Non-additive inheritance of glucose phosphate isomerase activity in mice heterozygous at the *Gpi*-1*s* structural locus

JOHN D. WEST AND JEAN H. FLOCKHART

Department of Obstetrics and Gynaecology, University of Edinburgh, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW

Summary

The activity of blood glucose phosphate isomerase (GPI-1) in mice heterozygous for various alleles at the *Gpi*-1*s* structural locus (heterozygotes a/b, a/c and b/c) was significantly higher than expected, on the basis of additive inheritance, from the levels in parental homozygotes. Moreover, the GPI-1 activity was higher in a/b heterozygotes than in either parent (heterosis). Studies of heat stability with kidney homogenates revealed that the relative stabilities of GPI-1 dimers was AA > AB > BB > AC ⩾ BC > CC. Differences in dimer stabilities *in vivo* would affect the total GPI-1 levels in heterozygotes and could account for non-additive inheritance but would be insufficient to explain heterosis for GPI-1 activity. Other possible contributing factors include unequal production or stability of monomers, or higher catalytic activity of heterodimers. Monomers could also associate non-randomly but this would not be sufficient to explain heterosis. It is clear that non-additive inheritance patterns may be produced by variants of either structural or regulatory genes.

1. Introduction

The *Gpi*-1*s^c^* allele of the structural gene that encodes mouse glucose phosphate isomerase, GPI-1 (EC 5.3.1.9) produces a heat labile GPI-1CC homodimer. Our previous study of *Gpi*-1*s^a^*/*Gpi*-1*s^c^* heterozygotes (West *et al.* 1987) revealed two opposing influences on GPI-1 activity. Three dimers were produced and the levels of GPI-1AC heterodimer (and probably also GPI-1CC homodimer) were initially enhanced relative to GPI-1AA homodimer, possibly as a result of high production of GPI-1C monomers. The initial ratio of dimers may be closer to 4 GPI-1CC:4 GPI-1AC:1 GPI-1AA than the more usual 1:2:1 ratio. However, the presumed 4:4:1 ratio was masked by the lability of GPI-1AC and the more extreme lability of GPI-1CC.

Differences in stabilities of allozymes could have a profound effect on the total enzyme activity in heterozygotes such that the activity may not be intermediate between the two homozygote levels. Padua, Bulfield & Peters (1978) concluded that *Gpi*-1*s^c^* heterozygotes had intermediate levels of erythrocyte glucose phosphate isomerase and showed co-dominance for enzyme activity. In contrast, our present results show that *Gpi*-1*s^c^* heterozygotes have higher GPI levels than expected for co-dominance.

2. Materials and Methods

Inbred C57BL/Ola and partially congenic C57BL/Ola.129-*Gpi*-1*s^a^*/Ws and C57BL/Ola-*Gpi*-1*s^c^*/Ws provided samples homozygous for the three *Gpi*-1*s* structural alleles (b/b, a/a and c/c respectively). Heterozygotes were produced by intercrossing the homozygous stocks. All mice were bred and maintained under conventional conditions in the Centre for Reproductive Biology.

(i) *Assays of blood glucose phosphate isomerase*

Blood samples for spectrophotometric assay of GPI activity were collected through a heparinised capillary tube from the retro-orbital sinus while the mouse was anaesthetised with ether. Samples were collected at 1 and 3 months of age from the same mice. Fifty microlitres of blood was added to 500 μl of lysis buffer (0·1 M triethanolamine, 1 mM-EDTA pH 7·5 buffer containing 5 mM dithiothreitol and 0·5 mg/ml saponin) and stored at −20 °C for up to 4 weeks. After the samples were thawed and centrifuged (6500 rpm in an MSE microcentaur centrifuge for 5 min), 250 μl of supernatant was diluted with 250 μl sample buffer (lysis buffer without saponin) and assayed. Assays were done as described by Charles & Pretsch (1987),

at room temperature (22–24 °C) in a Pye Unicam SP6-500 UV spectrophotometer. Samples were assayed in batches with, as far as possible, representatives of each genotype in each batch of approximately 30 samples.

(ii) *Heat inactivation and electrophoresis of kidney glucose phosphate isomerase*

Kidneys for electrophoretic analysis were homogenized (100 mg/ml) in water with a Polytron tissue homogenizer and centrifuged at high speed (13000 rpm; 11600 g) for 5 min in an MSE microcentaur centrifuge. The supernatant was diluted 1/10 with water (supernatants from c/c homozygotes were diluted 1/5), mixed and aliquots dispensed into ten 1·5 ml Eppendorf tubes. Two series of heat inactivation experiments were set up by placing tubes in a 48 °C water bath for various times. In the first series tubes were incubated at 48 °C for 0, 5, 10, 15 and 20 min. In the second series tubes were treated for 0, 15, 30, 45 and 60 min. After the appropriate time at 48 °C each tube was put on ice until the end of the heat treatments and then the series of tubes was stored at −20 °C before electrophoretic analysis.

