THE REGULATORY ROLE OF GLUCOSE 1,6-DIPHOSPHATE IN MUSCLE OF DYSTROPHIC MICE

Rivka BEITNER and Jardena NORDENBERG
Department of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel

Received 27 November 1978

1. Introduction

Glucose 1,6-diphosphate (Glc-1,6-P_2) was first shown [1–3] to be the coenzyme in the phosphoglucomutase reaction. Our experiments have revealed that this compound acts also as a deinhibitor of phosphoglucomutase [4–7]. Glc-1,6-P_2 has also been reported [8–10] to be one of the strongest deinhibitors of phosphofructokinase, the rate-limiting enzyme in glycolysis. Studies [4,7,11] have revealed that Glc-1,6-P_2 exerts a potent inhibitory action on muscle hexokinase. We have also shown that the levels of this regulator in muscle fluctuate under different physiological and hormonal conditions [7,12–14], leading to concomitant changes in the activities of the enzymes which are regulated by this modulator. All these experiments show that Glc-1,6-P_2 is a powerful regulator of glucose metabolism in muscle. The regulatory action of Glc-1,6-P_2 is not limited to muscle. This compound also plays an important role in the regulation of red cell glycolysis through its potent effects on the key glycolytic enzymes (reviewed [15]).

In [16] we found a striking decrease in the levels of Glc-1,6-P_2 in dystrophic muscle, which was associated with a marked reduction in the activity of phosphofructokinase. These findings prompted us to examine whether the reduction in Glc-1,6-P_2 in the dystrophic muscle is also associated with correlated changes in other enzymes which are affected by this regulator. The results here show that such correlation does indeed exist, reflecting the pathological changes in glucose metabolism in the dystrophic muscle.

2. Methods

Normal and genetically dystrophic mice of strain 129 ReJ, aged 8–12 weeks (except where otherwise indicated) were used. The animals were anesthetized with sodium pentobarbital, hind-leg muscles were rapidly removed and frozen between a pair of aluminium tongs pre-cooled in liquid N_2. Frozen muscle was powdered in a mortar cooled in liquid N_2 and the powder used for extraction of Glc-1,6-P_2 and enzymes.

Glc-1,6-P_2 was extracted as in [7], except that only 40–70 mg frozen powder was used. Glc-1,6-P_2 was measured by the fluorometric method in [17]. Hexokinase was extracted and assayed fluorometrically as in [7]. 1 mU hexokinase represents the amount of enzyme activity which forms 1 nmol NADPH in 1 min at 25°C. Phosphoglucomutase was extracted and assayed as in [7], except that ATP was not added to the extraction buffer. 1 mU phosphoglucomutase activity catalyzed the reduction of 1 nmol $NADP^+$/min at 25°C. 6-Phosphogluconate dehydrogenase was extracted by homogenizing ~100 mg frozen tissue powder in 4 vol. cold 0.05 M Tris–HCl buffer (pH 7.4) for 30 s in an ice bath using a Willems-Polytron homogenizer at a setting of 4. The homogenates were centrifuged at 27 000 × *g* at 4°C for 20 min. Activity was assayed in a reaction mixture which consisted of: 0.05 M Tris–HCl (pH 7.6), 1 mM Mg-acetate, 5 μM 6-phosphogluconate, 12.5 μM $NADP^+$, and 10–25 μl muscle extract, in final vol. 2.0 ml. Fluorescence of NADPH was read in Perkin Elmer (MPF-44) fluorescence spectrophotometer. 1 mU 6-phosphogluconate dehydrogenase represents

the amount of enzyme activity which forms 1 nmol NADPH in 1 min at 25°C. Controls in which $NADP^+$ was omitted, were recorded for each experiment.

Protein was measured by the method in [18] with crystalline bovine serum albumin as a standard. Non-collagen protein was determined by the method in [19].

3. Results

We have found [16] that the levels of Glc-1,6-P_2 in the muscle tissue of dystrophic mice, between 7 and 8 weeks of age, is reduced to 46% that of normal mice. In the experiments shown in table 1 we measured the concentration of this regulator in dystrophic and normal muscle at two stages of the disease, in mice aged 4 weeks, when the first signs of muscle weakness are observed, and in mice aged 12 weeks. The results show a significant decrease in Glc-1,6-P_2 level in the dystrophic muscle at 4 weeks and a slightly greater decrease at 12 weeks of age. The fact that the reduction in the Glc-1,6-P_2 levels occurs in the early stages of the disease, suggests that these changes may be primary and not secondary in relation to the dystrophic process.

