CHARACTERIZATION OF GLYCOGEN PHOSPHORYLASE ISOENZYMES PRESENT IN CULTURED SKELETAL MUSCLE FROM PATIENTS WITH MCARDLE'S DISEASE

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Summary: Muscle biopsy specimens from patients with McArdle's disease lack glycogen phosphorylase activity. Significant phosphorylase activity was detected in cultured muscle cells from these patients. The phosphorylase isoenzymes in the cells were identified electrophoretically and immunochemically. On polyacrylamide disc gel electrophoresis, two types of isoenzymes were separated in about equal amounts. Both differed the muscle type in migration, kinetic, and immunochemical properties. The first type corresponded to a fetal phosphorylase isoenzyme, and the second was a liver-like type which was completely absorbed with antibody against the rat liver isoenzyme. No adult skeletal muscle isoenzyme was detected.

INTRODUCTION

In patients with McArdle's disease, there is a genetic absence of the enzyme glycogen phosphorylase (EC 2.4.1.1) in skeletal muscle. However, in 1972, Roelofs et al (1) reported that phosphorylase could be demonstrated histochemically in regenerating and cultured muscle from patients with McArdle's disease. Although several hypotheses were presented to explain this observation, none have been confirmed. In 1975, Sato et al (2,3) demonstrated that there were at least three types of phosphorylase isoenzymes in rat tissues: a fetal type as well as the muscle and liver types previously described. The fetal type, which is the predominant type in many fetal rat tissues, is replaced by the muscle and liver types during development of the respective tissues and disappears in the normal adult skeletal muscle and liver. The fetal

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type remains as brain type or type I in the adult brain and heart.

Based on this work, we felt that the fetal type, and not the muscle type which is genetically lacking, might appear in regenerating and in cultured muscle fibers from the patients. In this paper, we present data showing that the fetal type phosphorylase isoenzyme together with the liver-like type, both of which seem to be different genetically from the muscle type, appear in the cultured muscle cells from patients with McArdle's disease.

MATERIALS AND METHODS

Human skeletal muscle culture. Muscle biopsy specimens were obtained aseptically during the course of diagnostic muscle biopsy. Muscle cells were cultured according to the method of Askanas and Engel (4) with slight modifications. After reaching maturity, the cells were scraped from the petri dishes, collected by centrifugation at 1500rpm for 10 min, frozen at -65°, and stored at that temperature. Two to seven months after harvesting they were packed in dry ice, shipped from Nashville to Hirosaki by air mail, and used immediately after their arrival. Muscle biopsy specimens obtained from the patients were frozen immediately in liquid nitrogen, stored at -65°, and shipped to Hirosaki along with the cultured cells.

Preparation of cell extract. The cultured cells and muscle biopsy or autopsy specimens were homogenized by sonication as previously described (3). After centrifugation at 105,000xg for 60 min, the supernatants were assayed for glycogen phosphorylase, creatine kinase, aldolase, and pyruvate kinase.

Enzyme assays. The phosphorylase assay was carried out as previously reported (5). [U-^{14}C]glucose 1-phosphate was used as substrate. Creatine kinase, aldolase, and pyruvate kinase activities were assayed according to the methods of Szasz et al (6), Blostein and Rutter (7), and Tanaka et al (8), respectively. Enzyme activities are expressed in units per mg protein, a unit being difined as the amount of enzyme catalysing one μmole of substrate per min. Protein concentrations were determined according to the method of Lowry et al (9).

Disc gel electrophoresis of phosphorylase. This was carried out as previously reported (3).

Preparation of rat phosphorylase antibodies. Antibodies against rat muscle and liver type isoenzymes were obtained from rabbits, as previously reported (3), and were tested for specificity by their ability to cross-react and make precipetates with human muscle and liver phosphorylase, respectively. No reaction was found with the other two isoenzymes, although the precipitate line between the antibody against the rat liver isoenzyme and the human liver isoenzyme fused with a spur with the line between the antibody and the rat liver isoenzyme, in Ouchterlony double diffusion test (not shown).

Chemicals. [U-^{14}C]glucose 1-phosphate was obtained from New England Nuclear Corp. The sources of other chemicals were described previously (3).

TABLE I. Enzyme activities in muscle cells cultured from patients with McArdle's disease. Assay procedures are given in the text. A unit of enzyme activity is defined as the amount of enzyme catalysing one μmole of substrate per min.