For electrophoretic analysis of the thermostability of different GPI-1 dimers (allozymes) samples were applied to Helena Titan III cellulose acetate electrophoresis plates using positions 2, 3, 4, 5 and 6 of a 0·25 μl Helena Super Z applicator. The different GPI-1 allozymes were separated and stained as previously described (West & Green, 1983; West & Fisher, 1984). The proportions of the different GPI-1 allozymes were quantified by scanning densitometry with a Helena Process-24 scanning densitometer fitted with a 4 mm slit and a 550 nm filter as previously described (West *et al.* 1987, 1989). For the sake of clarity each allozyme is referred to in the text by its dimeric designation: allozyme GPI-1A is homodimer AA; GPI-1AC is heterodimer AC, etc.

Tracks 2–6 were loaded with 0·25 μl samples treated either for 0, 5, 10, 15 and 20 min respectively, at 48 °C (series 1) or for 0, 15, 30, 45 and 60 min (series 2). After electrophoresis each of the five tracks were scanned in the normal anodal-to-cathodal direction to quantify the relative proportions of the different GPI-1 allozymes (dimers) in the sample. In order to compare the relative activities of a given dimer after various times at 48 °C the plate was scanned perpen-

Table 1. *GPI activity (μmol NADPH produced min^{-1} ml^{-1} blood at room temperature) in blood from mice of six different genotypes*

Gpi-1s genotype	No. of samples	1 month's activity ± S.E.	(expected)[a]	3 months' activity ± S.E.	(expected)	Statistical significance of difference between 1 and 3 months[b]	
						t	P
a/a	19	8·520 ± 0·237	—	8·182 ± 0·278	—	0·88	n.s.
b/b	24	8·322 ± 0·269	—	8·510 ± 0·301	—	0·48	n.s.
c/c	20	4·377 ± 0·187	—	2·667 ± 0·084	—	7·14	< 0·001
a/b	29	9·990 ± 0·205	(8·421)	9·519 ± 0·266	(8·346)	1·58	n.s.
a/c	22	8·980 ± 0·267	(6·449)	7·645 ± 0·239	(5·425)	2·88	< 0·01
b/c	31	8·004 ± 0·153	(6·350)	7·266 ± 0·216	(5·589)	2·79	< 0·01

[a] Expected values for the heterozygotes are calculated as the mean of the two homozygote values.
[b] Paired t tests (n.s., not significant).

Table 2. *Tests for genetic variability in GPI-1 activity by analysis of variance*

Genotypes compared	Mean activities (from Table 1)	Variation between genotypes
1-month blood samples		
a/a, a/b, b/b	8·52, 9·99, 8·32	$F(2, 69) = 14.28; P < 0.0001$
a/a, a/c, c/c	8·52, 8·98, 4·38	$F(2, 58) = 115.53; P < 0.0001$
b/b, b/c, c/c	8·32, 8·00, 4·38	$F(2, 72) = 111.93; P < 0.0001$
3-month blood samples		
a/a, a/b, b/b	8·18, 9·52, 8·51	$F(2, 69) = 5.82; P < 0.005$
a/a, a/c, c/c	8·18, 7·65, 2·67	$F(2, 58) = 195.43; P < 0.0001$
b/b, b/c, c/c	8·51, 7·27, 2·67	$F(2, 72) = 171.59; P < 0.0001$

dicularly to include the dimer in each of the five tracks.

Changes in total GPI-1 activity were quantified by applying 0·25 μl samples as above, staining for GPI-1 activity without electrophoresis and scanning perpendicularly from tracks 2–6. (Three rows of samples were applied per electrophoresis plate.) To control for variation in volume of sample applied in positions 2–6 of the applicator, the same sample (*a/a* homozygote supernatant) was applied to each of positions 2–6 and the GPI-1 activity compared by scanning all five tracks perpendicularly as above (without electrophoresis). This was repeated three times for each of 6 *a/a* samples. A second series of controls was set up with serial dilutions and analysed in the same way. The percentages of sample (diluted with water) in each track were as follows: Track 2, 100 %; 3, 80 %; 4, 60 %; 5, 40 %; 6, 20 %.

(iii) *Statistical analysis*

Statistical analysis was done on an Apple Macintosh Plus Computer using Statworks (Data Metrics, Inc.) and CLR ANOVA (Clear Lake Research) programmes to compute Student's *t*-test and analysis of variance.

3. Results

(i) *Comparison of GPI-*1 *activities in different genotypes*

Table 1 shows the glucose phosphate isomerase activity in blood of six genotypes at 1 and 3 months of age. The activity was significantly lower at 3 months for *c/c* homozygotes, and *a/c* and *b/c* heterozygotes but the other genotypes showed no significant change. This is consistent with our previous observation that the AC:AA dimer activity ratio falls between 1 and 3 months in *a/c* mice (West *et al.* 1987). The GPI-1 activities in heterozygote mice (*a/b*, *a/c* and *b/c*) were all higher than the mean of the two parental values.

Statistical analyses of these data are shown in Tables 2 and 3. Analysis of variance showed that there was significant variation in GPI-1 activity between genotypes for all three sets of heterozygote/homozygote comparisons in both 1- and 3-month blood samples. Three further tests (Table 3) were carried out by specifying contrasts among the means in the analysis of variance. For each of the six comparisons of genotypes, the distribution of activities differed significantly from that expected for additive inheritance (eg $a/c = \frac{1}{2}a/a + \frac{1}{2}c/c$). For the 1-month *b/c* comparison and for both *a/c* comparisons the data were adequately explained by dominant inheritance. The activity in 3-month *b/c* blood samaples was closest to that expected if BB and BC dimers had equal stability and specific activity (both higher than for CC homodimers) so that the expected *b/c* activity was $\frac{3}{4}b/b + \frac{1}{4}c/c$.

