PHOSPHORYLASE KINASE DEFICIENCY IN MICE

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Received 6 September 1970

1. Introduction

The absence of phosphorylase kinase* in the I-strain mouse was first described by Lyon et al. [1–4]. The trait shows X-chromosomal inheritance [5]. Lyon reported that phosphorylase kinase is completely absent from skeletal muscle of deficient mice. Brain, heart and kidney are affected to a lesser extent than skeletal muscle, showing approximately 9, 20 and 66% respectively of the activity found in normal control tissue [4]. Deficient mice show a normal activity of phosphorylase kinase in intestine [4] and liver [6].

Despite the complete absence of phosphorylase kinase in the skeletal muscle, glycogen breakdown occurs after administration of epinephrine or after electrical stimulation [1, 2].

We were able to confirm that in extracts of skeletal muscle of deficient mice, prepared as by Lyon [1, 5], no phosphorylase kinase activity can be detected. However, we will show here that phosphorylase kinase is not completely absent from the muscles of these mice. The enzyme is present in a particulate form which is sedimented upon centrifugation.

This observation may explain the effects noted above, of epinephrine and electrical stimulation on glycogen breakdown in the muscle of the I-strain mouse.

2. Methods

Small samples of mouse skeletal muscle (20–80 mg of femoral muscle) obtained during surgery under ether anesthesia or after decapitation of the animals, were quickly weighed, minced with scissors, transferred to a precooled glass homogenizer and homogenised with a motor-driven Teflon pestle in 50 volumes of ice-cold water. Approximately half of the homogenate was centrifuged for 10 min at 13,000 *g* (4°).

Phosphorylase was assayed by incubating 20 μl of homogenate or supernatant with 20 μl of 150 mM glucose-1-phosphate, pH 6.4, 20 mg/ml glycogen (nucleotide free) with or without 2 mM AMP, for 0 and 30 min at 30°. The reaction was stopped by addition of 1.0 ml of 36 mM H_2SO_4 and phosphate was determined by addition of 1.0 ml of a solution, freshly prepared by dissolving $FeSO_4$ (40 g/l) in 1% ammonium molybdate-0.5 M sulfuric acid. The blue color was read at 700 nm 2 min after addition of the ammonium molybdate reagent.

Phosphorylase kinase activity was determined by incubating 20 μl of homogenate or supernatant with 100 μl of a solution containing 40 mM tris, 40 mM β-glycerophosphate, 3.6 mM ATP, 12 mM magnesium acetate and approximately 12 U.* rabbit muscle phosphorylase *b*. The assay was performed at pH 6.8 or 8.2. The digest was incubated for 0 or 10 min at 30° and the kinase reaction was stopped by the addition of 2.0 ml of 40 mM glycerophosphate-30 mM cysteine-5 mM EDTA, pH 6.8. Phosphorylase *a* formed by the action of kinase is assayed in a 50 μl sample as indicated above. For assay at pH 8.2 the homogenate and the supernatant have to be diluted four-fold; at pH 6.8 the undiluted homogenate or supernatant are used.

* Phosphorylase kinase: ATP: phosphorylase phosphotransferase (EC 2.7.1.38);
phosphorylase: α-1,4-glucan: orthophosphate glucosyltransferase (EC 2.4.1.1).

* I.U. (unit) of phosphorylase is the amount which converts 1 μmole of substrate per min as assayed under the above described conditions.

Glycogen was determined in the crude homogenate by enzymic degradation and enzymic glucose estimation [7].

Phosphorylase *b* was isolated from rabbit muscle by the method of Fischer et al. [8].

Mice of the I/Hf strain were a generous gift of Dr. H.A.Hoffman (National Cancer Institute, Bethesda, Md.). Since the I strain mice are poor breeders, we are transferring the trait of phosphorylase kinase deficiency on the genetic background of the C57 black strain. Most of the animals used in the present studies were hybrids between the two strains.

