FEBS Letters 583 (2009) 1841–1845

FEBS Letters

journal homepage: www.FEBSLetters.org

Phosphoglycerate mutase in mammalian striated muscles: Subcellular localization and binding partners

Wojciech Kowalski, Agnieszka Gizak, Darek Rakus *

Department of Animal Molecular Physiology, Wroclaw University, ul. Cybulskiego 30, 50-205 Wroclaw, Poland

ARTICLE INFO

Article history:
Received 4 February 2009
Revised 22 April 2009
Accepted 1 May 2009
Available online 8 May 2009

Edited by Berend Wieringa

Keywords:
Glycolysis
Interaction
Localization
Muscle
Phosphoglycerate mutase

ABSTRACT

Contrary to previously published data, we have found that in mammalian skeletal muscles, phosphoglycerate mutase (PGM) is organized in a regular, striated fashion within the sarcomere. In the absence of the enzyme effectors, PGM localizes mainly at the M-line, but under conditions typical for contracting muscle, the enzyme accumulates within the I-band of the sarcomere. Searching for muscle PGM binding partners, we have found that PGM interacts with several enzymes of triose phosphate metabolism. It might suggest that PGM is a central structural element of the muscle glycolytic complex located within the isotropic region of the sarcomere.

Structured summary:
MINT-7034028: *PGM* (uniprotkb:P16290) *physically interacts* (MI:0218) with *lactate dehydrogenase B* (uniprotkb:P42123), *lactate dehydrogenase A* (uniprotkb:P04642), *G3PDH* (uniprotkb:P04797), *aldolase* (uniprotkb:P05065), *Creatine kinase* (uniprotkb:P07335), *phPhosphoglycerate kinase* (uniprotkb:P16617) and *Enolase* (uniprotkb:P04764) by *pull down* (MI:0096)

1. Introduction

Phosphoglycerate mutase (PGM, EC 5.4.2.1) catalyzing the interconversion of 3-phosphoglycerate and 2-phosphoglycerate is an indispensable enzyme of glycolysis and gluconeogenesis [1]. In mammals, PGM exists as a homodimer consisting of two muscle-type monomers (PGM-MM), two brain-type monomers (PGM-BB) or as a heterodimer (PGM-MB) [2]. PGM-BB is widely distributed in all mammalian tissues, except adult skeletal muscles where PGM-MM is present [3,4]. In fetal and neonatal skeletal muscles and heart, the PGM-MB heterodimer occurs [2,4].

Although the sequences of enzymatic reactions constituting the glycolytic and gluconeogenic pathways in skeletal muscle are well known, their regulation and spatial organization are not yet fully understood. Many studies indicate that glycolytic enzymes are associated with contractile proteins [5–7]. Such association not only alters their regulatory properties and kinetics [8,9], but may also facilitate channeling of substrates between metabolically sequential enzymes [7,9].

In contrast to well-established localization of majority of glycolytic enzymes within sarcomeric structures [5–7], the data on subcellular localization of muscle PGM is unclear.

In 1975, it was demonstrated that association of PGM with isolated structural proteins of rabbit striated muscles is relatively weak [5]. No evidence of such association was found in rat skeletal muscle, using immunocytochemistry [10]. The data from mammalian muscles are in conflict with results concerning insect muscles. It has been clearly demonstrated that in *Drosophila* flight muscle, PGM accumulates at both the M and the Z-line of sarcomere [11]. These results might suggest that in mammals and in insects, different organization of glycolytic enzymes within sarcomere and hence, different mode of muscle glycolysis regulation exist.

Nevertheless, since almost all glycolytic enzymes of mammalian muscle bind to the I-band of sarcomere it seems reasonable to expect that if the muscle glycolytic complex exists, PGM should localize within it (interacting, if not directly with sarcomere, at least with one of the enzymes which bind to sarcomeric structures).

