Journal of Structural Biology 194 (2016) 282–291

Contents lists available at ScienceDirect

Journal of Structural Biology

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

The molecular organization of the beta-sheet region in Corneous beta-proteins (beta-keratins) of sauropsids explains its stability and polymerization into filaments

Matteo Calvaresi [a,*], Leopold Eckhart [b], Lorenzo Alibardi [c]

[a] Department of Chemistry "Giacomo Ciamician", University of Bologna, Italy
[b] Research Division of Biology and Pathobiology of the Skin, Department of Dermatology, Medical University of Vienna, Austria
[c] Comparative Histolab and Department of Bigea, University of Bologna, Italy

ARTICLE INFO

Article history:
Received 5 December 2015
Received in revised form 29 February 2016
Accepted 5 March 2016
Available online 7 March 2016

Keywords:
Reptiles
Birds
Corneous beta-proteins
3D-modeling
Polymer formation

ABSTRACT

The hard corneous material of avian and reptilian scales, claws, beak and feathers is mainly derived from the presence of proteins formerly known as beta-keratins but now termed Corneous beta-proteins of sauropsids to distinguish them from keratins, which are members of the intermediate filament protein family. The modeling of the conserved 34 amino acid residues long central beta-sheet region of Corneous beta-proteins using an *ab initio* protein folding and structure prediction algorithm indicates that this region is formed by four antiparallel beta-sheets. Molecular dynamic simulations and Molecular Mechanics/Poisson Boltzmann Surface Area (MM–PBSA) analysis showed that the disposition of polar and apolar amino acids within the beta-region gives rise to an amphipathic core whose stability is further increased, especially in an aqueous environment, by the association into a dimer due to apolar interactions and specific amino-acid interactions. The dimers in turn polymerize into a 3 nm thick linear beta-filament due to van der Waals and hydrogen-bond interactions. It is suggested that once this nuclear core of anti-parallel sheets evolved in the genome of a reptilian ancestor of the extant reptiles and birds about 300 millions years ago, new properties emerged in the corneous material forming scales, claws, beaks and feathers in these amniotes based on the tendency of these unique corneous proteins to form stable filaments different from keratin intermediate filaments or sterical structures formed by other corneous proteins so far known.

1. Introduction

The process of formation of corneous material in the corneous layer of the epidermis and in skin derivatives (nails, hairs, claws, scales, feathers, etc.) in vertebrates is based on the deposition of specialized structural proteins, here referred to as corneous proteins. The basic cytoskeleton of cornifying cells is made up of the intermediate (10 nm diameter) filament proteins of the Type I and Type II keratin families. Keratins, also known as alpha-keratins or cytokeratins, have the classical intermediate filament structures with a central alpha-helical domain that facilitates heterodimerization and filament formation (Steinert and Freedberg, 1991; Kalinin et al., 2002; Rogers, 2004; Alibardi, 2006; Eckhart et al., 2013). Beta-sheets are either absent or very short in length in these keratins under physiological conditions (Hanukoglu and Fuchs, 1983; Fraser and Parry, 2009) but these regions increase in number and extension under heat or/and strong stretching conditions of the keratin material, as evidenced by X-ray diffraction analysis (Fraser et al., 1972; Kreplak et al., 2004; Paquin and Colomban, 2007; Chou and Buehler, 2012).

While keratins are broadly known in most vertebrates, much less knowledge is available on other filamentous or non-filamentous corneous proteins that associate to keratins during the process of cornification, especially in non-mammalian vertebrates. In mammals, corneous proteins such as involucrin and loricrin are encoded by a gene cluster in a locus indicated as Epidermal Differentiation Complex (EDC, Mischke et al., 1996). Keratin Associated Proteins (KAPs), representing another mammal-specific group of proteins that interact with keratins within hair and nails, are encoded by genes outside of the EDC (Rogers et al., 2006; Wu et al., 2008). Therefore, three main groups of proteins cooperate to form the corneous material of the skin and skin derivatives in

* Corresponding author at: Department of Chemistry G. Ciamician, via Selmi 2, University of Bologna, 40126 Bologna, Italy.
E-mail address: matteo.calvaresi3@unibo.it (M. Calvaresi).

http://dx.doi.org/10.1016/j.jsb.2016.03.004
1047-8477/

mammals: keratins, KAPs and Corneous EDC proteins. While the keratins form filaments, KAPs form the inter-filamentous matrix surrounding keratin filaments in mammalian skin derivatives, and are believed to establish true chemical bonds or a chemical–physical interaction with the filaments of keratins (Rogers, 2004; Rogers et al., 2006; Chou and Buehler, 2012). Corneous EDC proteins associate to keratins to form the corneous cell envelope of epidermal corneocytes (Kalinin et al., 2002; Eckhart et al., 2013).

