S3P13.WP

# COMPARATIVE STUDIES ON GLYCOGEN-PHOSPHORYLASE FROM PSE- AND NORMAL MUSCLES

P.L. LOPEZ BUESA, K.O. HONIKEL and F. SCHWÄGELE

Institute for Chemistry and Physics, Federal Centre for Meat Research, 8650 Kulmbach, Germany

Please refer to Folio 16.

## INTRODUCTION

The main biochemical characteristic of PSE-prone muscles is a fast metabolism of carbohydrates post-mortem. One theory, which may explain this observation is a failure of the regulation of $Ca2^{+}$-concentration in the sarcoplasm of PSE-muscles leading to an activation of glycogenolysis. Another theory deals with ATP consuming processes like active membrane transport. Nevertheless, there are many authors who report about differences between enzymes of the glycogenolytic and glycolytic pathway from PSE- and normal muscles. One of these enzymes is glycogen-phosphorylase (GP), which catalyses the degradation of glycogen transferring a glucose residue from the non-reducing end of the glycogen chain to inorganic phosphate. After isomerization from glucose-1-phosphate to glucose-6-phosphate the latter product enters glycolysis. GP is regulated by allosteric mechanisms and phosphorylation. Several authors reported about differences, sometimes contradictory, with respect to the activity of GP from normal and PSE-muscles. Ono *et al.* (1977) found an enhanced GP a (phosphorylated form) activity in PSE-tissue. Schwägele and Honikel (1989) reported about a higher total activity (GP a and GP b) in PSE-prone muscles, whereas Ensinger *et al.* (1982) could not find differences in the activities of GP a and GP b in muscles of normal and PSE-animals. As these results were obtained with muscle homogenates, we isolated GP from normal and PSE-tissue and compared its kinetic and structural properties.

## MATERIALS AND METHODS

*M.longissimus dorsi* (MLD) from pigs of the German Landrace breed was used for the isolation of the enzyme GP. The halothane-test, the pH1-value (45 minutes post-mortem) and the presence of a pyruvate kinase isoenzyme (Haschke, 1992), which is specific for PSE-prone muscles, served as criteria for the differentiation between normal and PSE-muscles. PSE-pigs showed in their MLD $pH_1$-values lower than 5.5, whereas in the case of normal animals the $pH_1$ was always above 6.4. For the isolation of the enzyme 0.5kg of MLD were ground and subsequently homogenized in 1.5l buffer containing 10mM KCl and 30mM potassium phosphate, pH7.0, using a Waring Blender. After a twofold $(NH_4)_2SO_4$-precipitation (40%) GP was separated by gel permeation chromatography on Sephacryl S-300. The activity of GP was determined according to Bergmeyer (1970) in the direction of glycogen degradation and according to Cori *et al.* (1950) in the direction of glycogen synthesis. Protein concentration was measured by application of a method of Whitaker and Granum (1980). SDS polyacrylamide gel electrophoresis (PAGE) was performed as described by Laemmli (1970). Not denaturating PAGE was performed using a modified method of SIGMA (1986). Analytical isoelectric focusing (IEF) was performed according to Frey *et al.* (1986) and preparative IEF according to a method described by PHARMACIA LKB (1989). GP b was phosphorylated with phosphorylase kinase (PK) from rabbit muscle according to Berndt and Rösen (1984).

## RESULTS AND DISCUSSION

The results of the isolation of GP show that normal and PSE-muscles have a similar GP activity which was determined

in the direction of glycogen degradation. The conclusion can be drawn that the prepared enzyme was present in the dephosphorylated form b, as the activity measured in absence of AMP was negligible. After gel permeation chromatography GP preparations showed a single band on SDS-PAGE with a densitometrically determined purity of more than 98%. No differences were found either for the specific or the total activity of GP isolated from normal and PSE-meat. The isoenzyme composition of GP from normal and PSE-tissue was analyzed by IEF techniques. As shown by analytical IEF (Figure 1) GP of both animal groups comprises several isoenzymes appearing in a narrow pH-range around 6.5. Similar results were obtained for GP, which was isolated in the presence of protease inhibitors, showing that the number of bands on IEF gels is not due to proteolytic artefacts during purification. The distribution of GP activity after separation of the isoenzymes by preparative IEF was also similar for both groups of pigs.

GP b of PSE- and normal meat was phosphorylated by phosphorylase kinase from rabbit muscle. The course of the reaction is depicted in Figure 2. GP a from both groups of animals showed about 25% higher specific activity than GP b.

Phosphorylation of GP b under catalysis of phosphorylase kinase affects the kinetic properties. In contrast to GP b the phosphorylated form GP a is active in the absence of AMP. The affinity of GP a to glycogen and glucose-1-phosphate is higher than in the case of GP b. In interaction with Pi, glycogen and glucose-1-phosphate, GP a showed the characteristics of a Michaelis-Menten kinetic.

The optimal pH-value for the enzymatic activity did not change by conversion from GP b to GP a. The determined optimum was around pH 7,0 for both forms of GP isolated from normal as well as PSE-meat.
Kinetic parameters of GP b and GP a were measured in interaction with the specific substrates and AMP. Interacting with glycogen and glucose-1-phosphate, GP b follows the characteristics of a Michaelis-Menten kinetic, whereas binding of inorganic phosphate and AMP proves to be allosteric. The apparent $K_m$- and $V_{max}$-values for the substrates glycogen and glucose-1-phosphate (Table 1) are similar to those as described by Will *et al.* (1970) and Oikonomakos *et al.* (1985). Taking these values in consideration no significant differences were found in comparison of GP b from normal and PSE-meat. The calculated Hill coefficients for the interaction of GP b isolated from normal and PSE-tissue with AMP and $P_i$ (Table 2) showed identical values.