Table 3. *Tests for additive inheritance of variation in GPI-*1 *activity by analysis of variance (F values)*

Genotypes compared	Mean activities (from Table 1)	Degrees of freedom	Deviation from simple additive inheritance (e.g. $a/c = 0·5a/a + 0·5c/c$)	Deviation from simple dominance (e.g. $a/c = a/a$)	Deviation from expected with fully stable heteropolymer (e.g. $a/c = 0·75a/a + 0·25c/c$)	Inheritance pattern
1-month blood samples						
a/a, a/b, b/b	8·52, 9·99, 8·32	$F(1,69)$	$F = 28·23^{d}$	18·32[d]	21·44[a]	Non-additive
a/a, a/c, c/c	8·52, 8·98, 4·38	$F(1,58)$	$F = 76·77^{d}$	1·91 n.s.	24·74[d]	Non-additive, dominant
b/b, b/c, c/c	8·32, 8·00, 4·38	$F(1,72)$	$F = 42·47^{d}$	1·33 n.s.	6·38[a]	Non-additive, dominant
3-month blood samples						
a/a, a/b, b/b	8·18, 9·52, 8·51	$F(1,69)$	$F = 10·99^{b}$	10·56[b]	11·62[b]	Non-additive
a/a, a/c, c/c	8·18, 7·65, 2·67	$F(1,58)$	$F = 69·46^{d}$	3·07 n.s.	9·21[b]	Non-additive, dominant
b/b, b/c, c/c	8·51, 7·27, 2·67	$F(1,72)$	$F = 33·98^{d}$	15·66[c]	0·52 n.s.	Non-additive

n.s., not significant ($P > 0·05$). [a] $P < 0·05$. [b] $P < 0·005$. [c] $P < 0·0005$. [d] $P < 0·0001$.

Table 4. *Tests for heterosis for glucose phosphate isomerse activity in* a/b *and* a/c *heterozygotes*

Genotypes compares	Statistical significance[a]		Inheritance pattern
	t	*P*	
1-month blood samples			
a/b, a/a	4·62	< 0·001	
a/b, b/b	5·01	< 0·001	Heterosis
a/c, a/a	1·27	n.s.	Dominance
a/c, c/c	13·88	< 0·001	(not heterosis)
3-month blood samples			
a/b, a/a	3·35	< 0·002	
a/b, b/b	2·52	< 0·05	Heterosis

[a] Student's *t* test.
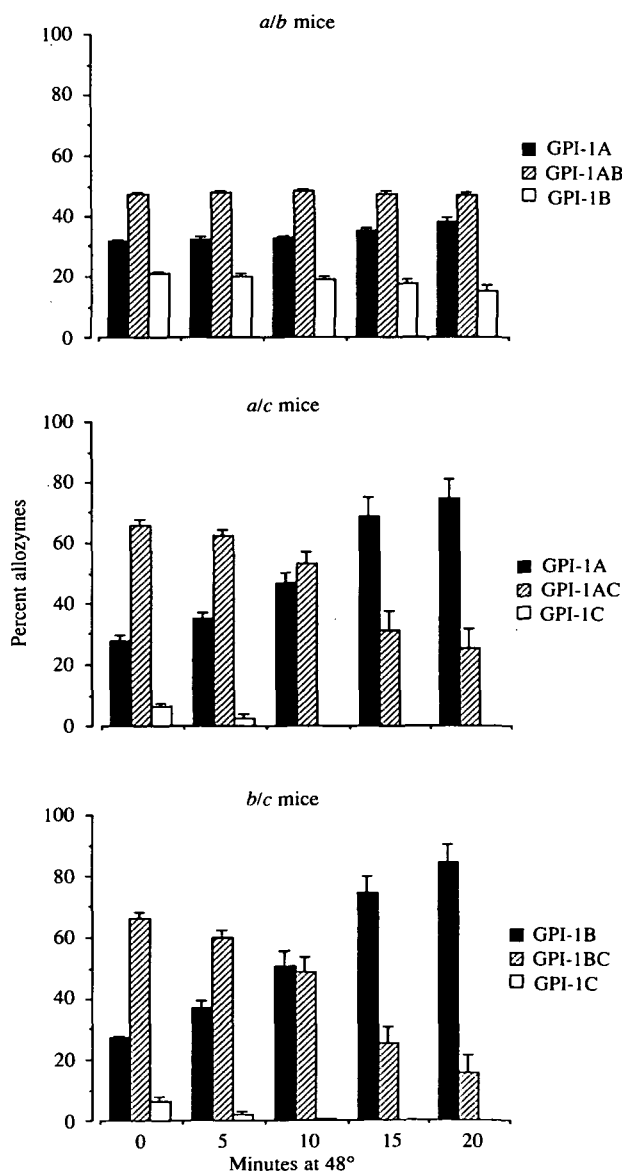n.s., not significant.

