GENETICS OF HYDROXYACID OXIDASE ISOZYMES IN THE MOUSE: LOCALISATION OF *Hao-2* ON LINKAGE GROUP XVI

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SUMMARY

Electrophoretic variants of individual isozymes of L- α -hydroxyacid oxidase (HAOX-B4) and amylase (AMY-A) in an Asian subspecies of mouse, *Mus musculus castaneus*, have been used to localise the gene encoding the HAOX B subunit. The structural gene loci for these isozymes (*Hao-2* and *Amy-1*) are apparently linked (4.9 ± 2.4 per cent recombinants) in this organism, which places *Hao-2* on linkage group XVI, since previous studies by Eicher and co-workers (1976) have localised *Amy-1* on this chromosome.

1. INTRODUCTION

L- α -HYDROXYACID OXIDASE (HAOX; E.C. 1.1.3.1) exists as two isozymes in mouse tissues which are tetrameric proteins in dilute solutions (designated A4 and B4) and are encoded by distinct genetic loci (*Hao-1* and *Hao-2* respectively) (Duley and Holmes, 1974; Phillips *et al.*, 1976; Holmes, 1977). HAOX-A4 preferentially oxidises short-chain aliphatic substrates and is commonly referred to as glycolate oxidase, whereas HAOX-B4 utilises long chain aliphatic and aromatic substrates (Kun *et al.*, 1954; Robinson *et al.*, 1962; Nakano *et al.*, 1968). These isozymes are differentially localised in mouse liver (A4) and kidney (B4) and are predominantly found within peroxisomes or in the extra-particulate cytoplasm of these tissues (see Masters and Holmes, 1977).

Previous genetic analyses of peroxisomal enzymes have demonstrated that *Hao-1* and *Cs* (the structural gene for mouse catalase) are linked on chromosome 2 of the mouse (Holmes and Duley, 1975). In contrast, however, *Hao-2* and *Dao* (the structural gene for D-amino acid oxidase) are not linked to these loci in this organism (Holmes, 1976; Holmes, 1977). In this communication, it has been demonstrated that *Hao-2* is localised on linkage group XVI near *Amy-1* (the locus encoding one of the isozymes of mouse amylase).

2. MATERIALS AND METHODS

Mice. Male C57BL/10 inbred mice and female F₁ hybrid mice derived from matings between male *Mus musculus castaneus* and female C57BL/10 inbred mice provided the necessary backcross offspring which were used to examine segregation and the possible linkage relationship between *Hao-2* and *Amy-1* loci.

(i) Chemicals

Phenazine methosulphate (PMS), methyl thiazolylblue (MTT), agarose, DL-p-hydroxyphenyllactate, Triton X-100, Tris (hydroxymethyl amino-methane), starch and glycine were purchased from Sigma Chemicals (St Louis, Missouri).

(ii) *Homogenate preparation*

Kidneys were excised from freshly killed mice, rinsed in cold distilled water and homogenised in 50 mM Tris HCl pH 8.0, 0.1 per cent Triton X-100 buffer (1 kidney + 0.5 ml buffer) using an Ultra-Turrax homogeniser. Animals were 6-8 weeks in age when examined. The homogenates were then centrifuged (45,000 $\times$ g, 30 min.) prior to electrophoresis.

(iii) *Gel electrophoresis and histochemical staining*

Kidney supernatants were subjected to zone electrophoresis on Titan III cellulose acetate plates (60 $\times$ 75 mm) (Helena Labs., Texas) with Tris-glycine buffer (25 mM Tris; 192 mM glycine) pH 8.5. A voltage of 250 V was applied for 25 min. The plates were then stained for activity and photographed.

HAOX-B4 was stained by modifying an unpublished agar-overlay technique of Eicher. Two ml. of an agarose solution (360 mg/25 ml) at 55°C was mixed with an equal volume of a solution containing buffer, substrate and histochemicals, poured over the cellulose acetate plate. Following incubation at 37°C, the agar overlay was washed from the plate with distilled water and the plate subsequently rinsed in distilled water to remove background stain and dried overnight at 37°C. The final concentrations in the 4 ml of agar overlay solution were Tris-HCl (pH 8.0), 100 mM; DL-p-hydroxyphenyllactate, 6.25 mM; PMS, 0.3 mM; MTT, 0.9 mM. A control stain was also used to reveal oxidase activity in the absence of substrate; Tris HCl (pH 8.0), 100 mM; PMS, 0.3 mM; MTT, 0.9 mM.