Table 1
Glucose 1,6-diphosphate levels in normal and dystrophic muscle

	Glc-1,6-P_2 (μmol/kg wet wt)	
	Mice aged 4 weeks	Mice aged 12 weeks
Normal	35.1 ± 1.4 (5)	42.6 ± 0.8 (5)
Dystrophic	21.1 ± 2.0 (5)	17.6 ± 1.8 (5)
% of normal	60	41
P value	<0.001	<0.001

Preparation of muscles, extraction and measurements of Glc-1,6-P_2 were as in section 2. Values are means ± SEM, with no. expts in parentheses

Since Glc-1,6-P_2 is a potent activator of muscle phosphoglucomutase and an inhibitor of muscle hexokinase, we examined whether a correlation exists between the reduction in the levels of Glc-1,6-P_2 in the dystrophic muscle and the activities of these enzymes. The experiments shown in table 2 reveal that concomitant to the decrease in Glc-1,6-P_2, the activity of phosphoglucomutase in dystrophic muscle was significantly reduced (whether expressed per mg protein or per mg non-collagen protein), whereas that of hexokinase was enhanced. Thus the activities of both enzymes correlate to the changes in the levels of Glc-1,6-P_2 which occur in the disease. Figure 1 shows that hexokinase from dystrophic muscle is extremely sensitive to inhibition by Glc-1,6-P_2.

The fact that the decrease in Glc-1,6-P_2 levels in the dystrophic muscle is associated with correlated changes in the activities of all three enzymes which are regulated by this modulator, namely, phosphofructokinase [16], phosphoglucomutase and hexokinase (table 2), strongly suggests that the decrease in the tissue levels of Glc-1,6-P_2 affects these activities.

Since our recent experiments (unpublished data) have revealed that Glc-1,6-P_2 is also a potent inhibitor of mammalian and yeast (Sigma) 6-phosphogluconate dehydrogenase, we measured the activity of this enzyme in the muscle of normal and dystrophic mice.

Table 2
Phosphoglucomutase and hexokinase activities in normal and dystrophic muscle

	Phosphoglucomutase activity		Hexokinase activity	
	mU/mg protein	mU/mg NCP[a]	mU/mg protein	mU/mg NCP[a]
Normal	366 ± 24 (7)	512 ± 34 (7)	4.23 ± 0.21 (17)	5.99 ± 0.36 (15)
Dystrophic	208 ± 22 (8)	327 ± 19 (8)	6.14 ± 0.50 (17)	9.18 ± 0.75 (15)
% of normal	57	64	145	153
P value	<0.0005	<0.001	<0.001	<0.001

[a] NCP, non-collagen protein

Enzymes were extracted and assayed as in section 2. Values are means ± SEM, with no. expts in parentheses

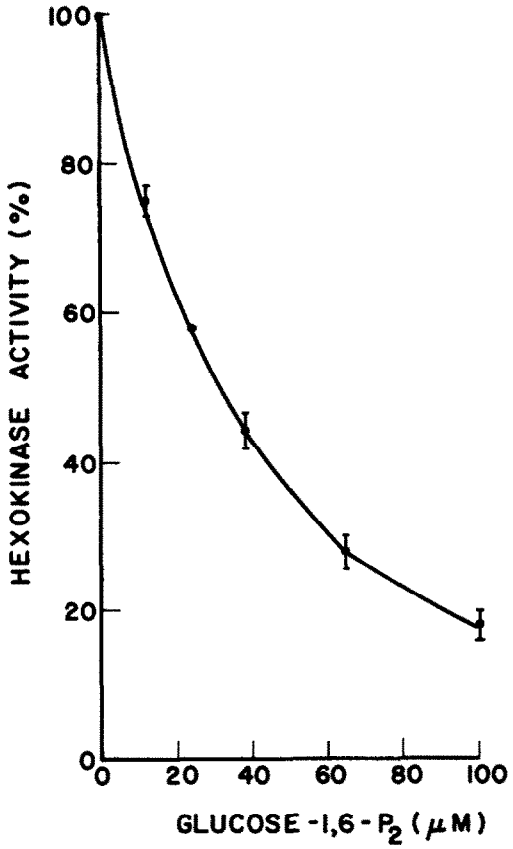

Fig.1. The inhibitory effect of Glc-1,6-P_2 on the activity of hexokinase from dystrophic muscle. Hexokinase was prepared from muscles of dystrophic mice and assayed as in section 2. Assays were carried out in the presence of variable Glc-1,6-P_2 concentrations. 100% hexokinase activity refers to 7.2 mU/mg protein. Vertical bars represent SEM for 4–6 expts.