Sample \ Enzyme	Glycogen phosphorylase*	Creatine kinase	Aldolase**	Pyruvate kinase
	munits/mg	munits/mg	munits/mg	units/mg
Muscle(autopsy) from				
adult	1.213	77,080	1.104	17.20
newborn	197	27,452	230	7.09
fetal(5M)	49.4	4,720	110.5	1.97
Muscle(biopsy) from				
patient A(McArdle's)	1.47	46,050	793.6	21.70
patient B(McArdle's)	1.52	31,780	395.7	12.99
Muscle cells cultured from				
normal A	6.22	25.92	8.88	1.58
normal B	7.75	232.2	25.08	2.20
patient A(McArdle's)	8.80	36.63	9.15	2.43
patient C(McArdle's)	8.29	25.20	4.17	1.17

* Values in the presence of 1mM AMP are shown. However, it has been evident recently that, in cultured muscle cells, about twice values are obtainable by addition of 0.5 M sodium sulfate together with 1 mM AMP.

** 1 mM fructose-1,6-bisphosphate as substrate.

RESULTS AND DISCUSSION

As shown in Table I, the total phosphorylase activity in the presence of 1 mM AMP in muscle biopsy specimens from patients with McArdle's disease were about 0.1% of the activity in control adult skeletal muscle. On polyacrylamide disc gel electrophoresis, negligible amounts of the enzyme were present, as shown in gels number 13 and 14 in Figure 1. Other muscle marker enzymes, such as creatine kinase, aldolase, and pyruvate kinase, were present at about 50% of the activity found in control muscle. However,

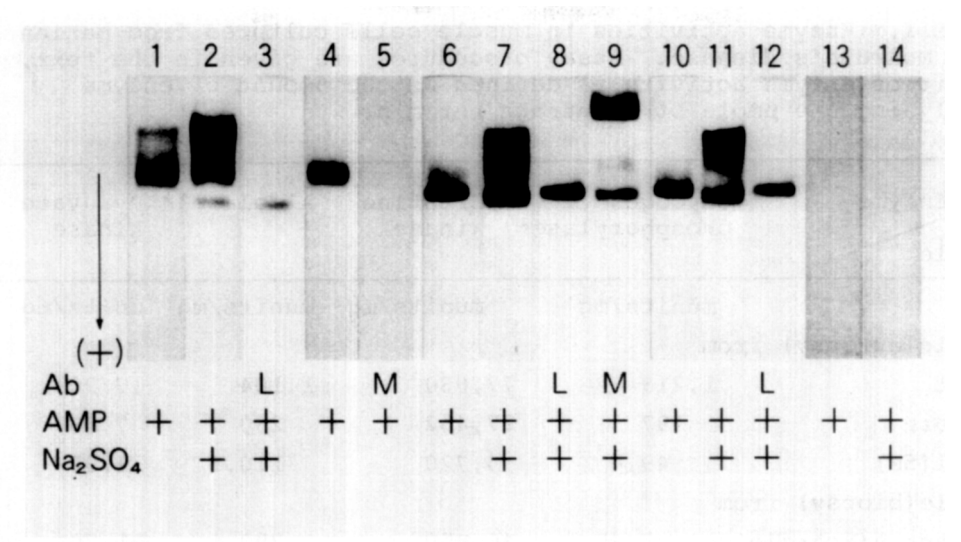

Figure 1. Polyacrylamide disc gel electrophoresis of phosphorylase in human liver, normal adult skeletal muscle, cultured muscle from normal biopsy material and from a patient with McArdle's disease.

About 0.2 munit of phosphorylase activity was applied each gel. The methods of electrophoresis and activity staining were the same as previously reported (3), except for overnight staining. On gels labelled (L) or (M), antibody against rat liver (L) or muscle (M) isoenzyme was mixed with an aliquot of the 105,000 g supernatant from each sample, and incubated at 30° for 10 min before electrophoresis. The requirement of AMP and Na_2SO_4 for activity is indicated by (+).