Fig. 1. Changes in allozyme (dimer) activity ratios induced by heating kidney homogenates from three types of heterozygotes (6 mice per group) at 48 °C for up to 20 min. The key refers to allozyme designations (allozyme GPI-1A = AA dimer, etc).

In three cases the heterozygote activity was higher than both of the parental values. Student's t tests (Table 4) showed that a/b heterozygote GPI-1 levels were significantly higher than a/a or b/b activities at both 1 and 3 months. This implies heterosis for glucose phosphate isomerase activity in this genotype.

(ii) *Heat-induced changes in allozyme (dimer) ratios*

Figure 1 shows the change in proportions of kidney GPI-1 allozymes for the three heterozygous genotypes during 20 min incubation at 48 °C. The a/c and b/c mice show a relative decline in GPI-1C and GPI-1AC or GPI-1BC allozymes (CC, AC and BC dimers). The a/b mice show little change in the allozyme proportions over this period but similar experiments conducted over 60 min show a relative increase in AA and a decrease in BB. The initial dimer activity ratio was 32·4±0·9AA:45·9±0·5AB:21·8±1·3BB. After 60 min at 48 °C this had changed to 47·0± 3·5AA:43·7±1·4AB:9·3±2·4BB. These changes imply relative dimer stabilities of AA > AB > BB, AA > AC and BB > BC. These kidney homogenates had relatively little CC dimer activity initially so it was difficult to assess the relative stabilities of the AC and CC or BC and CC dimers from Fig. 1. (Initially the dimer ratio in a/c mice was 28·0± 1·7AA:65·7±2·3AC:6·4±1·1CC and in b/c mice it was 27·3±0·7BB:66·4±1·7BC:6·2±1·6CC.)

(iii) *Heat-induced decline in total GPI-1 and individual dimer activities*

In order to monitor the decline in GPI-1 activity during heating, the electrophoresis plates were scanned perpendicularly across allozymes from all time points (as described in the Materials and Methods section) and normalized relative to a value of 100% for the untreated sample.

Six untreated a/a samples were each applied to tracks 2–6 and scanned in the same way to control for

Table 5. *Results of control experiment to validate quantification of six sets of serial diluted* a/a *kidney homogenates by scanning densitometry*

Track (applicator position)	Sample concn (%)	Total GPI-1 (%)		
		Expected	Observed	Normalized
2	100	33·3	31·1 ± 0·9	100
3	80	26·7	25·3 ± 1·1	81·8 ± 4·8
4	60	20·0	18·8 ± 0·7	60·6 ± 2·7
5	40	13·3	15·0 ± 0·7	48·4 ± 3·5
6	20	6·7	9·8 ± 0·5	31·7 ± 2·5

variation in applicator pick-up. This was repeated three times for each sample. If all five applicator positions (tracks 2–6) accurately applied 0·25 μl each track should have 20 % of the total activity. The results for these 18 scans showed the total activity to be distributed as follows: 20·5 ± 0·5 in track 2, 19·5 ± 0·4 in track 3, 18·4 ± 0·5 in track 4, 20·9 ± 0·4 in track 5 and 20·5 ± 0·6 in track 6. This showed that there were only small differences between applicator positions, so no correction was made for this in the subsequent analysis. In another control experiment, tracks 2–6 were loaded with serial dilutions of a/a samples and scanned to test the accuracy of the quantification. The results for six sets of serial dilutions are shown in Table 5. There was a tendancy for the GPI-1 activity in the most dilute samples (tracks 5 and 6) to be overestimated but this is consistent with previous control experiments when pairs of samples were compared (West & Green, 1983; West *et al.* 1989). Overall, the results show that the method of quantification is sufficiently accurate for the present comparative study.

Figure 2 shows the decline in total GPI-1 activity in kidney samples from 6 gentoypes heated at 48 °C for up to 60 min. From this it is clear that a/a, b/b and a/b mice have the most stable enzyme, c/c have the
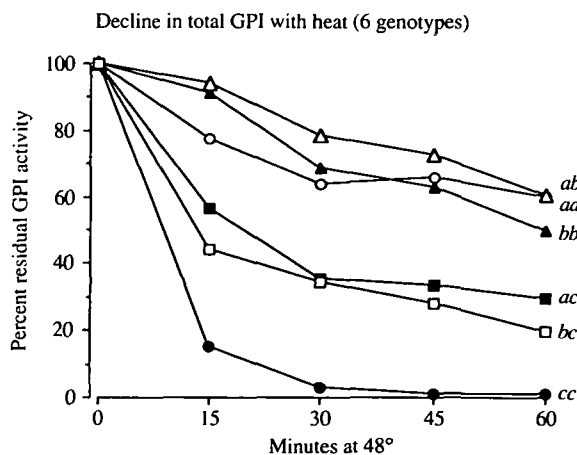
most unstable form and the GPI-1 in a/c and b/c mice is intermediate in thermostability.

