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Genetic analysis of a new haplotype of the histidine decarboxylase gene complex in C57BL/6 mice

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Summary

The histidine decarboxylase (HDC) gene complex, [*Hdc*], comprises the structural gene for mouse kidney HDC and closely linked regulatory elements which determine enzyme concentration and its response to hormones. One of these regulatory elements, *Hdc-e*, determines the response (induction or repression) of kidney HDC to oestrogen. HDC is oestrogen-inducible in C57BL/10 and oestrogen-repressible in DBA/2 and C57BL/6; alleles of *Hdc-e* segregate in crosses between C57BL/10 and DBA/2 and between the C57BL substrains. Two different haplotypes of [*Hdc*] have been defined previously, B.10 (*Hdc-s*^{b}, *Hdc-c*^{b}, *Hdc-e*^{b}) in C57BL/10 and D (*Hdc-s*^{d}, *Hdc-c*^{d}, *Hdc-e*^{d}) in DBA/2. C57BL/6 represents a third haplotype (B.6) (*Hdc-s*^{b}, *Hdc-c*^{b}, *Hdc-e*^{d}) which differs from both B.10 and D. *Hdc-e* may therefore be a component of the complex independent of *Hdc-s* and *Hdc-c*.

1. Introduction

The concentration of histidine decarboxylase (HDC; E.C. 4.1.1.22) in mouse kidney is under multihormonal control. In C57BL/10 mice HDC is inducible by thyroxine and oestrogen (and pregnancy) and is repressible by androgens (Rosengren, 1962; Kahlson & Rosengren, 1968; Henningsson & Rosengren, 1972; Grahn *et al.* 1973; Schayer & Reilly, 1975; Bulfield & Nahum, 1978). Variation in the response of HDC to hormones has been discovered amongst strains of mice (Martin, 1983; Martin & Bulfield, 1984*a*; R. J. Middleton and S. A. M. Martin, unpublished), as well as variation in the endogenous levels of the enzyme and its structure (Martin & Bulfield, 1984*b*; Martin *et al.* 1984). Analysis of the different phenotypes at the biochemical, genetical and molecular levels will reveal the various mechanisms by which HDC activity and concentration are regulated.

A gene complex, [*Hdc*], comprising a single structural gene and regulatory elements has been identified and mapped to chromosome 2 (Martin & Bulfield, 1984*a*, *b*; Martin *et al.* 1984) and other unlinked genes are also known to affect HDC expression (S. A. M. Martin and R. J. Middleton, unpublished). Two different combinations of alleles within the gene complex are known, represented by the strains C57BL/10 (B.10 haplotype; (*Hdc-s*^{b}, *Hdc-b*^{b} *Hdc-e*^{b})) and DBA/2 (D haplotype; (*Hdc-s*^{d}, *Hdc-c*^{d}, *Hdc-e*^{d})). Variation in response to oestrogen is widely distributed amongst the inbred strains. Some show oestrogen-induction and others oestrogen-repression of HDC (Martin & Bulfield, 1984*a*).

We report that alleles of *Hdc-e* differ between the substrains C57BL/10 and C57BL/6; HDC is oestrogen-inducible in the kidneys of C57BL/10 females and oestrogen-repressible in C57BL/6. Therefore C57BL/6 represents a third, different [*Hdc*] haplotype (*Hdc-s*^{b}, *Hdc-c*^{b}, *Hdc-e*^{d}). This is only the third reported genetic difference between the C57BL substrains, and the first one known to affect a regulatory function.

2. Materials and Methods

(i) *Animals and hormone treatment*

The inbred strains of mice C57BL/10ScSn (abbreviated to C57BL/10) and C57BL/6 were obtained from Bantin and Kingman Ltd, Grimston, Hull, U.K. and bred F_1–F_3 prior to use. The C57BL/Fa strain is maintained in the Department of Genetics, Edinburgh University. Females were used at 2–3 months of age. Mice were implanted with pellets of 17β-oestradiol for 14 days as described previously (Martin & Bulfield, 1984*a*).

(ii) *Histidine decarboxylase assay*

HDC activity was assayed by the release of $^{14}CO_2$ from D,L-(carboxyl-^{14}C) histidine (Amersham International, Amersham, U.K) as described previously

(Martin *et al.* 1984). Enzyme activity is expressed as nmols histidine utilized/min/g wet weight kidney tissue at 30 °C.

