JOURNAL OF PROTEOMICS 75 (2012) 3925–3937

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/jprot

The identification of a new role for LEKTI in the skin: The use of protein 'bait' arrays to detect defective trafficking of dermcidin in the skin of patients with Netherton syndrome

Kate Bennett[a], *Wendy Heywood*[a], *Wei-Li Di*[a], *John Harper*[a], *Gary L. Clayman*[b], *Arumugam Jayakumar*[b], *Robin Callard*[a], *Kevin Mills*[a,*]

[a]*Institute of Child Health & Great Ormond Street Hospital for Sick Children, University College London, 30 Guilford Street, London, WC1N 1EH, United Kingdom*

[b]*Department of Head and Neck Surgery, UTMD Anderson Cancer centre, 1515 Holcombe Blvd, Houston, TX, 77030, USA*

ARTICLE INFO

Article history:
Received 27 February 2012
Accepted 29 April 2012
Available online 12 May 2012

Keywords:
LEKTI
Kallikreins
Dermcidin
Cathepsin G
Mass spectrometry
QTOF MS

ABSTRACT

Lympho-Epithelial Kazal-Type-related Inhibitor (LEKTI) has been demonstrated to be an inhibitor of various kallikreins and is thought to play a role in the regulation of skin desquamation. In order to identify and investigate the potential of LEKTI to interact with other proteins, a method was developed using immobilised proteins onto arrays and nanoUPLC/MALDI-TOF MS. Using various domains of LEKTI, we demonstrated that these domains bound a number of kallikreins (5, 13 and 14) to varied extents on the array surface. Inhibitory assays confirmed that binding on the protein array surface corresponded directly to levels of inhibition. The method was then tested using skin epidermal extracts. All forms of rLEKTI with the exception of rLEKTI 12–15, demonstrated the binding of several potential candidate proteins. Surprisingly, the major binding partners of LEKTI were found to be the antimicrobial peptide dermcidin and the serine protease cathepsin G and no kallikreins. Using confocal microscopy and Netherton syndrome skin sections, we confirmed the co-localisation of LEKTI with dermcidin and demonstrated altered trafficking of dermcidin in these patients. This potential new role for LEKTI as a multifunctional protein in the protection and transport of proteins in the epidermis and its role in disease are discussed.

Crown Copyright © 2012 Published by Elsevier B.V. All rights reserved.

1. Introduction

LEKTI is a Kazal-type protease inhibitor expressed in the most differentiated layers of stratified epithelial tissues including the skin, where expression has been localised to the granular layer and stratum corneum [1,2]. LEKTI is synthesised in its full-length form (15 domains) and cleaved rapidly into a range of single or multidomain fragments that are secreted from the cell [1–4]. Recessive mutations in *SPINK5*, the gene that encodes LEKTI, have been identified as the cause of Netherton syndrome [5], a chronic skin disease characterised by accelerated stratum corneum shedding and loss of skin barrier function [6–8]. LEKTI has a recognised role as a serine protease inhibitor through its inhibitory activity against a range of serine proteases in vitro, including members of the kallikrein (KLK) family [4,9–12]. In Netherton syndrome, where LEKTI expression is either absent or reduced, KLK5- and KLK7-like activities are increased, which leads to premature degradation of corneodesmosomes, over-desquamation and stratum corneum thinning [2,13,14]. In our previous study, we showed that LEKTI was able to inhibit the cysteine protease caspase 14 in vitro [15], which indicated that LEKTI is a multifunctional protease inhibitor. Caspase 14 has

* *Corresponding author*. Tel.: +44 207 905 2873.
E-mail address: k.mills@ich.ucl.ac.uk (K. Mills).

1874-3919/$ – see front matter. Crown Copyright © 2012 Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.jprot.2012.04.045

been implicated in both epidermal differentiation and hydration through processing of the barrier-related protein, filaggrin [16,17]. This was the first study to report the inhibitory activity of LEKTI against a cysteine protease, which presents the possibility that LEKTI targets multiple protease families in the skin and suggests an involvement of LEKTI in novel biological pathways other than those associated with KLKs. In order to identify further potential proteases or targets for LEKTI, an immobilised protein array technique was developed. This technique uses protein arrays and immobilisation of LEKTI domains as 'baits'. Binding conditions on the array were optimised and confirmed using matrix-assisted laser desorption/ionisation quadrupole time-of-flight mass spectrometry (MALDI TOF MS) analysis of protein standards. Identification of unknown binding proteins in the skin was achieved by scaling up the procedure, extraction off the array surface, proteolytic digestion and Q-TOF MS sequencing. The method developed in this study has shown that the levels of KLKs binding to rLEKTI on the array were in an amount that corresponded to the levels of inhibitory activity against the KLKs. This technique was then used in vivo to probe potential binding partners of LEKTI in the skin using epidermal extracts and the two proteins dermcidin and cathepsin G were identified as binding partners of LEKTI. Confirmation of this discovery was substantiated by immunohistochemical staining of control and Netherton syndrome skin sections. This work describes the development of the technique, the identification of targets of LEKTI and a potential new function for LEKTI in a protection/transport role of antimicrobial peptides to the skin surface.

