris and Hopkinson 1976; Brown et al. 1978; Wilson et al. 1984).

Taking these features into account, peptidases in various mammalian species (Lewis and Truslove 1969; Saison 1973; Womack and Fitzgerald 1973), as well as in fishes (Frick 1983), were found to be homologous to human peptidases.

Interest in this particular group of markers has accrued from their increasing use in population studies, as well as from expanding mapping experiments involving the genes for peptidases in mammals (Table 1). In this paper, data are presented concerning the polymorphism of peptidases (B and D) and their genetic control in mink. The results obtained also allowed us to assign the gene for peptidase B to mink chromosome 9.

Materials and methods

Adult minks (*Mustela vison*) of both sexes were studied. These animals were bred at the Experimental farm of this Institute. The minks were of three coat colours: standard (genotype +/+), Hedlund white (genotype h/h) and black cross (genotype S/+). The standard or wild type mink is completely dark brown (Robinson 1975), the recessive Hedlund white (h/h) is completely white with dark eyes while the heterozygote (+/h) shows white markings on the head, feet, and tail tip, with streaks of white on the belly (Shackelford and Moore 1954). The black cross is a dominant white-spotting mutation (Shackelford 1949). The heterozygotes (S/+) are characterized by a marked depigmentation of the coat. However, stripes of pigmented hair remain over the back and shoulder blades; the rest of the body is white. The homozygotes (S/S) are almost white.

Blood was withdrawn from the tail vein, and the organs needed were procured from minks killed for commercial purposes.

Starch gel electrophoresis of peptidases A, B, C, D and S was done using the technique developed for peptidases A and C (Rubtsov et al. 1982). The activities of these peptidases were identified according to Rubtsov et al. (1982) using gly-leu (peptidases A and C), leu-gly-gly (peptidases B and S) and phe-pro (peptidase D) substrates.

Table 1. A synopsis of the biochemical genetics of peptidases A, B, C, D, E and S in mammals

Species	Polymorphism	Chromosome localization	References
Man	A, B, C, D	A, B, C, D, E, S	Lewis and Harris (1967); Ruddle et al. (1972); Chen et al. (1973); Creagan et al. (1973); McAlpine et al. (1975); Harris and Hopkinson (1976); Brown et al. (1978); Wilson et al. (1984)
Mouse	D, C, E, S	A, B, C, D, E, S	Lewis and Truslove (1969); Womack et al. (1975); Francke et al. (1977); Leinwand et al. (1978); Lalley et al. (1978a, b); Skow (1981); Peters et al. (1983)
Rat	C	D, C	Womack and Cramer (1980); Yoshida (1984)
Mink	D	A, C	Saison (1973); Rubtsov et al. (1982)
Chinese hamster		B, D, S	Stallings and Siciliano (1981); Siciliano et al. (1983)
Cat	A	B, S	O'Brien et al. (1980); O'Brien and Nash (1982)
Cattle	D	B, C	Saison (1973); Dain et al. (1984)
Pig	C	B	Saison (1973); Dolf (1984)
Rabbit		B	Soulie and Grouchy (1983)
Dog	D		Saison (1973)
Deer mouse	A, B		Dawson et al. (1983)
Tuco-tuco	B, D		Lewis (1972)
Monkeys[a]		A, B, C, S	Roderick et al. (1984)

[a] Chimpanzee, gorilla, orangutan, baboon, African green monkey, rhesus, capuchin monkey and mouse lemur

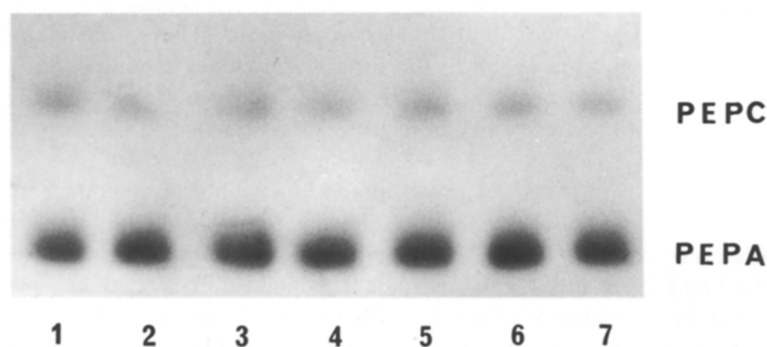

Fig. 1. Electrophoretic pattern of peptidase A (PEPA) and C (PEPC) in erythrocytes from American minks: *1–7* samples from seven standard minks

A panel of mink-Chinese hamster hybrid clones was used for assignment of the gene for peptidase B. The panel was set up taking into account statistical criteria (Wijnen et al. 1977; Cowmeadow and Ruddle 1978). Detailed descriptions of the 28 independent hybrid clones including their karyotypic characterization have been presented previously (Rubtsov et al. 1981a, b).

