ENZYME PATTERN OF PYRUVATE-KINASE FROM PSE-PORK AND MEAT OF NORMAL CHARACTERISTICS

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MMARY: During the development of the porcine-stress-syndrome in post mortem muscles of stress susceptible pigs an erated glycolysis is observed. For this reason pyruvate-kinase (PK) a key enzyme of the energy turnover in the glycolytic way was isolated from M. longissimus dorsi of normal and PSE-prone pigs.
pared to the enzyme from normal muscles, PK from PSE-muscles shows an increased specific activity, a lower K_m value for phosphoenol-pyruvate and a greater K_{cat}/K_m ratio.
ording to the results achieved by isoelectric focusing techniques, PK from PSE-muscles consists of three isoenzymes, eas the normal enzyme exhibits two bands only. The isoenzymes were isolated by preparative isoelectric focusing and pared with regard to their kinetic properties. Isoenzyme 3, which is specific for PSE-meat, shows a tenfold higher specific ity and a thirtyfold lower K_m value for phosphoenol-pyruvate than isoenzyme 1. Analysis of the amino acid composition did reveal differences between the isoenzymes 1 and 3.
phorylation and dephosphorylation experiments carried out with the isolated isoenzymes 1 and 3 from PSE-muscles and mplete PK preparations from normal and PSE-pigs show clearly that the PSE-specific isoenzyme 3 is the phosphorylated of isoenzyme 1. The kinetic properties of isoenzyme 1 are altered by phosphorylation with the consequence of a higher ity at more acidic pH values in the case of PK from PSE-meat.

RODUCTION:

s susceptible pigs experiencing stress shortly before or at slaughter show very fast biochemical changes in their muscles. n one hour the pH in some muscles drops down to values between 5,5 and 5,3, whereas in muscles of normal glycolysis pH within one hour after slaughter decreases only to values of 5,9 and higher. At prevailing high temperatures (>35°C) this -decline leads to denaturation of sarcoplasmic and myofibrillar proteins and to degeneration of membranes with the result PSE-pale, soft and exudative)-meat (BENDALL and WISMER-PEDERSEN, 1962).
PSE-syndrome, which is observed in the muscles of pigs post mortem, and the related malignant hyperthermia (MH) (ELLIS HEFFRON, 1985; ORDING, 1988) developing in the muscles of pigs and man after application of halothane are well-known e many years. Both syndromes show some similar symptoms, especially acidosis and increased body-temperature. An ac- ated glycogenolysis in muscle cells is accompanied by an accumulation of lactic acid and an increased ATP-turnover. There a number of hypothesis, which try to explain this high energy turnover, e.g. uncoupling of ATP formation in mitochondria, s consuming membrane transport systems, muscle contraction by increased Ca^{2+}-efflux (von FABER et al., 1983) and futile consuming ATP in the synthesis and breakdown of metabolites (CLARK et al., 1973). Changes in membranes and myofi- proteins were related to increased lipase activities and irregular activation of proteases (CHEAH et al., 1986). All these hy- ses have not been proved so far and are not conclusive in context. The sequence of biochemical reactions in the cell and nges in the membranes, leading to the porcine-stress-syndrome in the muscles of stress susceptible pigs after slaughter, to the malignant hyperthermia during anaesthesia are still unknown. For this reason we focused our investigations on the idation of the cell metabolic reasons of these muscle disorders, especially on glycolytic enzymes. Here we report about parative studies on PK (EC 2.7.1.40) from normal and PSE-muscles.

TERIALS AND METHODS:

ongissimus dorsi was obtained 45 min post mortem from "halothane-tested" pigs of "German Landrace". At this time the pH- es of halothane-negative animals were higher than 6,2, whereas halothane-positive pigs showed values below 5,5 and ssed the typical properties of PSE-meat.
ces after determination of the pH-value 1 kg of M. longissimus dorsi was excised from each carcass, the connective tissue oved, ground and finally 500 g of the muscle homogenized in 1,5 l buffer containing 10 mM KCl and 30 mM potassium sphate, pH 7,0. PK was isolated according to a method of SCOPES (1977). SDS polyacrylamide gelelectrophoresis (PAGE) performed as described by LAEMMLI (1970), whereas not-denaturating PAGE was performed by an own method.
electric focusing techniques were performed according to HOFMANN and BLÜCHEL (1986) as well as WINTER et al. (1980). er hydrolysis of PK (MOORE and STEIN, 1963) the resulting amino acids were analyzed on a Beckman Multichrom M. N- inal amino acids of the isoenzymes of PK were determined using dansylchloride.

