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Research paper

A novel approach for reliable detection of cathepsin S activities in mouse antigen presenting cells

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ABSTRACT

Cathepsin S (CTSS) is a eukaryotic protease mostly expressed in professional antigen presenting cells (APCs). Since CTSS activity regulation plays a role in the pathogenesis of various autoimmune diseases like multiple sclerosis, atherosclerosis, Sjögren's syndrome and psoriasis as well as in cancer progression, there is an ongoing interest in the reliable detection of cathepsin S activity. Various applications have been invented for specific detection of this enzyme. However, most of them have only been shown to be suitable for human samples, do not deliver quantitative results or the experimental procedure requires technical equipment that is not commonly available in a standard laboratory. We have tested a fluorogen substrate, Mca-GRWPPMGLPWE-Lys(Dnp)-DArg-NH_2, that has been described to specifically detect CTSS activities in human APCs for its potential use for mouse samples. We have modified the protocol and thereby offer a cheap, easy, reproducible and quick activity assay to detect CTSS activities in mouse APCs. Since most of basic research on CTSS is performed in mice, this method closes a gap and offers a possibility for reliable and quantitative CTSS activity detection that can be performed in almost every laboratory.

1. Introduction

Cathepsin S (CTSS) belongs to a group of eleven currently known human cysteine cathepsins (Turk et al., 2012). CTSS is a lysosomal and endosomal cysteine protease expressed in professional antigen presenting cells (APCs) such as macrophages, B cells and dendritic cells (Driessen et al., 1999). Though, also non-professional antigen presenting cells such as intestinal epithelial cells have been shown to express CTSS (Beers et al., 2005). Its main physiological role is the control of the maturation of MHC class II molecules by generating the class II-associated invariant chain peptide (CLIP) fragment through invariant chain (Ii) proteolysis. This proteolysis is a required step for the loading of antigenic peptide onto the MHC class II heterodimer and the subsequent transport to the cell surface where it is involved in T cell activation (Conus and Simon, 2010). This makes CTSS the main regulator for MHC class II surface expression in professional and non-professional APCs (Driessen et al., 1999). Therefore, CTSS is assigned to play a crucial role for the activation of MHC class II mediated immune responses. Hence, regulation of this protease seems to be of high importance for avoiding uncontrolled $CD4^+$ T cell activation. Indeed, enhanced CTSS protein expression, mRNA expression, protein secretion and activity dysregulation have an implication for the induction or progress of a variety of autoimmune diseases. CTSS dysregulation-associated autoimmune diseases are, i.e. atherosclerosis (Figueiredo et al., 2015), multiple sclerosis (Haves-Zburof et al., 2011; Fissolo et al., 2008), psoriasis (Schonefuss et al., 2010) and the Sjögren's syndrome (Hamm-Alvarez et al., 2014). Additionally, enhanced CTSS secretion into the intestinal lumen is reported to promote pain induction during inflammatory bowel disease (Cattaruzza et al., 2011). Furthermore, CTSS dysregulation is also involved in cancer progression (Sobotic et al., 2015). This involvement of CTSS in the pathogenesis of various diseases makes this protease an intensely studied drug target (Figueiredo et al., 2015; Kohl et al., 2015; Vazquez et al., 2015; Jadhav et al., 2014). In order to evaluate the precise role of CTSS function in this context, not only protein concentrations of CTSS should be detected but rather its catalytic activity as protein amount and enzymatic activity do not necessarily need to correlate. Cathepsins in general provide a broad and overall similar substrate specificity which makes it difficult to distinguish them in experimental settings (Choe et al., 2006).

Different methods have been used to detect specific CTSS activities in vitro or in vivo, all of them providing different advantages and

Abbreviations: APCs, antigen presenting cells; BMDCs, bone marrow derived dendritic cells; CTSB, cathepsin B; CTSD, cathepsin D; CTSL, cathepsin L; CTSS, cathepsin S; rh, recombinant human; PMGLP, Mca-GRWPPMGLPWE-Lys(Dnp)-DArg-NH_2; rm, recombinant mouse; RT, room temperature.

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disadvantages. Most of them focus on the detection of human CTSS. Here we present a method for in vitro detection of also mouse CTSS activities based on a fluorogen substrate that has previously been described to be exclusively cleaved by human CTSS (Lutzner and Kalbacher, 2008). This method is cheap, specific, reliable and easy to perform in a standard laboratory without the requirement of special technical equipment, besides the requisition of a fluorescence compatible photometer. We think this method could contribute to a progress in CTSS-related research.

2. Results

2.1. PMGLP is well recognized by recombinant mouse CTSS (rmCTSS)

The CTSS proteolysis activity detecting substrate Mca-GRWPPMGLPWE-Lys(Dnp)-DArg-NH_2 (PMGLP) was primarily designed for the detection of human CTSS activities (Lutzner and Kalbacher, 2008). Our aim was to determine whether PMGLP is also suitable for the detection of mouse CTSS. In a first step, we therefore tested several recombinant mouse cathepsins for their cleavage behavior towards PMGLP and compared them to recombinant human (rh) CTSS (Fig. 1). To assess the general enzymatic activity of the commercially purchased recombinant mouse (rm) cathepsins, we first looked for possible protein digestion in a Coomassie-stained 12% SDS gel (Fig. 1A, right panel). Since we could not detect any possible proteolysis-caused loss of function of rhCTSS, rmCTSS, rm cathepsin B (rmCTSB) and rm cathepsin D (rmCTSD), we next checked for their ability to cleave the substrate Z-FR-AMC. This substrate (Z-FR-AMC) is published as being recognized and cleaved by CTSS (Lutzner and Kalbacher, 2008), CTSB (Barrett, 1980) and CTSL (Malagon et al., 2010) and was used as a positive control to evaluate the proteolytic function and protein integrity of these cathepsins. However, rmCTSD integrity could not be checked by using Z-FR-AMC since this enzyme is not capable of cleavage this substrate. Both, PMGLP and Z-FR-AMC display fluorescent properties upon enzymatically catalyzed hydrolytic cleavage. The increase in detected fluorescence intensity change over time is therefore equivalent to the amount of proteolytically processed substrate. This enzymatically catalyzed substrate conversion into a fluorescent substance was monitored by measuring the fluorescence intensity change over time, therefore delivering substrate conversion curves (Fig. 1A, left panels). Fluorescence intensities are measured in dimensionless “arbitrary units” (AU). Absolute enzymatic activities were then calculated by performing a linear regression at the beginning of the substrate turnover curve (Fig. 1B). The slope magnitude of this linear regression delivers a value for the activity of the tested enzyme that can be expressed as the difference of fluorescence intensity in

