OPTIMAL CONDITIONS FOR THE ASSAY OF PORK MUSCLE DIPEPTIDYL PEPTIDASES I AND III.

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BACKGROUND AND OBJECTIVES

Dipeptidyl peptidases (DPP) are proteases that hydrolyze several dipeptide sequences from the NH- termini of peptides and proteins [McDonlad & Barrett, 1986]. Their role in regulation of peptide hormones and protein turnover is a matter of study, but little is known about their action during meat ageing and processing of meat products or how they contribute to the flavor development and final quality. In the present work, pH and temperature requirements of DPP I (3. 4. 14. 1) and DPP III (3. 4. 14. 4) from pork muscle have been studied, with the aim to facilitate its rapid assay in early postmortem meat and study their possible action in meat and meat products according to the processing conditions and meat characteristics.

METHODS

Enzyme assays. 0.5 mM of Gly-Arg-AMC in 50 mM sodium acetate/acetic acid buffer pH 5.5, containing 1 mM of DTT was used for the standard assay of DPP I activity and 0.5 mM of l-Arg-Arg-AMC in 50 mM sodium tetraborate/potassium phosphate buffer pH 8.0, containing 0.05 mM Co^{++} to determine DPP III activity. In the pH study, DPP I activity was determined in the pH range from 4.0 to 8.0 by using 100 mM citric acid/200 mM disodium phosphate as reaction buffer. For DPP III activity (pH range 5.5 – 9.5) 100 mM citric acid/200 mM disodium phosphate buffer (pH 5.5 – 9.5) and 50 mM sodium tetraborate/100 mM monopotassium phosphate buffer (pH 7.5 – 9.5) were employed as reaction buffer. The reaction mixture (300 µL) was incubated in a multiwell plate at 37°C and the generated fluorescence was determined in a multiscan fluorometer (Fluoroscan II, Labsystems™, Finland), using excitation and emission wavelenghts of 335 and 460 nm, respectively. Activity was expressed as percentage of activity at optimum pH. In the temperature assay, the standard reaction mixture was previously equilibrated in Eppendorf tubes. After incubation, the enzyme reaction was stopped by adding 300 µL of 100 mM glycine-NaOH buffer pH 10.5 for DPP I and by 300 µL of 0.25 M acetic acid solution in the case of DPP III assay. Activity was expressed in the same way as the pH assay.

Purification of the enzymes. Samples (16 g) of muscle *Biceps femoris* were homogenized 1/10 in 100 mM citric acid buffer pH 5.0 in a Polytron homogenizer. The homogenate was centrifuged and the supernatant filtered through glass wool. This soluble extract was then fractionated with ammonium sulfate. With DPP I, the precipitated protein was collected between 40-70 % saturation whereas for DPP III the 20-50 % ammonium sulfate cut was collected. The pellet was redissolved in a minimal volumen of 50 mM Tris-HCl, pH 6.0, containing 50 mM (DPP III) or 100 mM (DPP I) NaCl. From this, DPP I was applied to a Sephacryl S-200 gel

The mechanism of inactivation is not fully known but, in the presence of calcium ions, m-calpain is autolysed to at least 2 active forms. The first is converted to the second by internal cleavages which is then converted to the inactive form (Crawford *et al.*, 1987). The first form is reversibly inactivated by EDTA and is therefore measured by its activity on addition of sufficient calcium ion concentration. However, the 2nd form is irreversibly inactivated by EDTA and is not estimated by measuring activity. The rate of inactivation by EDTA, determined here with beef µ-calpain, was the same as that for chicken m-calpain (Crawford *et al.*, 1987). With this sequential autolysis, the effect of EDTA is temporal. In post-mortem muscle therefore, if calpain were activated, more loss would be induced by EDTA with increasing time post-mortem.

Using Western blotting also showed that losses may occur by non-extraction of calpain (Geesink and Goll, 1995) but the losses by non-extraction using routine extraction procedures were not quantified.

CONCLUSIONS

New methods should be sought to determine the active forms of calpain, particularly of µ-calpain, present in muscle and meat.

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stability of DPP I makes it possible to develop its peptidase activity in cooked-meat products, like cooked ham, submitted to high temperatures during processing. DPP III, from the data exposed, could still have a long-time action in processed meat products, but a relevant contribution during meat ageing or in cooked-meat products is not expected. In any case, both enzymes are interesting for their potential use as predictors of meat quality.

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Table 1: Purification of **DPP I** from porcine skeletal muscle.

Purification step	Specific activity (U/mg)	Purification (fold)
Crude extract	0.019	1
Soluble extract	0.09	4.66
40-70 % $(NH_4)_2SO_4$	0.393	20.3
Gel-filtration	2.22	114.9
Stron anion exchange 0-500 mM	11.29	584.2

Table 2: Purification of **DPP III** from porcine skeletal muscle.

Purification step	Specific activity (U/mg)	Purification (fold)
Soluble extract	0.04	1
20-50 % $(NH_4)_2SO_4$	0.77	18.6
Weak anion exchange 50-25 mM NaCl	16.7	404.8
Weak anion exchange 100-200 mM NaCl	96.07	2329.3

Figure 1: Influence of pH on DPP I and DPP III activity. Results are expressed as a percentage of the activity at optimum pH. (▲) : DPP I; (O): DPP III.

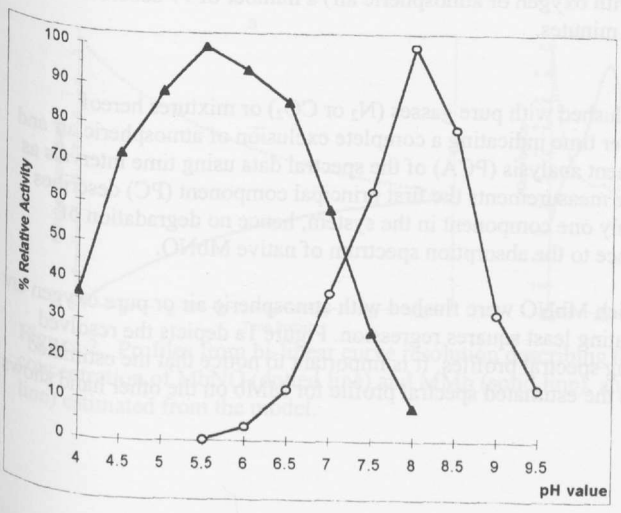

Figure 2: Effect of temperature on DPP I and DPP III activities. Activity was expressed as a percentage of the activity at optimum temperature. (▲) : DPP I; (O): DPP III.