Genet. Res., Camb. (1989), **53**, *pp.* 105–110 *With* 1 *text-figure Printed in Great Britain*

The liver/erythrocyte pyruvate kinase gene complex [*Pk-1*] in the mouse: structural gene mutations

LESLEY A. FITTON* AND GRAHAME BULFIELD†
Gene Expression Group, AFRC Institute of Animal Physiology and Genetics Research, Roslin, Midlothian EH25 9PS, U.K.

(Received 19 July 1988 and in revised form 21 October 1988)

Summary

Nine enzyme activity variants of liver/erythrocyte pyruvate kinase have been found amongst laboratory and wild mice. Four of these variants have been shown by biochemical and immunological criteria to be mutations of the structural gene, *Pk-1s*. These four structural gene mutations, and two regulatory gene mutations, define the gene complex, [*Pk-1*]. One allele of the structural gene, *Pk-1s*^{l}, found in the inbred strain C57BL, has an unusual phenotype and affects the expression of pyruvate kinase in the liver but not erythrocyte. A possible mechanism for this tissue-specific structural gene mutation is suggested.

1. Introduction

The glycolytic enzyme pyruvate kinase (PK, EC 2.7.1.40) is differentially expressed in mammals in a complex tissue specific manner. It is coded for in the mouse by two structural genes: *Pk-1* (chromosomal location unknown; Moore & Bulfield, 1981) and *Pk-3* (chromosome 9, Peters *et al.* 1981; Peters & Andrews, 1984); there is no *Pk-2* gene. There are four major isoenzymes of PK in mammals produced from four mRNAs, two from each structural gene possibly by RNA splicing: PK-R in erythrocytes and PK-L in adult liver from the *Pk-1* gene; PK-M_1, in heart, muscle, brain, etc. and PK-M_2 in kidney and foetal liver from the *Pk-3* gene (Imamura & Tanaka, 1972; Marie *et al.* 1976, 1981; Harada *et al.* 1978; Saheki *et al.* 1978; Noguchi *et al.* 1982; Saheki *et al.* 1982; Simon *et al.* 1982; Peters & Andrews, 1984). The cDNA sequences for both genes have been cloned (Noguchi *et al.* 1983, 1984, 1986; Simon *et al.* 1983; Inoue *et al.* 1986; Lone *et al.* 1986).

A number of inherited variants of *Pk-1* and *Pk-3* have been discovered in the mouse. For the *Pk-3* gene these include both on allele producing no measurable enzyme activity (*Pk-3*^{r}; Johnson *et al.* 1981) and an electrophoretic allele (*Pk-3*^{b}; Peters *et al.* 1981) and for *Pk-1*, nine enzyme activity variants (Bulfield *et al.* 1978; Moore & Bulfield, 1981; Bulfield *et al.* 1984; Charles & Pretsch, 1984, 1987) and an electrophoretic allele (found in *Mus spretus*; Bonhomme *et al.* 1984).

† Corresponding author.
* Present address: Department of Haematology, The Royal Victoria Infirmary, Newcastle-upon-Tyne, NE1 4LP, U.K.

In this paper we analyse four putative structural gene mutations of *Pk-1* found in inbred and wild mice and in a further paper we will analyse two putative regulatory gene mutations. These regulatory and structural gene mutations define the gene complex, [*Pk-1*].

Material and methods

(i) *Animals*

The inbred strains C57BL/6 and C3H/He were obtained from Bantin and Kingman Ltd, Grimston, Hull, U.K. The phenotypes W (*Pk-1*^{b}/*Pk-1*^{b}; Moore & Bulfield, 1981), PKD and PKL (Bulfield *et al.* 1984) originated as heterozygotes in wild-caught animals and are maintained as homozygous stocks. Livers from the BXH RI strains were a gift from Dr B. A. Taylor of the Jackson Laboratory (Bar Harbor, MA, 04609) and were sent to Roslin packed in dry ice; other tissues were stored at −70 °C prior to use at which temperature the enzyme from all phenotypes was stable.