Gel number 1-3, adult human liver (autopsy sample); gel number 4 and 5, adult human muscle (autopsy sample); gel number 6-9, cultured muscle cells from normal subject A; gel number 10-12, cultured muscle cells from patient A with McArdle's disease; gel number 13 and 14, adult human muscle from patient A with McArdle's disease (biopsy sample).

in cultured muscle cells from the patients phosphorylase activity appeared in the same range as that found in cultured muscle cells from normal muscle, even though the cultured muscle cells were immature biochemically.

In human tissues, as in rat tissues, we can demonstrate at least three phosphorylase isoenzymes, as shown in Figure 1. Adult human liver (gel number 1-3) obtained from a patient at autopsy contained a very small amount of the fastest migrating fetal type, a hybrid between the fetal and liver types, and the

slowest migrating predominant liver types (seen most densely on gel number 2). The latter isoenzyme depended on AMP and sodium sulfate (as the b-form) and was absorbed completely with the antibody against the rat liver isoenzyme (gel number 3). Normal adult human skeletal muscle (gel number 4 and 5) contained only the muscle type, which migrated between the fetal and liver types and was absorbed completely with antibody against the rat muscle type (gel number 5).

The cultured muscle cells obtained from normal patient A in Table I (gel number 6-9) contained two or three isoenzymes. The fastest migrating isoenzyme corresponded to the fetal type. The middle band may be a hybrid between the fetal and liver-like types. The slowest migrating isoenzyme was the liver-like type, whose activity depended on AMP and sodium sulfate (gel number 7) and was absorbed completely with antibody directed against liver type (gel number 8). The migration of the liver-like type was reduced by the addition of antibody against the muscle type; the reason for this is unclear. A small amount of the muscle type might be present in the cultured cells, and could complex with the antibody to disturb the migration of the liver-like type.

The cultured muscle cells from patient A with McArdle's disease (gel number 10-12) also contain both fetal and liver-like types. The muscle biopsy specimen taken from patient A showed no activity of phosphorylase before cultivation (gel number 13,14).

It is unclear why the liver-like type appeared in cultured muscle. Dreyfus and co-workers (10-12) have suggested that the liver-like type (or a hybrid between the brain and liver-like types) is the prevalent one in many human tissues including placenta, and is genetically different from the liver isoenzyme. Thus, the liver-like type may be another fetal type different

from the brain type in human tissues. If so, it is quite reasonable that the liver-like type (the b form which depends on both AMP and sodium sulfate, as the liver type does) appeared in human cultured muscle cells along with the brain type, as seen in other fetal or immature tissues.

No significant amount of a mucle type could be detected in the cultured muscle cells from either patients with McArdle's disease or normal muscle. The explanation for the lack of the skeletal muscle type isoenzyme might be the failure of these cultured muscle cells to mature biochemically. Although the cells were mature enough morphologically to fuse with each other to form myotubes, significant amounts of muscle marker enzymes did not appear.

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REFERENCES

1. Roelofs, R.I., Engel, W.K., and Chuvin, P.B. (1972) Science 177, 795-797.
2. Sato, K., Sato, T., Morris, H.P., and Weinhouse, S. (1975) Ann. N.Y. Acad. Sci. 259, 273-286.
3. Sato, K., Satoh, K., Sato, T., Imai, F., and Morris, H.P.(1976) Cancer Res. 36, 487-495.
4. Askanas, V., and Engel, W.K. (1975) Neurology 25, 58-67.
5. Sato, K., Morris, H.P., and Weinhouse, S. (1973) Cancer Res. 33. 724-733.
6. Szasz, G., Busch, E.W., and Farohs, H.B. (1970) Dtsch. med. Wschur. 95, 829-835.
7. Blostein, R., and Rutter, W.J. (1963) J. Biol. Chem. 238, 3280-3285.
8. Tanaka, T., Harano, Y., Sue, F., and Morimura, H. (1967) J. Biochem. (Tokyo) 62, 71-91.
9. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem. 193, 205-275.
10. Proux, D., and Dreyfus, J.C. (1973) Clin. Chim. Acta 48, 167-172.
11. Proux, D., Vibert, M., Meienhofer, M.C., and Dreyfus, J.C. (1974) Clin. Chim. Acta 57, 211-216.
12. Koster, J.F., Slee, R.G., Daegelen, D., Meienhofer, M.C., Dreyfus, J.C., Niermeyer, M.F., and Fernandes, J. (1976) Clin. Chim. Acta 69, 121-125.