This fact prompted us to re-evaluate the data on the subcellular localization of PGM in mammalian skeletal muscles. To achieve the goal we used microscopic techniques, subcellular fractionation and enzyme activity measurements. To extend our understanding of PGM-MM role in rat skeletal muscle we also searched for PGM-MM binding partners using affinity chromatography and MS analysis. Results of the experiments are presented and their physiological meaning is discussed.

Abbreviations: BSA, bovine serum albumin; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; PEG, polyethylene glycol; PK, pyruvate kinase; PMSF, phenylmethylsulfonyl fluoride; TIM, triosephosphate isomerase; TRIS, tris(hydroxymethyl)aminomethane; TRITC, tetramethyl rhodamine isothiocyanate

* Corresponding author. Fax: +48 071 375 92 13.
E-mail address: drakus@biol.uni.wroc.pl (D. Rakus).

0014-5793/$36.00 © 2009 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2009.05.004

2. Materials and methods

Phosphocellulose P-11 was purchased from Whatman (England). Other reagents were from Sigma (USA).

If it is not stated otherwise, all procedures were carried out at $T = 4$ °C.

2.1. Phosphoglycerate mutase purification and determination of its kinetic parameters

Phosphoglycerate mutase (PGM) from rabbit skeletal muscle was purified to homogeneity according to the method elaborated in our laboratory. The isolation procedure, SDS–PAGE and mass spectrometry analysis of purified PGM are presented as a supplementary data (Supplementary Table 1 and Fig. 1).

PGM activity was measured in the presence of coupling enzymes: enolase (1 unit/ml), pyruvate kinase (1 unit/ml) and lactic dehydrogenase (12 unit/ml), following the decrease of NADH absorbance. A total of 1 ml of the assay mixture contained: 100 mM tris(hydroxymethyl)aminomethane (TRIS), 100 mM KCl, 10 mM $MgCl_2$, 0.5 mM ethylenediaminetetraacetic acid (EDTA), 10 mM 3-phosphoglycerate (3-PG), 20 μM 2,3-bisphosphoglycerate (2,3-BPG), 1.5 mM ADP, 0.2 mM NADH, pH 7.4, at 37 °C. PGM was used to start the reaction.

The kinetic parameters of PGM were determined both in forward, glycolytic direction (with 3-PG as a substrate and 14 ng/ml of PGM), and in reverse, gluconeogenic direction (using 2-phosphoglycerate as a substrate and 56 ng/ml of PGM). The parameters were determined at pH 6.3 and at pH 7.4, $T = 37$ °C. Spectrophotometric measurements were performed with a HP 8452A diode array spectrophotometer. The calculation of k_m was performed using GraFit 4.0.12 program (Leatherbarrow, 2000).

2.2. Effect of Ca^{2+} and 3-PG on association of PGM with subcellular structures of muscle fibers

Fresh rabbit skeletal muscles were homogenized in the buffer: 20 mM TRIS, 0.4 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), 5% polyethylene glycol (PEG) 8000, pH 6.3 or 7.4, supplemented either with 10 μM $CaCl_2$, or with 100 μM 3-PG. The ratio of tissue to buffer (wt./vol.) was 1:2. Homogenates were centrifuged at 15 000×*g* for 20 min and the supernatants were used to measure the activity of cytosolic PGM. To release the cytoskeleton-bound PGM fraction, the pellets were homogenized in 9 volumes of the homogenization buffers supplemented with 0.5% Triton-X 100, incubated for 30 min on ice and centrifuged at 15 000×*g* for 20 min. The final supernatants were used to determine the activity of cytoskeleton-bound PGM.

2.3. Fluorescent labeling

Fluorescently labeled PGM (FITC-PGM) was obtained by modification of the protein with fluorescein isothiocyanate (FITC) as described by Goding [12]. The lack of proteolysis of fluorescently labeled protein was checked by 10% SDS–PAGE. The number of fluorochrome molecules conjugated to the enzyme was estimated spectrophotometrically to be 3 FITC molecules per monomer of phosphoglycerate mutase.

