STUDIES IN POST-MORTEM METABOLISM OF PSE-PRONE PORT MUSCLES

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SUMMARY

After an animal has experienced stress, PSE-prone muscles develop post mortem an extremely rapid glycogenolysis. This process leads to a fast drop in pH and is accompanied by an increased ATP-turnover. The sequence of biochemical events, leading to the PSE-syndrome is still unknown. For the elucidation of this problem glycogenolytic enzymes from porcine muscles were isolated, which are involved in the ATP-turnover showing pH_1-values (45 min p.m.) between 6.8 and 5.3 Phosphorylase, phosphofructokinase (PFK), glyceral-dehydephosphatedehydrogenase (GAPDH), phosphoglyceratekinase (PGK), pyruvatekinase (PK) and adenylatekinase) AK) were tested for their total activity/kg meat and their specific activity. Several alterations in these measured activities were determined in dependance of the pH_1-values. It is presumed, that the development of the PSE-syndrome among other parameters depends on the isoenzyme composition of these enzymes, which may be regulated by different mechanisms of modification.

INTRODUCTION

Stress susceptible pigs experiencing stress shortly before or at slaughter show rapid biochemical changes post mortem. Within one hour the pH in the muscles of these pigs drops down to values of 5.5 - 5.3; whereas normal pork muscles show at the same time pH-values between 6.5 and 5.9 At prevailing high temperatures these rapid pH-changes cause denaturation of sarcoplasmic and myofibrillar proteins and degeneration of membranes, leading to pale, soft, and exudative meats, called PSE-meat (Bendall and Wismer-Pedersen, 1962; Briskey, 1964; Honikel and Woltersdorf, 1984; Honikel and Kim, 1985).

An extremely accelerated glycogenolysis which is accompanied by an accumulation of lactic acid and an increased ATP-turnover, are involved in the development of the PSE-syndrome. The initiating process for the rapid break-down of glycogen is most probably a hormone induced malfunction of processes in the energy-turnover of the cell. There exist a number of hypotheses which try to explain this rapid energy-turnover, for instance uncoupling of ATP-formation in mitochondria, ATP consuming membrane transport systems, muscle contraction by increased Ca^{2+}-efflux and futile cycling of glycolytic metabolites e.g. fructose-6-phosphate and fructose-1,6-diphosphate under ATP-consumption (Eikelenboom and van den Bergh, 1973; Jordon et al., 1980; Cheah et al., 1984; Clark et al, 1973).

None of these hypotheses has been proven valid so far. Preliminary studies of Honikel and Kim (1985) have excluded the muscle contraction theory, but the sequence of biological events in the cell, leading to the PSE-syndrome is still unsolved and the biochemical regulatory mechanisms responsible for this extremely fast turnover of energy-rich compounds after slaughter are unknown.

To make a contribution for the elucidation of this problem, we isolated glycogenolytic enzymes, involved in the ATP-turnover from pork longissimus dorsi muscles showing pH_1- values, measured 45 minutes post mortem, between 6.8 and 5.3.

MATERIAL AND METHODS

Pigs of various breeds, mainly german Landrace and Pietrain with a slaughterweight of 80 - 100 kg were used in this study. The experiments were carried out exclusively with M. longissimus dorsi, which was taken from the carcass 40 to 45 min post mortem. At different points in the muscle the pH- values were measured. Exactly 100 g of the minced meat were homogenised in 300 ml of a phosphate-buffer, pH 7.0, and the cell-fragments removed by centrifugation. The soluble proteins of the homogenate were fractionated into five protein-pools by ammonium-sulphate precipitation. Subsequently the protein concentrations were determined by the method of Whitaker and Granum (1980). The resulting protein-fractions were analysed by

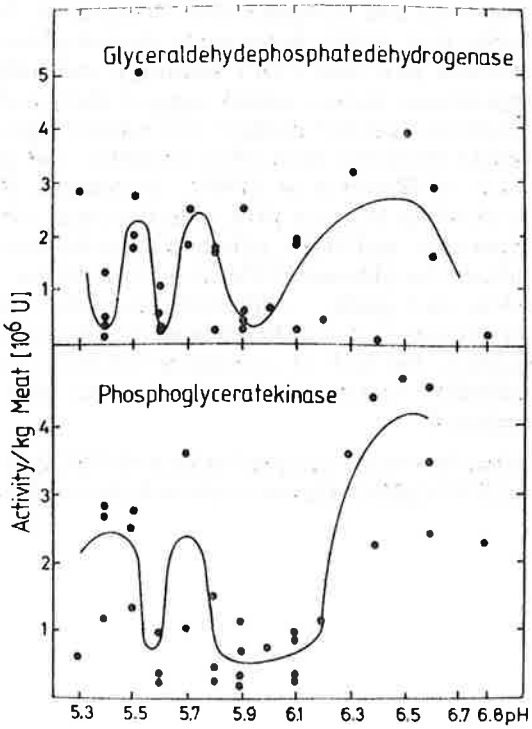

Figure 1: Activities per kg meat of glyceraldehydephosphate-dehydrogenase (GAPDH) and phosphoglyceratekinase (PGK) in dependance of the pH_1-values between 5.3 and 6.8. Enzymes were isolated from M.long.dorsi.