(iii) Immunoprecipitation

HDC concentration was titrated in kidney homogenates by immunoprecipitation with an anti-foetal rat HDC antiserum (the gift of Dr T. Watanabe, Osaka University School of Medicine, Japan). Kidneys were homogenized in 5 × v/w of 0·1 M potassium phosphate buffer, pH 6·8, 0·01% sodium azide, at 4 °C and the homogenates were centrifuged at 1000 g_{av} for 15 min. Increasing volumes of the supernatants were incubated with constant volumes of the antiserum in a total volume of 215 μl for 1 h at room temperature and then overnight at 4 °C. Ten μl of protein A (*S. aureus* cells; Sigma) in phosphate buffer was added and incubation continued at room temperature for 30 min with gentle shaking. The mixtures were centrifuged for 5 min in an Eppendorf minifuge to precipitate the *S. aureus* cells with the antigen-antibody complexes, and 200 μl of supernatant was assayed for residual HDC activity as described. The data was analysed by statistical regression analysis using a semi-log plot (Martin & Bulfield, 1984*a*), and the volume of antiserum required to precipitate 50% of the initial HDC activity was calculated.

3. Results

(i) *Effect of oestrogen on levels of histidine decarboxylase activity in the C57BL substrains*

Females of the substrains C57BL/10 and C57BL/6 have the same low HDC activity phenotype (Martin *et al.* 1984), but can be distinguished by their response to administered oestrogen. Whereas HDC is oestrogen-inducible in the kidneys of C57BL/10 animals, it is oestrogen-repressible in C57BL/6 (Table 1). As reported previously (Martin & Bulfield, 1984*a*), the magnitude of HDC induction and repression is not large but the changes in enzyme activity due to oestrogen are highly significant (Table 1).

The C57BL substrains were separated in the Jackson Laboratory prior to 1937 (Festing, 1979) though some years earlier some of the progenitor stock had been imported into Britain by Grüneberg. The line was passed to Falconer and is still maintained in this laboratory (C57BL/Fa). C57BL/Fa females have the same phenotype as C57BL/6 in the response of HDC to oestrogen.

(ii) *Immunoprecipitation of induced and repressed histidine decarboxylase*

Induction or repression of HDC levels in response to oestrogen could be due to changes either in specific activity or changes in enzyme concentration. An anti-foetal rat antiserum (Fukui, Watanabe & Wada, 1981) which crossreacts with mouse kidney HDC (Martin *et al.* 1984) was used to titrate the changes in HDC concentration due to oestrogen.

In C57BL/10 HDC activity is induced in response to oestrogen, and a correspondingly larger volume of the antiserum was required to precipitate 50% of the enzyme activity (Table 2). Conversely a proportionally smaller volume of antiserum precipitated 50% of the repressed enzyme activity in C57BL/6 homogenates. For both strains then, the ratio (oestrogen-treated/control) HDC activity was very close to the ratio of amount of antiserum required to inhibit the enzyme to 50% of its initial activity. Therefore induction of enzyme activity in C57BL/10 and its repression in C57BL/6 are consistent with changes in HDC concentration, due to altered rates of enzyme synthesis or degradation.

(iii) *Segregation analysis*

Both C57BL substrains have low HDC activity but they differ in their response to oestrogen. HDC activity in the kidneys of oestrogen-treated (C57BL/10 × C57BL/6) F_1 females is slightly higher than the control levels (Table 1); a large number of heterozygotes were scored and the difference was significant. The result is consistent with an additive mode of inheritance of the different responses to

Table 1. *Effect of oestrogen on histidine decarboxylase activity*[a] *in C57BL/10, C57BL/6 and (C57BL/10 × C57BL/6) F_1 females*

	Control	(*n*)	+ Oestrogen	(*n*)	Significance (P) (*t*-test)
C57BL/10	0·81 ± 0·22	(8)	1·37 ± 0·14	(20)	< 0·005
C57BL/6	0·68 ± 0·15	(9)	0·23 ± 0·06	(11)	< 0·001
F_1 (observed)	0·53 ± 0·04	(7)	0·66 ± 0·05	(48)	< 0·01
F_1 (expected)[b]			0·59		

[a] Mean ± S.E. expressed as nmols histidine/min/g tissue at 30 °C.
[b] Calculated on the basis of an additive mode of inheritance. If each chromosome contributes equally to the heterozygote phenotype then the expected change in HDC activity due to oestrogen treatment is 0·53 + [(1·37 − 0·81)/2)] − [(0·68 − 0·23)/ 2] = 0·59.

