DOES THE ACTIVITY OF GLYCOGEN DEBRANCHING ENZYME LIMIT THE RATE OF THE GLYCOLYSIS?

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Background

In living muscle glycogen provides local fuel storage for short-term energy consumption. After slaughter glycogen degradation to lactate causes *post mortem* pH to decline in muscles. The rate and the extent of the pH decrease affects several meat quality traits. The complete degradation of glycogen is achieved by two enzymes: glycogen phosphorylase (phosphorylase) and glycogen debranching enzyme (GDE) (Brown and Brown, 1966). Mammalian GDE is a monomeric protein containing two independent catalytic activities: a glycan transferase (EC2.4.1.25) (transferase), and amylo-1,6-glucosidase (EC3.2.1.33) (glucosidase).

Phosphorylase catalyses the sequential phosphorolysis of the outer chains of the glycogen molecule until it reaches the fourth glucose unit from the branch point of the molecule (Walker and Whelan, 1960). Glycogen with four glucose units in every branch is called the limit dextrin state. The outer layer of limit dextrin has symmetric structure and it is converted back to an asymmetric structure by the transferase activity of GDE. This occurs by transferring a maltotriosyl group from the side chain to the main chain. The glucosidase hydrolyses the remaining glucosyl branch, producing free glucose. The debranched dextrin formed has long outer chains which are again susceptible to further degradation by phosphorylase (Brown and Brown, 1966; Nelson, Kolb and Larner, 1969; Nelson and Larner, 1970).

Yurovitzky and Milman (1975) suggested that the rate of glycogenolysis is limited by the activity of GDE. Furthermore, Immonen (2000) showed that *post mortem* glycogenolysis may stop even if there is glycogen left in the muscle and speculated that GDE may play a role in this process. However, there is very little information available about the activity of GDE in meat production animals.

Objectives

The aim of the present study was to investigate the activity of GDE in relation to temperature in porcine light *longissimus dorsi* (LD) muscle and dark *masseter* (M) muscle. If temperature affects the activity of GDE, it may influence the rate of *post mortem* glycogenolysis and glycolysis and thus the ultimate pH of meat. The method for determining the activity of GDE used in the present study measures activities of both the transferase and the glucosidase, not the individual activities of the enzyme.

Materials and methods

Muscle samples (LD and M) from 10 pigs were obtained from a commercial abattoir. The LD sample was dissected from the last rib about 35 min after stunning. The samples were frozen and stored in liquid nitrogen. The analyses were performed within two days after sampling. The activity of GDE was first determined in the muscles of four animals at temperatures 4, 15, 25, 35, 39 and 42 °C and was further determined at higher temperatures (39, 42, 50 and 60 °C) in six additional animals.

The activity of GDE was determined using the method of Nelson, Palmer and Larner (1970) with minor modifications. The method follows the change in the iodine-complex spectrum of glycogen phosphorylase limit dextrin (limit dextrin), a natural substrate for GDE. In the present study the assay solution contained only 0.1 ml 1% limit dextrin and 0.020 ml 0.5 M sodium maleate (A24979, Sigma-Aldrich). The pH of the reaction mixture was adjusted to 6.3 ± 0.05 and then incubated at 4, 15, 25, 35, 39, 42, 50 or 60 °C before the reaction was started by adding 0.08 ml meat extract. The meat extract was prepared daily, using 2.5 ml buffer which contained 0.05% $KHCO_3$ and 0.004 M EDTA (pH approx. 7.8 at 25 °C) per 1 g wet weight muscle. The mixture was homogenised (Ultra-Turrax T25, Janke and Kunkel, Germany) and centrifuged (Sorvall Instruments RC5C) 10 min, 10 °C and 30000 G and the supernatant was used in the measurements.