The analysis of the decline in activity of specific dimers during 60 min at 48 °C is illustrated in Fig. 3.
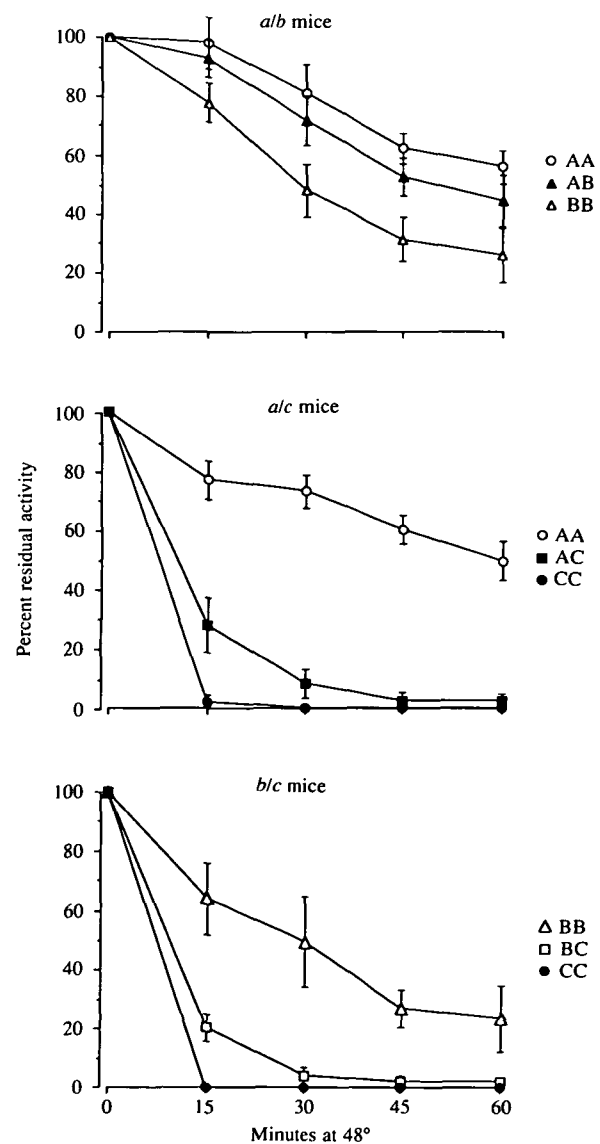

Fig. 3. Relative heat stability (at 48 °C) of different GPI-1 dimer activities produced by kidney homogenates from a/b, a/c and b/c heterozygote mice, (6 mice per group). The vertical bars represent the standard errors of the means.
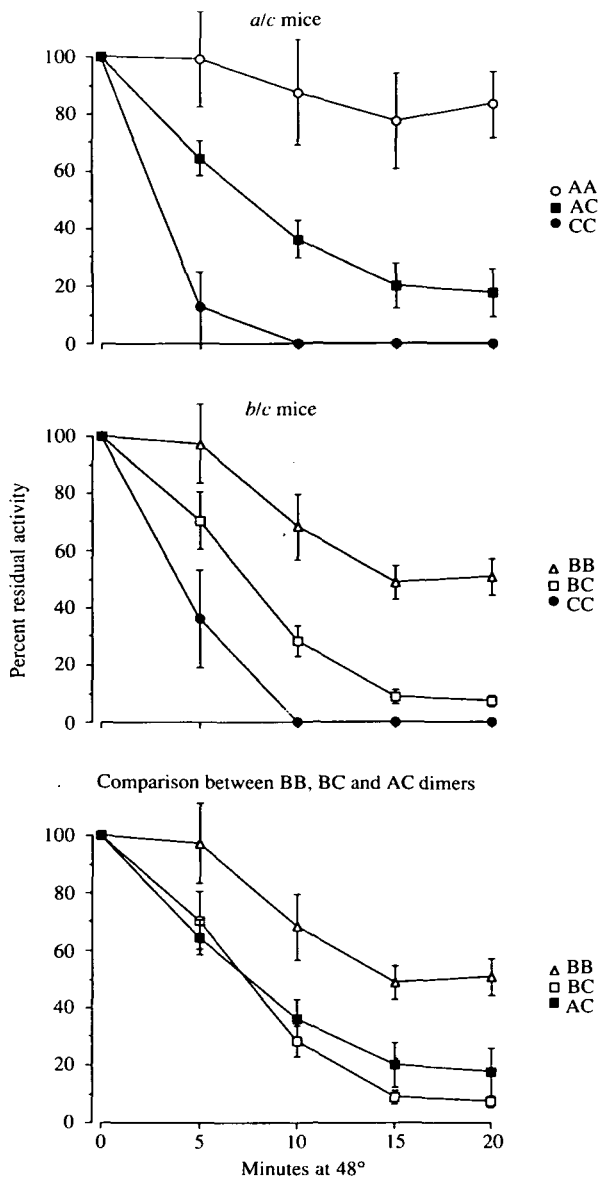

Fig. 2. Relative heat stability (at 48 °C) of total GPI-1 in kidney homogenates from mice of six genotypes. Six mice per group.

Fig. 4. Relative heat stability (48 °C for up to 20 min) of different GPI-1 dimer activities produced by kidney homogenates from a/c and b/c heterozygote mice (6 mice per group). The top two graphs represent the dimers produced by a/c and b/c mice respectively. The bottom graph compares the decline in AC (from a/c mice), BC and BB (from b/c mice) activity.