Summarizing all the determined data there are obviously no differences in the kinetic properties of GP a from PSE- and normal meat.

## CONCLUSIONS

No significant differences have been found in comparison of the kinetic and electrophoretic properties of GP from normal and PSE-meat. Several active isoenzymes of GP were observed in a narrow pH-range for both groups of animals. Differences in the properties of GP from PSE- and normal meat, which are described in the literature, can be due to non optimum criteria applied for the selection of the animal material and different phosphorylation degrees of the tested enzyme.

From the experiments reported it appears that GP does not permanently exist in different active isoenzymes in PSE- and normal pigs besides the existing GP a and GP b forms. Unlike pyruvate kinase in which Haschke (1992) could prove an additional more active and stable phosphorylated isoenzyme, GP is controlled in pig muscles in a similar way, but this control is continuously switched on and off according to the need of muscle, i.e., ATP consumption rate by metabolism. Thus the high energy turnover (ATP-turnover) in muscles of PSE-pigs is possibly caused by the turnover rate of GP in its usual forms. It seems unlikely that GP is the primary reason for the fast post mortem changes in PSE-prone muscles. The primary causes are the reaction(s) which show an accelerated ATP turnover. These reactions whatever they are, need further investigations.

REFERENCES


BERGMEYER, H.U. 1970. *Methoden der enzymatischen Analyse.* Verlag Chemie, Weinheim/Bergstr. pp.467-468.

BERNDT, N., and RÖSEN, P. 1984. Isolation and partial characterization of two forms of rat heart glycogen-phosphorylase. *Arch. Biochem. Biophys.* 228:143-154.

ENSINGER, U., ROGDAKIS, E., MÜLLER, E., and FABER, H. 1982. Stoffwechselparameter bei halothanpositiven und halothannegativen Schweinen. Z. Tierzüchtg. *Züchtungsbiol.* 99:26-32.

FREY, M.D., KINZHOFER, A., ATTA, B.M., and RADOLA, B.J. 1986.
Preparation of rehydratable polyacrylamide gels and their application in ultrathinlayer isoelectric focusing. *Electrophoresis.* 7:28-40.

HASCHKE, C. 1992. *Isoenzymmuster und Phosphorylierung der Pyruvatkinase aus PSE-Fleisch und Fleisch normaler Qualität.* Dissertation, Universit„t Bayreuth.

LAEMMLI, U.K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature.* 227: 680-685.

OIKONOMAKOS, N.G., MELPIDOU, A.E., and JOHNSON, N.L. 1985. Crystallization of pig skeletal phosphorylase b. Purification, physical and catalytic characterization. *Biochem. Biophys. Acta.* 832:248-256.

ONO, K., TOLPEL, D.G., CHRISTIAN, L.L., and ALTHEN, T.G. 1977. Relationship of cyclic-AMP and phosphorylase a in stress-susceptible and control pigs. *J. Food Sci.* 42:108-110.

PHARMACIA LKB. 1989. Preparative flat-bed electrofocusing in carrier ampholyte pH gradients. In: PHARMACIA LKB (ed). *2117 Multiphor II electrophoresis system.* Pharmacia LKB, Uppsala, Sweden. pp.87-96.

SCHWÄGELE, F., and HONIKEL, K.O. 1989. Studies in early post-mortem metabolism of PSE-prone pork muscle. In: HOFFMANN, J.G., and SCHMIDT, A. (eds). *Malignant Hyperthermia: An Update.* Verlag Volk und Gesundheit, Berlin. pp.307-311.

SIGMA. 1986. *Nondenatured Protein Molecular Weight Marker Kit.* Sigma techn. bulletin, St. Louis, Missouri. No. MKR-137.

WILL, H., KRAUSE, E.G., and WOLLENBERGER, A. 1970. A kinetic study of muscle phosphorylase b variants of the pig: heart specific isoenzyme and skeletal muscle enzyme. *Biochem. Biophys. Res. Commun.* 40:7-14.

WHITAKER, J.R., and GRANUM, P.E. 1980. An absolut method for protein determination based on difference in absorbance at 235 and 280 nm. *Anal. Biochem.* 109:156-159.

Table 1. Apparent Km- and Vmax-values for the interaction of GP b with glucose-1-phosphate and glycogen.

| Substrate | $P_i$ (mM) | AMP (AMP) | PSE-muscle $K_m$ (mM) | PSE-muscle $V_{max}$ (units/ml) | Normal muscle $K_m$ (mM) | Normal muscle $V_{max}$ (units/ml) |
|---|---|---|---|---|---|---|
| Glycogen | 75 | 0.5 | 0.26 | 28.6 | 0.22 | 26.7 |
| | 20 | 0.5 | 1.0 | 26.3 | 1.1 | 22.6 |
| | 5 | 0.5 | 2.2 | 9.1 | 2.9 | 9.1 |
| G1P | | 0.1 | 58 | 53 | 51 | 55 |
| | | 0.2 | 13 | 53 | 17 | 55 |
| | | 1.0 | 8 | 53 | 7 | 55 |

Table 2. Hill coefficients for the interaction of GP b with Pi and AMP.

| Constant substrate concentration | Hill-coefficient PSE-muscle | Hill-coefficient normal muscle |
|---|---|---|
| 5.55 mM glycogen 0,5 mM AMP | 1.49 ± 0.11 | 1.42 ± 0.04 |
| 0.83 mM glycogen 0,5 mM AMP | 1.48 ± 0.18 | 1.46 ± 0.07 |
| 5.55 mM glycogen 75 mM Pi | 1.85 ± 0.20 | 1.83 ± 0.14 |
| 0.83 mM glycogen 75 mM Pi | 1.86 ± 0.15 | 1.98 ± 0.10 |