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McARDLE'S DISEASE: A STUDY ON THE MOLECULAR BASIS OF TWO DIFFERENT ETIOLOGIES OF MYOPHOSPHORYLASE DEFICIENCY

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Summary

Three patients with myophosphorylase deficiency were investigated. Two had no detectable activity, while one had 1% residual activity. The patient with 1% residual activity had 40% of the normal amount of myophosphorylase protein. No myophosphorylase protein could be detected in the other two cases. A precipitin band in the Ouchterlony double immunodiffusion test was not present in any case. This study showed that modifying the normal enzyme (without changing the molecular weight) changed the immunoprecipitin activity of the phosphorylase protein. Therefore, immunoprecipitation is not a valid technique for differentiation of the variants of myophosphorylase deficiency, and another method, for example SDS-electrophoresis, should be applied.

Introduction

McArdle's disease is due to a deficiency of muscle phosphorylase activity [1,2]. The absence of phosphorylase activity is restricted to skeletal muscle, while in the other tissues normal phosphorylase activity is found. This implies that skeletal muscle contains a specific form of phosphorylase. The existence of a separate entity of hepatic phosphorylase deficiency supports this contention [3,4]. This suggests that these phosphorylases are under separate genetic control and differ in primary structure.

Based upon immunological experiments, it has been concluded that two variants of myophosphorylase deficiency exist [5–8], one in which cross-reacting material (CRM) can be detected and another in which no CRM is

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present. A residual activity of 5% of normal is found in the variant which has CRM [6].

Phosphorylase protein accounts for about 5% of the soluble protein content of muscle. For this reason a prominent band of phosphorylase protein should be detected by the sodium dodecyl sulphate (SDS) polyacrylamide electrophoresis. Using this method, Feit and Brooke [9] could discriminate between two variants: one in which no phosphorylase protein could be detected and another which contained a normal amount of phosphorylase protein. Both variants were devoid of myophosphorylase activity (histochemically) and the presence of CRM was not investigated.

In this contribution, we present the results obtained with three cases of myophosphorylase deficiency. Two patients have no detectable enzymatic activity, no phosphorylase protein and no CRM. One patient has a residual myophosphorylase activity ($\pm 1\%$), phosphorylase protein, but no CRM.

The meaning of absent CRM in enzymological enzyme deficiency studies and the resulting conclusions are discussed in relation to these findings.

Materials and methods

Biopsy samples from cases I and II were taken from the m. tibialis anterior and from case III from the m. quadriceps femoris. All samples were frozen in isopentane at -160°C . Until used, the samples were stored at -70°C . Tissue homogenates (10%) were prepared in 50 mmol/l Tris-HCl (pH 7.4) and centrifuged for 10 min at $10\,000 \times g$. The supernatants were used for the enzyme assays. Phosphorylase activity was measured in the presence of 5'AMP (Ref. 10). The activities of phosphofructokinase, phosphoglucosomerase, phosphoglucosomutase and pyruvate kinase were performed according to Layzer et al. [11]. Aldolase activity according to Ref. 12, and creatine kinase activity was determined with creatine [13] and with creatine phosphate [14] as substrate. Glycogen content was determined in the total homogenate as described by Huijing [15]. The SDS polyacrylamide electrophoresis was performed according to Weber and Osborn [16] and Feit et al. [17], with some modifications. These modifications implied that no urea was added and that the tissue extracts were not heated to 100°C . Under these conditions two bands of phosphorylase could be detected reproducibly. The electrophoresis was performed with 8 mA per gel, until the tracking dye (bromophenol blue) reached the bottom of the gel. The proteins were stained with Coomassie Brilliant Blue G-250 (Ref. 12). The electrophoretograms obtained were scanned with a Gilford Spectrophotometer G 2400.

Antiserum against phosphorylase from rabbit muscle was prepared according to Dreyfus and Alexandre [6]. This antiserum inhibited human myophosphorylase completely and in the Ouchterlony double diffusion plates one precipitin line was obtained with crude human muscle extract.

Results

The activities of phosphorylase, creatine kinase and various glycolytic enzymes in the muscle from the patients and controls are summarized in

Table I. With cases I and II no detectable phosphorylase activity is found, while for case III some residual activity is measureable (about 1% of the lowest control value). All the other glycolytic enzyme activities from the patients are somewhat elevated as compared to the controls. This fact is not the consequence of low protein content in the affected muscle, because the activity of creatine kinase in the affected muscle is within the normal range. The table also shows the glycogen content of the various muscle biopsies. Cases I and II have an increased amount of glycogen, while for case III the amount of glycogen found is at the upper limit of the control values. From other studies we know that muscle phosphofructokinase activity is one of the enzymes which can easily be lost on improper handling of the muscle samples. This is probably caused by the high sensitivity of phosphofructokinase to proteolytic enzymes. The high activity of phosphofructokinase found in the patients' muscles indicates that no enzyme proteolysis had occurred, which indicates that the absent phosphorylase activity is not due to post-biopsy alterations.