The AC and BC heterodimers are more stable than the CC homodimer (GPI-1C allozyme), but the loss of the CC dimer is almost complete within the first 15 min. In order to clarify this relationship, results from a similar analysis of GPI-1 dimers in a/c and b/c mice during 20 min at 48 °C are shown in Fig. 4. These results confirm that the relative stabilities of GPI-1 allozymes is AA > AB > BB; AA > AC > CC; BB > BC > CC.

Moreover, comparison between BB, AC and BC dimers (bottom graph in Fig. 4) show that BB is the most stable and that AC may be slightly more stable than BC. Comparison of graphs for AC and BC in Fig. 3 supports this conclusion. The relative stabilities

of GPI-1 dimer activities are therefore AA > AB > BB > AC $\geqslant$ BC > CC. In principle, the relative stabilities of AC and BC could be compared more directly by performing a heat inactivation experiment on a mixture of a/c and b/c homogenates and scanning each track separately (as in Fig. 1). However, in practice this would be difficult because AC and BB dimers co-migrate and are difficult to resolve by electrophoresis.

4. Discussion

The assays of total GPI-1 activity in blood showed that the levels in all three types of heterozygotes (a/b, a/c and b/c) were significantly higher than expected on the basis of additive inheritance. Moreover the activity in a/b heterozygotes was significantly higher than in either a/a or b/b homozygotes and therefore showed heterosis.

When appropriate corrections are made for differences in assay temperature (a 10 °C increase in assay temperature doubles the enzyme activity) the present GPI-1 blood activities are in the same range as those reported by Padua, Bulfield & Peters (1978). These authors did not study a/b heterozygotes but concluded that both a/c and b/c heterozygotes had blood GPI-1 levels consistent with additive inheritence, although the activity in b/c heterozygotes ($10\cdot55\pm0\cdot37$) was higher than predicted ($8\cdot95$) on the basis of additive inheritance. (The homozygote levels were $4\cdot84\pm0\cdot40$ and $13\cdot6\pm0\cdot47$ for c/c and b/b respectively.)

Three previous studies, with different inbred and congenic strains of mice, showed that erythrocyte GPI-1 activity was higher in a/a homozygotes than b/b mice (Padua *et al.* 1978; van Zant *et al.* 1983; Warner *et al.* 1985) but this difference was less marked with congenic strains (van Zant *et al.* 1983). Of these studies only Warner *et al.* (1985) studied a/b heterozygotes. In contrast to our present results with congenic strains, Warner *et al.* (1985) found that the mean GPI-1 level in a/b erythrocytes was intermediate between those for the two parental inbred strains (A/J and C57BL/6J). It is as yet unclear whether the difference between these results and the results of our present study are attributable to strain differences and our observation of heterosis requires independent confirmation.

The heat inactivation showed that the stability of total kidney GPI-1 activity in a/c and b/c heterozygotes was intermediate between parental stabilities in agreement with similar studies of mouse erythocyte GPI-1 described by Padua *et al.* (1978). Comparable results have also been reported for some human deficiencies of GPI (e.g. Whitelaw *et al.* 1979; Dallapiccola *et al.* 1986) and Padua *et al.* (1978) list 14 variants of GPI deficiency in man, all of which are characterized by heat labile enzyme. Although the thermostability pattern described by Whitelaw *et al.* (1979) and Dallapiccola *et al.* (1986) for human GPI

Table 6. *Predicted effect of difference in GPI-*1 *dimer specific activity (or stability) and primary ratio of dimers on total GPI activity in samples from homozygous* (a/a *and* c/c) *and heterozygous* (a/c) *mice*

Assumed relative specific activities (or stabilities) of GPI-1 dimers			Calculated total GPI-1 activity in mice of different genotypes[a]			Inheritance pattern
AA	AC	CC	*a/a* mice	*a/c* mice	*c/c* mice	
1. Assuming monomer ratio of 1A:1C (dimer ratio 1AA:2AC:1CC)						
(a) 10	10	10	100	100	100	Non-variant
(b) 10	5	0	100	50	0	Additive
(c) 10	6	2	100	60	20	Additive
(d) 10	10	2	100	80	20	Non-additive
(e) 10	8	2	100	70	20	Non-additive
(f) 10	2	2	100	40	20	Non-additive
(g) 10	12	2	100	90	20	Non-additive
(h) 10	20	10	100	145	80	Non-additive, heterosis
2. Assuming monomer ratio of 1A:2C (dimer ratio 1AA:4AC:4CC)						
(a) 10	10	10	100	150	200	Additive
(b) 10	5	0	100	50	0	Additive
(c) 10	6	2	100	70	40	Additive
(d) 10	10	2	100	97	40	Non-additive
(e) 10	8	2	100	83	40	Non-additive
(f) 10	2	2	100	43	40	Non-additive
(g) 10	12	2	100	110	40	Non-additive, heterosis
(h) 10	20	10	100	203	87	Non-additive, heterosis

[a] GPI-1 activities calculated and normalised so that homozygous *a/a* mice have activity of 100. When monomer ratio is 1A:1C activities calculated for *a/a* mice as 4AA; *a/c* mice, 1AA+2AC+1CC; *c/c* mice, 4CC. When monomer ratio is 1A:2C, *a/a* mice, 4AA; *a/c* mice, 6/9 (1AA+4AC+4CC); *c/c* mice, 8CC.

were similar to those seen in mouse *a/c* and *b/c* heterozygotes, the activity levels for the human heterozygotes were compatible with additive inheritance.