(ii) *Determination of enzyme activity*

Pyruvate kinase (EC 2.7.1.40) activity was determined using an LKB Reaction Rate Analyser as previously described (Ultrolab; Bulfield & Moore, 1978; Moore & Bulfield 1981) and interfaced with a Commodore 64 computer. Enzyme activity units were expressed as μmol/min/ml blood or μmol/min/g wet weight liver at 30 °C.

Table 3. *Equivalence points and slopes of liver and blood PK of five phenotypes determined by immunotitration*[a]

	Liver		Blood	
Phenotype	Equivalence point	Slope	Equivalence point	Slope
C57BL/6	13·5 ± 1·3	19·8 ± 0·90	18·5 ± 1·0	20·1 ± 1·30
C3H/He	10·7 ± 0·7	8·4 ± 0·80	21·0 ± 1·5	17·4 ± 1·80
W(*Pk-1*^{b}/*Pk-1*^{b})	10·1 ± 1·1	6·6 ± 1·15	17·2 ± 1·5	8·6 ± 0·75
PKD	11·9 ± 0·8	8·9 ± 0·25	19·5 ± 2·3	9·3 ± 0·90
PKL	10·8 ± 1·5	10·1 ± 1·00	18·2 ± 0·9	7·5 ± 0·55

[a] Mean ± S.E. of 10 animals, each animal assayed three times, for each phenotype.

but only in the specific activity of the enzyme, implying that they are caused by mutations at the structural gene, *Pk-1s*.

(iii) *The W, PKD and PKL phenotypes*

These three phenotypes have already been partially characterised. The W phenotype derived from a wild heterozygous male trapped in Edinburgh, and is caused by homozygosity of a structural gene mutation *Pk-1s*^{b}, that segregates from both C57BL/6 and normal wild-caught mice and affects PK activity, thermal stability and K_m for ADP in both liver and blood (Moore & Bulfield, 1981). The PKD and PKL phenotypes were derived from individual low PK activity wild-caught males from Edinburgh and Leicestershire, respectively, which were putative heterozygotes (Bulfield *et al.* 1984). No detailed segregation analysis has been performed with these two phenotypes but true breeding strains were established immediately after crossing the original putative heterozygote male to four of his low PK activity putative heterozygote daughters (from a cross with a C57BL/6 female). These strains have bred true for the low PK activity PKD and PKL phenotypes for over 30 generations and this strongly indicates that they are caused by homozygosity of a mutant allele. These phenotypes also have lower liver and blood PK activity than both normal wild-caught mice and C57BL/6 animals. As the PKD and PKL phenotypes were each derived from an individual mouse trapped over 300 miles apart and each only isolated once from their sites it is unlikely that they are caused by homozygosity of the same allele. The enzyme from both phenotypes also has a wild type heat stability and K_m for ADP and therefore differs from animals of the W phenotype (Bulfield *et al.* 1984); it is therefore likely that three different alleles cause these three phenotypes.

That the PKD and PKL phenotypes are caused by alleles of the structural gene, *Pk-1s*, is strongly suggested by the effect on PK specific activity and not PK concentration in these animals (Table 3, Fig. 1). These putative alleles of *Pk-1s* will be provisionally called *Pk-1s*^{c} causing the PKD phenotype and *Pk-1s*^{d} causing the PKL phenotype.

(iv) *The C57BL/6 and C3H/He phenotypes: the BXH RI strains*

In previous work (Moore & Bulfield, 1981; Bulfield *et al.* 1984) the C57BL inbred strain had been taken as the standard 'wild-type' strain. The C3H strain has, however, the most common phenotype amongst the inbred strains (Bulfield *et al.* 1978) and differs from C57BL/6 in a tissue specific manner having the same PK activity in the blood but with a two-fold difference in the liver activity (Table 1).

