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Research Paper

A potential role for endogenous proteins as sacrificial sunscreens and antioxidants in human tissues

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ABSTRACT

Excessive ultraviolet radiation (UVR) exposure of the skin is associated with adverse clinical outcomes. Although both exogenous sunscreens and endogenous tissue components (including melanins and tryptophan-derived compounds) reduce UVR penetration, the role of endogenous proteins in absorbing environmental UV wavelengths is poorly defined. Having previously demonstrated that proteins which are rich in UVR-absorbing amino acid residues are readily degraded by broadband UVB-radiation (containing UVA, UVB and UVC wavelengths) here we hypothesised that UV chromophore (Cys, Trp and Tyr) content can predict the susceptibility of structural proteins in skin and the eye to damage by physiologically relevant doses (up to 15.4 J/cm^2) of solar UVR (95% UVA, 5% UVB). We show that: i) purified suspensions of UV-chromophore-rich fibronectin dimers, fibrillin microfibrils and β- and γ-lens crystallins undergo solar simulated radiation (SSR)-induced aggregation and/or decomposition and ii) exposure to identical doses of SSR has minimal effect on the size or ultrastructure of UV chromophore-poor tropoelastin, collagen I, collagen VI microfibrils and α-crystallin. If UV chromophore content is a factor in determining protein stability *in vivo*, we would expect that the tissue distribution of Cys, Trp and Tyr-rich proteins would correlate with regional UVR exposure. From bioinformatic analysis of 244 key structural proteins we identified several biochemically distinct, yet UV chromophore-rich, protein families. The majority of these putative UV-absorbing proteins (including the late cornified envelope proteins, keratin associated proteins, elastic fibre-associated components and β- and γ-crystallins) are localised and/or particularly abundant in tissues that are exposed to the highest doses of environmental UVR, specifically the stratum corneum, hair, papillary dermis and lens. We therefore propose that UV chromophore-rich proteins are localised in regions of high UVR exposure as a consequence of an evolutionary pressure to express sacrificial protein sunscreens which reduce UVR penetration and hence mitigate tissue damage.

Introduction

Chronic exposure to ultraviolet radiation (UVR) produces dose-dependent changes in skin structure which have a profound impact on both its mechanical function and clinical appearance (see [1,2] for comprehensive reviews of this photoageing process). In the early stages of photoageing key elastic fibre-associated components such as fibrillin microfibrils and fibulin-5 are lost from the papillary dermis [3,4], whilst the latter stages are characterised by the deposition of disorganised elastotic material (solar elastosis: [5,6], the gain of glycosaminoglycans [7] and the loss of both fibrillar collagens [8,9] and collagen VII anchoring fibrils [10]. Although the potential role of cell-derived extracellular matrix (ECM) proteases in mediating dermal remodelling is well established [11–14] the substrate specificity of the UVR up-regulated

Abbreviations: AFM, atomic force microscopy; ECM, extracellular matrix; HDF, human dermal fibroblast; LTBP, latent transforming growth factor β-binding protein; MED, minimal erythemal dose; MMP, matrix metalloproteinase; ROS, reactive oxygen species; SSR, solar simulated radiation; TGFβ, transforming growth factor β; UVR, ultraviolet radiation.

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matrix metalloproteinases (MMPs-1, −2, −3, −7, −9 and −12) is low [15]. Collectively, these enzymes are capable of degrading many key ECM components including fibrillar collagens, elastic fibre constituents, proteoglycans, adhesive glycoproteins and dermal-epidermal junction components [16–18]. Therefore it is difficult to reconcile the concept of non-specific cell-derived ECM proteases as the sole mediators of matrix degradation with the complex spatial, compositional and temporal ECM remodelling which characterises chronically UV-exposed skin. In common with other groups [19,20] we have therefore suggested that the direct photochemical decomposition of ECM proteins may be an important factor in mediating differential remodelling of the dermis in photoaged skin [15,21,22].

Whilst exposure to UVR can profoundly influence collagen structure and function, such changes have been induced using sources which emit large doses of UVC (< 280 nm) radiation (for example [23,24]). In contrast, the wavelengths which comprise sunlight at the Earth's surface and are capable of penetrating to the dermis (UVA and UVB: Fig. 1) [25] have a minimal effect on collagen structure and function [15,20,26,27] even when the doses employed are more than two orders of magnitude greater than the minimal erythemal doses (MEDs) of the respective wavebands (see Watson et al. [22] for a comprehensive review). Although collagen is largely devoid of sulphur- (cysteine [Cys]) and aromatic ring-containing (Histidine [His], Phenylalanine [Phe], Tryptophan [Trp] and Tyrosine [Tyr]) amino acid residues other ECM components contain a much larger percentage of these UV chromophores [28]. We have previously demonstrated that fibrillin microfibrils (essential elastic fibre components) and the ubiquitous adhesive glycoprotein fibronectin (with UV chromophore contents of 21% and 13% respectively) are susceptible to low doses of broadband UVB radiation (Philips TL-12 source) which have no effect on the electrophoretic mobility of collagen I (UV chromophore content 2%) [15]. However, whilst this broadband UVB source is used extensively in photobiology research, its spectral output is dissimilar to terrestrial solar UVR having a small UVC content and crucially a larger UVB:UVA ratio (Fig. 1a) [29]. Studies on skin optics predict that longer wavelength UVR will penetrate the skin more deeply and there is a consensus that UVA (as opposed to UVB) radiation is the key wave band responsible for photoageing (Fig. 1b) [1,30,31].