The results summarized in table 3 show a striking elevation of the activity of this enzyme in the dystrophic muscle. Figure 2 shows that 6-phosphogluconate dehydrogenase from dystrophic muscle is extremely sensitive to inhibition by Glc-1,6-P_2. Thus the reduction in the level of this inhibitor in dystrophic muscle (table 1) may most probably account, at least partially, for the activation of this enzyme.

4. Discussion

Previous studies from this laboratory have revealed that the Glc-1,6-P_2 levels in muscle fluctuates under

Table 3
The activity of 6-phosphogluconate dehydrogenase in normal and dystrophic muscle

	6-Phosphogluconate dehydrogenase	
	mU/mg protein	mU/mg NCP[a]
Normal	2.16 ± 0.18 (6)	2.90 ± 0.26 (5)
Dystrophic	5.62 ± 0.61 (8)	7.48 ± 1.30 (6)
% of normal	260	258
P value	<0.0005	<0.02

[a] NCP, Non-collagen protein

The enzyme was extracted and assayed as in section 2. Values are means ± SEM, with no. expts in parentheses

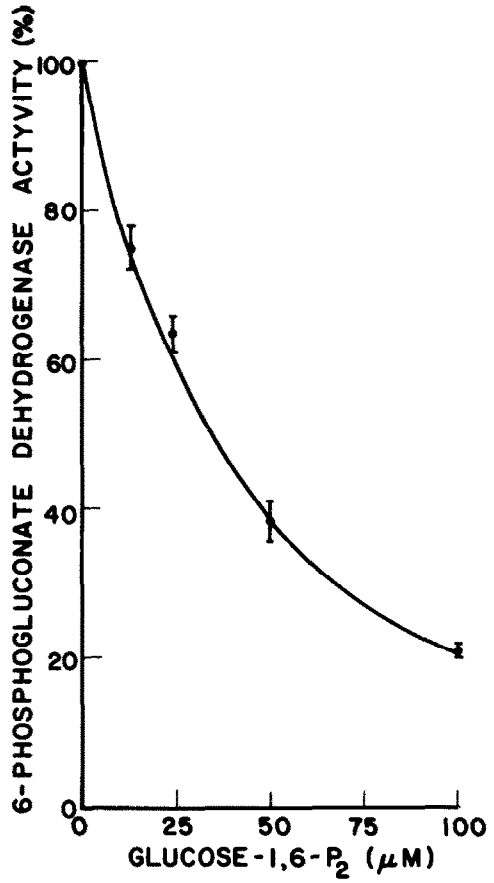

Fig.2. The inhibitory effect of Glc-1,6-P_2 on the activity of 6-phosphogluconate dehydrogenase from dystrophic muscle. The enzyme was extracted from muscles of dystrophic mice and assayed as in section 2. Assays were carried out at variable Glc-1,6-P_2 concentrations. 100% activity refers to 5.9 mU/mg protein. Values are means ± SEM for 4–6 expts.

different physiological and hormonal conditions, leading to concomitant changes in the activities of the enzymes which are regulated by this modulator. For example, epinephrine and dibutyryl cyclic AMP were found [7] to increase Glc-1,6-P_2 levels in rat diaphragm muscle and this increase was accompanied by a reduction in the activity of hexokinase and activation (deinhibition) of phosphofructokinase and phosphoglucomutase. The present and previous [16] results obtained from dystrophic muscle provide an additional example of the striking correlation between the tissue level of Glc-1,6-P_2 and the activities of the key enzymes of glucose metabolism which are affected by this modulator. The decrease in the level of Glc-1,6-P_2 in the dystrophic muscle, which is a common regulator of phosphoglucomutase, phosphofructokinase, hexokinase, and 6-phosphogluconate dehydrogenase, was associated with correlated changes in the activities of all four enzymes. Through its regulatory action on the activities of the key enzymes in glucose metabolism, this modulator may regulate the different pathways of glucose metabolism. Considering the reduction in the activity of phosphofructokinase and glycolysis, as well as the reduction in the activity of phosphoglucomutase and in the glycogen content [20] of dystrophic muscle, it seems likely that the glucose-6-phosphate, formed through the accelerated hexokinase reaction, shifts towards the pentose phosphate pathway due to the enchancement of 6-phosphogluconate dehydrogenase, one of the rate-limiting enzymes of this pathway. The activity of glucose-6-phosphate dehydrogenase was also reported to increase in dystrophic muscle [21]. The enhancement of glucose metabolism via the pentose phosphate pathway, which provides reducing equivalents for lipid synthesis and pentoses for nucleic acid synthesis [22], may account for the increases in lipid, and RNA and DNA content which were found [23–26] in the dystrophic muscle.

