ETICS OF CATHEPSINS FROM DIFFERENT PORCINE MUSCLES

RTIGAS, M.GIL and C.SARRAGA

stitut de Recerca i Tecnologia Agroalimentàries (IRTA). Centre de Tecnologia de la Carn. 17121 Monells. Girona. Spain.

MMARY

he present study, the kinetic parameters of cathepsins B, L and D present in semipurified extracts from skeletal (*Longissimus* (LD) and cardiac porcine muscles are compared, in order to analyze the possibility of using these enzymes as additives in the industry.

epsins from cardiac muscle show higher affinity for their substrates than the enzymes obtained from *Longissimus dorsi* muscle 50% for cathepsins B and L, and 90% for cathepsin D).

TRODUCTION

eral biochemical and structural modifications take place in the myofibrils during the postmortem changes of muscle as a food. e changes have been associated with the activity of certain endogenous proteolytic enzymes, specially calpains and cathepsins itani et al., 1981; Ouali et al. 1984; Ouali, 1990).

enzymes used in the meat industry are from vegetal origin or from microorganisms. The characterization and purification of cle proteinases and their physiological inhibitors could lead to a higher effectiveness and a better control of the results in the strial processes. Their study opens a possibility for a new biotechnological approach to the strategies for optimization and ardization of the quality of meat and meat products in which proteolysis has an important role.

purified cathepsins now available commercially are for laboratory use only, but not for human consumption. This is due to the fication methodologies, which imply risks for health and very high costs.

mparison between the kinetic parameters of cathepsins B, L and D from cardiac and *Longissimus dorsi* (LD) muscles has been e before starting the purification of these enzymes and studying their utility as additives.

TERIAL AND METHODS

cles preparation.

et and LD muscle were dissected from normal quality carcasses selected by measuring the pH at 45 min and 24h post-mortem meter Crison with Ingold 4 o 6 penetration electrode), and the electric conductivity (Quality meater, Digi 550, nenschoftlichtechmishe, Weilheim, Germany).

auricles of the heart were removed to make the dissection easier. Both muscles were trimmed of fat and connective tissue and cut into portions of about 60 g., which were vacuum packed in dark bags and stored frozen until used.

ation of muscles extracts.

steps were performed at 4ºC.

grounded muscle was processed according to the method of Okitani et al. (1980) adapted to our working conditions. Briefly,

the muscle was homogenized in 2 vol. of 15 mM HCl, 3% NaCl; the homogenate was adjusted to pH 3.7 and stirred for 2h at 4ºC. After centrifugation for 15 min at 6500 x g, the pellet was resuspended in 1 vol. of the former solution and the extraction repeated. The two supernatants were fractionated with ammonium sulfate between the 25% and the 65% of the salt. The resulting pellet was dissolved in 10 mM sodium acetate buffer, pH 5.0, containing 0.1 M NaCl and dialysed overnight against the same buffer. The dialysed constitutes the enzymatic source.

Assay of enzyme activities.

Cathepsins B and L were assayed together with the common substrate, N-CBZ-L-phenylalanyl-L-arginine 7-amido-4-methyl-coumarin (Z-Phe-Arg-NHMeC) (Bachem), and cathepsin B with the specific substrate N-CBZ-L-arginyl-L-arginine 7-amido-4-methyl-coumarin (Z-Arg-Arg-NHMeC) (Bachem), according to Etherington and Wardale (1982).

The range of substrate concentration was 1-50 μM for the cardiac muscle, and 1-15 μM for the LD muscle. One unit of activity was defined as the amount of enzyme hydrolising 1nmol of substrate per minute at 37ºC.

Cathepsin D activity was determined against denatured haemoglobin in the range 0.1-5.0% (w/v) for the cardiac muscle, and 0.25-2.75 % (w/v) for the LD muscle, following the method of Etherington et al. (1990). The TCA soluble peptides were quantified by the method of Lowry et al. (1951), using Tyrosine as standard. One unit of activity was defined as the amount of enzyme releasing 1 μg of tyrosine per minute at 45ºC.

Dependence of proteolytic activity with enzyme concentration was studied prior to the assays with different substrate concentrations (results not shown) in order to optimize the results.

RESULTS AND DISCUSSION

The Lineweaver-Burk plots for cathepsin B and cathepsin B+L activities show the differences in K_m and v_{max} between the enzymes from the two muscles studied (Fig.1). Thus, affinity of cathepsin B for its specific substrate in cardiac muscle is 3.4-fold higher than in the LD muscle (K_m LD = 28.60 μM, K_m Cardiac = 8.37 μM). Similar results were observed for cathepsins B and L against their common substrate: cathepsins from cardiac muscle present an apparent K_m value for Z-Phe-Arg-NHMeC 1.7-fold lower than cathepsins from the striated muscle LD (K_m LD = 50.00 μM, K_m Cardiac = 29.85 μM).