Results and discussion

The electrophoretic patterns of mink peptidases obtained when using gly-leu as substrate is given in Fig. 1. The patterns consist of two bands: one major, the other minor. The major band is possibly peptidase A with stronger activity than peptidase C as judged by its utilization of the substrate (Harris and Hopkinson 1976). It is noteworthy that a heteropolymeric isozyme is present in mink-Chinese hamster hybrid clones positive for mink peptidase A (Rubtsov et al. 1982). It is suggested that mink peptidase A is a dimer. In contrast to peptidase A, mink peptidase C behaves as a monomer in mink-Chinese hamster hybrid clones (Rubtsov et al. 1982). Peptidase A is a dimer in both man and mouse (Harris and Hopkinson 1976; Francke et al. 1977), while peptidase C is a monomer in man, mouse and rat (Lewis and Truslove 1969; Harris and Hopkinson 1976; Womack and Cramer 1980).

No electrophoretic variants of peptidases A and C were observed amongst the 150 minks examined.

The patterns of peptidase D observed in mink erythrocytes are shown in Fig. 2. The enzyme has two bands, one major and the other minor which was not consistently detected.

Three electrophoretic variants of peptidase D were detected among the 335 minks of three coat colour types: fast F, slow S and FS (Fig. 2). The latter had

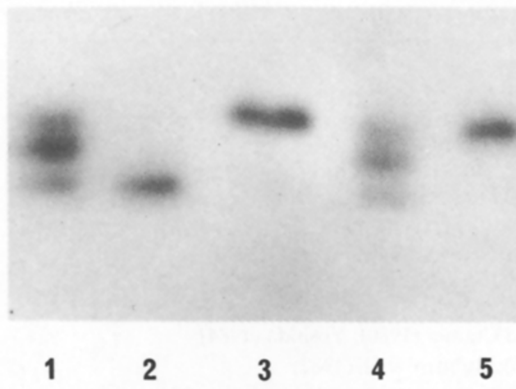

Fig. 2. Electrophoretic variants of peptidase D in the American mink. *1, 4* variant FS; *2* variant S; *3, 5* variant F

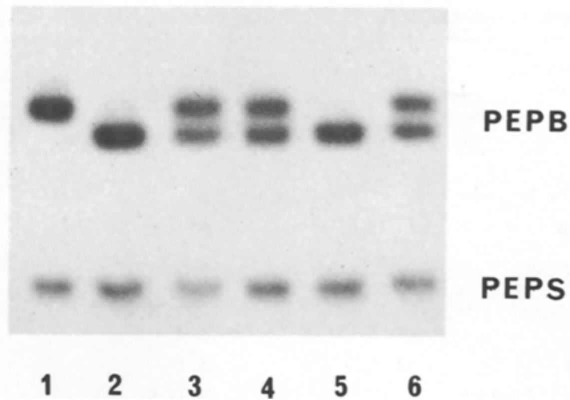

Fig. 3. Starch gel electrophoresis of peptidase B (PEPB) and S (PEPS) from the heart muscle of the American mink. *1* F, the fast variant of peptidase B; *2, 5* S, the slow variant of peptidase B; *3, 4, 6* variant FS of peptidase B. There are clearly no variations in electrophoretic mobility of peptidase S

Table 2. Results of crosses between minks having different variants of peptidase D

Parental phenotype	No. of		Peptidase D variants in progenies		
	Crosses	Progenies	F	FS	S
F×FS	2	7	2	5	–
FS×FS	3	17	3	10	4
FS×S	6	38	–	18	20
F×S	2	14	–	14	–
S×S	8	34	–	–	34

three bands of the enzyme, of which two had the same electrophoretic mobilities as F and S, and the third had a mobility intermediate with respect to F and S (Fig. 2). The activity of this fraction of peptidase D is higher than that of the other two (Fig. 2). Similar electrophoretic variants of peptidase D have been observed amongst 63 minks studied by Saison (1973).