Table 1
Phosphorylase kinase activity at pH 6.8 in mouse muscle homogenate and 13,000 *g* supernatant.

	Phosphorylase kinase activity (U. of phosphorylase activated/min/g muscle)		
Sex	Homogenate	13,000 *g* supernatant	% in supernatant
Normal mice			
m	515	474	92
m	631	515	82
m	556	298	53
m	871	717	82
m	833	603	72
m	966	620	64
f	802	610	67
f	630	492	78
m	583	589	100
m	412	345	80
m	479	474	99
m	514	466	90
Mean ± S.D.	649±169	517±114	80±13%
Deficient mice			
m	26	0	0
m	32	0	0
m	47	0	0
m	36	0	0
m	56	0	0
m	60	0	0
m	49	0	0
m	28	0	0
m	0	0	0
m	13	0	0
m	12	0	0
Mean ± S.D.	33±18	0	0

3. Results and discussion

It can be seen from table 1 that there is definitely some phosphorylase kinase activity in homogenates of deficient mice when assayed at pH 6.8. On the average this activity is 5% of the normal activity. In normal mice most of the phosphorylase kinase activity is found in the supernatant after centrifugation of the homogenate at 13,000 *g* for 10 min (4°). However, in the deficient mice no activity remains in the supernatant. This is also true when the homogenate is centrifuged for 10 min at 5,000 *g*. This explains the finding of Lyon and Porter [1], that the kinase was completely absent from the muscle of the mice because they used a 5,000 or 10,000 *g* supernatant for the assay.

In our experiments all, or almost all, of the phosphorylase kinase activity can be found in the resuspended 13,000 *g* precipitate.

Table 2
Phosphorylase kinase activity at pH 8.2 in muscle homogenates and 13,000 *g* supernatant of male mice.

	Phosphorylase kinase activity (U. of phosphorylase activated/min/g muscle)		
	Homogenate	Supernatant	% in supernatant
Normal mice	2465	2280	93
	2070	384	18
	1548	1092	71
	769	366	48
	1428	435	30
	2795	2208	79
	2318	1703	74
Mean ± S.D.	1913±649	1210±792	
Deficient mice	330	0	0
	303	0	0
	448	0	0
	338	0	0
	269	0	0
	247	0	0
	416	0	0
	121	0	0
	313	0	0
	302	0	0
Mean ±	308±85		

Table 3
Phosphorylase activity in muscle homogenates and 13,000 *g* supernatant of male mice.

	Phosphorylase activity (μmoles/min/g muscle)				
	In homogenate		In 13,000 *g* supernatant		% of total phosphorylase in supernatant
	−AMP	+AMP	−AMP	+AMP	
Normal mice	12	79	8	70	89
	20	75	20	59	79
	12	79	12	79	100
	7	75	12	68	91
	43	72	48	71	99
	54	76	59	76	100
					93±7
	32	55			
	16	79			
	39	54			
	43	57			
	32	42			
Mean ± S.D.	28±15	68±12			
Deficient mice	11	83	11	75	90
	6	31	4	31	100
	10	89	3	77	87
	7	77	6	59	77
	7	71	–	–	–
	6	63			
	8	66			
	9	51			
	6	67			
Mean ± S.D.	8±2	66±16		mean	89

Table 2 shows that at pH 8.2 muscle homogenates of deficient mice show considerable activity of phosphorylase kinase (16% of the normal mean) when compared with normal mice. Again the supernatant shows no activity. The values found in normal mice show a much larger variation at pH 8.2 than at pH 6.8, as does the percentage of enzyme found in the supernatant.

Table 3 shows that the total phosphorylase activity in the normal and the deficient mice is comparable. The amount of phosphorylase *a* in deficient mice (as assayed in the absence of AMP) is below normal, comfirming Lyon et al. [1–4].