Proteolytic digests of the enzyme were obtained using a method described by KAMP (1986). Chromatographic separation of resulting peptides was achieved on a HPLC equipped with a reversed-phase C-18 column (HASCHKE, 1992).
Enzymatic phosphorylation of the isoenzymes was performed with cAMP-dependent protein kinase according to HJELMQUIST et al. (1974). The degree of phosphorylation of isoenzyme 1 was determined according to a method described by COOPER (1980) with an assay containing γ-^{32}P-ATP. Dephosphorylation of isoenzyme 3 and PK from PSE-muscle was achieved by application of alkaline or acidic phosphatase.

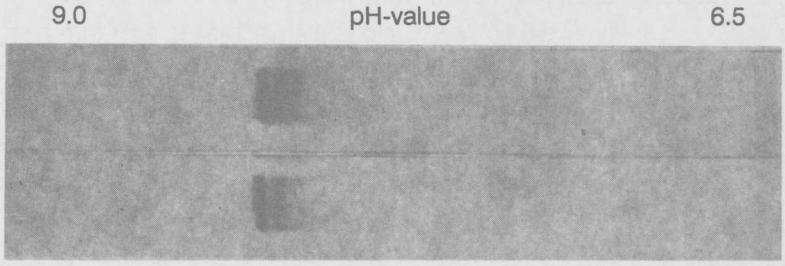
RESULTS AND DISCUSSION:

The investigations were carried out with 10 pigs. As expected, the five selected halothane-positive animals developed within min post mortem the PSE-syndrome in M. longissimus dorsi, whereas the halothane-negative pigs showed the characteristics normal pork.
The total and specific activities of pure PK isolated from PSE-meat were up to 4 times higher than for the enzyme preparation from normal meat, although there were no differences in the amount of protein. The kinetic parameters for the interaction of phosphoenol-pyruvate (PEP) with PK prepared from M. longissimus dorsi of two pigs with different pH_1-values determined 45 min after slaughter are shown in table 1. The K_m of the enzyme from PSE-meat (pH_1 5,3) was about 5 times lower than for PK from normal meat (pH_1 6,6). The effectiveness of an enzyme, which can be expressed by K_{cat}/K_m, is in the case of PK from PSE-meat more than ten times higher. This implies that in the muscle cells the kinetic properties of PK are responsible for the turnover of PEP and not the concentration of the enzyme.

Table 1: Kinetic data of total pyruvate-kinase isolated from PSE- and normal meat

kinetic parameters	PSE-meat	normal meat
K_m [µM]	17	91
V_{max} [µM min^{-1}]	0,67	0,36
K_{cat} [s^{-1}]	1075	445
K_{cat}/K_m [$\mu M^{-1}s^{-1}$]	63	4,9

Figure 1: Analysis of pyruvate-kinase from normal and PSE-muscles by isoelectric focusing techniques.



The two isolated PK species were very different with regard to their activities in dependency upon the pH-value. PK prepared from normal meat is inactive at pH-values between 5,0 and 5,5. The enzyme isolated from PSE-meat shows already two third of its maximal activity at the latter pH-value in the test medium.
The molecular weight of the enzyme, which consists of four identical subunits, was determined by SDS-PAGE using several protein standards. With an extrapolated value of 52000 D for the subunit the molecular weight for the intact quarternary PK was calculated with 208000 D in total. PSE and normal pork showed subunits with identical molecular weights.
The isoenzyme composition of PK isolated from PSE- and normal meat was analysed by isoelectric focusing techniques. As shown in figure 1 the PK preparation from PSE-muscle comprises three isoenzymes. In the case of PK from normal muscle the third isoenzyme band with the lowest isoelectric point appears very weak and represents only 5% of the enzyme, whereas the share of isoenzyme 3 of PK isolated from PSE-muscle is about 20%. Because of these results we concluded that the differences in the kinetic properties of the prepared PK species are caused by different active isoenzymes. Therefore it was necessary to isolate these isoenzymes by preparative isoelectric focusing techniques using horizontal granulated gels.
The kinetic data for the isoenzymes of PK from PSE- and normal meat are summarized in table 2.

2: Kinetic data of the isoenzymes of pyruvate-kinase from PSE- and normal muscle.

ic meters	PSE-meat isoenzyme 1	isoenzyme 2	isoenzyme 3	normal meat isoenzyme 1	isoenzyme 2
[µM]	142	67	5	162	72
x [µM min^{-1}]	0,53	0,35	0,33	0,46	0,34
s^{-1}]	459	798	1505	415	719
/ K_m [μM^{-1} s^{-1}]	3,2	12	300	2,6	10

parison of the PK preparations from PSE- and normal meat the isolated isoenzymes 1 and 2 show very similar properties egard to their kinetic data. Isoenzyme 3, which can only be found in PSE-muscles, has in contrast to isoenzyme 1 a old lower K_m value for phosphoenol-pyruvate and a 100 fold higher effectiveness in the turnover of this substrate. Thus me 3 is responsible for the changes in the kinetic behaviour of PK from PSE-muscle.