With the knowledge of these data it is most intriguing to investigate if, and how much, phosphorylase protein and CRM are present in the muscle of the various patients. Therefore, the muscle supernatants were subjected to SDS polyacrylamide electrophoresis. Under the conditions described in the Methods section, two protein bands of phosphorylase were obtained with control rabbit myophosphorylase (Boehringer phosphorylase *a*). Probably these bands reflect the tetramer and dimer form of the molecule. Under our conditions, the protein is not split into its subunits, due to the omission of urea and heating to 100°C. The results are reproducible and the intensity of the protein staining is linear with the amount of protein layered on the gel, at least within the applied protein range (20–60 µg). Fig. 1 shows the results of the polyacrylamide electrophoresis of cases I, II, control tissue and rabbit myophosphorylase *a* (Boehringer enzyme). It is evident that the two phosphorylase bands are missing in both cases I and II. By scanning the gels it is possible to measure the intensity of the protein bands. Fig. 2 shows the results in which cases I and III

TABLE I

THE ACTIVITIES OF VARIOUS GLYCOLYTIC ENZYMES IN MUSCLE HOMOGENATES FROM CONTROLS AND PHOSPHORYLASE DEFICIENT PATIENTS

The activities are expressed as µmol/min/mg protein. The amount of glycogen is expressed as µg/mg protein. N.D.: not detectable.

	Patients			Controls	
	I	II	III		
Phosphorylase	N.D.	N.D.	1.14	88	—332 (n = 8)
Phosphofructokinase	0.63	0.62	1.00	0.33—	0.54 (n = 5)
Phosphoglucosomerase	1.34	1.37	—	0.69—	1.34 (n = 7)
Phosphoglucomutase	1.11	1.09	—	0.55—	0.90 (n = 5)
Pyruvate kinase	2.38	1.85	—	0.56—	1.53 (n = 7)
Aldolase	0.51	0.57	—	0.33—	0.45 (n = 3)
Creatine kinase (creatine)	1.89	1.94	—	1.32—	3.19 (n = 7)
Creatine kinase (creatine phosphate)	21.14	23.47	—	15.08—	38.64 (n = 7)
Glycogen	153	116	75	32	— 79 (n = 13)

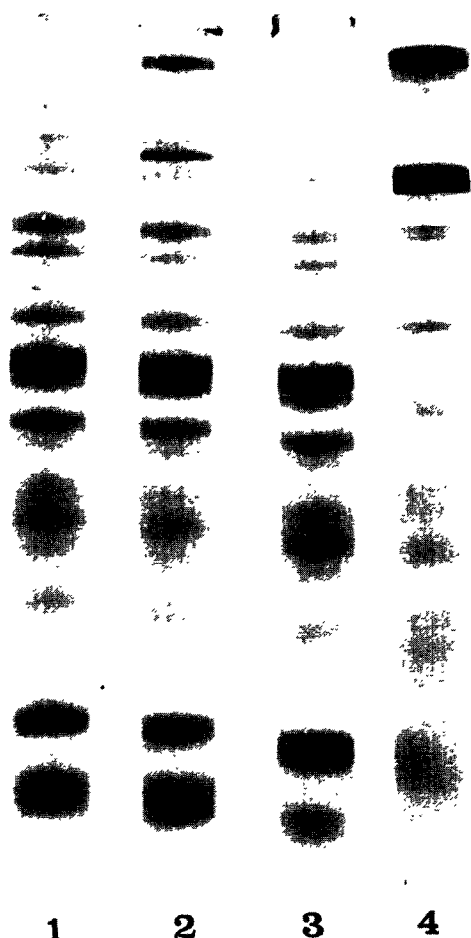


Fig. 1. SDS polyacrylamide electrophoresis of soluble human muscle protein. 1, case I; 2, control; 3, case II; and 4, phosphorylase a (Boehringer). Conditions as described in Materials and Methods.

are compared with control muscle extract. For case I there is no phosphorylase protein, but with case III significant peaks in the position of phosphorylase are found. To quantitate the data obtained, the areas underneath the two peaks were measured and related to the amount of protein used for the electrophoresis. To compare the various peaks the areas are expressed as $\text{mm}^2/\mu\text{g}$ protein. This is permissible in view of the linearity of the staining intensity and the amount of protein applied for electrophoresis. The results of these calculations are presented in Table II. Both the separate values and the sum of peak I and II are indicated. A comparison of the control values shows that by this method reproducible and reliable data can be obtained. In comparison with control

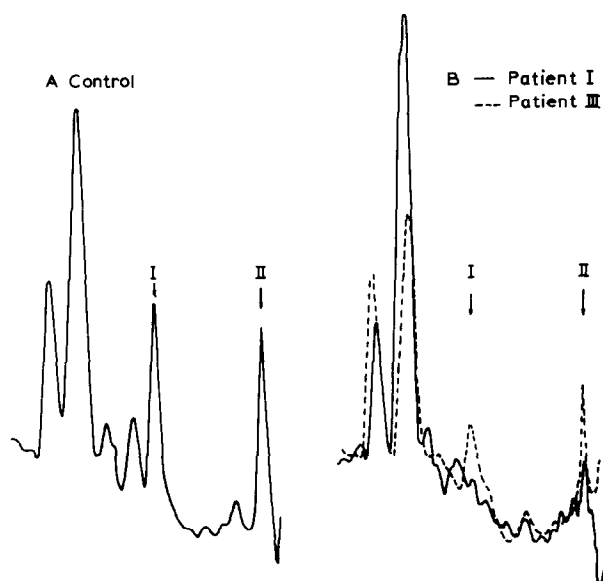


Fig. 2. Scanning pattern of the upper peak of the SDS polyacrylamide electrophoresis. A, control; B, solid line, case I and broken line, case III. Peaks I and II are the phosphorylase protein bands.