Figure 2 shows the Michaelis-Menten plots of cathepsin D activity against haemoglobin as substrate for both muscles. The affinity of the enzyme from cardiac muscle for the substrate is 14-fold higher than the affinity of the enzyme from the LD. It can be also observed that cathepsin D activity decreases at concentrations of haemoglobin higher than 1% (w/v) for both muscles. This fact has been reported previously for the enzyme from porcine *Biceps femoris* muscle (Rico et al., 1991).

According to the results obtained, the substrate concentrations selected for subsequent assays were as follows: 10 μM of Z-Phe-Arg-NHMeC and Z-Arg-Arg-NHMeC for cathepsins B+L and B from both muscles; 0.5% (w/v) haemoglobin for cathepsin D from the LD muscle, and 0.025% (w/v) haemoglobin for cathepsin D from cardiac muscle.

Previous reports of kinetics parameters for purified cathepsins B, L and D show notable variations between organs and muscles of different species (Barret, 1980; Mason, 1985; Draper & Zeece, 1989; Koohmaraie, 1990). Our results indicate that differences between muscles within the same species exist, too. On the other hand, the K_m values obtained in this study are higher that most

Fig.1. Lineweaver–Burk plots for Cathepsins B+L and B activities in (A) LD muscle and (B) Cardiac muscle.

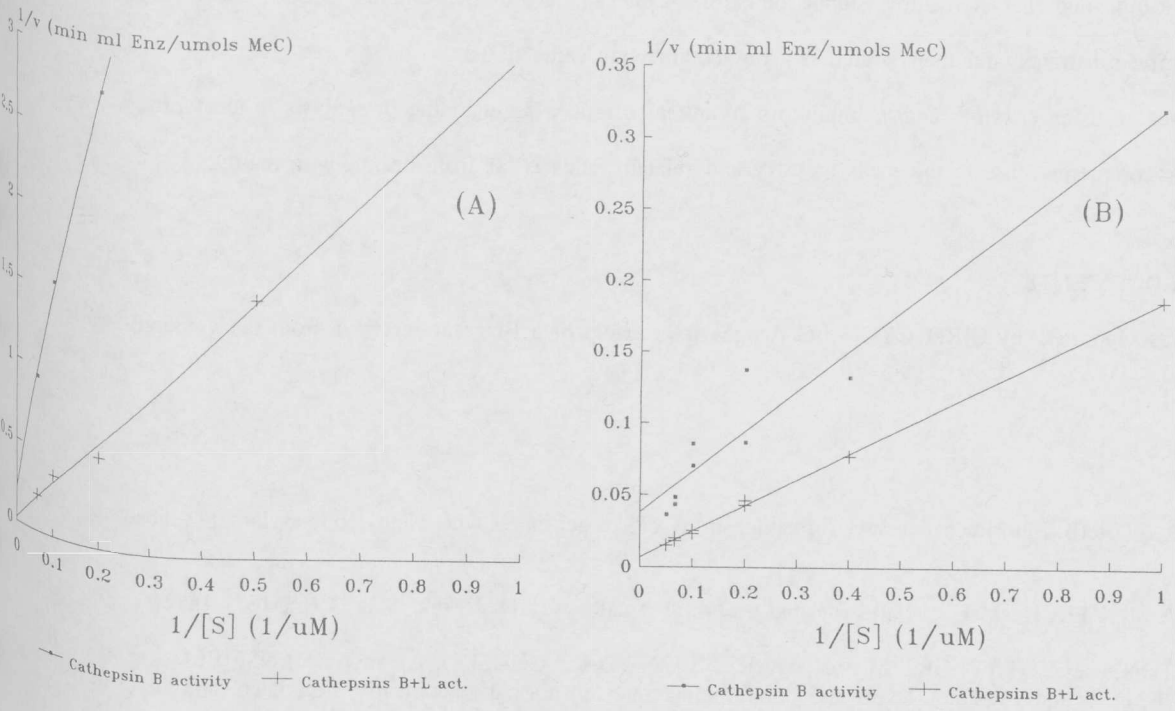
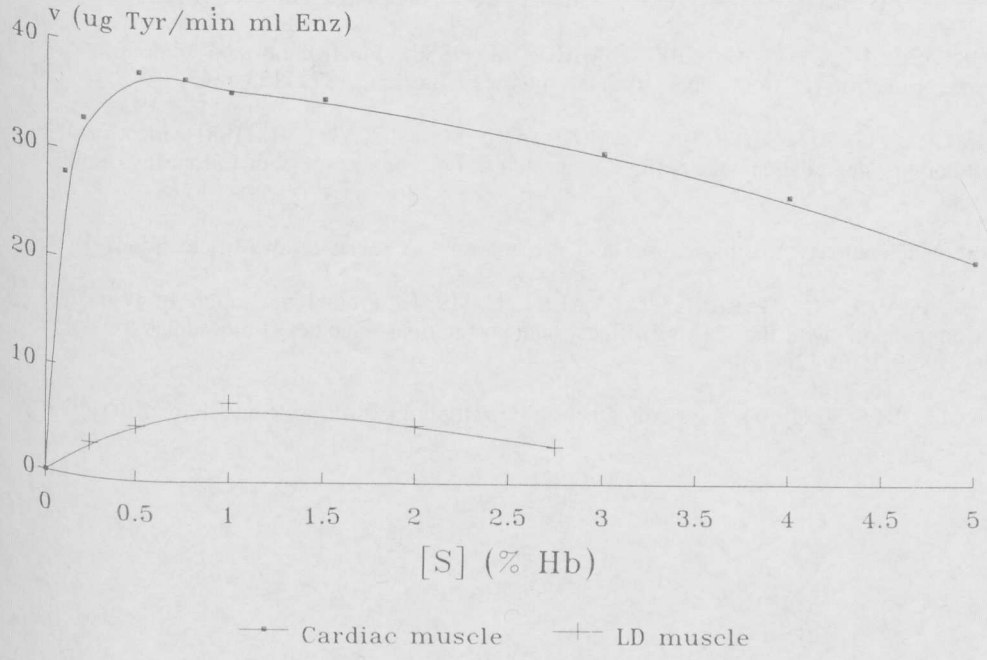