pendency of the activities of isoenzyme 1, 2 and 3 upon the pH-value in the test medium with respect to the turnover of phoenol-pyruvate is depicted in figure 2. At pH 5,5 the activities of the isoenzymes 1 and 2 are very low, whereas yme 3 shows already 40% of its maximal turnover rate. The pH-optimum for the latter isoenzyme was determined at pH as it can be seen in figure 2 the maximal activity of the other isoenzymes occur at a higher pH-value.

alysis of the amino acid composition of the isoenzymes 1 and 3, which was performed after hydrolysis of the esponding proteins on a cation exchange resin using the method of post column derivatisation, did not reveal differences. rmore for both isoenzymes serine was determined to be the N-terminal amino acid.

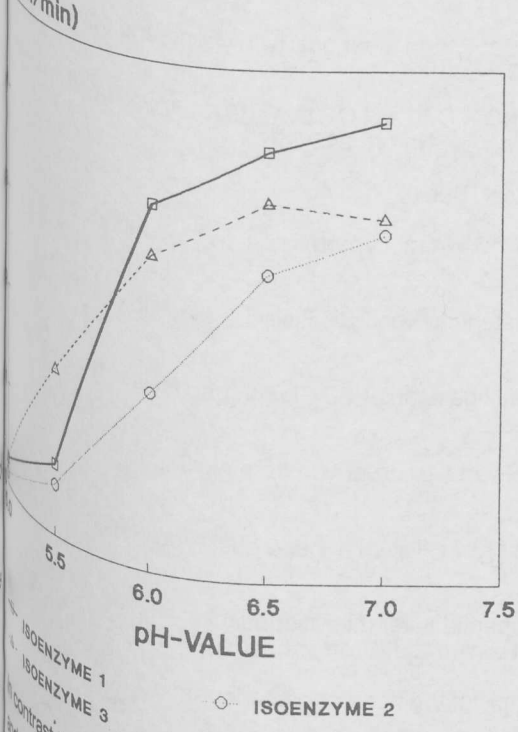


Figure 2: Dependence of the activity of isoenzyme 1,2 and 3 of PK isolated from PSE-muscle upon the pH-value in the test medium

ntrast to these results not-denaturating (native) PAGE showed significant differences in comparison between isoenzyme 1 d 3. The migration of the latter isoenzyme in the electric field towards the anode was faster than in the case of isoenzyme 1. ferent charged proteins can be the reason for this behaviour, which was already observed by means of isoelectric focusing hniques. According to the results in both applied electrophoretic methods isoenzyme 3 should be more negatively charged n isoenzyme 1.

gative charges can be introduced into proteins by phosphate groups. Therefore phosphorylation and dephosphorylation

experiments were carried out with the isolated isoenzymes 1 and 3 of PK from PSE-muscles and the complete PK preparation from normal and PSE-meat. The resulting products of both assays were analysed by means of native PAGE, HPLC, determination of the kinetic data and the stochiometry of incorporated γ-^{32}P-ATP in PK. It could be shown clearly by phosphorylation, which was catalysed by a cAMP-dependent protein kinase from porcine heart that the PSE-specific isoenzyme 3 is the phosphorylated form of isoenzyme 1. Only one subunit of this isoenzyme binds a phosphate group. Dephosphorylation of isoenzyme 3 results in the less active isoenzyme 1. If the phosphorylation experiment is performed with the complete preparation of PK from normal meat, which normally contains two isoenzymes, isoenzyme 3 appears. After dephosphorylation PK isolated from PSE-meat, which consists of three isoenzymes, only two isoenzymes are found by means of isoelectric focusing techniques. Thus the kinetic properties of isoenzyme 1 are altered by phosphorylation with the consequence of a higher activity at more acidic pH-values in the case of PK from PSE-meat.

CONCLUSION:

In dependence of the pH-value in porcine muscle at 45 min post mortem, different isoenzyme pattern of PK were found. PK from PSE-muscle is composed of three isoenzymes, the preparation from normal meat contains two isoenzymes. The specific activity of total PK isolated from PSE-meat is about four times higher than that from normal meat and shows in addition a lower K_m for phosphoenol-pyruvate and a higher K_{cat}/K_m ratio, which is an equivalent for the effectiveness of the turnover of the latter substrate. These differences in the kinetic data for both enzyme preparations are caused by a phosphorylation mechanism, which transforms isoenzyme 1 into isoenzyme 3 which is the monophophorylated product of isoenzyme 1. Isoenzyme 3 exists only in PSE-meat.

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