In this study we have used a combination of biochemical and ultrastructural analyses to test the hypothesis that environmentally relevant doses of solar simulated radiation (SSR: 95.0% UVA, 5.0% UVB) and UVA radiation (filtered SSR: 99.6% UVA, 0.4% UVB) are capable of differentially degrading key dermal ECM components (collagen I, tropoelastin, collagen VI, fibrillin microfibrils, fibronectin) and lens proteins ((α-, β- and γ-crystallins) according to their terrestrial UV chromophore (Cys, Trp and Tyr) [32–34] content. As a consequence of their high molecular weight, ECM proteins such as fibrillin-1 and fibronectin contain many potential amino acid UV chromophores (fibrillin-1 [Accession number: P3555] for example contains 362 Cys, 13 Trp and 92 Tyr residues). This compositional complexity (which in the case of fibrillin microfibrils is combined with a resistance to dissociation into component monomers), makes it impractical to characterise the effects of UVR exposure on individual amino acid residues. Instead we employed biochemical approaches, including gel electrophoresis which has been used extensively by ourselves, and others, to probe the effects of UVR on the structure of fibronectin, collagen I and tropoelastin and the lens crystallins (see [24] and our comprehensive review of collagen and UVR [22]). For the remaining assemblies, fibrillin and collagen VI microfibrils we characterised the effects of UVR on their ultrastructure. It is clear that the beads-on-a-string morphology of these microfibrils is susceptible to single amino acid substitutions and also to the local micro-

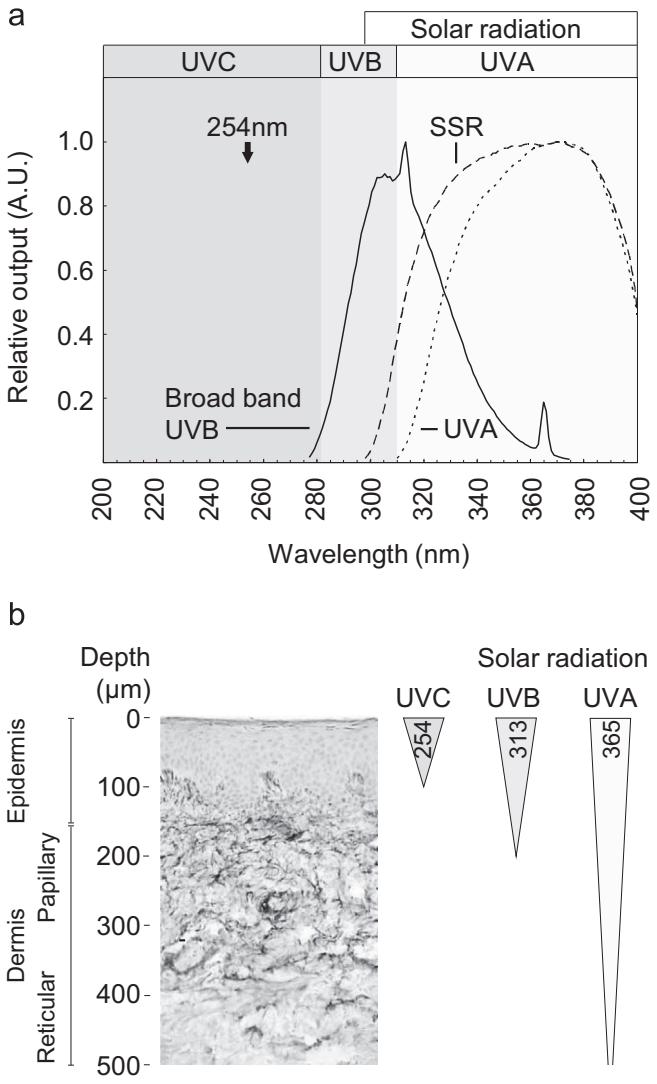

Fig. 1. Solar simulated radiation is primarily composed of penetrating, yet low energy, UVA wavelengths. (a) Normalised spectral outputs of broadband UVB, solar simulated radiation (SSR) and filtered SSR (UVA) sources. The spectral output of SSR (WG320 filtered xenon arc lamp) is composed of 5.0% UVB radiation. Further filtration (with WG345) removes the majority of the UVB component (UVA: 99.6%, UVB: 0.4%). In contrast, the spectral output of a broadband UVB source such as the Philips TL-12 [15] contains UVA (44.3%), UVB (55.3%) and UVC (0.4%) components whilst the output of many UVC-rich sources peaks at 254 nm. (b) Although such 254 nm UVC radiation can influence the epidermis, solar UVB and UVA radiations can penetrate the papillary dermis and subcutaneous tissue respectively (adapted from [31]).

environment [15,16,35–37]. Finally we have related UV chromophore content to tissue location (within skin and eyes) and formulated a secondary hypothesis that amino acid chromophore-rich proteins compliment the sunscreen activity of other endogenous chromophores including melanins and tryptophan-derived components such as N′-formylkynurenine and 6-formylindolo[3,2-b]carbazole [38].

Materials and methods

All chemicals were of analytical grade and obtained from Sigma-Aldrich Co. Ltd. (Poole, UK) unless otherwise stated.