Non-mammalian amniotes, i.e. reptiles and birds (together known as sauropsids) have another type of corneous skin and skin appendage proteins, traditionally indicated as beta-keratins (Baden and Maderson, 1970; Fraser et al., 1972; Wyld and Brush, 1979, 1983; Sawyer et al., 2000; Greenwold and Sawyer, 2011, 2013; Greenwold et al., 2014). Biomolecular studies on these proteins in birds (Gregg et al., 1984; Presland et al., 1989; Whitbread et al., 1991) and reptiles (Dalla Valle et al., 2005, 2007a,b, 2008, 2009a,b, 2010; Alibardi et al., 2007, 2009; Hallahan et al., 2008), have indicated that they present a central region of 34 amino acids of high homology among sauropsids. Different from the known structural domains of the three types of aforementioned corneous proteins, the central region of Corneous beta proteins is predicted to form beta-sheets and therefore has been termed beta-sheet region (Fraser and Parry, 1996, 2011a; Alibardi et al., 2007, 2009). This unique protein segment is thought to allow Corneous beta proteins to form filaments with a 3 nm diameter, indicated as beta-filaments, and part of the inter-filamentous matrix in scales, claws, beaks and feathers (Brush, 1983; Fraser and Parry, 1996, 2011a,b, 2014; Fig. 1). Genomic studies have shown that the genes coding for these non-keratin proteins are arranged within the EDC that is present in both bird and reptile genomes, besides genes encoding other corneous proteins such as loricrin, cornulin, and trichohyalin-like scaffoldin (Vanhoutteghem et al., 2008; Mlitz et al., 2014; Strasser et al., 2014; Holthaus et al., 2015; Fig. 1). The sauropsidian EDC is present in a chromosome location distant from those containing the alpha-keratin genes (Vandebergh and Bossuyt, 2012).

Previous X-ray diffraction studies indicated the general structure of the β-rich central domain (Fraser et al., 1972; Fraser and Parry, 1996), but could not determine the precise structure of the filament-matrix composite by conventional analytical methods. After sequencing of emu feather proteins (O'Donnell, 1973), infrared studies showed that a large fragment from the interior of the molecule was rich in β-sheet regions. The Fourier analysis of the distribution of the β-favoring and turn-favoring residues in this sequence indicated that there was a central domain in which the chain looped backwards and forwards with a periodicity of eight residues resulting in an axial length around 2.4 nm for this domain, a value equal to that of the spacing of a very prominent meridional reflection in the X-ray diffraction pattern of feathers (Fraser and MacRae, 1976). A similar reflection in the X-ray patterns of a range of avian and reptilian hard keratins suggested that similar central domains might be present in corneous material from birds and reptiles (Fraser et al., 1972; Stewart, 1977). This was later determined when the amino acid sequence of a reptilian claw keratin became available (Fraser and Parry, 1996). Further extensive molecular and proteome studies of scale corneous proteins from all non-avian reptilian groups (Dalla Valle et al., 2005, 2007a,b, 2008, 2009a,b,, 2010; Alibardi and Toni, 2006; Alibardi et al., 2007, 2009; Toni et al., 2007), showed that this homology is maintained over the entire range of hard β-keratins in birds and reptiles (Alibardi et al., 2009; Fraser and Parry, 2011a, 2014).

In order to determine the chemical–physical characteristics at the origin of the unique properties of mechanical resistance, inflexibility (but elasticity in some cases) and hydrophobicity of the Corneous beta proteins of sauropsids, the present study is focused on the analysis of the atomistic and molecular assembly responsible for the formation of the stable filamentous polymers of 3 nm indicated as beta-filament. This is achieved by a computational analysis that defines a structure of the central domain of Corneous beta proteins in representative sauropsids. This modeling study points out the atomistic reasons for the high stability of the central amino acid region present in these proteins and supports its role as the

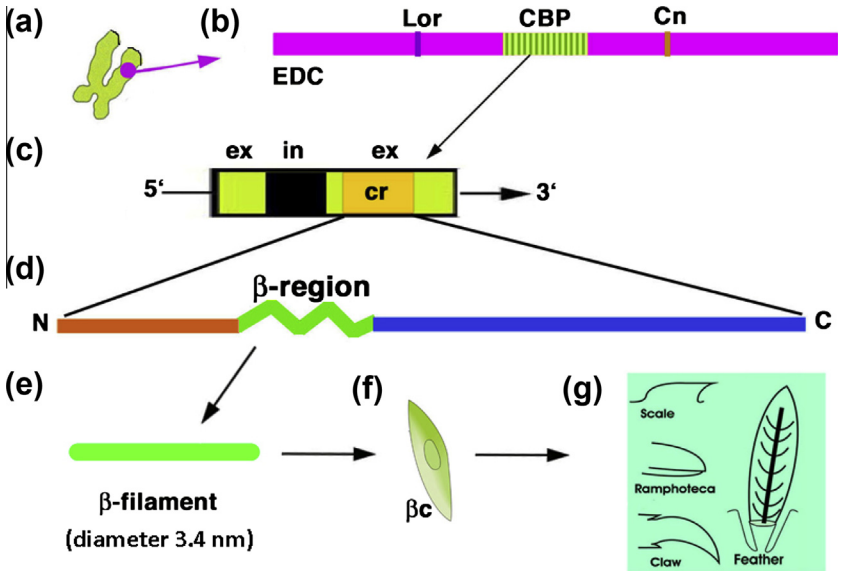

Fig. 1. Schematic representation of the EDC (Epidermal Differentiation Complex) in a chromosome locus (pink dot in A), which is enlarged in B, showing the localization of the cluster of Corneous beta protein (CBP) genes between the genes for Loricrin (Lor) and Cornulin (Cn) (Strasser et al., 2014; Holthaus et al., 2015). In C the general structure of one gene shows the two exons (ex), the intron (in) and the coding region (cr) located within the second exon, and that is translated in Corneous beta proteins (D and E). The beta-region is located between the N (red) and C (blue) terminal regions, and is located anteriorly in beta-proteins of turtles, crocodilians, and birds and more posteriorly in those of lizards and snakes (D). Beta-filaments (E) consisting of CBPs contribute to the dense corneous material within the beta-corneocytes (βc) (F) forming skin appendages such as scales, claws, beak (rhamphotheca) and feathers (G).