Amylase isozymes were stained according to the unpublished procedure of Eicher. An agar-starch gel (2 per cent agarose; 0.5 per cent soluble starch; 0.85 per cent NaCl) was prepared in a Petri dish, and the cellulose acetate plate after electrophoresis placed face down on to the gel. Following incubation at 37°C for 20 min., the plate-gel interface was exposed and the gel treated with 0.5 per cent iodine solution to reveal zones of amylase isozyme activity.

3. RESULTS AND DISCUSSION

Electrophoretic variants of α -amylase from the saliva and pancreas of feral and inbred *Mus musculus* have been reported by Sick and Nielsen (1964) and Kaplan and co-workers (1973). Three loci have been postulated for this enzyme which are apparently differentially expressed in tissues of the mouse. The locus encoding salivary (submaxillary gland) amylase has been designated *Amy-1*, whereas two additional loci have been proposed for the pancreatic isozymes (*Amy-2*, *Amy-3*). Only the *Amy-1* and *Amy-2* loci exhibited electrophoretic variation between inbred strains, and genetic analyses have demonstrated tight linkage for these genes at the centromeric end of linkage group XVI (Sick and Nielsen, 1964; Eicher *et al.*, 1976). In the present study, both *Amy-1* and *Amy-2* gene products were observed in kidney extracts and the genetic variation previously reported for *Amy-1* in *castaneus* mice (Kaplan *et al.*, 1973) was used as a gene marker for chromosome 12.

Eighty-two progeny from a backcross of (C57BL/10 ♀ $\times$ *castaneus* ♂) F_1 ♀ $\times$ C57BL/10 ♂ were examined for segregation at the *Hao-2* and *Amy-1* loci

(table 1). The variants were inherited in a normal Mendelian fashion with two alleles in each case showing co-dominant expression. The loci exhibited a recombination frequency of 4.9 ± 2.4 per cent and the variation observed from the expected 1 : 1 : 1 : 1 ratio for unlinked loci was significant ($\chi^2 = 67.0$; 3 d.f.), thus indicating that these loci are co-ordinately localised on linkage group XVI.

A previous study of the genetic control of kidney HAOX (B4 isozyme) has demonstrated linkage of *Hao-2* with *Adh-3*, the locus encoding the C_2 isozyme of alcohol dehydrogenase (ADH) in mouse tissue (Holmes, 1977).

TABLE 1
Two-point recombination cross between *Hao-2* and *Amy-1**

Parents (♀ × ♂)	Parentals				Recombinants			
	<i>Hao-2</i> ^a <i>Hao-2</i> ^b	<i>Amy-1</i> ^a <i>Amy-1</i> ^b	<i>Hao-2</i> ^a <i>Hao-2</i> ^a	<i>Amy-1</i> ^a <i>Amy-1</i> ^a	<i>Hao-2</i> ^a <i>Hao-2</i> ^a	<i>Amy-1</i> ^a <i>Amy-1</i> ^b	<i>Hao-2</i> ^a <i>Hao-2</i> ^b	<i>Amy-1</i> ^a <i>Amy-1</i> ^a
(C57BL/10 × <i>castaneus</i>) × C57BL/10	40		38		3		4	1
Total	78							

* Recombination frequency = 4.9 ± 2.4 per cent; variation from 1 : 1 : 1 : 1 ratio for unlinked loci was significant ($\chi^2 = 67.0$ 3 d.f.; $P \sim 2.1 \times 10^{-14}$), indicating that the two loci are linked.

The localisation of these genes on linkage group XVI has been confirmed by other experiments which have demonstrated linkage between *Va* (*varitint*—a coat colour gene on linkage group XVI) Cattanaach and Moseley, 1973), and *Adh-3* in mice (Holmes, unpublished results). Further genetic experiments are under way in this laboratory to establish the gene order of these biochemical loci, *Hao-2*, *Amy-1* and *Adh-3*, with respect to previously mapped genes, *Va* and *de* (*droopy ear*).

It may be concluded from this and a previous study (Duley and Holmes, 1974) that the A4 and B4 isozymes of mouse HAOX are encoded by separate loci (*Hao-1* and *Hao-2* respectively) localised on separate chromosomes on the mouse genome. *Hao-1* has been mapped within 8 centiMorgans of the *Cs* gene encoding catalase on chromosome 2 (Holmes and Duley, 1975) whereas *Hao-2* is localised on linkage group XVI near *Amy-1*.

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