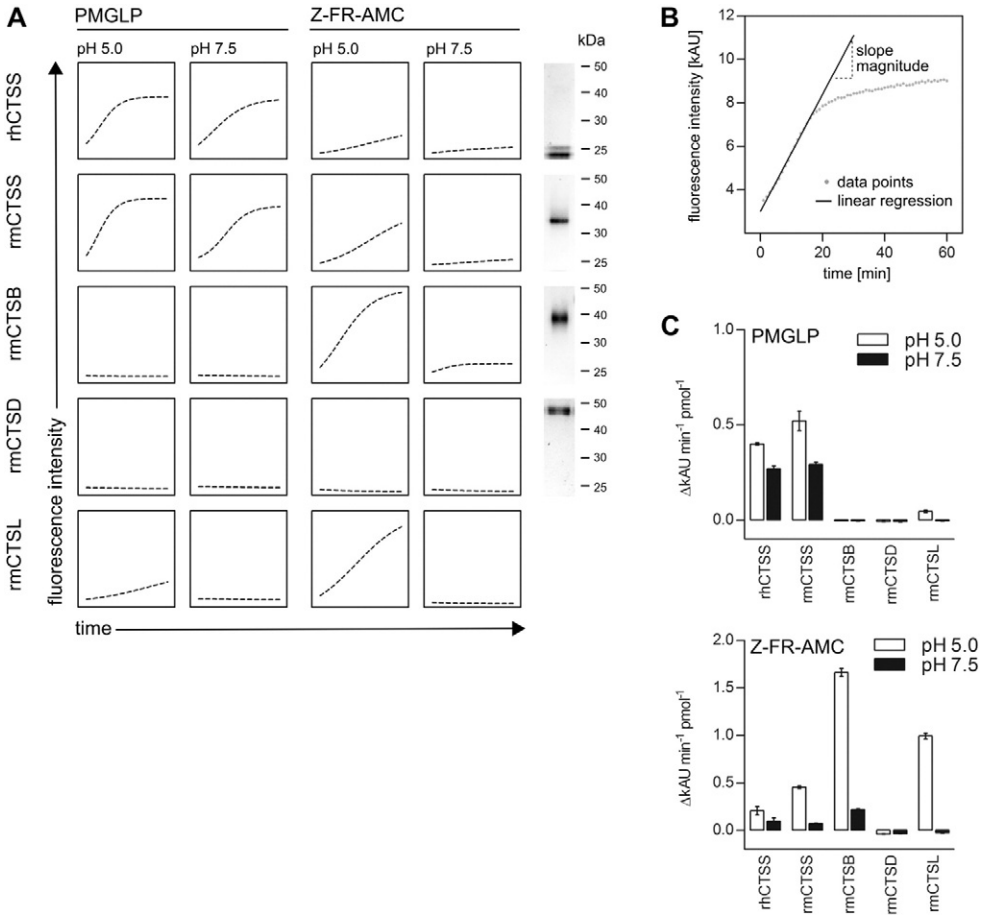

Fig. 1. Recombinant mouse CTSS efficiently cleaves PMGLP. Various recombinant mouse (rm) and human (rh) cathepsins (CTS) were tested for their cleavage behavior towards human cathepsin S (rhCTSS) substrate PMGLP. Z-FR-AMC was used as an activity control since it is efficiently cleaved by cathepsin B (CTSB), cathepsin S (CTSS) and cathepsin L (CTSL) but not cathepsin D (CTSD). Equimolar amounts of recombinant cathepsins were preincubated in either a phosphate buffer at pH 7.5 or a sodium citrate buffer at pH 5.0. After addition of the fluorogen substrates PMGLP or Z-FR-AMC, fluorescence increase due to substrate conversion was monitored for 60 min at 37 °C. (A) Monitoring of substrate conversion. Fluorescence increase was recorded for 60 min. Fluorescence intensities were adjusted to the conversion curve with the highest fluorescence increase. Illustrated conversion curves are averaged curves of three independent experiments. On the right, one representative Coomassie staining of each tested recombinant cathepsin that was separated on a 12% SDS gel. (B) Illustration of the way how enzymatic activities are calculated. Gray dots represent data points of the substrate conversion that results in an increase of fluorescence intensity. The solid line indicates the linear regression at the beginning of the enzymatic reaction. The broken line represents the slope magnitude of the linear regression. For details on how the linearization was performed, see the Materials and methods section. (C) Substrate conversion rates in $\Delta AU\ min^{-1}$ (pmol enzyme)$^{-1}$ were computed from the substrate conversion curves in (A) as described in (B) (see text for further information) (n = 3), error bars represent standard deviation (SD).