The data obtained when minks with different variants of peptidase D were intercrossed are given in Table 2. From these data it follows that polymorphism for peptidase D in minks is under the control of two codominant alleles at the autosomal locus designated as PEPD: alleles $PEPD^a$ controls F and allele $PEPD^b$, S variants. The frequencies of the genes $PEPD^a$ and $PEPD^b$ for standard minks (n = 179) were 0.33 and 0.67, those for Hedlund white (genotype h/h) (n = 75) were 0.21 and 0.79, respectively. The respective estimates were 0.22 and 0.78 for black cross (genotype S/+) (n = 48).

The presence of an additional band of peptidase D in $PEPD^a/PEPD^b$ individuals is an indication of their dimeric structure. Peptidase D is also dimeric in man (Harris and Hopkinson 1976) and mouse (Lalley et al., 1978 b).

The pattern obtained from peptidases B and S in heart muscles is given in Fig. 3. The slower band, well-visualized in the heart muscles and in trace amounts in the erythrocytes, is presumably peptidase S. This type of expression of peptidase S in the tissues has been reported for its human counterpart (Harris and Hopkinson 1976). In the case of tripeptides serving as substrates, peptidase S is weakly active and peptidase B preferentially digests tripeptides (Harris and Hopkinson 1976).

The three electrophoretic variants of peptidase B detected among 166 minks were fast F, slow S, and FS consisting of two bands in a 1 : 1 ratio (Fig. 3). The results of an analysis of peptidase B carried out on progeny of minks with one of its variants are summarized in Table 3. From these results it was inferred that polymorphism for peptidase B is determined by two codominant alleles at the autosomal locus designated as PEPB. Thus, allele $PEPB^a$ is responsible for F and $PEPB^b$ for S variants. The frequencies of $PEPB^a$ and $PEPB^b$ genes were estimated as 0.44 and 0.56 (n = 59) for standards, 0.08 and 0.92 (n = 75) for Hedlund whites, and 0.37 and 0.63 (n = 32) for black crosses, respectively. The low frequency of allele $PEPB^a$ in Hedlund whites is noteworthy. This may be attributed to a founder effect or close linkage of the PEPB and h genes.

Judging by the patterns obtained from $PEPB^a$/$PEPB^b$ individuals, this enzyme is monomeric in mink, as it is in man (Harris and Hopkinson 1976) and cattle (Dain et al. 1984).

No variants of peptidase S were observed amongst the 166 minks examined.

The patterns of peptidase B yielded by mink fibroblasts (MV cells), Chinese hamster (B14 and V79 cells) as well as the mink-Chinese hamster hybrid clones

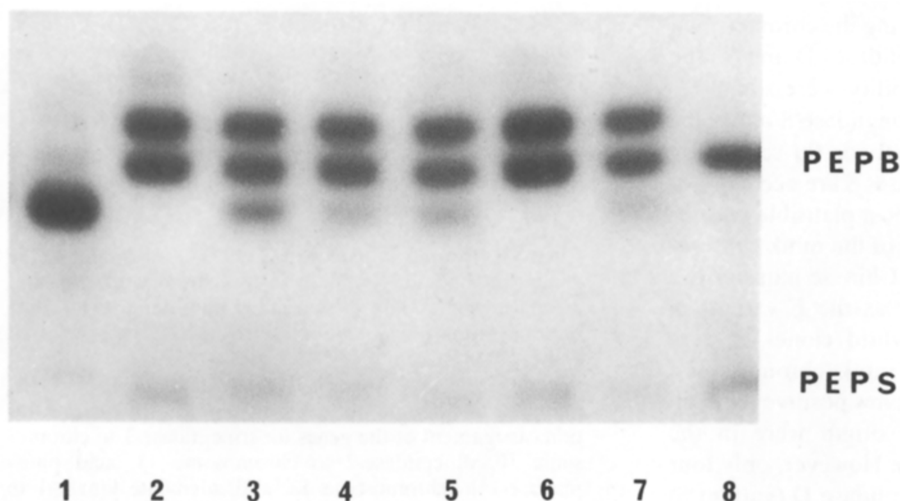

Fig. 4. Electrophoretic patterns of peptidase B (PEPB) and S (PEPS) yielded by mink fibroblasts, MV cells (*1*), Chinese hamster B14 (*2*) and V79 (*8*) cells, clones positive for mink peptidase B (*3, 4, 5, 7*) and hybrid clone negative for peptidase B of mink origin (*6*)

Table 3. Results of crosses between minks having different variants of peptidase B

