Journal of Chromatography A, xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Review article

Use of chromatographic and electrophoretic tools for assaying elastase, collagenase, hyaluronidase, and tyrosinase activity

Syntia Fayad, Philippe Morin, Reine Nehmé*

Institut de Chimie Organique et Analytique (ICOA), Université d'Orléans – CNRS, UMR 7311, Orléans, France

ARTICLE INFO

Article history:
Received 15 June 2017
Received in revised form 20 October 2017
Accepted 1 November 2017
Available online xxx

Keywords:
Capillary electrophoresis
enzymatic assays
high performance liquid chromatography
microscale thermophoresis
kinetic constants

ABSTRACT

Elastase, collagenase, hyaluronidase and tyrosinase, are very interesting enzymes due to their direct implication in skin aging and as therapeutic hits. Different techniques can be used to study these enzymes and to evaluate the influence of effectors on their kinetics. Nowadays, analytical techniques have become frequently used tools for miniaturizing enzyme assays. The main intention of this article is to review chromatographic and electrophoretic tools that study the four enzymes above mentioned. More specifically, the use of high-performance liquid chromatography and capillary electrophoresis and their derivative techniques for monitoring these enzymes will be investigated. The advantages and limitations of these assays will also be discussed. The original use of microscale thermophoresis and thin layer chromatography in this domain will also be covered.

Contents

1. Introduction

The skin is composed of three layers; the epidermis, dermis and the subcutaneous layer [1]. The extracellular matrix (ECM) is the outermost part of the skin and contains essential components for wound healing [2]. The molecules associated with the ECM of each tissue, including collagens, proteoglycans, laminins and fibronectin, and the manner in which they are assembled determine the structure and the organization of the ECM [3]. The degradation of ECM is thus linked to skin aging. Different intrinsic and extrinsic factors can affect the skin and cause its damage [4,5]. Intrinsic structural changes occur as a natural consequence of aging

Abbreviations: BGE, background electrolyte; BTH, bovine testicular hyaluronidase; CS, chondroitin sulfate; ECM, extracellular matrix; EMMA, electrophoretically mediated microanalysis; FAC, frontal analysis chromatography; HA, hyaluronic acid; HNE, human neutrophil elastase; Hyal, hyaluronidase; IB, incubation buffer; LIF, laser induced fluorescence; MMP, matrix metalloproteinases; MST, microscale thermophoresis; PMMA, pressure-mediated microanalysis; SFE, skin fibroblast elastase; TDLFP, transverse diffusion of laminar flow profiles; Tyr, tyrosinase.

* Corresponding author at: Institut de Chimie Organique et Analytique (ICOA), Université d'Orléans, BP 6759, rue de Chartres, 45067 Orléans cedex 2, France.
E-mail address: reine.nehme@univ-orleans.fr (R. Nehmé).

https://doi.org/10.1016/j.chroma.2017.11.003
0021-9673/

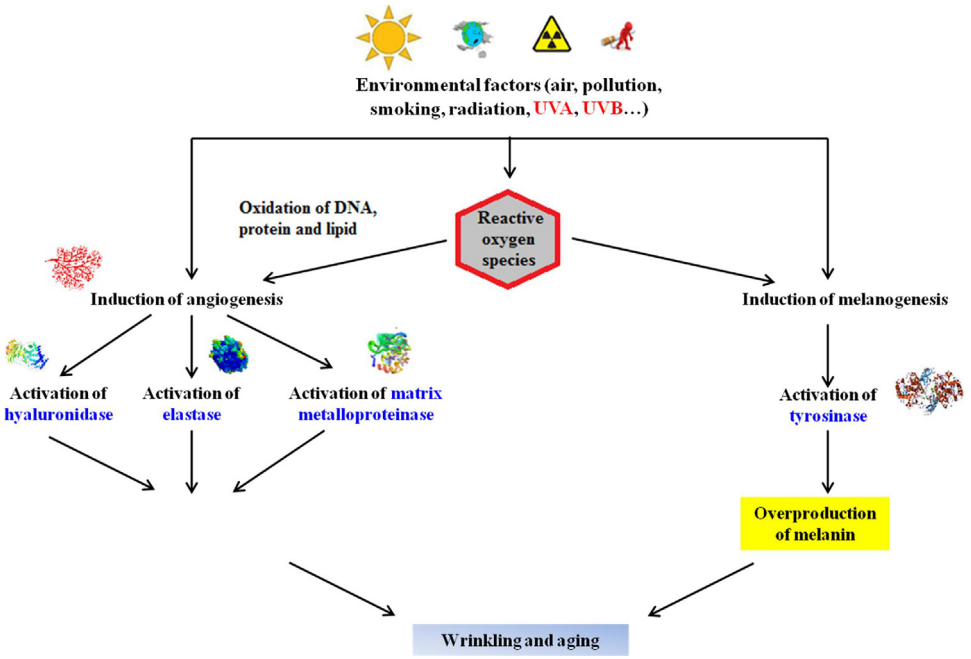

Fig. 1. Schematic representation of skin aging.