The heat inactivation studies revealed that the relative stabilities of dimeric activities are AA > AB > BB > AC ⩾ BC > CC although the difference between AC and BC is slight. This is consistent with our previous finding of AA > AC > CC for GPI-1 dimers in unfertilized eggs (West *et al.* 1987).

Differences in stabilities among the various GPI-1 dimers may contribute towards the higher than predicted enzyme activities in *a/c*, *b/c* and *a/b* heterozygotes. *In vivo* differences in stability may parallel those seen in the heat stability experiments or may be the result of other variation that affects the turnover of GPI-1 molecules in blood. Beutler (1983) has argued for differential sensitivity to proteases and Johnson and colleagues have discussed differences in turnover of mouse hypoxanthine phosphoribosyl-transferase variants during reticulocyte maturation (Johnson & Chapman, 1987; Johnson *et al.* 1985, 1988).

Another possible contributing influence is elevated production of GPI-1C monomers. Evidence for this was presented by West *et al.* (1987) and the GPI-1 allozyme ratios shown in Fig. 1 of the present paper are also consistent with this view. The ratio of AC/AA and BC/BB in untreated *a/c* and *b/c* mice were 2·35 (65·7 ± 2·3/28·0 ± 1·7) and 2·43 (66·4 ±

1·7/27·3 ± 0·7) respectively. In contrast, the ratio of AB/AA in untreated *a/b* heterozygotes was only 1·50 (47·5 ± 0·5/31·7 ± 0·6). Since AC and BC dimers are both more labile than AB the magnitude of this difference was probably originally greater. We previously reported AC/AA ratios in *a/c* heterozygotes of up to 3·7 for 6½ day embryos, 4·26 for adult liver, and kidney ratios (2·21) similar to the present study (West *et al.* 1987).

If elevated production of GPI-1C monomers occurs this may explain why *c/c* mice show only mild haematologic symptoms (Padua *et al.* 1978) when many of the human GPI deficiences cause severe haematolytic anaemia. It may also help to explain why non-additive inheritance occurs in *a/c* and *b/c* mice but was not apparent in the two human GPI deficiences cited above.

The statistical tests (Tables 3 and 4) indicate that the data is better explained by dominance or heterosis than by additive inheritance. These are descriptions of the observations but do not explain the data at a functional biochemical level. Examples of how variation in specific activity (or stability) of different GPI-1 dimers could produce higher than expected enzyme activities in heterozygous *a/c* mice are shown in Table 6, assuming an initial monomer ratio of either 1A:1C or 1A:2C and random association of monomers to form enzymatically active dimers.

When each dimer has equal specific activity or stability [examples 1(a), 2(a) in Table 6] then the total

GPI-1 activities in a/a, a/c and c/c mice are consistent with additive inheritance. This is also true when the specific activity of the AC dimer is exactly mid-way between those of the AA and CC dimers [examples 1(b,c), 2(b,c)]. However, any deviation from this results in non additive inheritance with enzyme activity levels in the a/c heterozygote that are either higher [1(d,e,g,h), 2(d,e,g,h] or lower [1(f), 2(f)] than the mean of the activities in a/a and c/c homozygotes. [Our data was statistically tested (Table 3) against expectations of example 1(d) in Table 6.]

From this it can be seen that variation in dimer stability could generate non-additive inheritance patterns. However, our results for blood GPI-1 activities (Tables 1 and 4) revealed that a/b heterozygotes had levels that exceeded the parental level. The only examples of this type of heterosis that we generated theoretically [Table 6, examples 1(h), 2(g, h)] were when the AC heterodimers had a higher specific activity (or were more stable *in vivo*) than either of the homodimers. It is also possible that monomers dimerise non-randomly but this would not be sufficient to account for heterosis without variation in activity or stability between dimers.

Whatever the cause of the observed non-additive inheritance of GPI-1 activity it is clear that, on theoretical grounds (Table 6), structural gene alterations that cause variation in stabilities among dimers could result in non-additive inheritance patterns. This is an important point because non-additive inheritance is expected for *trans*-acting regulatory genes but not for *cis*-acting regulatory genes or structural genes [see Paigen, (1971, 1979) for discussion].

At least two other examples of non-additive inheritance of mammalian enzyme levels have been reported. Fleisher *et al.* (1973) showed that human skin fibroblasts from obligate heterozygotes for cystathionine synthase had 4.40 ± 0.92 nmol mg^{-1} protein h^{-1} of this enzyme, homozygote deficients had 0.77 ± 0.42 and controls had 20.97 ± 1.81. Kaufman, Max & Kang (1975) found that human liver biopsies from patients with hyperphenylalaninaemia had a mean phenylalanine hydroxylase activity of 3.73 ± 0.58 μmol mg protein^{-1} h^{-1}; parents (heterozygotes) had 9.92 ± 2.79 and controls had 75.5 ± 7.86. In contrast to our present results, both of these examples illustrate non-additive inheritance for multimeric enzymes with lower than expected heterozygote activities. For cystathionine synthase, the authors postulated a 1:2:1 ratio of dimers in the heterozygote but with no catalytic activity from the heterodimer and very little activity from the mutant homodimer. For phenylalanine hydroxylase Kaufman *et al.* (1975) assumed that the normal genotype AABB produced normal dimer $\alpha\beta$ and that hyperphenylalaninaemia patients were homozygous at one locus (say A'A'BB) producing $\alpha'\beta$ dimers with low activity. Heterozygotes (AA'BB) would be expected to show additive inheritance with equal proportions of $\alpha\beta$ and $\alpha'\beta$ dimers.