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Table II. *Immunoprecipitation of oestrogen-induced and oestrogen-repressed histidine decarboxylase activity*

	HDC activity[a]	μl antiserum[b]
C57BL/10 control	0·78	0·19
C57BL/10 +oest	1·67	0·38
Ratio +oest/control	2·14	1·96
C57BL/6 control	0·70	0·19
C57BL +oest	0·33	0·09
Ratio +oest/control	0·48	0·47

[a] nmols histidine/min/g tissue at 30 °C.
[b] volume of anti-foetal rat HDC antiserum required to inhibit the enzyme by 50% of its activity in kidney homogenates from control and oestrogen-treated mice.

oestrogen: the observed HDC activity in oestrogen-treated heterozygotes (0·66 nmol/min/g) is not significantly different from the amount expected for additive inheritance (0·59 nmol/min/g; $\chi^2 = 0{\cdot}0083$, N.S.; Table 1).

Codominant monogenic inheritance is confirmed by the distribution of phenotypes observed in progeny from a backcross of the F_1 to C57BL/10 (Fig. 1). HDC activities in oestrogen-treated F_1 animals are intermediate between those of the parental strains. Backcross animals comprise a bimodal distribution of HDC activities about the F_1 and parental mean values. This is consistent with segregation of codominant alleles of a single gene, *Hdc-e*, which differ between the substrains C57BL/10 and C57BL/6.

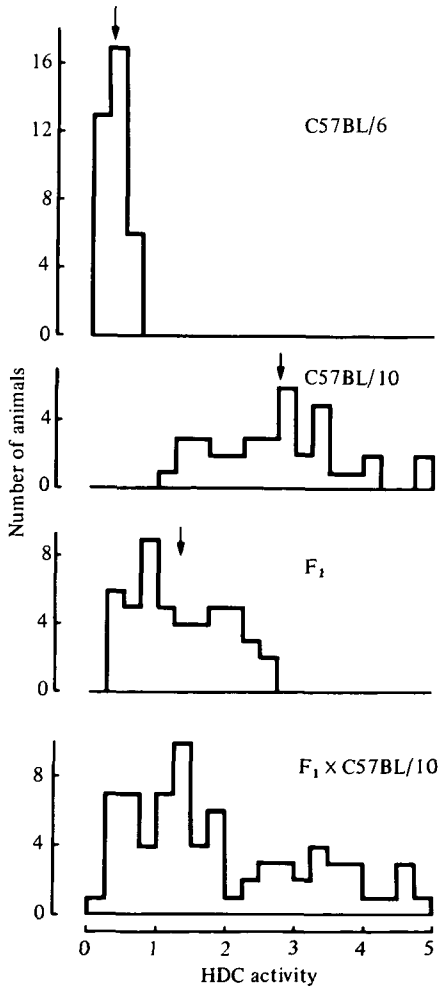

Fig. 1. Segregation analysis: distributions of kidney histidine decarboxylase activities in oestrogen-treated animals. Arrows show mean values.

(iv) *Nomenclature*

Monogenic segregation of alleles of *Hdc-e* was originally observed in crosses between C57BL/10 (*Hdc-e*^{b}; oestrogen-inducible) and DBA/2 (*Hdc-e*^{d}; oestrogen-repressible; Martin & Bulfield, 1984*a*). The allele determining oestrogen-repression of HDC in C57BL/6 cannot be distinguished from the DBA/2 allele and according to the rules of the Committee on Standardized Genetic Nomenclature for Mice must therefore be given the same nomenclature, *Hdc-e*^{d} (Lyon, 1981).