Acknowledgements

This research was carried out under a grant from the Muscular Dystrophy Association of America, and in part by the Research Committee, Bar-Ilan University. The skillful technical assistance of Mrs H. Morgenstern is highly appreciated. Mr Adi Kan is acknowledged for highly professional care of the mice.

References

[1] Leloir, L. F., Trucco, R. E., Cardini, C. E., Paladini, A. C. and Capputo, R. (1948) Arch. Biochem. 19, 339–340.
[2] Capputo, R., Leloir, L. F., Trucco, R. E., Cardini, C. E. and Paladini, A. C. (1948) Arch. Biochem. 18, 201–203.
[3] Cardini, C. E., Paladini, A. C., Caputto, R., Leloir, L. F. and Trucco, R. E. (1949) Arch. Biochem. 22, 87–99.
[4] Beitner, R., Haberman, S. and Livni, L. (1975) Biochim. Biophys. Acta 397, 355–369.
[5] Beitner, R. and Haberman, S. (1974) Biochim. Biophys. Acta 334, 237–241.
[6] Livni, L. and Beitner, R. (1975) Isr. J. Med. Sci. 11, 1181.
[7] Beitner, R., Haberman, S. and Nordenberg, J. (1978) Mol. Cell. Endocrinol. 10, 135–147.
[8] Hofer, H. W. and Pette, D. (1968) Hoppe-Seyler's Z. Physiol. Chem. 349, 1378–1392.
[9] Krzanowsky, J. and Matschinsky, F. M. (1969) Biochem. Biophys. Res. Commun. 34, 816–823.
[10] Beitner, R., Haberman, S. and Cycowitz, T. (1977) Biochim. Biophys. Acta 482, 330–340.
[11] Haberman, S. and Beitner, R. (1975) Isr. J. Med. Sci. 11, 1180.
[12] Haberman, S., Nordenberg, J. and Beitner, R. (1977) Isr. J. Med. Sci. 13, 971.
[13] Haberman, S., Nordenberg, J. and Beitner, R. (1978) Isr. J. Med. Sci. 14, 406.
[14] Cohen, T. J., Nordenberg, J., Haberman, S. and Beitner, R. (1979) Isr. J. Med. Sci. in press.
[15] Rapoport, S. (1974) in: The Human Red Cell in Vitro (Greenwalt, T. J. and Jamieson, G. A. eds) pp. 153–178, Grune and Stratton Inc., NY.
[16] Beitner, R., Haberman, S., Nordenberg, J. and Cohen, T. J. (1978) Biochim. Biophys. Acta 542, 537–541.
[17] Passonneau, J. V., Lowry, O. H., Schulz, D. W. and Brown, J. G. (1969) J. Biol. Chem. 244, 902–909.
[18] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[19] Mawatari, S., Takagi, A. and Rowland, L. P. (1974) Arch. Neurol. 30, 96–102.
[20] Vignos, P. J., jr and Warner, J. L. (1963) J. Lab. Clin. Med. 62, 579–590.
[21] Kitahara, A., Imai, F., Takaya, S. and Sato, K. (1977) Biochim. Biophys. Acta 500, 256–266.
[22] Beaconsfield, P. and Reading, H. W. (1964) Nature 202, 464–466.
[23] Srivastava, U., Devi, A. and Sarkar, N. (1963) Exp. Cell. Res. 29, 289–297.
[24] Srivastava, U. (1968) Can. J. Biochem. 46, 35–41.
[25] Goldspink, D. F. and Goldspink, G. (1977) Biochem. J. 162, 191–194.
[26] Goldspink, D. F. (1977) Life Sci. 20, 57–64.