However, occasionally higher values are found. In one mouse, intentionally subjected to stress, 45% of the phosphorylase was active in the absence of AMP.

Table 4
Glycogen content of mouse muscle homogenate and supernatant.

	Glycogen (mg/g muscle)	
	Homogenate	Supernatant
Normal mice	0.9	1.0
	2.0	1.9
	0.8	0.8
	0.5	0.6
Deficient mice	8.7	8.3
	7.5	6.3
	10.1	9.0
	8.8	7.1

Table 5
Phosphorylase kinase in skeletal muscle of heterozygous mice.

	pH 6.8		pH 8.2	
	Activity in homogenate (U./min/g muscle)	Percent activity in 13,000 *g* supernatant	Activity in homogenate (U./min/g muscle)	Percent activity in 13,000 *g* supernatant
Expt.				
1	401	63	1256	12
2	390	54	1550	5
3	261	86	1436	49
4	299	89	1564	67
Normal	649±169	80±13	1913±649	18–93

It is clear from table 3 that all the phosphorylase activity of the homogenate, was recovered in the 13,000 *g* supernatant. This applies also to phosphoglucomutase (EC 2.7.5.1).

Glycogen content of the muscle of the deficient mice was considerably higher than in control mice. All or almost all of the glycogen remains in the supernatant upon centrifugation (table 4). The fact that all our values for the glycogen concentration in normal and deficient animals are somewhat lower than those reported by Lyon [4, 5] reflects the fact that we do not freeze the muscle.

The present experiments show that phosphorylase kinase is not completely absent in the I-strain mice and in hybrids derived from this strain. Kinetic properties of the phosphorylase kinase such as the K_m for ATP and phosphorylase in the deficient mice are comparable to those found in normal control mice. The residual activity may explain the glycogen breakdown occurring after administration of epinephrine or after electrical stimulation [1, 2].

It remains to be shown whether the different properties of the phosphorylase kinase in these mice is due to a mutation in the structural gene or e.g. to a defect in a gene coding for the localization of the enzyme, as is known for glucuronidase [9]. In this respect it is of interest that heterozygous female mice do not differ significantly from normal with respect to the activity of phosphorylase kinase in a 5,000 *g* supernatant [4, 5]. Table 5 shows that the kinase activity of homogenates assayed at pH 6.8 or 8.2 does not differ significantly from normal values nor does the percentage of the enzyme remaining in the supernatant after centrifugation at 13,000 *g* for 10 min.

In the liver of the deficient mice a normal phosphorylase kinase activity was found and as in the case of normal control livers all the activity remains in the supernatant of a 16% homogenate in 0.25 M sucrose upon centrifugation at 41,000 *g* for 30 min.

Comparison of the phosphorylase kinase defect and its effects in mice, and the glycogen storage disease in human patients, which is characterized by a defect in phosphorylase kinase [10–12] shows many similarities [13, 14], such as X-chromosomal inheritance and absence of symptoms or signs in adult mice and adult patients. However, two differences are immediately apparent. In the mouse, abnormalities can be detected in skeletal muscle, brain, heart and kidney, while intestine and liver appear to be normal. In human patients the defect seems to be generalized since it has been found in leucocytes [10, 11], erythrocytes [12], liver [15, 16] and muscle [17]. In both species the deficiency is incomplete i.e. some residual activity can be found. In humans the low activity is due to a high K_m of the phosphorylase kinase for phosphorylase [18] Sedimentation characteristics of the enzyme in humans have not been investigated but it is routinely assayed in 14,000 *g* supernatant of a leucocyte homogenate. In the deficient mice the activity of phosphorylase kinase is below normal in the muscle and the enzyme is present in a particulate form which is sedimented upon centrifugation.

Acknowledgements

This investigation is supported by PHS grant AM

13359 of the National Institute of Arthritis and Metabolic Diseases. The technical assistance of Mrs. Dity Naab is greatfully acknowledged.

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