values, case I shows very low values both for peak I and II. However, in contrast to case I, case III seems to have a normal value for peak II but a decreased value for peak I. Taking the sum, case III possesses about 40% of the mean control value.

In cases I and III the presence of CRM was determined by the Ouchterlony double diffusion technique (Fig. 3, hole 2, 4). Though 40% of myophosphorylase protein is present in case III, precipitin lines are obtained neither in case I nor in case III. This indicates that no CRM is present in these cases. A control sample (hole 1) shows, as expected, a single precipitin line. The finding in case III that, although phosphorylase protein is present, no CRM is detectable, focussed our attention upon the possibility that slight enzyme modifications

TABLE II

THE AREA UNDERNEATH PEAK I AND II OF MUSCLE PHOSPHORYLASE OBTAINED FROM CONTROLS AND PHOSPHORYLASE DEFICIENT PATIENTS

The areas are expressed as mm^2 and calculated on basis of $1 \mu\text{g}$ of protein. The areas are calculated from $\frac{1}{2} \times \text{width} \times \text{height}$ of the peaks.

	Peak		
	I	II	I + II
Control a	3.8	1.1	4.9
a	4.5	1.1	5.6
b	5.1	2.0	7.1
c	2.9	3.0	5.9
d	6.0	1.0	7.0
Patient I	0.1	0.3	0.4
III	1.6	1.0	2.6

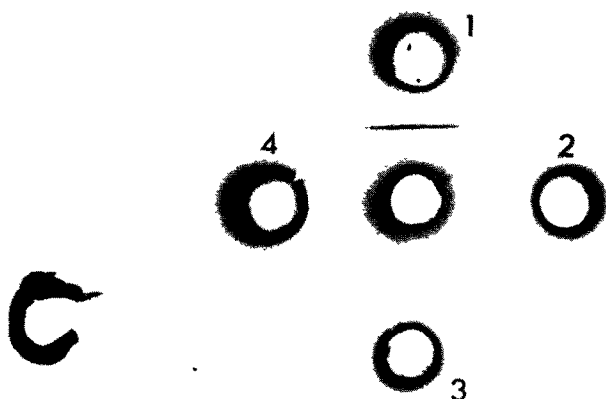


Fig. 3. Ouchterlony double diffusion plate with agar (1% in 0.15 mol/l NaCl). Center hole, antibody: hole 1, control; 2, case I; 3, control, with lost activity; and 4, case III. 5 μ l of antibody were used and 10 μ l of supernatant of 10% homogenate.

(without changing the molecular weight) can change not only the enzymatic activity but also the precipitation behaviour of the enzyme antibody complex. To check this possibility a control sample with normal activity was frozen and thawed several times and stored at -70°C . This procedure leads to a loss of phosphorylase activity. After this procedure normal phosphorylase protein is still present as determined by the method used in Table II, while no precipitin line is found in the Ouchterlony plate (Fig. 3, hole 3).

Discussion

In any disease, due to a lack of enzyme activity, the molecular lesion can be based on various mechanisms. One of the first things that one investigates is the presence (or absence) of CRM. The finding that no CRM is detected is often used as evidence for the absence of the mutant protein. In this report, we show that the antigenicity of phosphorylase can be changed (at least the immunoprecipitation is lost) under conditions that the enzyme activity has vanished, while the SDS polyacrylamide electrophoresis shows that no detectable change in the molecular weight and amount of the protein has occurred. These findings indicate that immunoprecipitation is not a reliable method for the detection of absence or presence of mutant proteins.

The two variants of myophosphorylase described earlier, are originally based on the ability or inability of immunoprecipitation with the antibody [6]. Our experiments show that such a procedure is not suitable, and that a decision has to be based on the detection of protein by SDS polyacrylamide electrophoresis technique for example. The absence of protein with the latter technique does not prove unequivocally, however, that the mutant protein is absent. It is still possible that the protein mutates in such a way that the molecular weight changes and takes up another position.

It is well known that muscle contains an abundant amount of phosphorylase. The physiological importance of this is discussed by Newsholme and Start [19]. In cases I and II no detectable phosphorylase activity can be detected and

consequently the glycogen content is raised. However, in case III the amount of glycogen is much less, if not normal, while the patient has only a residual activity of 1%. The low residual activity seems to be adequate to degrade the glycogen. This is not surprising when residual phosphorylase activity is compared to the activities of the other glycolytic enzymes of the controls (Table I). It should be noticed that the residual phosphorylase activity is still 2–3 times higher than most of the glycolytic enzymes measured.

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