



Effects of L- and iso-ascorbic acid on meat protein hydrolyzing activity of four commercial plant and three microbial protease preparations



Minh Ha^{a,*}, Alaa El-Din Bekhit^b, Alan Carne^a

^a Department of Biochemistry, University of Otago, PO Box 56, Dunedin, New Zealand

^b Department of Food Science, University of Otago, PO Box 56, Dunedin, New Zealand

ARTICLE INFO

Article history:

Received 10 June 2013

Received in revised form 4 September 2013

Accepted 17 October 2013

Available online 26 October 2013

Keywords:

Meat

Proteases

Ascorbic acid

Plant

Bacterial

Fungal

ABSTRACT

The present study investigated the effects of both L- and iso-ascorbic acid (AA) on the activity of four plant proteases (papain, bromelain, actinidin and zingibain) and three microbial proteases (Bacterial Protease G, Fungal 31,000 and Fungal 60,000) preparations using fluorescent-labelled casein, meat myofibrillar and connective tissue extracts to explore their effects on meat structure components upon treatment with individual proteases. While L-AA in the range 0.8–3.2 mM inhibited the activity of papain, bromelain and zingibain, iso-AA acted as an inhibitor of papain but as an activator of zingibain and had no significant effect on bromelain. Both AA isoforms acted as an activator of the actinidin protease and the concentration of AA isoforms appeared to affect the level of activation of the protease. The effect of the two AA isoforms on collagen and myofibrillar protein hydrolyzing activity varied depending on the concentration of the two AA isoforms. The results indicate the ability to up and down regulate the activity of the investigated proteases by using an appropriate concentration of the AA isoform.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

The production of meat with consistent tenderness is an important goal for the meat industry due to tenderness being an important sensory and marketing property. In addition to the use of various physical and chemical interventions to produce tender meat, the addition of exogenous proteases has been demonstrated to have potential application in meat tenderization to add value to tougher meat cuts and improve the palatability of meat (Ashie, Sorensen, & Nielsen, 2002; Lewis & Luh, 1988).

Several proteases have been evaluated for this purpose, including proteases which belong to the plant cysteine protease class, namely papain, bromelain, actinidin and zingibain. Meat block injection or marination with some of these proteases has been shown to result in a decrease in shear force, suggesting improved meat tenderness (Ashie et al., 2002; Lewis & Luh, 1988). However, treatment with proteases such as papain and bromelain, which have a broad specificity for protein hydrolysis, resulted in over-tenderized meat and an undesirable mushy texture. Proteases from kiwifruit, ginger, bacterial and fungal sources have been shown to exhibit more specifically targeted protein hydrolysis, and a narrower range of conditions for their hydrolytic activities. Our previous studies on commercial preparations of the four plant proteases mentioned above (Ha, Bekhit, Carne, & Hopkins, 2012) and three

commercially available microbial protease preparations which have been granted GRAS status (Ha, Bekhit, Carne, & Hopkins, 2013) were focused towards evaluating comparatively the capability of these proteases to hydrolyse meat myofibrillar and connective tissues, whose hydrolysis is primarily responsible for meat tenderness. In these studies, we demonstrated that the newly available commercial actinidin and zingibain protease preparations are promising candidates for targeting specific tenderizing applications as opposed to papain and bromelain which exhibited a broad specificity towards meat myofibrillar and connective tissue proteins. Furthermore, we found that the microbial proteases are promising meat tenderizers with lower activation temperature and more specific hydrolyzing specificities towards meat proteins when compared with papain. Regulating the activities of meat tenderizing enzymes applied to different meat cuts with varying intrinsic protein composition is important to ensure a desired meat tenderness level is achieved without creating mushy meat.

Apart from varying the amount of proteases in meat tenderizer formulations, the addition of food-grade compounds, such as ascorbic acid (AA), has been proposed to control the activity of meat tenderizing proteases such as papain (Ockerman, Harnsawes, & Yetim, 1993). AA is commonly used as an antioxidant to improve the activity of cysteine proteases by maintaining the active site of the protease in reduced form. For example, the proteolytic activity of zingibain was stabilized during storage and the half-life of the enzyme at 60 °C was increased about 10-fold in the presence of 0.2% ascorbate (Adulyatham & Owusu Apenten, 2005; Bhaskar,

* Corresponding author. Tel.: +64 3 479 7849.

E-mail address: minh.ha@otago.ac.nz (M. Ha).

Sachindra, Modi, Sakhare, & Mahendrakar, 2006), suggesting that the addition of AA to meat tenderizers containing proteases is therefore of interest in development of such formulations. On the other hand, studies evaluated the effect of L-AA on the hydrolytic activity of ficin (Fukal, Kas, Sova, & Rauch, 1986) or papain (Kanazawa, Fujimoto, & Ohara, 1993) using synthetic substrates reported inhibition of these proteases which was dependent on the concentration of AA. The reported results indicate potential differential effects on plant proteases which appear to be dependent on AA concentration and the protease investigated. Such information on bacterial and fungal proteases is not available in the public domain. The investigation of the effects of AA on the protein degradation of meat proteins has not been reported. This type of investigation could pinpoint the actions of the AA, as well as generate new information that could potentially lead to better application of AA to regulate the activity of proteases.

In this study, the addition of both L- and iso-AA was investigated to evaluate comparatively their effects on the activity of four plant proteases and three microbial proteases with a focus on hydrolysis of meat myofibrillar and connective tissue extracts to explore their effects on specific meat structural proteins upon treatment with proteases.