2.4. The protein exchange

Single skeletal muscle fibers were prepared as described by Kraft et al. [13].

The FITC-labeled PGM was dialyzed against relaxing solution [13], pH 6.3 or 7.4. The muscle fibers were incubated in a drop of the relaxing solution with anti-α-actinin antibodies, secondary tetramethyl rhodamine isothiocyanate (TRITC)-conjugated antibodies and then with 0.2 mg/ml FITC-PGM (overnight). All fibers were washed with the relaxing solution, mounted on slides and examined using confocal microscopy (Olympus IX71 microscope with FluoView 500 confocal scanner unit).

As a control, a competition experiment was performed: the muscle fibers were incubated with 0.2 mg/ml FITC-PGM and with increasing concentrations (0–1 mg/ml) of unlabeld PGM. Alternatively, the muscle fibers were incubated with FITC-labeled bovine serum albumin (BSA) (0.2–0.5 mg/ml).

To check the effect of Ca^{2+} and 3-PG on PGM localization, the fibers containing cytoskeleton-associated FITC-PGM were incubated in the relaxing solutions containing 10 μM $CaCl_2$ or 50 μM 3-PG, respectively.

2.5. Sepharose 4B-PGM column

CNBr-activated Sepharose 4B powder (600 mg) was swollen in 1 mM HCl and washed with 10 volumes of 1 mM HCl. Then the resin was washed with coupling buffer (CB; 25 mM sodium pyrophosphate, 0.5 M NaCl, pH 8.4). After overnight dialysis in CB, PGM (3 mg) was incubated with Sepharose 4B resin for 24 h with gentle stirring. Unbound PGM was washed away with the CB. The amount of PGM immobilized on the resin (0.8 mg) was calculated as a difference between the amount applied and unbound PGM. Remaining active imidocarbonate groups of the Sepharose were blocked for 16 h with 1 M ethanolamine, pH 8.0. PGM-Sepharose was loaded into a column, washed with CB and equilibrated with 20 mM TRIS, 0.5 mM EDTA, pH 7.4.

2.6. Identification of PGM-interacting proteins

Fresh rat skeletal muscles were homogenized in a buffer containing 20 mM TRIS, 150 mM NaCl, 0.5 mM EDTA, 1 mM PMSF, 1 mM dithiothreitol (DTT), 1% Triton-X 100, pH 7.4. Homogenate was centrifuged at 15 000×*g* for 20 min. and the obtained supernatant was dialyzed overnight against the 20 mM TRIS, 0.5 mM EDTA, pH 7.4. Then the supernatant was passed ten times through pre-equilibrated PGM-Sepharose column. To remove proteins unspecifically associated with PGM-Sepharose, the column was washed extensively with equilibrating buffer until the absorbance ($\lambda = 280$ nm) dropped below 0.01. PGM-interacting proteins were eluted with 1 M NaCl in equilibrating buffer. The samples were precipitated with TCA and resolved by SDS–PAGE. The gel was silver-stained and the protein bands were excised and analyzed commercially by ESI-MS at Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland.

The activities of pyruvate kinase (PK) and triose phosphate isomerase (TIM) in the eluent containing proteins interacting with PGM-CNBr-Sepharose were measured, respectively, according to [14,15].

2.7. Statistical analysis

Statistical analysis of protein exchange section was performed by the Cell Imaging Software (Olympus Soft Imaging Solution GmbH, Germany).

3. Results and discussion

It is well known that in striated muscles, almost all glycolytic enzymes and creatine kinase bind to actin filaments within the I-band and, supposedly, to the Z-line of sarcomere [6,7,11,13,16]. It

is hypothesized that such localization enables them to function as a glycolytic multi-enzyme complex, directly providing ATP to myosin ATPase [11,13]. Although this complex cannot be active in the absence of phosphoglycerate mutase, there is no data confirming localization of PGM within the sarcomere of vertebrate muscles. Instead, it has been shown in vitro that PGM does not associate with muscle contractile proteins [10].

