Aminoacylase-1 isoenzymes: A comparative study*

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Analysis of aminoacylase-1 (ACY-1) zymograms from 21 species indicated one structural locus coding for one dimeric enzyme. Among the various strains of five laboratory mammals (*Mesocricetus auratus*, *Cricetulus griseus*, *Mus musculus*, *Rattus norvegicus*, *Cavia porcellus*) only in *Mus musculus*-strains genetic variability was found. Crosses segregating for *ACY-1^a/ACY-1^b* reveal a three-banded banding pattern typical for the heterozygous state of a dimeric enzyme. Electrophoretic comparisons of all species studied indicate a high degree of phylogenetic variability at this locus.

INTRODUCTION

The enzyme aminocyclase-1 (ACY-1; E.C. 3.5.1.14) catalyses the hydrolysis of acylated amino acids into L-amino acids and acyl groups, while L-aspartate derivatives are cleaved by aminoacylase-2 (Birnbau *et al.*, 1952; Krdel and Schneider, 1976).

Using cellogel electrophoresis and bioautographic assay Naylor *et al.* (1979) detected a genetic polymorphism in the electrophoretic mobility of this enzyme in mouse strains. In all tissues studied *ACY-1^a/ACY-1^b* heterozygotes produce three electrophoretically distinct allozymes, indicating a dimeric structure for this enzyme.

A biochemical detection method described by Qavi and Kit (1980) allowed the mapping of ACY-1 to the short arm of chromosome 3 in man (Kit *et al.*, 1980). Among several cell lines screened for ACY-1 activity by the former authors, extracts from African green monkey kidney cells (CV-1) expressed two and three bands indicating the possible existence of two genetic loci for ACY-1 in vertebrates. By means of the biochemical detection method mentioned above we studied the electrophoretic patterns of this enzyme from a variety of animal species. The existence of only one elec-

trophoretic band both in vertebrate and invertebrate species under investigation confirm that only one locus exists for this enzyme. A comparison of our data with the published ones reveal a species-specific pattern.

MATERIAL AND METHODS

Mice-, rat-, guinea pig-strains, Chinese and Syrian hamsters were bred in the animal station of our institute or at the Institute of Animal Breeding, Himberg. *Apodemus sp.*, and *Sorex sp.* were caught in the North-eastern part of the Burgenland, Austria. *Solenodon paradoxus* liver and kidney were taken from a deceased animal by W. Poduschka, Vienna. The other specimen listed in fig. 1 were a gift of H. Nopp and H. Schwammer, both Institute of Zoology, Vienna.

Preparation of samples and electrophoretic procedures were done according to established methods (Csaikl 1984, Csaikl *et al.*, 1980). For specific enzyme detection the biochemical assay of Qavi and Kit (1980) was used.

RESULTS AND DISCUSSION

Tissue distribution

Among the tissues listed in table 1 serum and whole blood cells showed no activity for ACY-1. All other

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Figure 1 Systematic position of the animals studied, sample sizes and the electrophoretic banding pattern.

Table 1 Activity of Aminoacylase-1 in mouse (strain: CBY/Ry), rat (strain: B/N) and guinea pig (strain: 13). The staining intensity was designated optically

	Organs											
	Liver	Kidney	Adrenal gland	Brain	Heart	Skeletal muscle	Testes	Lung	Pancreas	Spleen	Serum	Blood cells
Activity	+++	+++	+	++	+	+	+	+	+	+	-	-

tissues screened in three laboratory strains of mouse, rat and guinea pig species had a single anodally migrating band. The staining activity of the enzyme was estimated optically by us and was used for a crude classification (table 1). This classification confirmed the published values of Naylor *et al.* (1979) in all but one organ. While these authors found the enzyme activity of liver to be only 25 per cent of that of kidney, with this staining procedure, in our hands both organs showed the same intensity. Whether this discrepancy is the result of a physiological based altered enzyme production or of an electrophoretically undetected variability remains obscure.

Genetic variability and phylogenetic considerations

The species screened and their systematic position are specified in fig. 1.

Screening of mouse strains revealed two different phenotypes. The strains C57Bl, C57Bl/6 and CBA/Ry were homozygous for allele b, while strains A/J, A, AKR, Balb/c, C3H, C3H/OuJ, DBA, DBA2, NZB/BinJ and 129/terSV were homozygous for allele c (allele determination after Naylor *et al.* 1979). Rat (B/N, F344, hr/hr, LE, SpD, W) and guinea pig strains (Dunkin-Hartley,

C4-deficient, 2, 13), Chinese and Syrian hamsters (both random bred) were homozygous for one species-specific allele (fig. 1). Specimen of an F_1 cross of two mouse strains, namely C57Bl $\times$ 129/terSV showed three electrophoretic bands, typically for a heterozygous state of a dimeric enzyme (fig. 2). The dimeric structure was ascertained originally by Naylor *et al.* (1979) with the use of a bioautographic assay and confirmed the physiological studies of porcine kidney ACY-1. The enzyme consists of two nearly identical subunits and has a molecular weight of 86·000 daltons (Kördel and Schneider, 1976). In the other mammal species only one homozygous species-specific phenotype was found (fig. 1).

The high genetic variability between the phylogenetic old insectivora species (*Solenodon paradoxus*, *Erinaceus europeus* and *Sorex araneus*) suggests that ACY-1 subunits have diverged rather early in phylogenetic times. The species-specific banding pattern in the other species under study may serve as an additional hint. In the phylogenetic rather young Muridae genus *Apodemus* (three species) no genetic difference was detected. The existence of this enzyme in invertebrate species like *Blatta orientalis*, *Helix pomatia*, *Daphnia sp.*, *Chironomus sp.* and *Tubifex sp.* is mentioned with interest.

Figure 2 Electropherograms of the various laboratory animals including the heterozygous $ACY-1^b/ACY-1^c$ phenotype of *Mus musculus*-cross C57Bl $\times$ 129/terSV. (R.n. = *Rattus norvegicus*, C.p. = *Cavia procellus*, M.a. = *Mesocricetus auratus*, C.g. = *Cricetulus griseus*, M.m. = *Mus musculus*)

The high incidence of species-specific patterns described by Qavi and Kit (1980) and in this paper leads to the conclusion of a rather high intrinsic mutation rate. Although our sample numbers are low, intraspecific variability seems to be minimal. In a study of 54 wild boars revealing polymorphisms of 12 per cent of the studied loci no variability was found for ACY-1 (Hartl and Csaikl, in preparation).

The existence of only one electrophoretical band in the studied specimen serves as a good evidence for the acceptance of a single locus for ACY-1. Under the light of this result the two and three bands described by Qavi and Kit (1980) in African green monkey kidney cells (CV-1) are rather the result of two alleles segregating in this cell line.

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