2. Materials and methods

All chemicals used were of analytical reagent grade unless otherwise stated. Protease preparations were kind gifts from international suppliers. Papain 25,000 mg (a papaya latex powder preparation), bromelain 110 (a pineapple fruit powder preparation), Bacterial Protease G, Fungal 31,000 (31 K) and Fungal 60,000 (60 K) were from Enzyme Solutions Pty. Ltd. (Victoria, Australia). Actinidin KFPE 2 Fold (a kiwifruit powder preparation) was from Ingredient Resources Pty. Ltd. (New South Wales, Australia). Zingibain protease (a ginger rhizome solution preparation) was from Biohawk Foundation (Queensland, Australia).

2.1. Total protein determination

The total protein contents of the commercial protease preparations were determined using a 2D Quant Kit (GE Healthcare, #80-6484-51, Auckland, New Zealand) according to the manufacturer's instructions.

2.2. Caseinolytic activity assay

The ability of the proteases to hydrolyse fluorescent-labelled casein was investigated using the assay of Thompson, Saldana, Cong, and Goll (2000) with modifications. The substrate used was a green fluorescent-labelled (BODIPY-FL) casein (Molecular Probes, #E6638) and the measurements were performed using a fluorescence microtiter plate reader (POLARstar OPTIMA, BMG labtech). The substrate was dissolved in K_2HPO_4 buffer (140 mM, pH 6.0) containing L-AA or iso-AA (0.8, 1.6, 2.5 and 3.2 mM) and assays were conducted at 45 °C.

The total amount of protein of each of the commercial plant protease preparations added to the assays was 0.02, 0.01, 0.5 and 0.2 µg for the papain, bromelain, actinidin and zingibain protease preparations, respectively. The total amount of protein of each of the commercial microbial protease preparations added to the assays was 2.3, 4.3 and 1.4 µg for the protease G, fungal 31 K and fungal 60 K protease preparations, respectively.

2.3. Connective tissue protein extraction from bovine Achilles tendon

Extraction of connective tissue proteins was performed according to the procedure of Ha et al. (2012). This extract was found on

analysis to contain mainly collagen and it is referred to as "Achilles tendon collagen protein extract". Bovine Achilles tendon obtained from cull dairy cattle >6 years old was used.

2.4. Achilles tendon collagen protein extract hydrolysis assays

Achilles tendon collagen protein extract was diluted with Milli-Q water (1:1). Assays were performed as described by Ha et al. (2012). Aliquots (200 µl) of the stock connective tissue extract (pH 6.0) containing either L-AA or iso-AA (0.8, 1.6, 2.5 and 3.2 mM, respectively) were adjusted to pH 6.0 using 4 M NaOH and equilibrated to the assay temperature (45 °C) in a Hybaid mini-oven (Hybaid Ltd., Teddington, UK). Commercial protease solution (40 µl) was added to the tubes which were then tumbled in the oven. Hydrolysis was allowed to occur for 5 min with the papain, bromelain, zingibain, protease G, 31 and 60 K preparations, and for 30 min with the actinidin preparation. Samples of the hydrolysis assay were obtained by transferring aliquots (4 µl) from the tumbled assay tube to 0.6 ml tubes containing Invitrogen SDS sample buffer (3 µl) (#NP0007), Invitrogen reducing agent (1 µl) (#NP0004) and Milli-Q water (6 µl). The samples were then mixed and immediately placed on dry ice and frozen. The samples were stored at –20 °C until analysis by SDS–PAGE. The total protein amount of the protease preparations added to the assays was 0.2, 0.02, 2.0 and 17.9 µg for the papain, bromelain, actinidin and zingibain protease preparations, respectively. The total protein amount of the microbial protease preparations added to the assays was 0.9, 0.3 and 0.6 µg for the protease G, fungal 31 K and fungal 60 K protease preparations, respectively.

2.5. Bovine *M. semimembranosus* myofibrillar protein extract preparation

The myofibrillar protein extraction procedure used was that described by Ha et al. (2012). Bovine *M. semimembranosus* muscle tissue obtained from cull dairy cattle >6 years old was used.

2.6. Bovine *M. semimembranosus* myofibrillar protein extract hydrolysis assays

Assays were performed as described in Ha et al. (2012) with minor modifications. Aliquots (200 µl) of the above stock meat myofibrillar extract (5 mg ml^{–1}) containing either L-AA or iso-AA (0.8, 1.6, 2.5 and 3.2 mM, respectively) were adjusted to pH 6.0 using 4 M NaOH and equilibrated to the assay temperature of 45 °C for 5 min in a Hybaid mini-oven, followed by the addition of an aliquot of a commercial protease solution (40 µl). The tubes were then tumbled in the oven. Hydrolysis was allowed to occur for 5 min with the papain, bromelain, zingibain, protease G and 31 K preparations, and for 2 h with the actinidin and 60 K preparations. Time course samples of the hydrolysis assay were obtained by transferring aliquots (10 µl) from the tumbled assay tube to 0.6 ml tubes containing Invitrogen SDS sample buffer (3 µl) (#NP0007) and Invitrogen reducing agent (1 µl) (#NP0004). The samples were then mixed and immediately placed on dry ice and frozen. The samples were stored at –20 °C until analysed by SDS–PAGE using Invitrogen (Auckland, New Zealand) gradient (4–12%) Bis-Tris gels (#NP0322BOX). Electrophoresis was performed as described by Ha et al. (2012). The total protein amount added of the protease preparations to the assays was adjusted in order to display a gradual hydrolysis of meat myofibrillar proteins on SDS gels. The total protein added was 3.7, 1.6, 4.0 and 17.9 µg for the papain, bromelain, actinidin and zingibain protease preparations, respectively. The total protein added was 9.2, 17.2 and 5.6 µg for the protease G, fungal 31 K and fungal 60 K protease preparations, respectively.

Download English Version:

<https://daneshyari.com/en/article/7598793>

Download Persian Version:

<https://daneshyari.com/article/7598793>

[Daneshyari.com](https://daneshyari.com)