In contrast to vertebrate skeletal muscle, in *Drosophila* flight muscle, PGM (as well as aldolase, glycerol 3-phosphate dehydrogenase, glyceraldehyde 3-phosphate dehydrogenase, TIM and phosphoglycerate kinase) binds both to the M- and to the Z-line [11].

Taking this into account, we attempted to re-evaluate few and relatively old in vitro data on the association of PGM with subcellular structures of mammalian skeletal muscles.

The results of protein exchange experiments presented here clearly demonstrate that in rat skeletal muscle, FITC-PGM is organized in a regular, striated fashion within the sarcomere (Fig. 1). After introduction of FITC-BSA to the muscle fibers (control reaction) we could not detect any binding of the protein to the sarcomere (data not shown), what suggests that PGM was specifically associated with subsarcomeric structures. Moreover, in the presence of increasing concentrations of unlabeled PGM the amount of FITC-PGM bound to the sarcomeric structures decrease, suggesting that the native enzyme binds to the same structural proteins as the modified form (Supplementary Fig. 2).

The striated pattern of PGM localization (as well as its diffuse presence in cytoplasm and in some of the nuclei) was also confirmed by immunohistochemistry (Supplementary Fig. 3).

Our results are in discrepancy with those of Urena et al. [10], who could not observe any association of PGM to contractile proteins. Presumably it arises from the method of the antibody preparation used by the authors (affinity chromatography in a CNBr-activated Sepharose 4B coupled with PGM). Coupling of PGM to the matrix may mask epitopes which are exposed on the surface of PGM associated with the sarcomeric structures. Thus, it is feasible that they had prepared and used antibodies highly specific to PGM epitopes normally hidden upon the enzyme association with the sarcomere.

The activity of PGM bound to the sarcomeric structures of rat skeletal muscle represents about 10% of the total PGM. This amount seems to be constant and not influenced by changes in concentration of Ca^{2+}, 3-PG (a PGM substrate) or pH value (Table 1). Nevertheless, an elevated concentration of Ca^{2+} and/or 3-PG causes significant translocation of this cytoskeleton-bound PGM fraction within the sarcomere (Fig. 2). In the absence of the enzyme effectors, PGM localizes mainly at the M-line (Fig. 1). Supplementation of the incubation medium with elevated concentrations of the effectors results in the accumulation of PGM within the isotropic region of sarcomere (i.e. within the actin filaments, around the Z-line) (Fig. 2). This suggests that under conditions typical for contracting muscle PGM participates in the formation of the glycolytic complex within the I-band. The F-actin-based localization of PGM might therefore reflect an increased requirement of myosin ATPase for energy during contractile activity.

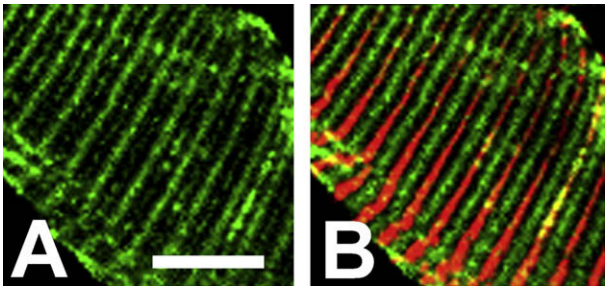

Fig. 1. Localization of FITC-conjugated PGM in isolated rat skeletal muscle fibers. In the absence of Ca^{2+} and 3-PG, PGM (green; A and B) accumulates mainly on the M-line and much weaker within the isotropic region of the sarcomere, as marked by α-actinin staining (red; B). Bar = 10 μm.

Table 1
The effect of Ca^{2+} and 3-PG on the association of phosphoglycerate mutase with subcellular structures of muscle fibers.