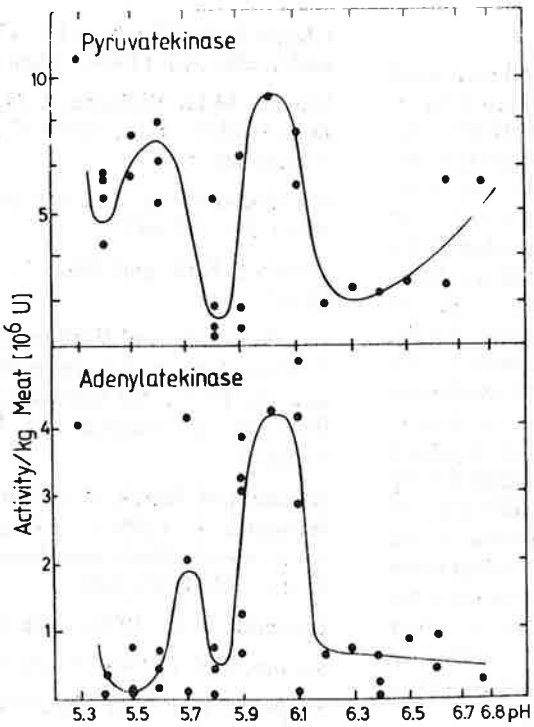

Figure 2: Course of activities per kg meat of pyruvatekinase (PK) and adenylatekinase (AK) according to the pH_1-values. Both enzymes were isolated from M.long. dorsi.

SDS polyacrylamide gelelectrophoresis. Polymerisation-mixtures for the gels contained 10% acrylamide and 0.1% bisacrylamide. Electrophoresis was performed according to Laemmli (1970). The activity per kg meat and the specific activity of the above mentioned enzymes were photometrically determined as described by Scopes (1976) using coupled enzymatic reactions.

RESULTS AND DISCUSSION

Normal and PSE-prone pork muscles contain in the living animal energy-rich compounds like creatinephosphate, ATP and glycogen. These energy-rick compounds are consumed post mortem for maintaining a quasi-living state for some time. Without oxygen supply lactic acid accumulates. The velocity of the lactic acid production depends on the ATP-turnover (Scopes, 1974). In glycogenolysis the most important enzymes with regulatory functions are phosphorylase, phosphofructokinase (PFK) and pyruvatekinase (PK). As the glycogenolysis post mortem in PSE-prone pork muscles is much faster than in normal muscles the question arises, if one set of enzymes is generally more active than the other.

The total and specific activities of phosphorylase a + b from muscles with pH_1-values between 5.5 and 6.1 are five times higher than in the pH-range above 6.1. This is in correspondence with the accelerated break-down of glycogen in PSE-muscles.

The activity spectrum of PFK shows no significant alterations. There are only slight changes in the activities per kg meat and specific activities in dependence of the pH_1-values. Only at very high and low pH_1-values the activities of this enzyme are slightly increasing. PFK plays a key role in controlling glycolysis. PFK catalyses the conversion of fructose-6-phosphate to fructose-1,6-diphosphate under ATP consumption. Clark et al. (1973) reported, that halothane sensitive pigs, which develop the PSE-syndrome show a very high ATP-turnover, because of a futile cycling from fructose-6-phosphate to fructose-1,6-diphosphate and back to fructose-6-phosphate after hydrolysis of the phosphate-group. Because of our results it seems to be so, that the enzymatic activities of PFK do not change from PSE to normal muscles. This again does not exclude an allosteric irregular behaviour of this enzyme in the intact cell, which contributes to the expression of the PSE-syndrome. Such a mistake in regulation must be seen in connection with a change in the amino acid sequence of the enzyme.

The enzymes GAPDH and PGK are very similar in their activities with the exception that GAPDH shows a higher activity in the pH_1-range between 6.2 and 6.0 than PGK. Generally the total activities of both enzymes are higher at pH_1-values above 6.3 than in the range below it (figure 1). GAPDH catalyses the synthesis of 1,3- diphosphoglycerate from glyceraldehyde- 3-phosphate and PGK converts 1, 3-disphosphoglycerate to 3-phosphoglycerate. In the lower pH_1-range there are two maxima at 5.5 and 5.7 respectively and one minima at pH1 6.0 and 5.6 As the two enzymes act successively in the reaction-sequence of the glycolysis the activities of these enzymes must be coordinated.

As expected in the case of PK and AK the course of activities according to the pH_1-values is very similar, which corresponds with the ATP-turnover rates (figure 2). AK phosphorylates AMP to ADP, which is a substrate for phosphoenol-pyruvate to pyruvate. There are several minima and maxima in the activity/kg meat and the specific activity. Between these extremes the differences in the activities are up to five-fold. Both enzymes are more active in pH_1-range below 6.1, than in the range above. This again is in correspondence with an increased energy-turnover in PSE-prone pork muscle and an accumulation of lactic acid.

In summary the following picture appears: In the pH_1-range below 6.1 the enzymatic activities of phosphorylase are high, AK and PK and GAPDH and PGK show very variable activities over the pH range. The activity of PFK is without alterations over the range of measured pH_1-values.

CONCLUSIONS

It is important to mention that the determined increased enzyme activities may be caused by different amounts of enzymes or by alterations in their specific acitivites. As the course of enzyme activity/kg meat and their specific activity in dependance with the pH_1-values are often congruent the total activities are supposed to be regulated by a specific mechanism of modification. This finding is also confirmed by denaturating SDS polyacrylamide gelelectrophoresis, because there are no significant changes in the intensity of enzyme bands, isolated at different pH_1-values. Because of these results we assume, that the development of the PSE-syndrome among other parameters depends on the isoenzyme composition of these enzymes, which are involved directly in the turnover of energy-rich compounds during glycogenolysis. We presume that the modification of isoenzymes is regulated by different mechanisms, which can vary the specific activities of enzymes and adapt them to the cellular conditions. Therefore at the moment we isolate these enzymes to analyse the characterise their isoenzyme patterns. In the near future we want to investigate the different modifications and to learn about the regulation of these modifying mechanisms.

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