The authors postulated that low heterozygote activity was a result of either (1) more rapid synthesis (or slower degradation) of α' than α subunits or (2) non-random combination of subunits leading to selective formation of defective $\alpha'\beta$ dimers.

Our results and the examples cited show that non-additive inheritance does not necessarily imply the involvement of a *trans*-acting regulatory gene.

References

Beutler, E. (1983). Selectivity of proteases as a basis for tissue distribution of enzymes in hereditary deficiencies. *Proceedings of the National Academy Sciences USA* **80**, 3767–3768.

Charles, D. J. & Pretsch W. (1987). Linear dose–response relationship of erythrocyte enzyme-activity mutations in offspring of ethylnitrosurea-treated mice. *Mutation Research* **176**, 81–91.

Dallopiccola, B., Novelli, G., Ferranti, G., Pachi, A., Cristiani, M. L. & Magnani, M. (1986). First trimster monitoring of a pregnancy at risk for glucose phosphate isomerase deficiency. *Prenatal Diagnosis* **6**, 101–107.

Fleisher, L. D., Tallan, H. H., Beratis, N. G., Hirschhorn, K. & Gaull, G. E. (1973). Cystathionine synthase deficiency: heterozygote detection using cultured skin fibroblasts. *Biochemical and Biophysical Research Communications* **55**, 38–44.

Johnson, G. G. & Chapman, V. M. (1987). Altered turnover of hypoxanthine phosphoribosyltransferase in erythroid cells of mice expressing *Hprt a* and *Hprt b* alleles. *Genetics* **116**, 313–320.

Johnson, G. G., Kroner, W. A., Bernstein, S. I., Chapman, V. M. & Smith, K. D. (1988). Altered turnover of allelic variants of hypoxanthine phosphoribosyltransferase is associated with N-terminal amino acid sequence variation. *Journal of Biological Chemistry* **263**, 9079–9082.

Johnson, G. G., Larsen, T. A., Blakely, P. & Chapman, V. M. (1985). Elevated levels of erythrocyte hypoxanthine phosphoribosyltransferase associated with allelic variation of murine *Hprt*. *Biochemistry* **24**, 5083–5089.

Kaufman, S., Max, E. E. & Kang, E. S. (1975). Phenylalanine hydroxylase activity in liver biopsies from hyperphenylalaninemia heterozygotes: deviation from proportionality with gene dosage. *Pediatric Research* **9**, 632–634.

Padua, R. A., Bulfield, G. & Peters, J. (1978). Biochmeical genetics of a new glucosephosphate isomerase allele (*Gpi-1ᶜ*) from wild mice. *Biochemical Genetics* **16**, 127–143.

Paigen, K. (1971). The genetics of enzyme realisation. In *Enzyme Synthesis and Degradation in Mammalian Systems* (ed. M. Rechcigl), pp. 1–47. Basel: Karger.

Paigen, K. (1979). Acid hydrolases as models of genetic control. *Annual Review of Genetics* **13**, 417–466.

van Zant, G., Eldridge, P. W., Behringer, R. R. & Dewey, M. J. (1983). Genetic control of hematopoietic kinetics revealed by analyses of allophenic mice and stem cell suicide. *Cell* **35**, 639–645.

Warner, C. M., Meyer, T. E., Balinsky, D. & Briggs, C. J. (1985). Variations in the amount of glucose phosphate isomerase in lymphocytes and erythrocytes from A/J and C57BL/6J mice. *Biochemical Genetics*, **23**, 815–825.

West, J. D. & Fisher, G. (1984). A new allele of the *Gpi*-1*t* temporal gene that regulates the expression of glucose phosphate isomerase in mouse oocytes. *Genetical Research* **44**, 169–181.

West, J. D., Flockhart, J. H., Angell, R. R., Hillier, S. G., Thatcher, S. S., Glasier, A. F., Rodger, M. W. & Baird, D. T. (1989). Glucose phosphate isomerse activity in mouse and human eggs and pre-embryos. *Human Reproduction* **4**, 82–85.

West, J. D. & Green, J. F. (1983). The transition from ooctyte-coded to embryo-coded glucose phosphate iso-merase in the early mouse embryo. *Journal of Embryology and Experimental Morphology* **78**, 127–140.

West, J. D., Leask, R., Flockhart, J. H. & Fisher, G. (1987). High activity of an unstable form of glucose phosphate isomerase in the mouse. *Biochemical Genetics* **25**, 543–561.

Whitelaw, A. G. L., Rogers, P. A., Hopkinson, D. A., Gordon, H., Emerson, P. M., Darley, J. H., Reid, C. & Crawfurd, M.d'A. (1979). Congenital haemolytic anaemia resulting·from glucose phosphate isomerase deficiency. *Journal of Medical Genetics* **16**, 189–196.

3-2