Buffer	PGM associated with subcellular structures (%)
pH 6.3 in the absence of effectors	10.7 ± 1.8
pH 6.3 in the presence of 10 μM Ca^{2+}	12.0 ± 2.0
pH 6.3 in the presence of 100 μM 3-PG	11.3 ± 1.0
pH 7.4 in the absence of effectors	8.9 ± 3.7
pH 7.4 in the presence of 10 μM Ca^{2+}	8.5 ± 0.8
pH 7.4 in the presence of 100 μM 3-PG	10.9 ± 0.1

Such mode of regulation of the PGM subsarcomeric localization is in agreement with the results obtained by Ramizel group [11] who has found that the association of glycolytic enzymes (e.g. PGM) with the Z-line of thoracic muscles of *Drosophila* sp. depends on calcium concentration.

Many glycolytic enzymes like phosphofructokinase, aldolase, glyceraldehyde 3-phosphate dehydrogenase and PK bind to F-actin-tropomyosin at acidic pH (pH values below 7.0). Studying the effect of pH changes on PGM properties we did not observe differences neither in affinity of the enzyme to muscle subcellular structures nor in its localization. However, in contrast to above-mentioned glycolytic enzymes, PGM does not seem to interact with sarcomeric proteins directly but rather through other enzymes [17].

Using affinity chromatography and subsequent MS analysis we found that in skeletal muscle, PGM may interact with several enzymes of triose phosphates metabolism, with creatine kinase and with gluconeogenic enzyme – malate dehydrogenase (Fig. 3). Among PGM-interacting enzymes we also found lactate dehydrogenase which had been earlier hypothesized to be a linker between the F-actin-tropomyosin complex and rabbit muscle PGM in vitro [17]. Although we were not able to detect PK and TIM protein using affinity chromatography-MS analysis, the measurement of the enzymatic activity clearly showed that both of the enzymes are among PGM-interacting proteins. The activity of PK and TIM in the fractions eluted with 1 M NaCl from PGM-CNBr-Sepharose was, respectively, 1.62 ± 0.57 and 2.94 ± 1.28 U/mg of PGM-interacting proteins.

The ability of PGM to associate with presumably all enzymes of triose phosphates metabolism might suggest that PGM is a central structural element of the glycolytic complex located within the isotropic region of the sarcomere. The kinetic properties of PGM support this hypothesis: although changes in pH influence both k_m and k_{cat}, they do not affect significantly the enzyme catalytic efficiency (k_{cat}/k_m) (Table 2). This may ensure a constant activity of the glycolytic complex, and thus a constant rate of ATP delivery to myosin ATPase during prolonged muscle contraction.

On the other hand, the decrease of pH significantly increases PGM catalytic efficiency ($P < 0.05$) towards 2-PG which serves as a substrate of the enzyme in gluconeogenic direction (Table 2). Such kinetic properties of PGM supports the hypothesis of Bonen and colleagues [18] that re-synthesis of glucose from carbohydrate precursors in skeletal muscles is more efficient at acidic pH, which occur after high intensity exercise.