Fig.2. Michaelis–Menten plot for Cathepsin D activity against denatured haemoglobin.



of the values found in the literature, although, it has to be noted that we have not used purified enzymes, but partially purifie extracts.

Further purification and characterization studies on cardiac cathepsins are of great interest because these enzymes show high affinit for their specific substrates and their source is a low commercial value tissue.

The advantages of using enzymes and/or inhibitors of animal origin to regulate the proteolysis in meat processing are quite clear But still their commercial use in the meat industry and related fields is far from becoming a reality.

ACKNOWLEDGEMENTS

This work was supported by CIRIT-CYCIT. G.Artigas is grateful for a FI grant received from the Generalitat de Catalunya.

REFERENCES

BARRET, A.J. (1980). Fluorimetric assays for cathepsin B and cathepsin H with Methylcoumarylamide substrates. Biochem.J. 187 909-912.

DRAPER, A.M.; ZEECE, M.E. (1989). Thermal stability of cathepsin D. J.Food Sci. 54 (6), 1651-1652.

ETHERINGTON, D.J.; TAYLOR, M.A.J.; WAKEFIELD, D.K.; COUSIN, A. and DRANSFIELD, E. (1990). Proteinase (Cathepsins B, D, L and calpains) levels and conditioning rates in normal, electrically stimulated and high-ultimate pH chicken muscle. Meat Science 28, 99-109.

ETHERINGTON, D.J.; WARDALE, R.J.(1982). The mononuclear cell population in rat leg muscle. Its contribution to the lysosomal enzyme activities of whole muscle extracts. J.Cell.Sci. 58, 139-148.

KOOHMARAIE, M.; KRETCHMAR, D.H. (1990). Comparisons of four methods for quantification of lysosomal cysteine proteinase activities. J.Anim.Sci. 68, 2362-2370.

LOWRY, O.H.;ROSENBROUGH, N.J.;FARR, A.L.and RANDALL, R.J. (1951). Protein measurement with Folin-Phenol reagent. J.Biol.Chem. 193, 265.

MASON, R.W.; GREEN, G.D.L.; BARRET, A.J. (1985). Human liver cathepsin L. Biochem.J. 226, 233-241.

OKITANI, A.; MATSUKURA, U.;KATO, H. and FUJIMAKI, M. (1980). Purification and some properties of a myofibrillar protein-degrading protease, cathepsin L, from rabbit skeletal muscle. J.Biochem. 87, 1133-1143.

OKITANI, A.; MATSUMURA, U.; MATSUMOTO, T.; FUJIMAKI, M. and KATO, H. (1981). Identification of the proteinases responsibles for the postmortem degradation of myofibrillar proteins. Proceedings of 27th European Congress of Meat Research Workers. Wien 24-28 Aug.

OUALI, A. (1990). Meat-tenderization: possible causes and mechanisms. A review. J.of Muscle Foods 1, 129-165.

OUALI, A.; OBLED, A.; DEVAL, C.; GARREL, N.; VALIN, C. (1984). Proteolytic action of lysosomal proteinases on the myofibrillar structure. Comparation with the CaANP effects and postmortem changes. Proceedings of the European Meeting of Meat Research Workers. nº30,3(16) 126-128.

RICO, E.; TOLDRA, F.; FLORES, J. (1991). Assay of cathepsin D activity in fresh pork muscle and dry-cured ham. Meat Science 29, 287-293.