The reaction was stopped in a boiling-water bath followed by immersion in an ice bath. The reaction times were 1, 1.5 and 2.5 min for LD muscle and 1, 2.5 and 4.0 min for M muscle. Iodine reagent (2.6 ml) was added to the stopped reaction mixture and the absorbance (525 nm) recorded after 20 min. The iodine reagent was prepared according to Nelson et al. (1970). The pH value (6.3) and the reaction times used for determining the temperature-activity profiles of GDE in both muscles were determined in preliminary experiments. Method blanks (zero time controls) were prepared by denaturing the meat extract protein in the boiling-water bath before adding the other reagents.

The conversion of limit dextrin to glycogen was ensured from the absorption spectra between 375 - 800 nm. Samples and the method blank were diluted 5-fold with additional iodine reagent. All the absorbance and absorption spectra measurements were obtained with a Lambda 2 spectrometer (Perkin Elmer, Ueberlinger, Germany).

Phosphorylase limit dextrin was synthesised in our laboratory according to the method of Werries, Franz and Geisemeyer (1990) as it is not available commercially. Commercially available phosphorylase *a* was passed through a column of ω-aminobutyl agarose (Werries et al. 1990). The activity of purified, dried phosphorylase *a* was measured spectrophotometrically according to Bass, Brdiczka, Eyer, Hofer and Pette (1969). Purified phosphorylase a was added to 350 mg glycogen (bovine liver, G0885, Sigma-Aldrich) in 5 ml 0.05 M phosphate buffer (pH 6.8). In the present study the solution also contained 0.1 mM 5'AMP (01930, Fluga). The solution was dialysed at 37 °C against 150 ml of the same buffer. The digest was mixed with trichloroacetic acid (final concentration 10% v/v), dialysed against water and centrifuged for 10 min at 1000 G. The limit dextrin formed was precipitated with 4 volumes of ethanol, centrifuged (5 min, 1000 G), washed twice with ethanol and dried.

Statistical analysis was performed with the Statistical Analysis System version 8.02 (SAS, 1990). The mixed procedure with Bonferroni adjustment was applied when calculating the least squares means of the variables in the temperature-activity profiles of GDE. The curves were fitted using SAS/insight and the curves were plotted with Microsoft Excell 97 SR-2 (XY(scatter), polynomial trendline). The temperature values for maximum enzymatic activity were read from the curves if possible.

Results and discussion

In both muscles the activity of GDE was higher ($P < 0.001$) at the temperatures found in the carcass just after slaughter (39 °C and 42 °C) than at temperatures found during cooling (4 °C and 15 °C) (Figure 1). In M muscle significant ($P < 0.01$) difference was also shown in activity of GDE between temperatures of 39 °C and 25 °C. The temperature-activity profiles indicated that GDE was more active in light LD muscle than in dark M muscle.

In LD muscle the optimum temperature for GDE activity was 39 °C. The activity began rapidly to fall when the temperature decreased to below 35 °C, and the enzyme was practically inactive at temperatures below 15 °C. The other glycogen degrading enzyme, phosphorylase, is also most active at normal body temperatures (Cori, Cori and Green, 1943).

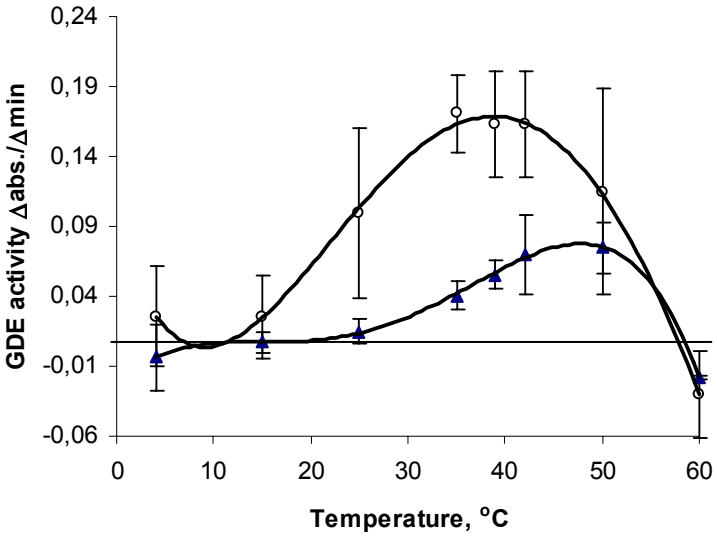

Figure 1. The activity of GDE in porcine *longissimus dorsi* (o) and *masseter* (▲) muscles in relation to temperature.