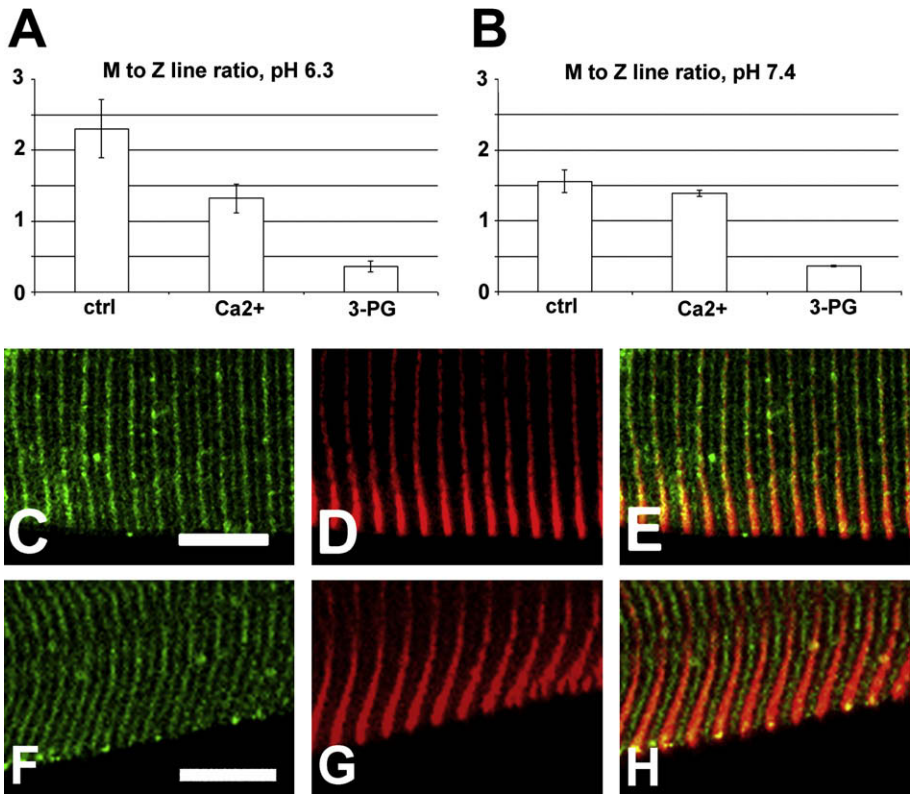

Fig. 2. The effect of Ca^{2+} (10 μM) and 3-PG (50 μM) on the sub-sarcomeric localization of PGM in pH 6.3 (A) and pH 7.4 (B). The accumulation of FITC-labeled PGM within subsarcomeric domains was measured as a sum of all green intensities around the M-line and the Z-line (within the I-band). 3-PG- (C–E) and Ca^{2+}-evoked (F–H) shift of PGM (green) from the M-line to the isotropic region of sarcomere marked by α-actinin (red). Bar = 10 μm.

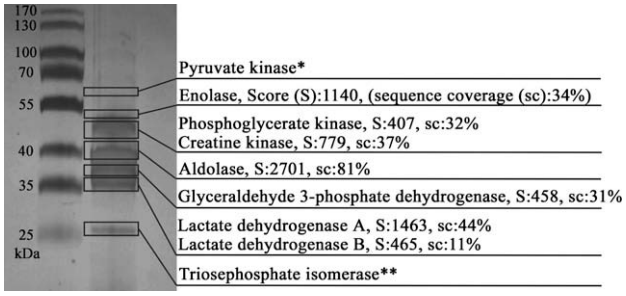

Fig. 3. SDS–PAGE of PGM-interacting proteins from rat skeletal muscle. The proteins interacting with PGM-Sepharose were separated by SDS–PAGE and silver-stained. The indicated protein bands were excised and analyzed by ESI-MS. Although pyruvate kinase and triosephosphate isomerase were not detected using ESI-MS analysis, we found significant activities of both the enzymes in the mixture of PGM-interacting proteins.

Table 2
The kinetic parameters of rabbit muscle phosphoglycerate mutase.

Parameter	3-PG as a substrate	2-PG as a substrate
k_m (mM), pH 7.4	0.58 ± 0.04	0.01 ± 0.003
k_m (mM), pH 6.3	1.84 ± 0.1	0.076 ± 0.02
k_{cat} (s^{-1}), pH 7.4	1408 ± 15	105 ± 18.4
k_{cat} (s^{-1}), pH 6.3	2663 ± 31	131 ± 26.0

Acknowledgement

This work was supported by Zoological Institute of Wroclaw University Research Grant.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2009.05.004.