It was not possible to analyse the difference between these two phenotypes with a classical segregation experiment so animals from the 12 BXH recombinant inbred strains (Taylor, 1978) were used. Three of the BXH strains had the C57BL/6 phenotype and eight had the C3H phenotype (Table 4); no unusual phenotypes were observed. Although the ratio of phenotypes amongst the RI strains is not 1:1, the deviation is not significant with such small numbers; the data indicates the segregation of a major gene

Table 4. *Liver PK activity between the BXH recombinant inbred strains*[a]

Strain	PK activity	Phenotype	*n*
C57BL/6J	57·0 ± 2·8	B	5
C3H/J	36·6 ± 1·1	H	5
BXH 2	37·1 ± 1·4	H	6
BXH 3	38·4 ± 2·0	H	6
BXH 4	37·4 ± 4·4	H	6
BXH 6	80·3 ± 1·7	B	9
BXH 7	35·3 ± 3·0	H	7
BXH 8	68·8 ± 3·5	B	8
BXH 9	60·1 ± 1·7	B	6
BXH10	38·5 ± 2·5	H	6
BXH11	43·1 ± 1·5	H	7
BXH12	42·5 ± 3·5	H	3
BXH14	—	—	—
BXH19	35·0 ± 0·7	H	8

[a] Mean ± S.E.; each animal assayed three times.

https://doi.org/10.1017/S0016672300027981 Published online by Cambridge University Press

amongst the BXH strains. Unfortunately this strain distribution pattern does not give any information on linkage (B. A. Taylor, personal communication).

The C3H/He and the C57BL/6 phenotypes only differ in measurable PK activity in the liver; they have the same measurable PK activity in the blood (Table 1). This liver difference is not due to enzyme concentration as the equivalence points are the same (Table 3, Fig. 1) but is due to a difference in enzyme specific activity (slopes; Table 3, Fig. 1) and is therefore likely to be due to an allelic difference at the structural gene, *Pk-1s*. We therefore name the allele in C3H/He mice (and most inbred strains; Bulfield *et al.* 1978) *Pk-1s*^{a} and that in C57BL/6, Pk-*1s*^{l} (liver-specific). Note that this is a change in allelic designation of C57BL/6 from our previous publication (Moore & Bulfield, 1981). A possible mechanism whereby an allelic difference of the structural gene can cause a tissue specific alteration in enzyme activity is discussed later.

4. Discussion

There are two structural genes for the glycolytic enzyme, pyruvate kinase in mammals: *Pk-1* codes for the PK-L and PK-R isoenzymes of adult liver and erythrocytes respectively and *Pk-3*, coding for both the PK-M_1 isoenzyme of muscle, brain etc. and the PK-M_2 isoenzyme of kidney and foetal liver (Imamura *et al.* 1978; Marie *et al.* 1976, 1981; Harada *et al.* 1978; Saheki *et al.* 1978, 1982; Noguchi *et al.* 1982; Simon *et al.* 1982; Peters & Andrews, 1984). By direct screening of inbred strains, mutagenised mice and feral animals, nine enzyme activity variants and an electrophoretic variant of PK-L and PK-R have been discovered (Bulfield *et al.* 1978; Moore & Bulfield, 1981; Bonhomme *et al.* 1984; Charles & Pretsch, 1984, 1987). In this paper we have subjected 5 of the enzyme activity phenotypes to genetical and biochemical analysis.

Three of the five phenotypes, W, PKD and PKL, each derived from separate populations of wild-caught mice, had reduced enzyme activity in both liver and blood (Table 1) without any change in enzyme concentration (Fig. 1 and Table 3); the enzyme in the W phenotype also has an altered K_m for ADP (Moore & Bulfield, 1984). The W phenotype segregated as a single gene and identifies the structural gene, *Pk-1s* (Moore & Bulfield, 1984) and breeding analysis of the PKD and PKL phenotypes also strongly suggests these are caused by single genes. It is therefore concluded that all three phenotypes are caused by separate allelic differences at the structural gene, *Pk-1s*.

Animals from the C57BL/6 and C3H/He inbred strains also have different phenotypes but in this case the variation is tissue-specific. PK activity in the blood is the same whereas C57BL/6 animals have two-fold greater PK activity in the liver (Table 1). In fact, the C3H/He phenotype is the standard amongst inbred strains (e.g. SM, A, 129; Bulfield *et al.* 1978) and C57BL/6 unique; the C3H/He phenotype is also a more logical basis for comparison with the three wild derived phenotypes (Table 1) and therefore has been given the wild-type allelic designation, *Pk-1s*^{a}.