For *masseter* muscle the optimum temperature for GDE was near 50 °C and the decrease in activity began before the temperature had decreased to below the body temperature (38.5±0.65 °C; Hannon, Bossone and Wade, 1990). The decrease in activity of LD muscle GDE did not begin until the temperature had decreased to below 35 °C. Nelson and Watts (1974) have showed that the temperature optimum for rabbit muscle GDE activity is near 50 °C and that the activity sharply decreases when the temperature decreases to 20 °C which is consistent with the results of the present study. It seems that the activity of GDE does not block rapid glycolysis and pH decrease when the temperature is high since the enzyme remains activity even at temperatures above 45 °C. This may be important in pale soft and exudative (PSE) meat, where the pH decreases rapidly at high temperatures. Rapid cooling could decrease GDE activity and thus the rate of glycolysis, so reducing the formation of PSE meat.

We assume that the decrease in the activity of GDE caused by the temperature decrease also takes place in carcasses after slaughter. During the normal chilling procedure used in Finland, the core temperature of porcine LD muscle decreases to below 35 °C in about 1 h *post mortem*. At that time the pH has decreased to about 6.3 and the glycogen (assumed concentration at the time of slaughter has been normal or high) is still in a state which is susceptible for the degradation of phosphorylase. The temperature decrease of the muscles goes on due to cooling of the carcass and, therefore, when the activity of GDE is needed to continue glycogenolysis and glycolysis, its activity is not maximal. Thus, the decrease in the activity of GDE may delay the rate of glycogenolysis and glycolysis *post mortem*.

In dark M muscle, the decrease in the activity of GDE and in the rate of glycogenolysis was faster than in the light LD muscle. This is supported by the findings that M muscle temperature decreases faster and that even a slight decrease in temperature significantly reduces the activity of GDE. Mélendez-Hevia et al. (1993) have estimated that at most 34.6% of glycogen molecule is directly susceptible to the degradation of phosphorylase. We estimated that this amount is enough to cause pH to decrease from 7.0 to about 6 in dark porcine M muscle *post mortem*. After that the activity of GDE is needed so that glycogenolysis can continue. However, the ultimate pH of M muscle does not usually decrease to below 6, which may be due to the temperature decrease in M muscle during cooling which inhibits the activity of GDE and leads to delayed glycogenolysis and thus to high ultimate pH. It is not known in which state the glycogen molecules are, in other words, how many glucose units are in the outer layer of the glycogen molecule at the moment of slaughter. Also is unclear, are all the glycogen molecules within a muscle in the same state.

Beecher, Briskey and Hoekstra (1965) showed that *post mortem* glycolysis is faster at 37 °C than at 4 °C both in light and dark parts of porcine *semitendinosus* muscle. Their results showed that the glycolytic and glycogenolytic enzymes remained active at low temperatures but were slower. The present study showed that

the activity of GDE was very slow at low temperatures (below 15° C). Beecher et al. (1965) found no significant differences between the ultimate pH values of the light parts of *semitendinosus* muscle held at 37 °C or at 4 °C, while the ultimate pH of the dark part of the *semitendinosus* muscle was significantly lower in muscles held at 37 °C than at 4 °C. This is also consistent with our results where the light LD muscle GDE also showed activity at temperatures below 25 °C but the dark M muscle enzyme did not. Thus, the decrease in the activity of GDE due to temperature decrease may stop the glycolysis earlier in dark M muscle than in light LD muscle.

Conclusions

It can be concluded that GDE is more active in porcine light LD muscle than in dark M muscle. In both muscles, the *post mortem* decrease in temperature strongly reduces the activity of GDE, which may in turn regulate the rate of glycolysis. The eventual impact of activity of GDE on PSE pork and cold shortening in beef warrants further study.

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