(v) *The HDC gene complex*

C57BL/6 represents a third haplotype (B.6) of [*Hdc*] (Table 3). The B.6 gene complex contains one allele peculiar to the D (DBA/2) haplotype, *Hdc-e*^{d}, and two alleles characteristic of the B.10 (C57BL/10) haplotype, *Hdc-c*^{b}, and *Hdc-s*^{b} which determine the concentration of HDC in mouse kidneys and properties affected by the enzyme's structure respectively (Martin *et al.* 1984; Martin & Bulfield, 1984*b*).

4. Discussion

A locus, *Hdc-e*, determines the response (induction or repression) of HDC to administered oestrogen. The effect of oestrogen on HDC activity is specific to kidney tissue. Immunoprecipitation with a specific anti-HDC antibody has shown that induction and repression of enzyme activity correspond to changes in HDC concentration and therefore to changes in the rate of enzyme synthesis or degradation. *Hdc-e* is linked to the HDC structural gene on chromosome 2 (Martin & Bulfield, 1984*a*, *b*); together these and other loci (Martin *et al.* 1984; R. J. Middleton and S. A. M. Martin, unpublished) comprise the HDC gene complex, [*Hdc*].

Two alleles of *Hdc-e* have been identified; both are widely distributed amongst the inbred strains. HDC is oestrogen-inducible in the kidneys of C57BL/10 mice and 6 other strains (*Hdc-e*^{b}) and oestrogen-repressible in C57BL/6, C57BL/Fa, DBA/2 and 5 other strains (*Hdc-e*^{d}; Martin & Bulfield, 1984*a*). This is only the third genetic difference between C57BL/10 and C57BL/6 to have been discovered since the strains were separated around 1937 (Festing, 1979). The other allelic differences are in *Lv* (affecting delta-aminolevulinate dehydratase activity; Hutton & Coleman, 1969) and H-9 (histocompatability locus, Graff, Polinsky & Snell, 1971).

Other than response to oestrogen, no difference between the C57BL substrains in HDC phenotype has

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Table 3. *The histidine decarboxylase gene complex*

	Hdc-s			*Hdc-c*		*Hdc-e*			
Haplotype	Allele[a]	Stability	K_m (PLP)	Allele[b]	Kidney HDC concentration	Allele[c]	Oestrogen	Type strain	Other strains
B.10	b	Stable	Low	b	Low	b	Inducible	C57BL/10	A2G,SWR
D	d	Labile	High	d	High	d	Repressible	DBA/2	C3H/He
B.6	b	Stable	Low	b	Low	d	Repressible	C57BL/6	C57BL/Fa, A, DBA/1 F/St

[a] Martin & Bulfield, 1984 *b*.
[b] Martin *et al.* 1984.
[c] Martin & Bulfield, 1984*a*; this paper.

been discovered, in HDC enzyme structure or in the regulation of its activity and concentration, either temporally or in response to the various hormone effectors. The B.10 and B.6 haplotypes are distinguished only by the allelic difference at *Hdc-e* (Table 3). In mammals, genetic analyses of tightly linked genes and gene complexes can be limited by the availability of mixed or recombinant haplotypes (see Pfister *et al.* 1982), though recombinants have been discovered in one case (Breen, Lusis & Paigen, 1977). Therefore it is important that *Hdc-e* alleles vary independently of the other components of [*Hdc*], and this in turn implies that *Hdc-e* might be an independent component of the gene complex. In the mouse fine structure mapping is limited, largely by breeding practicalities, to obtaining recombination between elements more than about 0·1 cM apart, or about 200 kilobases of DNA apart. Therefore analysis at the genetical level cannot reveal the details of the molecular organization of the HDC gene complex.

Variation within [*Hdc*] is less likely to be due to recombination than to new mutation of *Hdc-e* since the geneological relatedness of the strains do not correlate with *Hdc-e* allele (Table 3, Festing, 1979; Martin & Bulfield, 1984*a*). The phenotype of C57BL/Fa implies that a mutation occurred in C57BL/10. The genetic relatedness of the C57BL substrains and the lack of any other difference in HDC phenotype means that the variant *Hdc-e* allele is essentially congenic on the C57BL background. This is especially important for the analysis of the gene complex at the molecular level, to identify the specific DNA sequence encoding *Hdc-e* and to determine the mechanism of its regulation of the structural gene's expression.

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