and are genetically determined while extrinsic factors are, to varying degrees, controllable. The extrinsic agents include exposure to UV sunlight, pollution or nicotine, repetitive muscle movements like squinting or frowning, and miscellaneous lifestyle components such as diet, sleeping position, and overall health [6–8]. Solar radiation can damage human skin such as an acute overexposure that causes clinical sunburn. Chronic exposure can lead to skin changes such as plaque-like thickening, loss of skin tone, deep furrowing and fine wrinkle formation. Collectively, these changes may be termed 'photoaged skin' or 'solar scar'. The solar UV radiation has particularly a negative effect on the health of the skin, since they involve the generation of reactive oxygen (ROS) species and cellular oxidative damage [9]. Fig. 1 shows the induction of skin aging caused by environmental factors and more specifically by solar UV. The generated ROS induce angiogenesis, which is the process of generating new blood vessels [10,11]. However, the blood vessels are also decreased in chronically UV-damaged human skin. This damage is known to result in marked degenerative changes of the upper dermal connective tissue with degradation of elastic fibers, collagen fibers and hyaluronic acid (Fig. 2). This is probably due to increased expression of elastase, matrix metalloproteinase (MMP), and hyaluronidase (hyal) produced in keratinocytes, fibroblasts and various inflammatory cells (Fig. 1). On the other hand, UV sunlight and ROS activates melanogenesis, which is the process of melanin production and distribution [12–14]. Tyrosinase (tyr), the key protein involved in skin pigmentation due to the presence of melanin pigment, is activated and a hyperpigmentation is produced [12]. Therefore, understanding the evolution of enzymes in the human body is important to understand and to find new therapeutics for a broad range of biological phenomena [15,16].

Elastase, known for its capacity to cleave proteins, prefers the amino acids valine and alanine (Fig. 2.a). Available evidence suggests that there are at least two main types of elastases implicated in the skin degradation, human neutrophil elastase (HNE) and skin fibroblast elastase (SFE). HNE is derived from neutrophil cells and SFE from fibroblasts and these two differ in their substrate specificity [17]. HNE is a 29 kD serine endoprotease of the proteinase S1 family. It exists as a single 238 amino acid-peptide chain with four disulfide bonds and contains two or three N-linked glycans of variable composition that account for its three major isoforms with an isoelectric point of 8.77 – 9.55. Natural substrates include elastin, cartilage proteoglycans, collagen types I, II, III and IV, and fibronectin [18]. SFE has been characterized as a membrane-bound metalloproteinase with little information about its structure, amino acid sequence, and encoding gene. It acts on oxytalan fibers and elaunin fibers but has limited effects on mature elastic fibers. Several studies suggests that SFE is identical to neprilysin, (neutral endopeptidase 24.11 or NEP) due to similarities in their molecular weights and activity profiles [19,20]. HNE and SFE are also implicated in the metabolism of elastic fibers in various types of tissues during inflammation or diseases [21,22].

The matrix metalloproteinases (MMPs) are a family of zinc and calcium dependent enzymes which are important in the resorption of ECM in normal physiological processes and pathological states [7]. These enzymes are responsible for the degradation of ECM molecules and are also implicated in the tissue remodeling, which accompanies inflammation, bone resorption, atherosclerosis, and the invasion of tumors [23]. Most of collagnases belongs to the MMP family. Collagenases are responsible for collagen degradation by breaking the peptide bonds (Fig. 2.b). Collagen is the major insoluble fibrous protein in the ECM and the connective tissue and its primary function is to maintain skin firmness. Sixteen types of collagen exist and around 80 – 90% of the collagen in human body only consists of types I, II, and III. Different types of collagenases exist like gelatinase B, which cleaves gelatine type I and collagen types IV and V, gelatinase A which cleaves gelatine type I and collagen types IV, V, VII and X and neutrophil collagenase which cleaves interstitial collagens with a preference for collagen type I [24].

Skin aging is also associated with loss of skin moisture where the most important key molecule involved is hyaluronic acid (HA). This high molecular weight mucopolysaccharide ($1.5\text{-}1.8 \times 10^6$ Da) was discovered in 1934 by Karl Meyer in the vitreous of bovine eyes [25]. This non-sulphated glycosaminoglycan is composed of repeating polymeric disaccharides of D-glucuronic acid and N-acetyl-D-glucosamine linked by a glucuronidic β $(1 \rightarrow 3)$ bond (Fig. 2.c) [26]. Its function is, amongst other things, to bind water and to lubricate movable parts of the body, such as joints and muscles. Its consistency and tissue-friendliness allow it therefore to be

Please cite this article in press as: S. Fayad, et al., Use of chromatographic and electrophoretic tools for assaying elastase, collagenase, hyaluronidase, and tyrosinase activity, J. Chromatogr. A (2017), https://doi.org/10.1016/j.chroma.2017.11.003