BLOCKING AGENTS FOR ELISA QUANTIFICATION OF CATHEPSIN L IN BOVINE AND PORCINE MUSCLE CRUDE EXTRACTS

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Conclusions

Skimmed milk revealed to be the most efficient of the assayed blockers, as also observed by other authors. In the case of pork, this would be the only suitable blocking agent but avoiding the highest assayed muscle crude extract concentration. All the same attention must be paid, since some authors pointed out the possibility that skimmed milk may interfere the antigen-antibody reaction under certain conditions [Vogt et al., 1987]. For ELISA quantification of cathepsin L from bovine species, goat serum and BSA, together with skimmed milk, could be used as effective blockers always that a surcoating steps be included in addition to be present in the assay buffer.

Background

The possibility to accurately quantify levels of endogenous muscle proteolytic enzymes has been proposed as a way to explain and predict meat texture variability. Cathepsin L is one of these target enzymes but quantification based on its endopeptidase activity actually is imprecise due to absence of a specific substrate. Immunochemical methods such as Enzyme Linked Immunosorbent Assay (ELISA) can be an interesting alternative for a more specific, sensitive and faster quantification of this peptidase directly in crude extracts. However, attention must be paid because ELISA can display important sources of error when not working in the appropriate conditions. Development of an ELISA test normally includes a first coating step consisting in the adsorption of antigens or antibodies to a plastic surface by non-specific binding (NSB). However, NSB of other undesired protein components during subsequent steps of the assay can give an overestimation of the signal not corresponding with the desired antigen-antibody reaction, which is detrimental for its sensitivity and specificity. Undesirable NSB may be minimized by saturating the remaining binding sites of the plastic surface with different protein additives that must not interfere with the immunoassay. Not all blocking proteins that are commonly used for that purpose are adequate for each particular ELISA, being necessary to determine the suitable one by empirical testing.

Objectives

The present work had as main objective the study of various blocking agents in their ability to prevent NSB of the reactants, other that capture IgG in the coating step, that will be utilized for the quantification of cathepsin L from both bovine and porcine muscle crude extracts by sandwich ELISA.

Methods

Development of specific IgG against bovine cathepsin L: Polyclonal antibodies against purified bovine cathepsin L were raised in rabbits as described in a previous work [Sentandreu et al., 2004]. The IgG fraction was obtained by chromatography on Q-Sepharose Fast Flow, being a part of this IgG fraction biotinylated.

ELISA protocol:

A) Incubation with the different blocking agents (surcoating, "SC") or with PBS only (no surcoating)

B) Addition of bovine or porcine muscle crude extracts

C) Incubation with biotinylated IgG specific of cathepsin L

D) Revelation with Extravidin®-peroxidase conjugate: Development of a colored reaction

References

Sentandreu, M. A., Aubry, L. & Ouali, A. (2004). A rapid purification procedure for Cathepsin L from bovine species and production of specific antibodies. *J. Sci. Food Agric.* In press.

Vogt, R. F., Jr., Philips, D. L., Henderson, O., Whitfield, W., & Spierto, W. (1987). Quantitative differences among various proteins as blocking agents for ELISA microtiter plates. *J. Immunol. Methods*, 101(1), 43-50.

Results

The experimental protocol design presented here is basically the sandwich ELISA developed for cathepsin L quantification but avoiding the initial coating step of binding the capture antibody. In these conditions, the effectiveness of blocking agents will be proved by their ability to avoid any kind of binding to the plastic surface, which will be reflected in the absence of O. D. at 655 nm due to peroxidase activity. Obtained results are presented in figure 1:

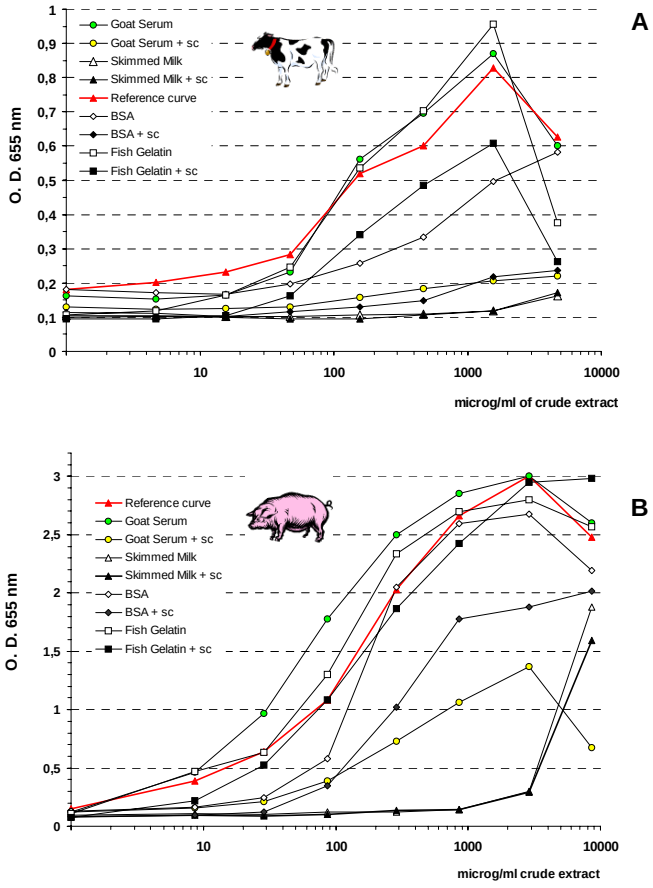

Figure 1: ELISA for cathepsin L using increasing concentrations of A) bovine or B) porcine muscle crude extracts. Legends showing "+ sc" indicate the curves in which a surcoating step has been carried out with the corresponding blocker. Those not showing "+ sc" belong to curves for which the surcoating step was not carried out, the corresponding blocking agent being only contained in the reaction buffer. Experimental points placed on the Y-axis correspond to 0 microg/ml of crude extract.

Achievements obtained in the present work can be summarized as follows:

Blocking agent:	BOVINE	PORCINE
Goat serum	OK	-----
Skimmed milk	OK	OK
BSA	OK	-----
Fish gelatin	-----	-----

: A surcoating step IS REQUIRED to achieve an effective blocking effect

: No need of a surcoating step to achieve an effective blocking effect

Acknowledgements

This work was supported by a Marie Curie Individual Fellowship (Q1-CT-2002-51527) attributed to Sentandreu, M. A..