2. Materials and methods

2.1. Materials

All standard reagents were of analar grade or equivalent and obtained from Sigma-Aldrich Company (Gillingham, Dorset, UK). RS100 arrays were obtained from Bio-Rad Laboratories (Hemel Hempstead, UK). Trypsin was of sequencing grade and was obtained from Promega UK Ltd. (Southampton, Hants, UK). The fluorogenic peptide substrate (Boc-V-P-R-AMC) was obtained from R&D Systems Europe Ltd (Abingdon, UK). Endopeptidase Lys-C was obtained from Sigma-Aldrich Company (Gillingham, Dorset, UK).

2.2. Proteins

The rLEKTI forms containing intact domains 1–6 (rLEKTI 1–6), domains 6–8 and partial domain 9 (rLEKTI 6–9′), domains 9–12 (rLEKTI 9–12), domains 12–15 (rLEKTI 12–15) and domains 1–15 (full-length rLEKTI) were created by Dr. A. Jayakumar, University of Texas, M.D. Anderson Cancer Centre. Each protein was cloned and expressed in Sf9 cells using the baculovirus expression vector system as reported previously [9,18]. Recombinant human KLK standard proteins 1, 5, 13 and 14 were obtained from R&D Systems Europe Ltd (Abingdon, UK).

2.3. Patients and skin biopsies

Skin biopsies were obtained from three patients with a clinical diagnosis of Netherton syndrome and from eight children with normal skin who attended the plastic surgery unit at Great Ormond Street Hospital (London, UK). Local ethics committee approval and consent from the parents were obtained prior to the procedure. Skin samples were either snap frozen or paraffin embedded and stored at −80 °C.

2.4. Protein extraction from skin

Control skin was obtained from age- and anatomy-matched biopsies from patients admitted to the hospital for routine surgery. The dermis was removed from all skin biopsies and the remaining epidermis washed with 3×1 ml of ice-cold phosphate buffered saline solution (pH 7.4) to remove all contaminating blood-derived proteins. The epidermis was sectioned into 10 μm pieces using a cryostat. The samples were then resuspended in lysis buffer (50 mM Tris pH 8.0, 150 mM NaCl, 5 mM EDTA, 1% Triton) and left on ice for 1 h and disruption of the tissue was achieved using ultra sonication (Soniprep 150, 3 cycles of 10 amplitudes for 10 s). After this time the sample was left on ice for a further 30 min and the suspension was spun down using a bench-top centrifuge at 16,100 *g* for 10 min at 4 °C. The protein supernatant was aliquoted into separate Eppendorf tubes and stored at −80 °C prior to analysis.

2.5. Immobilisation of proteins to create 'bait' arrays

2.5.1. Coupling rLEKTI to the array surface

Recombinant LEKTI (rLEKTI) was diluted in phosphate buffered saline (PBS, pH 7.4) to a final concentration of ~1 pmol/μl. A total of 4.3 pmol was added to each spot on RS100 arrays (BioRad, UK). The array was incubated overnight at 4 °C in a humidity chamber. Excess buffers were removed using a clean tissue. Any unreacted carbonyldiimidazole sites present on the surface of the array were blocked by the addition of 5 μl of 0.5 M ethanolamine/50 mM Tris, pH 8.0, followed by incubation at room temperature (20 °C), for 30 min in a humidity chamber. After this time, the excess blocking solution was removed and the array washed for 15 min with 8 ml of PBS, pH 7.4, 0.1% Triton X-100. The array was washed for a further 15 min in 8 ml PBS containing no Triton X-100, followed by a final 10 minute wash with 10 ml of PBS, pH 7.4 containing no Triton. Excess PBS wash buffer was removed from the areas around the spots using a tissue. Care was taken not to touch the surface of the spots containing bound rLEKTI. All protein arrays were used immediately.

2.5.2. Optimisation of the affinity capture of serine proteases using immobilised LEKTI arrays

All proteases were diluted to a final concentration of 2.2 pmol/μl using 50 mM ammonium bicarbonate, pH 7.8 and 5 μl of the diluted sample (10.8 pmol) was added to the array surface containing the immobilised rLEKTI. The array was incubated for 2 h at room temperature (20 °C) in a humidity chamber. Excess sample solution was removed from the spots and the array was washed twice in 8 ml of PBS, pH 7.4 containing 0.1% Triton X-100 for 15 min. The array was transferred to a fresh tube and washed in 8 ml PBS, containing no Triton, for 5 min. The buffer was poured off and the array was rinsed briefly (10 s) 3 times in 8 ml of 5 mM ammonium acetate, pH 7 to

Download English Version:

https://daneshyari.com/en/article/1225828

Download Persian Version:

https://daneshyari.com/article/1225828

Daneshyari.com