References

[1] Fothergill-Gilmore, L.A. and Watson, H.C. (1989) The phosphoglycerate mutases. Adv. Enzymol. Relat. Areas Mol. Biol. 62, 227–313.
[2] Omenn, G.S. and Cheung, S.C. (1974) Phosphoglycerate mutase isozyme marker for tissue differentiation in man. Am. J. Human Genet. 26, 393–399.
[3] Chen, S.H., Anderson, J.E. and Giblett, E.R. (1977) Human red cell 2,3-diphosphoglymutase mutase and monophosphoglyceratemutase mutase: genetic evidence for two separate loci. Am. J. Human Genet. 29, 405–407.
[4] Prehu, M.O., Calvin, M.C., Prehu, C. and Rosa, R. (1984) Biochemical and immunological arguments for homology between red cell and liver phosphoglyceromutase isozymes. Biochim. Biophys. Acta 787, 270–274.
[5] Clarke, F.M. and Masters, C.J. (1975) On the association of glycolytic enzymes with structural proteins of skeletal muscle. Biochim. Biophys. Acta 381, 37–46.
[6] Masters, C.J., Reid, S. and Don, M. (1987) Glycolysis – new concepts in an old pathway. Mol. Cell. Biochem. 76, 3–14.
[7] Ovadi, J. and Srere, P.A. (2000) Macromolecular compartmentation and channelling. Int. Rev. Cytol. 192, 255–280.
[8] Liou, R.S. and Anderson, S. (1980) Activation of rabbit muscle phosphofructokinase by F-actin and reconstituted thin filaments. Biochemistry 19, 2684–2688.
[9] Rakus, D., Pasek, M., Krotkiewski, H. and Dzugaj, A. (2004) Interaction between muscle aldolase and muscle fructose 1,6-bisphosphatase results in the substrate channeling. Biochemistry 43, 14948–14957.
[10] Urena, J.M., Egea, G., Grana, X., Castella, J., Marsal, J., Carreras, J. and Climent, F. (1990) Location of phosphoglycerate mutase in rat skeletal muscle. An immunocytochemical and biochemical study. Eur. J. Cell Biol. 51 (1), 151–156.
[11] Sullivan, D.T., MacIntyre, R., Fuda, N., Fiori, J., Barrilla, J. and Ramizel, L. (2003) Analysis of glycolytic enzyme co-localization in *Drosophila* flight muscle. J. Exp. Biol. 206, 2031–2038.
[12] Goding, J.W. (1976) Conjugation of antibodies with fluorochromes: modifications to the standard methods. J. Immunol. Meth. 13, 215–226.
[13] Kraft, T., Hornemann, T., Stolz, M., Nier, V. and Wallimann, T. (2000) Coupling of creatine kinase to glycolytic enzymes at the sarcomeric I-band of skeletal muscle: a biochemical study in situ. J. Muscle Res. Cell Motil. 21, 691–703.

[14] Fujii, H. and Miwa, S. (1983) Pyruvate kinase in: Methods of Enzymatic Analysis (Bergmeyer, H.U., Ed.), pp. 496–502, Berlag Chemie Weinhein, Academic Press, New York.
[15] Merkle, S. and Pretsch, W. (1989) Characterization of triosephosphate isomerase mutants with reduced enzyme activity in Mus musculus. Genetics. 123, 837–844.
[16] Pette, D. (1975) Some aspects of supramolecular organization of glycogenolytic and glycolytic enzymes in muscle. Acta Histochem. Suppl. 14, 47–68.
[17] Bronstein, W.W. and Knull, H.R. (1981) Interaction of muscle glycolytic enzymes with thin filament proteins. Can. J. Biochem. 59 (7), 494–499.
[18] Bonen, A., McDermott, J.C. and Tan, M.H. (1990) Glycogenesis and glyconeogenesis in skeletal muscle: effects of pH and hormones. Am. J. Physiol. 258, E693–E700.