The high-liver PK activity phenotype of C57BL/6 animals is not due to an alteration in enzyme concentration but rather in enzyme activity (Fig. 1, Table 3). The phenotypic difference between C57BL/6 and C3H/He animals appear to segregate as a single major gene amongst the BXH RI strains (Table 4). This data indicates that C57BL/6 animals have a tissue-specific mutation in the structural gene *Pk-1s* which has been called *Pk-1s*^{l}. A molecular mechanism is available for a tissue-specific mutation in *Pk-1s* in that the PK-L and PK-R isoenzymes are encoded in separate RNAs produced by the same gene (Marie *et al.* 1981; Saheki *et al.* 1982). Therefore the mutation in C57BL/6 animals could be in a DNA sequence specific for the PK-L isoenzyme. With the cloning of the *Pk-1s* gene (Noguchi *et al.* 1983; Simon *et al.* 1983; Inoue *et al.* 1986; Lone *et al.* 1986) this suggestion can be directly tested.

There are now up to six alleles of the *Pk-1s*

Table 5. *Alleles of the Pk-1s structural gene in mice*

Allele	Alterations	Strain or phenotype	Reference
Pk-1s^{a}	—	C3H and other inbred strains	Bulfield *et al.* 1978; and this paper
Pk-1s^{b}	Activity, stability K_m for ADP	W (feral)	Moore & Bulfield, 1981; and this paper
Pk-1s^{c}	Activity, stability	PKD (feral)	Bulfield *et al.* 1984; and this paper
Pk-1s^{d}	Activity, stability	PKL (feral)	Bulfield *et al.* 1984; and this paper
Pk-1s^{e}	Electrophoretic mobility	*Mus spretus*	Bonhomme *et al.* 1984
Pk-1s^{l}	Tissue specific activity	C57BL	Bulfield *et al.* 1978; and this paper

[a] Note that the *c* and *d* are considered non-identical only on recovery from far distant trapping sites (see Results).

https://doi.org/10.1017/S0016672300027981 Published online by Cambridge University Press

structural gene in mice (Table 5). The identification of this locus will now allow the genetical and biochemical analysis of two further phenotypes affecting the levels of the PK-L and PK-R isoenzymes caused by putative regulatory gene mutations. This analysis will be the subject of a further publication.

We should like to thank Mr J. Parker, Systems Support Engineer, Racal-Redac Systems Ltd, for designing and building the interface and software for the LKB Kinetic Analyser with the Commodore 64 computer, and the SERC for a research studentship to L.A.F.

References

Bonhomme, F., Catalan, J., Britton-Davidian, J., Chapman, V. M., Moriwaki, K., Nevo, E. & Thaler, L. (1984). Biochemical diversity and evolution in the genes *Mus*. *Biochemical Genetics* **22**, 275–303.

Bulfield, G., Hall, J. M. & Tsakas, S. (1984). Incidence of inherited enzyme activity variants in feral mouse populations. *Biochemical Genetics* **22**, 133–138.

Bulfield, G. & Moore, E. A. (1974). Semi-automated assays for enzymopathies of carbohydrate metabolism in liver and erythrocytes using a reaction rate analyser. *Clinica Chemica Acta* **53**, 265–271.

Bulfield, G., Moore, E. A. & Kacser, H. (1978). Genetic variation in activity of the enzymes of glycolysis and gluconeogenesis between inbred strains of mice. *Genetics* **89**, 551–561.

Charles, D. J. & Pretsch, W. (1984). A new pyruvate kinase mutation with hyperactivity in the mouse. *Biochemical Genetics* **22**, 743–750.

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

Goding, J. W. (1978). Conjugation of antibodies with fluorochrome modifications to the standard methods. *Journal of Immunological Methods* **13**, 215–226.

Harada, K., Saheki, S., Wada, K. & Tanaka, T. (1978). Purification of four pyruvate kinase isozymes of rats by affinity chromatography. *Biochimica et Biophysica Acta* **524**, 327–339.

Imamura, K. & Tanaka, T. (1972). Multi-molecular forms of pyruvate kinase from rat and other mammalian tissues. 1. Electrophoretic studies. *Journal of Biochemistry* **71**, 1043–1051.

Inoue, H., Noguchi, T. & Tanaka, T. (1986). Complete amino acid sequence of the rat L-type pyruvate kinase deduced from the cDNA sequence. *European Journal of Biochemistry* **154**, 465–469.