Parental phenotype	No. of		Peptidase D variants in progenies		
	Crosses	Progenies	F	FS	S
FS×F	2	7	3	4	–
FS×FS	2	7	1	3	3
F×S	1	4	–	4	–
FS×S	3	15	–	7	8
S×S	15	41	–	–	41

are shown in Fig. 4. Peptidase B pattern of Chinese hamster is represented by a single band in V79 cells and by two bands of equal activities in B14 (Fig. 4). It can be suggested that B14 cells were derived from an animal heterozygous for peptidase B, while V79 cells were derivatives from an animal homozygous for the slow-migrating variant of peptidase B. It should be noted that the panel of mink-Chinese hamster hybrid clones was obtained by fusion of B14 cells (previously referred to as M-15-1) with mink cells (Rubtsov et al. 1981a). The peptidase B in mink MV cells is a variant S which moves more slowly to the anode than both variants of Chinese hamster peptidase B (Fig. 4). However, the mink F variant of peptidase B has the same electrophoretic mobility as the slow band of Chinese hamster. The hybrid clones positive for mink peptidase B (variant S) are easily identified (Fig. 4); the identification of variant F of mink peptidase B in positive clones is based on the enhanced activity of the slow-migrating fraction of Chinese hamster peptidase B. Two positive clones of such a type were observed in the panel of 24 mink-Chinese hamster clones. The data for the segregation of mink chromosomes and the expression of mink peptidase B in 24 mink-Chinese hamster hybrid clones are summarized

Table 4. Segregation of mink chromosomes and mink peptidase B in 24 independent mink-Chinese hamster hybrid clones[a]

Mink chromosome	Mink PEPB/mink chromosome			
	No. of concordant clones		No. of discordant clones	
	+/+	–/–	+/–	–/+
1	7	5	9	3
2	5	6	11	2
3	6	4	10	4
4	8	5	8	3
5	8	6	8	2
6	10	3	6	5
7	3	5	13	3
8	4	12	5	3
9	16	7	0	1
10	10	1	6	7
11	7	6	9	2
12	8	3	8	5
13	8	3	8	5
14	11	3	5	5
X	14	0	2	8

[a] The first panel consisted of 25 clones (Rubtsov et al. 1981a, b). Clone FD16B was omitted from their number because subsequent gene localization experiments demonstrated the presence of mink chromosome markers 5, 9 and 12 in this clone although it contains, according to our cytogenetic data only mink chromosome 14 and X-chromosome

in Table 4. The segregation of mink chromosome 9 and the expression of peptidase B was concordant in all the clones except one. Clone F10B was discordant possibly due to a cytogenetically unidentifiable deletion carried by mink chromosome 9. Thus, based on the data set out in Table 4, the most likely candidate for assignment of the gene for mink peptidase B is mink chromosome 9.

We did not succeed in determining the chromosomal location of the genes for mink peptidases D and S. The differences in electrophoretic mobility were slight between mink and Chinese hamster peptidase S under the conditions used (Fig. 4). Other methods for separating mink and Chinese hamster peptidases S are needed.

As to peptidase D, one of the most plausible reasons why it was not associated with any of the mink chromosomes was that peptidase D from Chinese hamster has the same electrophoretic mobility as the F variant of mink peptidase D. Among 28 hybrid clones, 3 were found to have the whole set of mink chromosomes. Moreover, no less than 7 hybrid clones positive for each and every chromosome of mink origin were in the clone panel (Rubtsov et al. 1981a). However, only four positive hybrid clones for mink peptidase D (variant S) were observed in the panel.

It is suggested that the remaining clones, putatively positive for mink peptidase D, were unrevealed because they carried mink variant F. Cells for setting up min-Chinese hamster clones were procured from 6 animals (Rubtsov et al. 1981a); most of these donors, in all probability, had the F variant of peptidase D since the allele $PEPD^{a}$ frequently occurred in the mink population. We are now developing a method for separation of peptidase D that would allow us to distinguish either of the variants of mink peptidase D from Chinese hamster peptidase D.

The previous assignments of the genes for peptidases A and C were mink chromosome 4 and 13, respectively (Rubtsov et al. 1982). Thus, the genes for peptidases A, B and C are located on different chromosomes in the American mink. The homologous genes in man (Ruddle et al. 1972; Creagan et al. 1973; Chen et al. 1973) and mouse (Lewis and Truslove 1969; Francke et al. 1977; Leinwand et al. 1979) are also located on different chromosomes.

The results of population analysis demonstrated genetic variations for peptidases B and D in mink. Polymorphism for peptidase D (Saison 1973), lactate dehydrogenase-B (Saison 1971) and anti-trypsin (Juneja et al. 1981) in mink has been described. Thus, the variants of peptidase B are new markers among those offered for population studies.

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