Johnson, F. M., Chasalow, F., Anderson, G., MacDougal, P., Hendnen, R. W. & Lewis, S. E. (1981). A variant in mouse kidney pyruvate kinase activity determined by a mutant gene on chromosome 9. *Genetical Research* **37**, 123–131.

Lone, Y.-C., Simon, M.-P., Kahn, A. & Marie, J. (1986). Complete nucleotide and deduced amino acid sequences of the rat L-type pyruvate kinase. *FEBS letters* **195**, 97–100.

Marie, J., Kahn, A. & Boivin, P. (1976). Pyruvate kinase isozymes in man. 1. M type isozymes in adult and foetal tissues, electrofocusing and immunological studies *Human Genetics* **31**, 35–45.

Marie, J., Simon, M.-P., Dreyfus, J.-C. & Kahn, A. (1981). One gene but two messenger RNAs encode liver L and red cell Li pyruvate kinase subunits. *Nature* **292**, 70–72.

Moore, K. J. & Bulfield, G. (1981). An allele (*Pk-1*^{b}) from wild-caught mice that effects the activity and kinetics of erythrocyte and liver pyruvate kinase. *Biochemical Genetics* **19**, 771–781.

Noguchi, T., Inoue, H., Chen, H.-L., Matsubara, K. & Tanaka, T. (1983). Molecular cloning of DNA complementary to rat L-type pyruvate kinase on RNA. Nutritional and hormonal regulation of L-type pyruvate kinase mRNA concentration. *Journal of Biological Chemistry* **258**, 15220–15223.

Noguchi, T., Inoue, H., Nakamura, Y., Chen, H.-L., Matsubara, K. & Tanaka, T. (1984). Molecular cloning of cDNA sequences for rat M_2-type pyruvate kinase and regulation of its mRNA. *Journal of Biological Chemistry* **259**, 2651–2655.

Noguchi, T., Inoue, H. & Tanaka, T. (1986). The M_1 and M_2-type isozymes of rat pyruvate kinase are produced from the same gene by alternative RNA splicing. *Journal of Biological Chemistry* **261**, 13807–13812.

Noguchi, T. & Tanaka, T. (1982). The M_1 and M_2 subunits of rat pyruvate kinase are encoded by different messenger RNAs. *Journal of Biological Chemistry* **257**, 1110–1113.

Peters, J. & Andrews, S. J. (1984. The Pk-*3* gene determines both the heart, M_1, and the kidney M_2, pyruvate kinase isozymes in the mouse; and a simple electrophoretic method for separating phosphoglucomutase-3. *Biochemical Genetics* **22**, 1047–1063.

Peters, J., Nash, H. R., Eicher, E. M. & Bulfield, G. (1981). Polymorphism of kidney pyruvate kinase in the mouse is determined by a gene, *Pk-3*, on chromosome 9. *Biochemical Genetics* **19**, 757–769.

Saheki, S., Harada, K., Sanno, Y. & Tanaka, T. (1978). Hybrid isozymes of rat pyruvate kinase. Their subunit structure and developmental changes in the liver. *Biochimica et Biophysica Acta* **526**, 116–128.

Saheki, S., Saheki, K. & Tanaka, T. (1982). Peptide structures of pyruvate kinase isozymes 1. Comparison of the four pyruvate kinase isozymes of the rat. *Biochimica et Biophysica Acta* **704**, 484–493.

Simon, M.-P., Besmond, Cottreau, D., Weber, A., Chaumet-Riffaud, P., Dreyfus, J.-C., Thepat, J. S., Marie, J. & Kahn, A. (1983). Molecular cloning of cDNA rat L-type pyruvate kinase and aldolase B. *Journal of Biological Chemistry* **259**, 1798–1802.

Simon, M.-P., Marie, J., Bertrand, O. & Kahn, A. (1982). Molecular organisation of the human Li and L pyruvate kinase. *Biochimica et Biophysica Acta* **709**, 1–7.

Taylor, B. A. (1978). Recombinant inbred strains: use in gene mapping. In *Origins of Inbred Mice* (ed. H. C. Morse), pp. 423–438. London: Academic Press.